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Original Article

Stat3 activation in epidermal keratinocytes induces Langerhans cell activation to form an essential circuit for psoriasis via IL-23 production

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ABSTRACT

Background: Psoriasis is an inflammatory disease associated with aberrant crosstalk between the epidermis and immune system. However, the role of Langerhans cells (LCs) in psoriasis remains controversial.
Objectives: To elucidate whether LCs are functionally involved in the development of psoriasis using a mouse model.
Methods: Two lines of transgenic mice were used and crossed. They included K5.Stat3C, the psoriasis-model mouse and langerin DTR knock-in (KI) mouse. We performed immunofluorescence staining for LCs in psoriatic lesion of human and model mice. Flow cytometric analyses were performed to compare between dendritic cells (DCs) and LCs in the epidermis and skin-draining lymph nodes (sDLNs). To assess cytokine/chemokine expression in the skin lesion or primary cultured keratinocytes, we performed RT-PCR, microarray analysis or intracellular staining on the flow cytometer.
Results: LCs were activated in psoriatic lesion of patients with psoriasis and K5.Stat3C mice. Compared with non-transgenic mice, K5.Stat3C mice constitutively showed an increased number of LCs in the sDLNs before psoriasis-like lesion developed. Stat3C transgenic keratinocytes expressed an elevated level of IL-1α. Psoriasis-like lesion in K5.Stat3C mice were attenuated in the absence of LCs, indicating that LCs were essential to the development of psoriasis-like lesion. Furthermore, we also recognized that epidermal LCs in psoriatic lesion of not only K5.Stat3C mice but also psoriasis patients produced IL-23.
Conclusions: Our study suggests that Stat3 activation in keratinocytes may impact on LC activation in situ via IL-1α stimulation, at least in part, and that their presence may be essential for the pathogenesis of psoriasis through producing IL-23.

1. Introduction

The pathogenesis of psoriasis is multifactorial composed of genetic, environmental and immunological factors. Indeed, psoriasis is formed with dynamic interactions between the immune system and the epidermal keratinocytes. Recent studies have demonstrated that the IL-23/Th17 axis plays an important role as immunological factors in the pathogenesis of psoriasis [1]. The most distinct evidence for the role of IL-23/Th17 in psoriasis comes from clinical studies. The critical role of IL-23 in the pathogenesis of psoriasis has been validated by the therapeutic efficacy of antibodies against IL-23, including those against either p40 or p19 subunits [2,3]. In addition, inhibition of the IL-23/Th17 pathway through the administration of tumor necrosis factor (TNF) and direct targeting of the IL-17A by monoclonal antibodies or the IL-17 receptor inhibitors attenuate psoriasis [4,5]. We previously reported that Stat3 is activated in keratinocytes in the majority of human psoriatic lesion [6]. K5.Stat3C transgenic mice, in which Stat3 is constitutively activated in keratinocytes, develop psoriasis-like lesion spontaneously or in response to wound stimuli or topical treatment with the 12-*O*-tetradecanoylphobol-13-acetate (TPA) [6]. K5.Stat3C mice meet pharmacological criteria and represent one of the most physiologically relevant animal models of psoriasis [7]. Psoriasis-like lesion in K5.Stat3C mice exhibit cytokine profiles similar to those of human psoriatic plaques [8], and both activated Stat3 in keratinocytes and activated T cells are required for the development of psoriasis-like lesion [6]. Further, we also have demonstrated that psoriasis-like lesion in K5.Stat3C mice respond to treatment with antibodies against IL-17A, IL-12/23p40, and IL-23p19 [8]. These findings validate the important role

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of IL-23/Th17 pathway in the development of psoriasis-like lesion in K5.Stat3C mice. In addition, we have demonstrated several crucial findings in the pathogenesis of psoriasis using K5.Stat3C mice [9–14].

Langerhans cells (LCs) are the dendritic cells (DCs) that reside in the epidermal layer of the skin [15]. LCs are activated in response to various stimuli, including microbial products, chemical sensitizers (i.e. haptens) and UV light [16]. LCs arrive in skin draining lymph nodes (sDLNs) as fully mature DCs and function as dedicated antigen-presenting cells (APCs) to efficiently stimulate T lymphocytes [17]. Mature LCs also provide additional signals to drive T-cell polarization through the secretion of appropriate cytokines, to ensure the immune response specifically tailored to the invading pathogen.

In order to examine the role of LCs in various disease processes, murine models, in which LCs can be experimentally ablated, have been established [18–21]. These include langerin-DTR KI mice, which express enhanced green fluorescent protein (eGFP) fused to the diphtheria toxin (DT) receptor (DTR) under the control of the langerin promoter [19]. Both LCs and langerin$^+$ dermal dendritic cells (dDCs) can be depleted by administration of DT in this mouse model.

The role of LCs play in the pathogenesis of psoriasis remains controversial, since previous studies have reported conflicting results [22–27]. In the present study, we investigated whether LCs were involved in the development of psoriasis-like lesion in K5.Stat3C:langerin DTR-KI mice. We show that LCs, but not langerin$^+$ dDCs, are essential to the development of psoriasis-like lesion in K5.Stat3C mice and Stat3 activation in keratinocytes leads to LC activation and migration to sDLNs.

2. Materials and methods

2.1. Patient with psoriasis

The study protocol was conducted in accordance with the guidelines of the World Medical Association's Declaration of Helsinki and was approved by the Institute Ethical Review Board of the Kochi Medical School, Kochi University. Three healthy volunteers and three patients with psoriasis were recruited. Skin biopsy specimens were taken from lesion of patients with psoriasis and healthy volunteers.

2.2. Mice

All experimental procedures performed on mice were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School. K5.Stat3C mice were generated as previously reported, and hemizygous transgenic mice were used in all experiments. TPA treatment was assessed following topical treatment on days 1 and 3 with 0.68 nmol TPA (Sigma) in 20 μl acetone on the right ear and 20 μl acetone alone on the left ear as the vehicle control. The ear skins and sDLNs were sampled on day 4. Thickness of epidermis was measured at 10 areas in the interfollicular epidermis stained with H&E. Langerin DTR-KI mice were kindly provided by Dr. Bernard Malissen.

2.3. Generation of K5.Stat3C:langerin DTR KI mice

Langerin DTR-KI mice expressed the human diphtheria toxin (DT) receptor under the control of the langerin promoter. To deplete langerin$^+$cells, 1 μg of DT (Sigma-Aldrich) was injected to the mice intraperitoneally, 1day before TPA treatment. DT treatment ablated LCs and langerin$^+$ dermal dendritic cells (DCs). We crossed the langerin DTR-KI mice with the K5.Stat3C mice to generate K5.Stat3C:langerin DTR KI mice.

2.4. Immunofluorescence microscopy

For detection of Langerhans cells and IL-23 in skin of human psoriatic lesion and mouse psoriasis-like lesion, snap-frozen sections were treated with a blocking reagent (Protein Block Serum-Free; Dako) for an hour at room temperature and followed incubation with first antibody for over night at 4 °C. The mouse anti-CD1a mAb (clone NA1/34, Dako) was used for human skin, the rat anti-CD207 Ab (clone eBioRMUL.2, eBioscience) was used for mouse skin, and anti-mouse and human IL-23p19 Ab (clone ab45420, abcam), followed by anti-mouse IgG-Alexa 488 (Invitrogen), anti-rat IgG-Alexa 488 (Invitrogen), and anti-rabbit IgG-Alexa 594 (abcam).

2.5. Preparation of epidermal cell and dermal cell suspensions

Epidermal and dermal cell suspensions were prepared as follows; briefly, ear skins were harvested from mice. After removal of subcutaneous tissues, the tissues were floated with epidermal side up onto 0.5%diapase solution for 45 min at 37 °C. The epidermis and dermis were separated and epidermal sheets were put in 0.3% trypsin / PBS with 0.1% DNase. After incubation with shaking for 10 min at RT, RPMI1640 medium and 0.1% DNase were added and shaked vigorously for several seconds. Epidermal cell suspension was filtered through a 40-μm cell strainer and collected. Dermal sheets were minsed in HBSS supplemented with 1000 U/ml collagenase type 4 (Worthington), 1000 U/ml hyaluronidase (SIGMA) and DNase and incubated for 60 min at 37 °C. Dermal cell suspension was filtered through a 40-μm cell strainer and collected.

2.6. Keratinocyte culture

Primary keratinocytes were isolated from the skin of K5.Stat3C newborn mice and non-transgenic mice as described [6]. In brief, epidermis was isolated by overnight digestion of dorsal skin from K5.Stat3C newborn mice with dispase (Becton Dickinson) at 4 °C. The epidermis was incubated with 0.25% trypsin (Life Technologies) for 5 min at 37 °C. After stopping the trypsin reaction with FBS, the keratinocyte suspension was passed through a 70 μm cell strainer. Freshly isolated primary keratinocytes were plated at 2.5×10^5cells/well in 24-well plates in Epilife (Life Technologies) supplemented with Hu-Media KG (Kurabo). Cells were cultured until they were subconfluent, then used for isolation of RNA.

2.7. Flow cytometric analysis

For dead cells exclusion, single-cell suspensions from sDLNs or epidermal and dermal suspensions were stained LIVE/DEAD Fixable Near IR Dead Cell Stain Kit (Life Technologies) prior to incubation with anti-CD16/32 for blocking FcR (BD Biosciences). Cells of sDLNs were stained for 20 min with anti-CD11c, anti-MHC class II, anti-CD326, anti-CD103, anti-CD80, and anti-CD86, followed by permeabilization with Cytofix/Cytoperm buffer (BD Phamingen) and intracellular staining with anti-CD207 mouse antibody (BioLegend). The detail of antibodies used in this study was listed in Supplementary Table 1. For intracellular staining with IL-17A, cells were stimulated for 4 h with 0.2 μg ml^{-1} PMA (Wako Chemicals), 2.7 μM ionomycin (Sigma-Aldrich), and 40 μg ml^{-1} Brefeldin A (Sigma-Aldrich) and stained by anti-CD4 and anti-CD8. For intracellular staining with anti–IL-23p19, cells of sDLNs were stimulated for 3 h with 10 μg ml^{-1} Brefeldin A (Sigma-Aldrich). Then cells were stained for 20 min with anti CD11c, anti MHC class II, followed by permeabilization with Cytofix/Cytoperm buffer (BD Pharmingen) and intracellular staining with anti-p19IL-23 and anti-CD207. Samples were acquired on a FACSCalibur flow

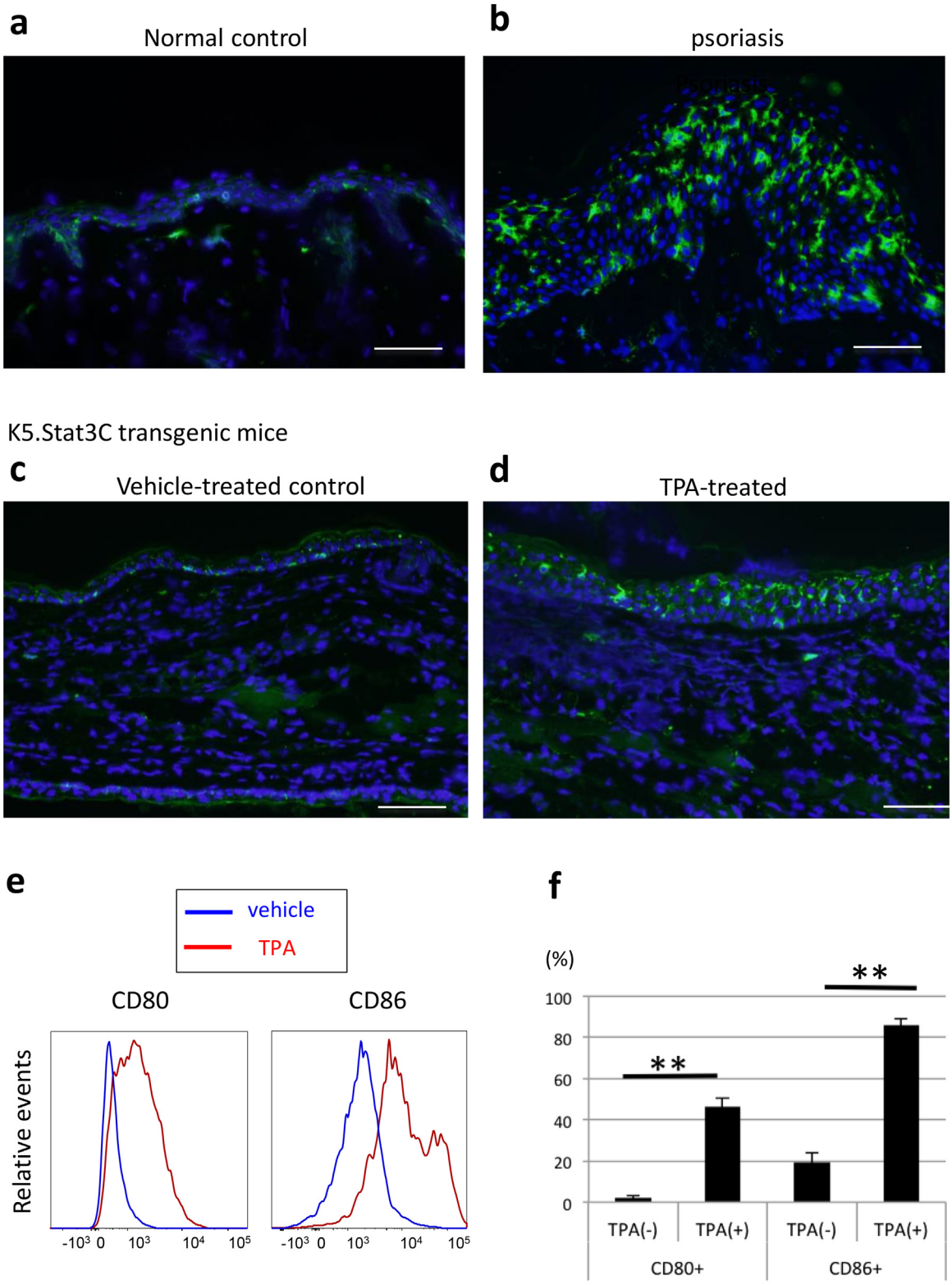

Fig. 1. Langerhans cells are activated in psoriatic lesion of human and K5.Stat3C mice.
(a, b) Representative anti-CD1a immunostaining of normal human skin (a) and psoriatic lesion from a patient with PASI of 11 (b). (c, d) K5.Stat3C mouse ear skin treated with acetone as vehicle (c) and TPA (d), showing psoriatic change, stained with anti-CD207 (langerin, green). Scale bars = 100 μm (a, c), 50 μm (b, d). (e) A representative histogram on flow cytometric analysis with anti-CD80 and CD86 of the epidermal LCs (% of the gated by $CD11c^{+}$MHC $classII^{+}CD207^{+}CD326^{+}$cells) from K5.Stat3C mice topically treated with vehicle (black lines) and TPA (red lines). (f) The percentages are shown for the expression of CD80 and CD86 in LCs from vehicle-treated (n = 5) and TPA-treated mice (n = 5). **, $p < 0.01$.

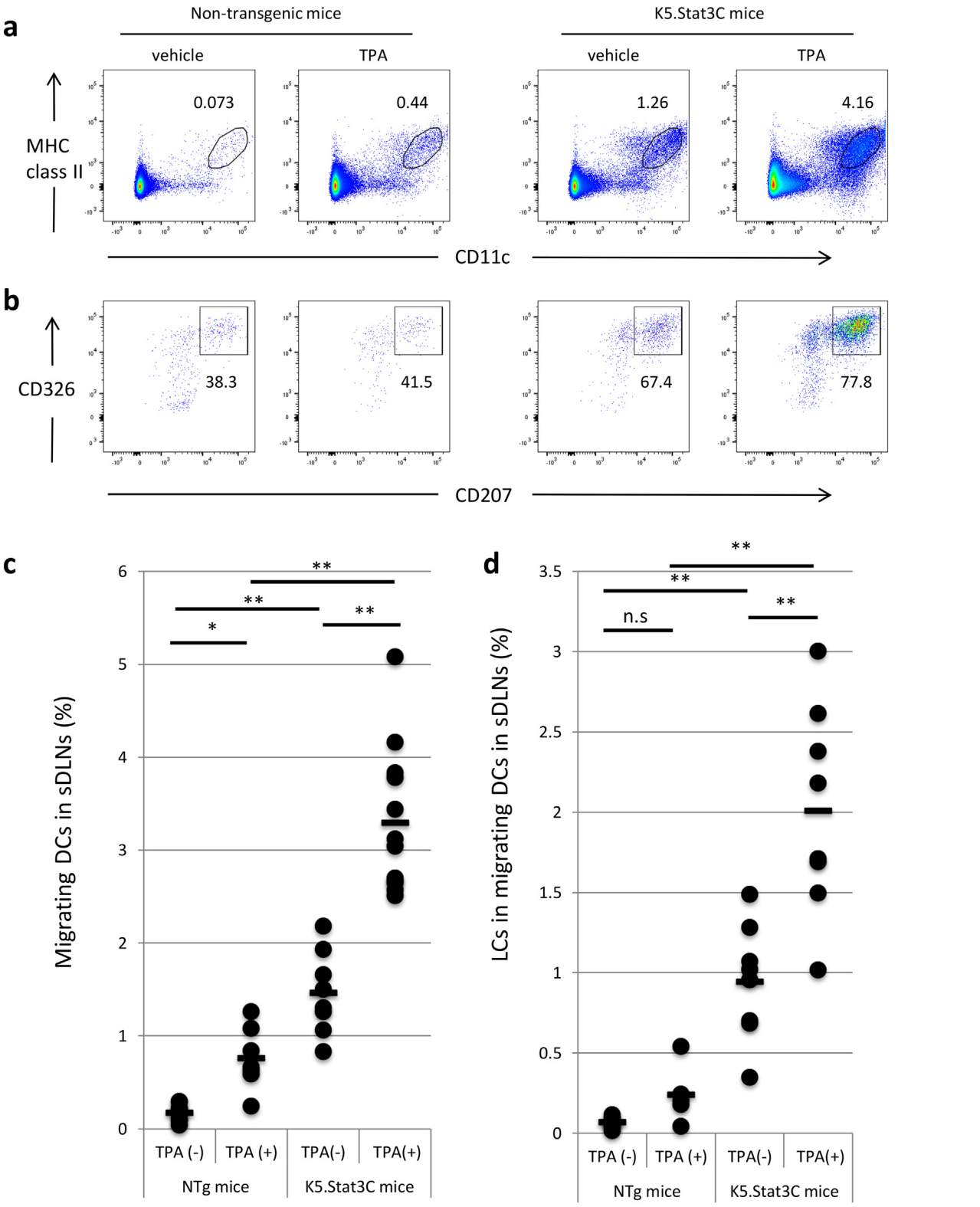

Fig. 2. Langerhans cells in Stat3 activated keratinocytes migrate to skin-draining lymph nodes (sDLNs).
Flow cytometric analysis of migrating DCs, ($CD11c^{+}$MHC-class II^{high} population) and LCs ($CD207^{+}CD326^{+}$ cells in subset of $CD11c^{+}$MHC-class II^{high} population) in sDLNs of non-transgenic and K5.Stat3C mice treated with acetone (vehicle control) and TPA. (a, b) The representative plots with migrating DCs (oval areas in a, % shown by number) and LCs (square areas in b, % shown by number in the gated oval areas in Fig. 2a). (c, d) Quantification of the rate of migrating DCs (%, oval area) and LCs (multiplying % in oval area in Fig. 2a by % in square area in Fig. 2b). Non-transgenic (NTg) mice treated with acetone (n = 9) or TPA (n = 7); K5.Stat3C mice treated with acetone (n = 8) or TPA (n = 13). Mean values were shown by horizontal bars. **, $p < 0.01$; *, $p < 0.05$; n.s, not significant; the one-way ANOVA followed by Bonferroni's test.

cytometer (BD Bioscience), and data analysis was conducted using FlowJo software (TreeStar).

2.8. RNA isolation and real-time RT-PCR

Skin biopsy specimens from mice were minced with scissors into small pieces on ice, then disrupted by ultrasonic sonication in buffer. Total RNAs were extracted using an RNA isolation kit (Promega) according to the manufacturer's protocol and were reverse transcribed using M-MLV reverse transcriptase (Invitrogen) with random oligonucle- otide hexamers (Invitrogen). PCR reactions were performed using Power SYBER Green PCR Master Mix (Applied Biosystems), and amplification conditions were as follows: 50 °C for 2 min, 90 °C for 10 min for 1 cycle, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The primers used were described in Supplementary Table 2. The quantity of each transcript was analyzed using the 7300 Fast System Software (Applied Biosystems) and was normalized to hypoxanthine phosphoribosyltransferase (HPRT) according to the ΔΔCt method.

2.9. Cell sorting

The LCs were separated by centrifugation at 1600 rpm for 15 min with discontinuous density-gradient consist of 60, 40% Percoll (GH Heathcare) layer. LCs were concentrated above 60% Percoll layers, whereas keratinocytes moved to the lower layer (higher density). For cell sorting, cells were blocked Fc with saturating amounts of anti-CD16/CD32 (2.4G2), then the following antibodies were used for surface staining: anti-mouse CD11c (clone N418 labeled with BV 421, BioLegend), anti-mouse I-A/I-E

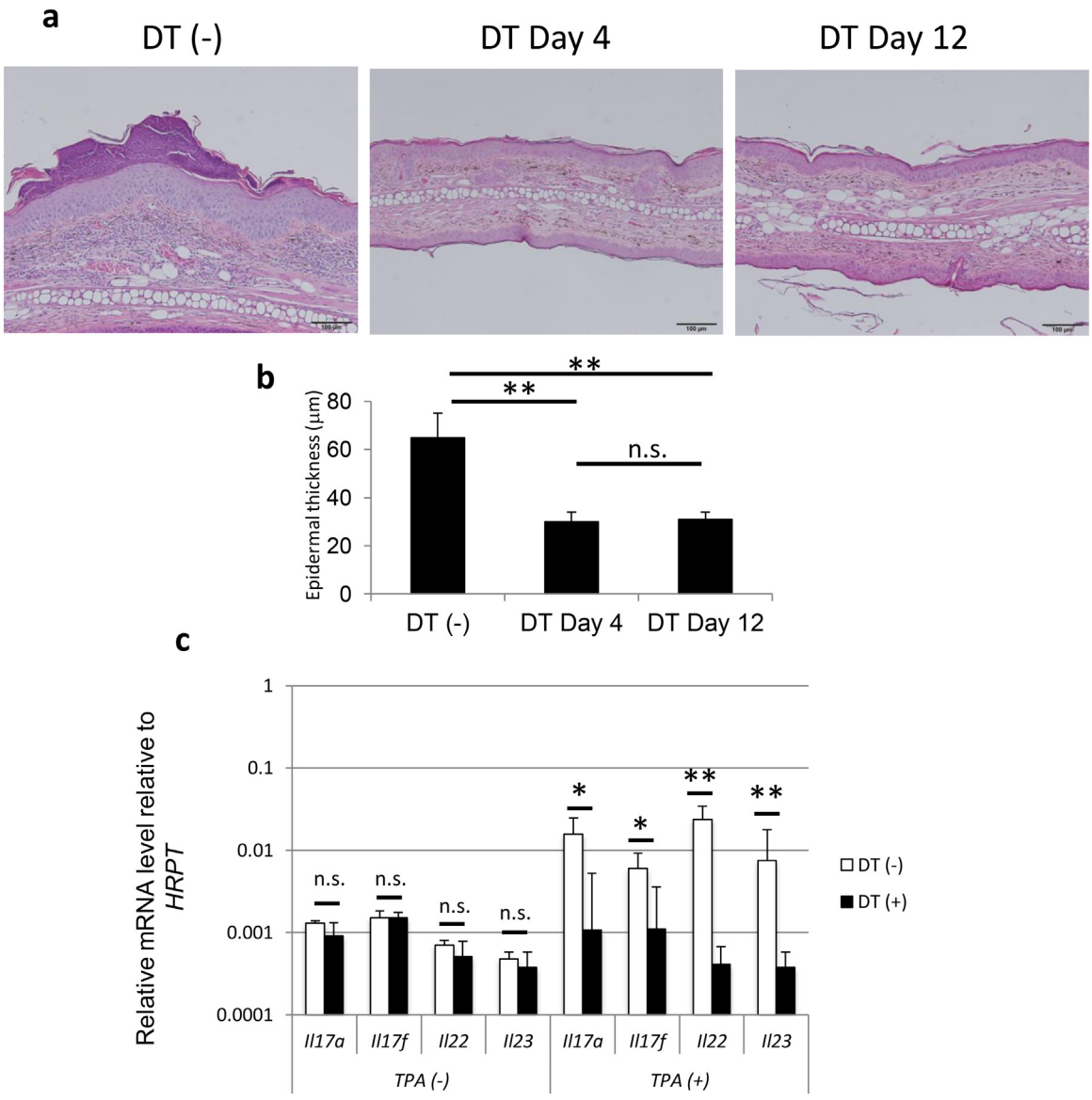

Fig. 3. Development of psoriasis-like skin lesion depends on the presence of LCs.
(a) Representative histological features of TPA-treated ear skin on day 4 and 12 post- DT treatment. Development of TPA-induced psoriasis-like lesion was inhibited in K5. Stat3C:langerin-DTR KI mice on day 4, 12 post treatments. H&E staining. Scale bars = 100 μm. (b) Epidermal thickness (mean ± s.d. μm) of the mice administrated of vehicle control (n = 19) at day 4 (n = 8) and day12 (n = 9) of DT. **, $p < 0.01$; n.s., not significant by Mann-Whitney *U* test. (c) Quantitative RT-PCR analysis for detection of Th17-related cytokine mRNA levels in TPA treated or untreated skin after DT administration (at day 14, black bars, n = 6) or control (white bars, n = 7). Mean value ± s.d. **$p < 0.01$, *$p < 0.05$, Mann-Whitney *U* test). In TPA-untreated group, DT-non-administrated mice (white bars, n = 5) and DT-administrated mice (black bars, n = 5) remained baseline with no significant (n. s.) difference in mRNA levels.

mAb (clone M5/114.15.2 labeled with PerCP-Cy5.5, BD Pharmingen) and anti-mouse CD207 (Langerin) mAb (clone caa8-28H10 labeled with PE, Miltenyi). Dead cell exclusion was performed using SYTOX Red Dead Cell Stain (Invitrogen). CD207+ cells gated on CD11c + I-A/I-E + cells were sorted using FACSAriaII (BD). Flow cytometry data were analysed with FlowJo software (TreeStar).

2.10. DNA microarray analysis

Total RNAs were extracted from cells using the NucleoSPin®RNAXS according to the manufacturer's instructions (MACHEREY-NAGEL). The DNA microarray analysis was performed using SurePrint G3 Human GE Human GE 8 × 60 K Microarray Ver2.0 according to the manufacturers' instructions (Agilent). The scanned images were analyzed with Feature Extraction Software 11.5.1.1(10.10.1.1)(Agilent) using default parameters to obtain background subtracted and spatially detrended Processed Signal intensities. Transcripts with at least a 2.0-fold change difference in expression level between untreated Stat3C mice and control samples were selected and used for further analysis.

2.11. Statistical analysis

Statistical analysis of significance was calculated using the Mann–Whitney *U* test or the one-way ANOVA followed by Bonferroni's test. A p value <0.05 was considered significant.

3. Results

3.1. Langerhans cells are activated in psoriatic lesion of human and K5. Stat3C mice

In psoriatic lesion, LCs appeared large with elongated dendrites compared with those in healthy control (Fig. 1a and b), suggesting that they were activated and mature in psoriatic epidermis. Most of LCs in psoriasis moved up to the all layers of thickened epidermis (Fig. 1b). Similar to human psoriasis, LCs in psoriasis-like lesion of K5.Stat3C mice exhibited larger in size, showing elongated dendrites to the upper layer of epidermis (Fig. 1d, TPA-treated). In contrast, LCs of vehicle-treated controls were confined to the basal layer (Fig. 1c). Moreover, we confirmed the activation status of epidermal LCs in TPA-treated K5. Stat3C mice with increased expression of CD80 and CD86 (Fig. 1e, f).

3.2. Langerhans cells migrated to lymph nodes at an increased percentage in K5.Stat3C transgenic mice compared with non-transgenic mice

To determine whether LCs migrated to sDLNs, we performed flow cytometry analyses. The migratory DCs in sDLNs are defined as $CD11c^{+}MHC\text{-}II^{high}$ population whereas resident conventional DCs are defined as $CD11c^{high}MHC\text{-}class\ II^{+}$ population. Notably, the percentage of migrating DCs including LCs was higher in K5.Stat3C mice than wild-type mice even without TPA treatment (Fig. 2a–d). Topical TPA treatment further increased the percentage of DCs including LCs with the increment being much larger in K5.Stat3C than control mice (Fig. 2a–d). Together, the results suggested that keratinocyte Stat3 activation, which was further facilitated with TPA treatment [28], might lead to LCs activation and migration to sDLNs.

3.3. Keratinocytes in K5.Stat3C mice constitutively stimulate LCs with IL-1α

Given that LC migration to lymph nodes was facilitated in K5.Stat3C mice even in the absence of topical TPA treatment, there should be a constitutive impact on LCs from the transgenic keratinocytes. To this end, we performed differential real-time RT-PCR analysis using primary keratinocytes from between control and K5.Stat3C mice. In Stat3C transgenic keratinocytes, IL-1α was up-regulated among the molecules that have been known to activate LCs (Supplementary Fig. S1). The result suggests that increased level of IL-1α by the Stat3C transgenic keratinocytes might, at least in part, impact on LC activation.

3.4. LCs are required for the development of psoriasis-like lesion in K5. Stat3C mice

To investigate the role of LCs in psoriasis, we generated K5. Stat3C:langerin DTR KI mice, in which langerin-expressing cells were deleted by injection of diphtheria toxin (DT) [19]. As previously found, the KI mice were lacking $langerin^{+}$ cells both in the epidermis and dermis at day 4 of DT treatment; however, at day 12, $langerin^{+}$ cells redistributed in the dermis but not in the epidermis (Supplementary Fig. S2a, b). Strikingly, we found that development of psoriasis-like lesion was remarkably inhibited in K5.Stat3C mice:langerin DTR KI mice equally at day 4 and 12 of DT treatment, suggesting that psoriasis strictly requires the epidermal LCs but not dermal $langerin^{+}$ cells (Fig. 3a, b). K5.Stat3C:langerin-DTR KI mice exhibited less epidermal thickness and reduced inflammatory cell infiltrates (Fig. 3a, b), and accordingly, psoriasis-related cytokines including IL-17A, IL-17F, IL-22 and IL-23 were down-regulated in the ear skin (Fig. 3c). It should be also noted that K5.Stat3C mice predominantly generated Th17 cells over IL-17-producing TCR-γδ cells (Supplementary Fig. S3a, b). This finding warranted our model to be immunologically relevant to human psoriasis, in contrast to other models including one with imiquimod treatment, in which TCR-γδ cells were the main IL-17 producer [29].

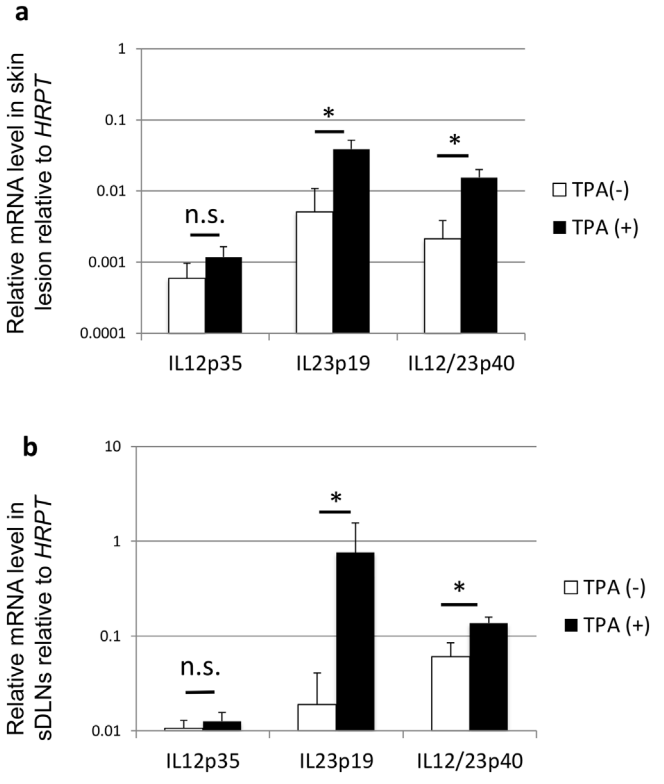

Fig. 4. IL-23mRNA is up-regulated in psoriasis-like lesion and sDLNs of K5.Stat3C mice.
Compared with vehicle-treated mice (white bars, n = 5), the mRNA levels of IL-23p19 and IL-12/23p40 were up-regulated in psoriasis-like lesion (a) and sDLNs (b) of TPA- treated K5.Stat3C mice (black bar, n = 5), whereas IL-12p35mRNA was not up-regulated. *, $p < 0.05$; n.s., not significant by Mann-Whitney *U* test.

3.5. LCs secrete IL-23 in K5.Stat3C mice

To examine whether any alteration occurred in LCs in K5.Stat3C mice under the static state condition, we performed DNA microarray analysis by comparing LC gene expression in between transgenic and non-transgenic mice. The result revealed the expression of *Il12b*,*Ccl8*, *Il6*, *Cxcl10*, and *Tgfa* were significantly higher in LCs isolated from K5.Stat3C mice than those from control mice (Supplementary Fig. S4). Interestingly, the highest up-regulated gene was *Il12b*, which encodes the shared component of IL-12 and IL-23. These data indicated that epidermal LCs in this transgenic mouse may be constitutively affected by the surrounding Stat3C transgenic keratinocytes.

It has been recognized that psoriasis development requires IL-23, evidenced by human and mouse model [1,2,4,8]. In line with this finding, transgenic mice, when treated with TPA, showed increased mRNA levels of IL-23p19 and IL-12/23p40 not only in psoriasis-like skin but also in sDLNs (Fig. 4a, b, respectively), whereas no significant increase in IL-12p35, indicating that IL-23 but not IL-12 was associated with the generation of psoriatic phenotype in this model as previously shown [8]. Intracellular flow cytometric analysis revealed an increase of epidermal LC that produced IL-23 upon TPA treatment (Fig. 5a, b). However, in the sDLNs, TPA treatment increased the percentage of IL-23-expressing subpopulation in langerin$^-$ DCs as well as LCs (Fig. 5c, d), although the percentage of the latter was higher than the former (3. 45 ± 1.43% verusus 1.86 ± 0.56%; p = 0.0062 by Mann Whitney U-test, Fig. 5d). Furthermore, most of the IL-23-producing LCs in sDLNs were positive for CD326 (Supplementary Fig. S5), conforming that they emigrated from the epidermis. Since LCs might potentially have a role in producing IL-23, the presence of epidermal LCs, was essential for the development of psoriasis-like phenotype as clearly demonstrated in the deletion experiment with langerin DTR KI mice (Fig. 3).

3.6. IL-23 producing LCs in psoriasis

The results with the mouse model prompted us to explore human psoriasis with immunostaining analysis. Strikingly, in psoriatic epidermis there were a number of IL-23-producing LCs (Fig. 6c, d), whereas no LCs were co-stained with anti-IL-23 in the control healthy epidermis (Fig. 6a, b).

In conclusion, Stat3 activation in keratinocytes may impact on LC activation via IL-1α stimulation, at least in part, and that their presence may be essential for the pathogenesis of psoriasis through producing IL-23. This study elucidates the intraepidermal vicious circuit with keratinocyte and Langerhans cells, which may trigger the essential IL-23-Th17 axis for psoriasis.

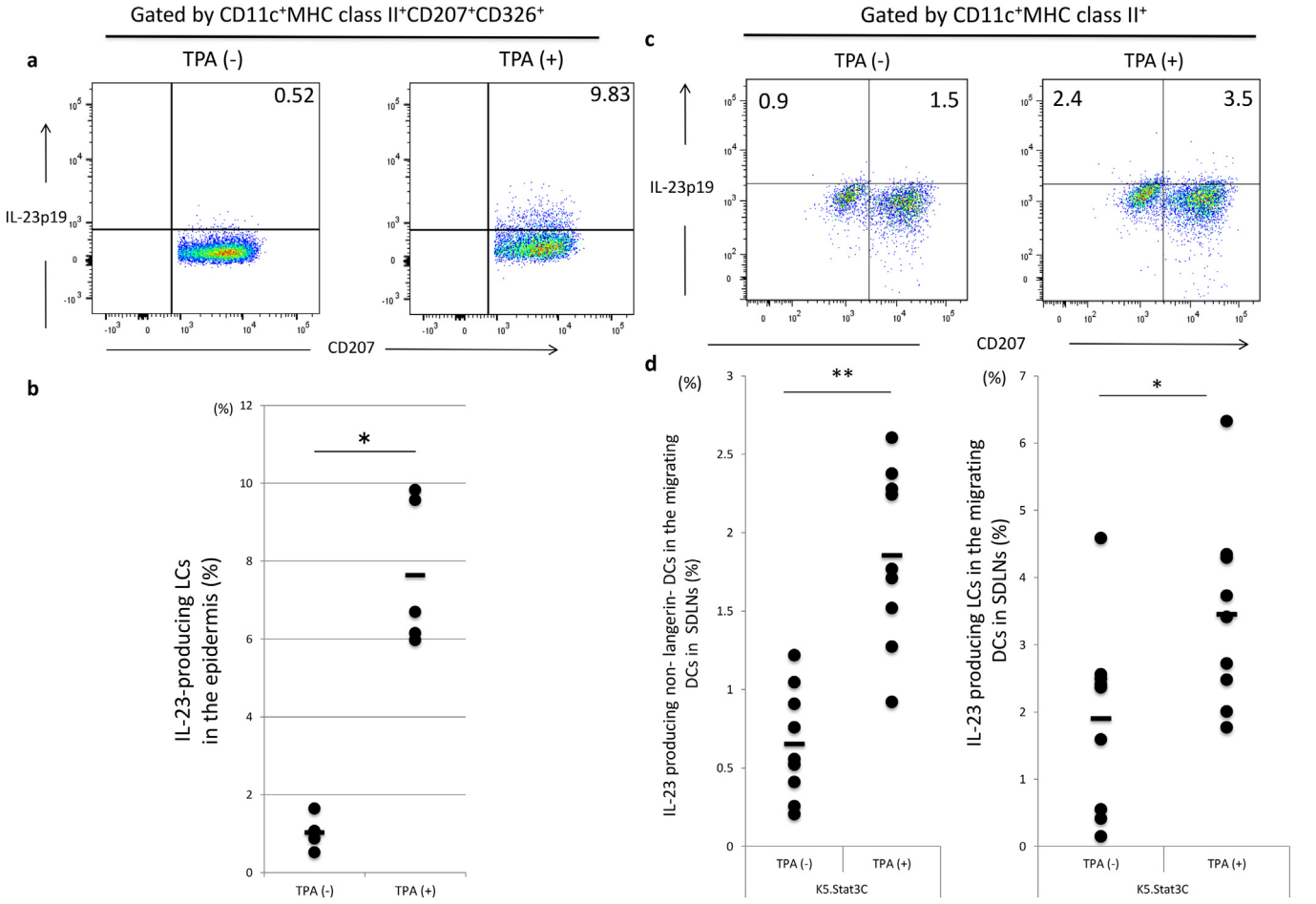

Fig. 5. Langerhans cells secrete IL-23 in psoriasis-like lesion and sDLNs of K5.Stat3C mice.
(a, b) Flow cytometric analysis of IL-23-expression in epidermal LCs from TPA-untreated and treated K5.Stat3C mice. (a) Representative plots for IL-23$^+$CD207$^+$ cells with % shown by number out of CD11c$^+$MHC class II$^+$CD207$^+$CD326$^+$. (b) Quantitative comparison of LCs (%) between TPA-untreated (n = 5) and treated groups (n = 5). *, $p < 0.05$ by the Mann–Whitney U test. (c, d) Flow cytometric analysis of IL-23$^+$migrating DCs to sDLNs from TPA-untreated and treated K5.Stat3C mice. The rate of IL-23$^+$LCs and IL-23$^+$langerin^{neg} migrating DCs were both increased in sDLNs of K5.Stat3C mice when treated with TPA. (c) Representative plots for IL-23$^+$ cells with % by number out of migrating DCs (CD11c$^+$MHC class II$^+$). (d) Quantitative comparison of IL-23$^+$ cells (%) of langerin$^-$ DCs and LCs from between TPA-untreated (n = 8) and treated K5.Stat3C mice (n = 8). **, $p < 0.01$; *, $p < 0.05$ by the Mann–Whitney U test.

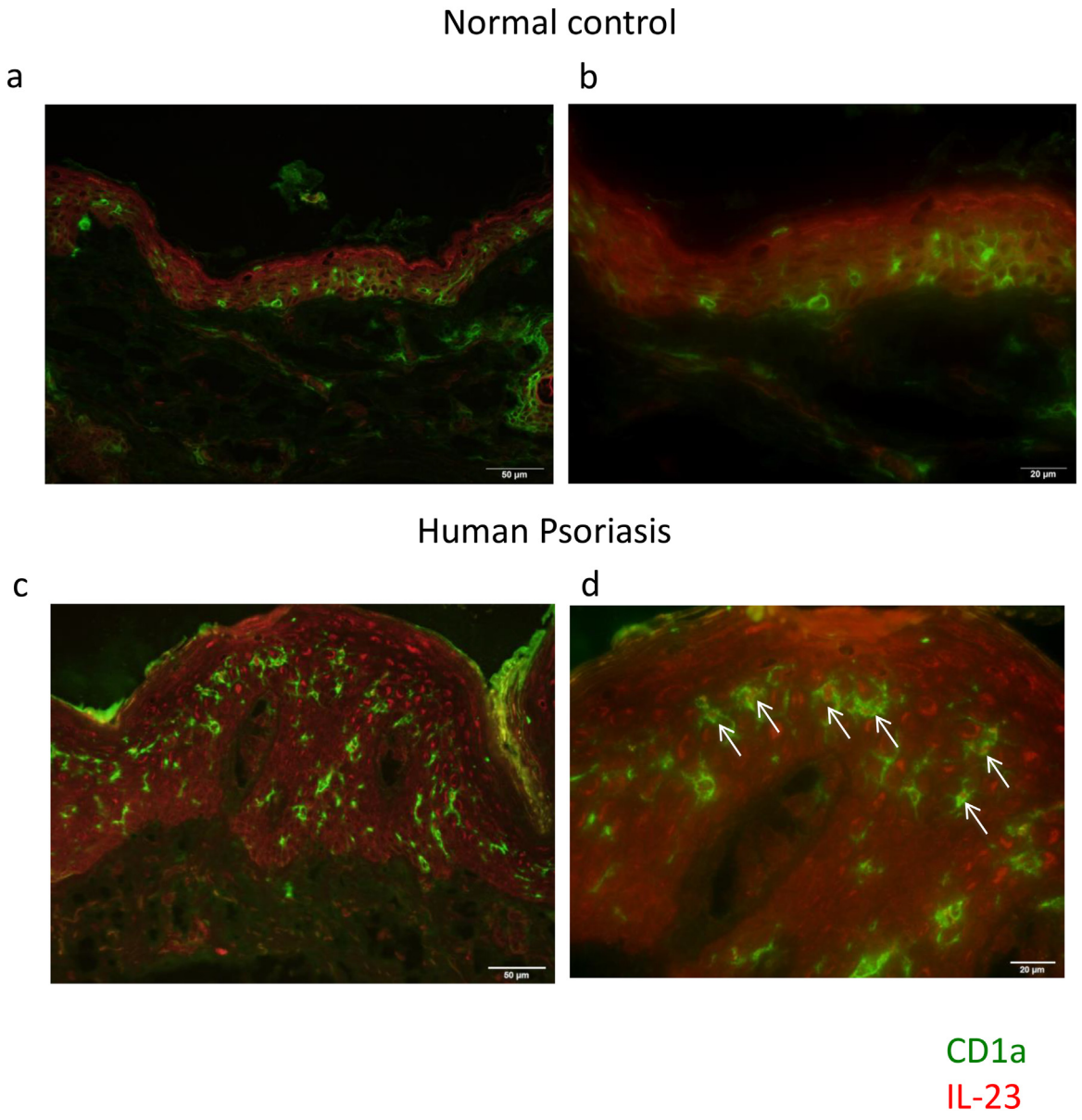

Fig. 6. LCs in human psoriatic lesion produce IL-23.
Representative immunostaining of normal skin (a, b) and psoriatic lesion (c, d) from a patient with PASI of 11 with anti-CD1a (green) and anti-IL-23p19 (red). Arrows indicate IL-23p19-expressing LCs. Scale bars = 50 μm (a, c), 20 μm (b, d).

4. Discussion

Although DCs and T cells are known to play important roles in the pathogenesis of psoriasis, the role of LCs in mouse models of psoriasis-like inflammation is controversial [22–27]. LCs were reported to function of a negative immunoregulatory role via IL-10 and programmed cell death ligand-1 during the initiation and progression of psoriasis [22]. Proliferating and activated LCs were also reported to have negative immune functions in langerin-DTR KI mice treated with long-term imiquimod (IMQ) application [23]. In contrast, LCs were required for the development of IMQ-treated psoriasis like inflammation in langerin-DTR KI mice [24,25]. In addition, human β-defensin 3 (HBD3) sustained and amplified psoriasis inflammation and HBD3 drived the production of IL-23 by LCs in psoriasis, which contributed to the pathogenesis of psoriasis [26]. Therefore, to elucidate the role of LCs in the pathogenesis of psoriasis, we used K5.Stat3C:langerin DTR KI mice, in which LCs could be depleted. Notably, the development of psoriasis-like lesion was inhibited in K5.Stat3C:langerin DTR KI mice during the time frame when LCs but not langerin$^+$ dDCs were depleted.

A number of recent studies have demonstrated that IL-23, which is essential for the development of Th17 cells, is functionally involved in the pathogenesis of psoriasis [1,2,4,8]. However, the cellular source of IL-23 in IMQ-treated mouse model of psoriasis was controversial. Wohn et al. reported langerin negative DCs in the skin, which produced IL-23, were responsible for IMQ-induced psoriatic plaque formation [24], whereas Yoshiki et al. showed that LCs were major DC subsets to produce IL-23 in the skin and sDLNs of IMQ-treated mouse model and that IL-23 promoted the infiltration of IL-17-producing V$\gamma4^+\gamma\delta$TCR^{mid+} cells to the epidermis [25]. In this study, we demonstrated that IL-23p19 and IL-12/23p40 were up-regulated in psoriasis-like lesion and sDLNs of K5.Stat3C mice. Specifically, we also confirmed that epidermal LCs produced IL-23 in the lesion, while other langerin$^-$ DC population produced IL-23 as well. Interestingly, it was recently demonstrated that CD301b$^+$ dermal DCs represented a distinct population that

was capable of producing IL-23, and played a key role in IL-17-mediated psoriasiform dermatitis [30]. Further study will be required to determine which are the main IL-23 producer for psoriasis development, LCs or CD301b$^+$ dermal DCs. A recent study showed that psoriasis-like skin inflammation required monocyte-derived DCs, including MHC-II highly positive LCs in IL-23-injection- and imiquimod-induced models [31]. Similarly, activated LCs from TPA-treated K5.Stat3C mice also expressed high levels of MHC class II (data not shown). However, it remains unclear whether the inflammatory LCs in our experimental setting are derived from monocytes.

In this study, we showed that CD4$^+$ T cells, CD8$^+$ T cells, and γδ T cells produced IL-17 and major source of IL-17 producing T cells was Th17 cell. It has been demonstrated that other murine models for psoriasis, including one by subcutaneous injection of IL-23 and the other with topical treatment with IMQ are associated with an increase of γδ T cells that produced IL-17A [32,33]. Since Th17 cells were predominant over γδ T cells in K5.Stat3C mice, this indicates the advantage of this murine model, being more likely relevant to human psoriasis.

As we noted in this study, the percentage of migrating DCs, including LCs, showed increase in sDLNs of K5.Stat3C mice even before skin lesion developed following TPA treatment, indicating that Stat3C expression in KCs may lead to constitutive LCs activation and migration to sDLNs. Indeed, an increased homeostatic DC migration might lead to an increase of IL-17-producing cells in sDLNs of K5.Stat3C mice compared with those in non-transgenic mice; however, no substantial difference was shown in the rate of IL-17$^+$ T cell population under the steady-state condition between them ($0.59 \pm 0.16\%$, K5.Stat3C mice, $n = 5$; $0.62 \pm 0.26\%$, non-transgenic mice, $n = 5$, $p = 1.0$ by Mann-Whitney U test).

Stat3 plays critical roles in KC proliferation, migration and survival, and more importantly, TPA treatment leads the activation of Stat3 via transactivation of EGFR [28,34]. Therefore, it is possible that Stat3-activated KCs might further stimulate LC activation through cytokines including GM-CSF, IL-1α, IL-1β, IL-18, TNF-α and TGF-β [35–39]. Here we determined IL-1α as the highest up-regulated KC cytokine in K5.Stat3C mice compared with non-transgenic mice and might, at least in part, stimulate LC activation to produce IL-23. In addition, IL-36 cytokines, the IL-1 family, are produced by KCs and play critical role in stimulating DCs as well (paper in preparation). It would be noteworthy to examine whether IL-36 cytokines stimulate LCs.

The absence of LCs hindered the development of psoriatic lesion following topical TPA treatment, suggesting the essential role of LCs. It remained unknown how migratory LCs to lymph nodes contributed to the development of psoriatic lesion, although they expressed increased level of IL-23 as well. In sum, Stat3 activation in KCs occurs in response to wounding stimuli or other environmental perturbation could be relevant to the initiation or exacerbation of psoriasis known as the Koebner phenomenon [34], leading to the intra-epidermal circuit of KC-LC and resulting the IL-23/IL-17 axis activation towards full-fledged establishment of psoriasis.

Conflict of interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2018.11.007.

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