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CD30L/CD30 protects against psoriasiform skin inflammation by suppressing Th17-related cytokine production by Vγ4$^+$ γδ T cells

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ABSTRACT

Psoriasis is a common, autoimmune, chronic inflammatory skin disease. It has been demonstrated that cutaneous T17 cells play an important pro-inflammatory role in the pathogenesis of psoriasis, through the production of various Th17-related cytokines. Our previous studies have demonstrated that CD30L/CD30 signal plays a pivotal role in the differentiation of CD4$^+$ Th17 cells and Vγ6$^+$γδ T17 cells in the gut-associated lymphoid tissues of mouse. However, its effect on the pathogenesis of psoriasis is unknown. Here, we fully prove that CD30L/CD30 signaling plays a novel protective role in the development of psoriasis in mice, through selective inhibition of CCR6 expression and Th17-related cytokine synthesis in the Vγ4$^+$γδ T17 cell subset. Meanwhile, treatment with agonistic anti-CD30 mAb had a significant therapeutic effect on our psoriasis mouse model. Therefore, the CD30L/CD30 signaling pathway is an ideal target for antibody therapy, which may become a new approach for the immunobiological treatment of psoriasis.

1. Introduction

Psoriasis is a prevalent, chronic inflammatory skin disease that affects approximately 0.5%–1% of children and 2%–3% of the world's population [1]. Although the pathogenesis of psoriasis is unclear, it is generally thought that several factors including genetics, infections, the endocrine system, environmental factors, and the immune system all contribute to its development [2]. Currently, numerous studies have shown that psoriasis is an autoimmune disease characterized by inflammation and injury to the skin tissue, which is induced by a disturbance of immune response resulting from several factors including external pathogens, internal autoantigens, and interactions with keratinocytes (KCs) [1–4].

In the early stage of psoriasis, epidermal KCs play an important role. When stimulated by several risk signals, they can synthesize and secrete a large number of antimicrobial peptides (AMPs), β-defensin, S100 protein, and immune effectors. These effectors can activate CD11c$^+$ myeloid dendritic cells (mDCs), which initiate the innate immune response; then, the effectors induce activation of cutaneous "resident T cells" and the subsequent production of proinflammatory cytokines such as TNFα, IFN-γ, IL-17A, and IL-22 [3,5,6]. Various cytokines can induce KCs to secrete different types of chemokines, thus recruiting more inflammatory cells for infiltration [3]. Under such circumstances, activated Th1 cells secrete IFN-γ and induce the secretion of CXCL9, CXCL10, and CXCL11, thus recruiting more Th1 cells; IL-17A-producing Th17 cells are activated and then secrete a high amount of IL-17A, inducing increased synthesis of CCL20, CXCL1, CXCL2, and CXCL8/IL-8 and thereby recruiting more T17 cells and neutrophils to migrate toward the skin lesions [7,8]. Similarly, increased secretion of IL-22 via the activation of Th22 cells is the major cause of the excessive proliferation of KCs [9]. Therefore, during the innate and adaptive immune response in the local skin lesions of psoriasis, KCs and T cells form a

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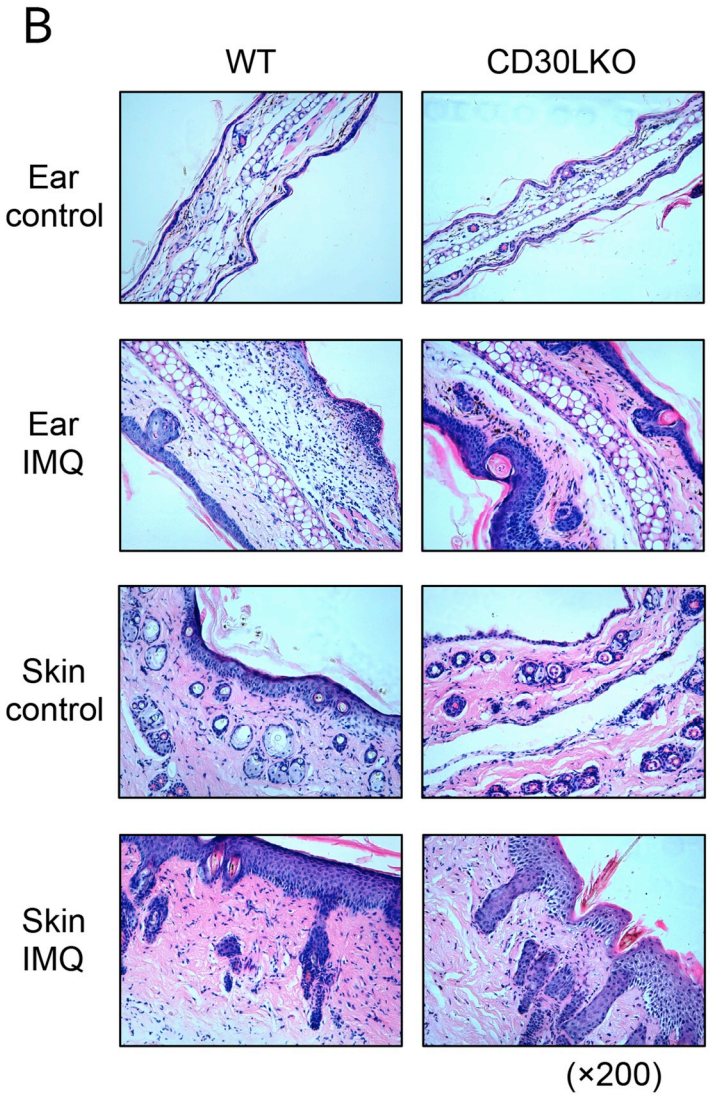

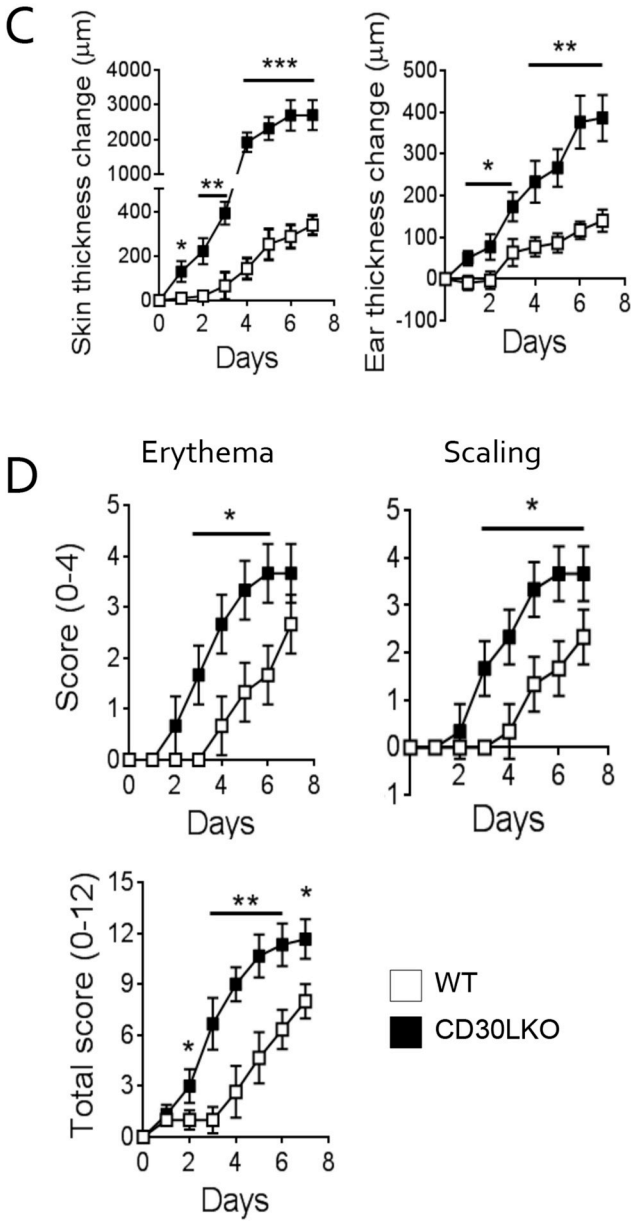

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Fig. 1. CD30L deficiency increases IMQ-induced skin inflammation and acanthosis. (**a**) Phenotypic presentation of mouse back skin for WT or CD30LKO mice treated with IMQ or vehicle for 5 days. (**b**) Histological presentation of WT or CD30LKO mice back or ear skin induced by IMQ or vehicle at day 5 (× 200). (**c**) Back and ear skin thickness of WT or CD30LKO mice was measured on the days indicated. Data are presented as mean ± s.d. (n = 8–10 mice per group) obtained from a representative of three separate experiments. (**d**) Clinical scores during IMQ treatment. WT or CD30LKO mice was treated with IMQ for 7 days. Erythema, scaling, and thickness of the back skin were scored on a scale from 0 to 4; scores were obtained from 8 to 10 mice per group. Data are presented as mean ± s.d. obtained from a representative of three separate experiments. Statistically significant differences between WT and CD30LKO mice are shown (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

positive feedback mechanism via the interactions described above, accelerate the activation and proliferation of KCs, amplify the immune effects, and eventually form a “malignant feedback loop” in the immunological pathogenesis of psoriasis [3].

According to previous studies, $CCR6^+$ Th17 cells, as the major effector cells, migrate to the skin lesions in response to CCL20, then produce IL-17A promoting the formation of a “malignant loop” in the immunological pathogenesis of psoriasis [10]. However, recent studies have shown that γδ T cells in the dermis are an important source for IL-17A, IL-22, and IL-17F; they also widely participate in the occurrence and development of psoriasis [11–15]. Basic scientific and clinical studies on the immunobiological effects of cutaneous γδ T cells have become a new focus in the research on the pathogenesis of psoriasis. Epidermal proliferation, dermal inflammation, and IL-17A production were markedly reduced in T cell receptor δ-deficient ($TCR\delta^{-/-}$) mice with psoriasis [13]. In $CCR6^{-/-}$ mice, dermal γδ T cells migrating to skin lesions was evidently exhibited reduced IL-22 secretion. Both the epidermal thickening and the activation and excessive proliferation of KCs in the psoriatic skin lesions of $CCR6^{-/-}$ mice were also significantly inhibited, and the disease symptoms in the IL-23-induced psoriasis mouse model were significantly alleviated [16]. Some recent reports have confirmed that PD-L1/PD-1 and adiponectin can effectively relieve inflammatory psoriatic symptoms in skin tissue by inhibiting IL-17A production by γδ T cells [14,17]. Thus, T17 cells, including Th17 cells and γδT17 cells, along with their associated cytokines, play essential roles in the pathogenesis of psoriasis.

CD30 and CD30 ligand (CD30L, also known as CD153) belong to the TNF receptor (TNFR)/TNF superfamily [18]. CD30 is mainly expressed on the surface of activated effector or memory T cells and B cells, while CD30L is primarily expressed on activated T cells and also on macrophages and special dendritic cell (DC) subsets [19]. CD30 expression is significantly increased in the skin lesions of psoriasis patients, and CD30L can be highly expressed on mast cells in the skin lesions of patients with atopic dermatitis and psoriasis [20]. CD30/CD30L signaling can activate the MAPK/ERK signaling pathway via CD30L-induced reverse signal, promote the secretion of IL-8 and participating in the immune response in inflamed skin [20]. In addition, blocking the CD30L/CD30 signal can down-regulate the migration of $CD4^+$ T cells into skin tissue [21]. Consequently, it has been suggested that CD30L/CD30 signal may play a potentially important role in triggering the inflammatory response in skin. Our previous has shown that CD30L/CD30 signals can promote the activation and differentiation of $CD4^+$ Th17 cells and IL-$17A^+V\gamma6^+$γδ T cells (according to Heilig and Tonegawa nomenclature) [22] in the intestinal mucosa and mucosa-associated tissues [23–25]. The immunological characteristics of skin tissue are different from those of intestinal mucosal tissues. Therefore, it is still not clear whether CD30L/CD30 signal transduction has the similarly effects on T17 cell mediated inflammatory immune response in the skin lesions of psoriasis.

In this study, we investigated the role of CD30L/CD30 signaling in the pathogenesis of psoriasis, using *Cd30l* gene deletion mice (CD30LKO mice) to establish a psoriasis model induced by imiquimod (IMQ)-treatment. We found that *Cd30l* gene deletion resulted in significantly enhanced susceptibility to IMQ-induced psoriasis. Further, we found the inflammatory symptoms of psoriatic skin lesions in mice were effectively improved through stimulating CD30L/CD30 signaling transduction with agonistic anti-CD30 antibody. These results demonstrate that CD30L/CD30 signal plays an important and novel protective role in the pathogenesis of psoriasis mediated by cutaneous γδT17 cells.

2. Materials and methods

2.1. Mice

Age- and sex-matched C57BL/6J (B6, as WT mice) male mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The generation and preliminary characterization of CD30LKO mice were described previously [23] with eight or more generations backcrossed onto B6 mice. All mice were maintained under specific pathogen-free conditions and were offered food and water *ad libitum*. All mice were used at 6–8 wk of age. This study was approved by the Animal Welfare and Ethics Committee of China Medical University (IACUC No. 16090 M). Experiments were performed under the control of the Guidelines for Animal Experiments.

2.2. Antibodies

Mouse biotin anti-CD16/32 (2.4G2); FITC-*anti*-TCR β chain; PE-*anti*-CD4 (GK1.5); APC-*anti*-CD8α (53-6.7); APC-, BV421-, PE-, and PereCP-*anti*-TCRγδ (GL3); FITC *anti*-TCR Vγ1.1/Cr4 (2.11); FITC-, PE-, and APC-*anti*-TCR Vγ2 (UC3-10A6); APC-*anti*-TCR Vγ3 (536); FITC- and APC-*anti*-F4/80 (BM8); PE- and APC-*anti*-Ly-6G/Ly-6C (RB6-8C5); FITC-*anti*-CD11b (M1/70); PE-*anti*-CXCR2 (SA044G4); PerCP-*anti*-I-A/I-E (M5/114.15.2); APC-*anti*-CCR6 (29-2L17); PE Cy7-*anti*-CD27 (LG.3A10); PE-*anti*-CD30 (mCD30.1); PE- and APC-*anti*-CD30L (RM153); PE-*anti*-IL-22 (Poly5164); and Alexa Fluor 488- and APC-*anti*-IL-17A (TC11-18H10.1) were purchased from Biolegend (San Diego, CA). Mouse FITC-*anti*-TCR Vγ3 (536), APC Cy7-*anti*-CD11b (M1/70), BV510-*anti*-I-A/I-E (M5/114.15.2), Alexa Fluor 647-*anti*-CD196 (140706), and PE-*anti*-IL-17A (TC11-18H10) were obtained from BD Biosciences (San Diego, CA). Mouse PE-*anti*-Ki-67 (SolA15), PerCP-*anti*-IL-22 (1H8PWSR), and PE-*anti*-IL-17F (eBio18F10) were obtained purchased from e-Bioscience (San Diego, CA). Purified anti-mouse CD3ε antibody (145-2C11) was obtained from Biolegend. Purified rat anti-Mouse CD16/CD32 (2.4G2) was obtained from BD Biosciences.

2.3. Induction of psoriasiform skin inflammation by IMQ

Male WT or CD30LKO mice were given a daily topical dose of 62.5 mg of IMQ cream (5%) (Aldara, INOVA Pharmaceuticals, Singapore) on the shaved back or ear for 5–7 consecutive days. Control mice were treated with the same dose of a vehicle cream (Vaseline Lanette cream, Fagron). To ameliorate any suffering of mice observed throughout these experimental studies, mice were euthanized by CO_2 inhalation on day 5 after IMQ-treatment and the tissues of back skin or ear were removed. Sections were taken for cell culture, flow cytometry, or histology. All procedures were approved and supervised by the Institutional Animal Care and Use Committee of China Medical University. Disease severity was assessed by visual assessment for the affected skin area. To be precise, erythema, scaling and thickening were scored independently on a scale from 0 to 4 (0, none; 1, slight; 2, moderate; 3, marked; 4, very marked), and the total score (erythema plus scaling plus thickness) served as the measure of the severity (scale 0–12).

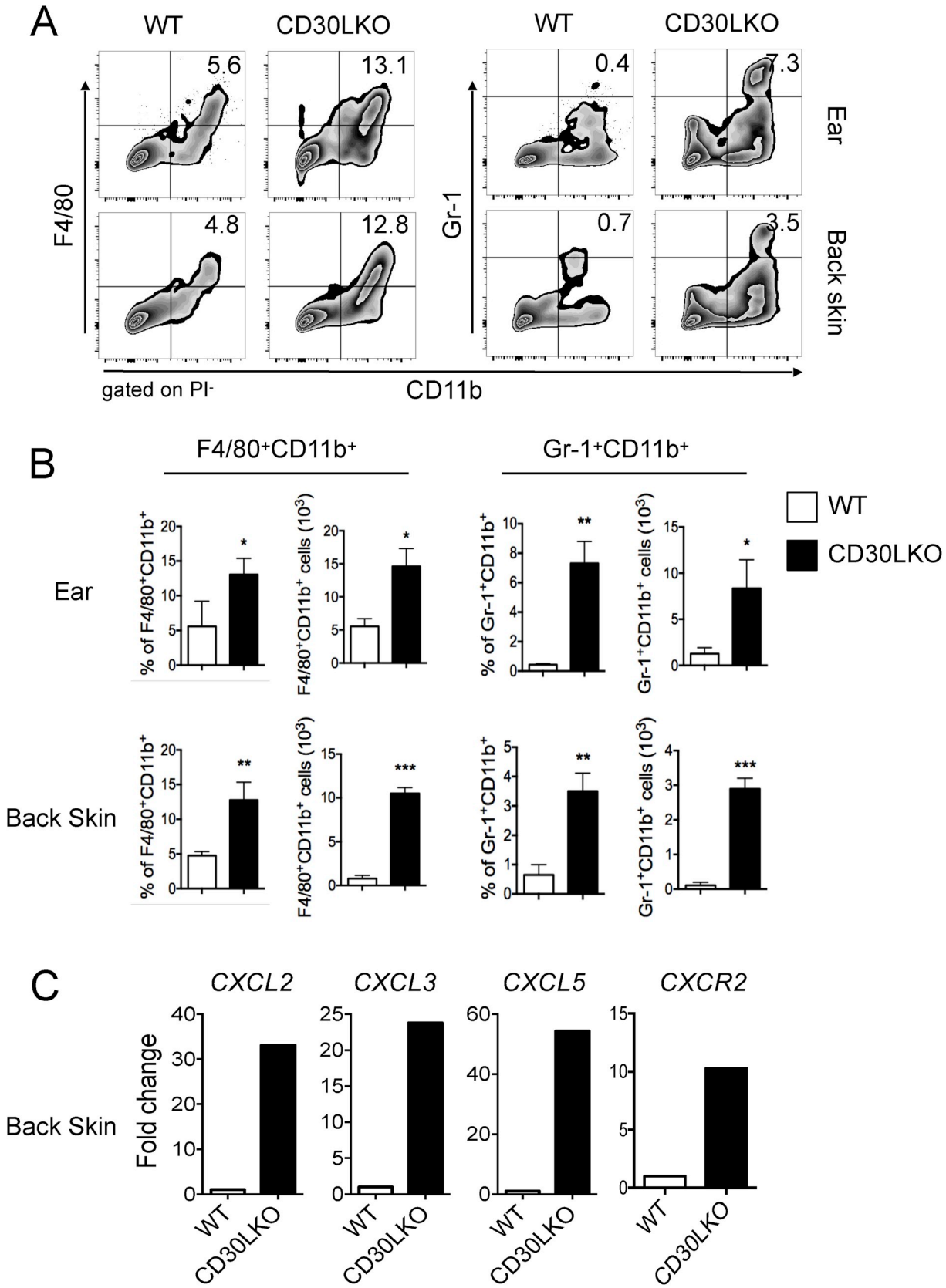

Fig. 2. The accumulation of neutrophils and macrophages in the skin lesions. Single cells were isolated from ear or back skin of WT or CD30LKO mice treated with IMQ for 5 days. The frequency (**a**) and the absolute number (**b**) of neutrophils ($CD11b^+Gr\text{-}1^+F4/80^-$) or macrophage ($CD11b^+F4/80^+Gr\text{-}1^-$) in ear or back skin were analyzed by flow cytometer. Data indicates the mean ± s.d. (n = 3 to 5 mice per group) from a representative of two separate experiments. Statistically significant differences between WT and CD30LKO mice are shown (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). (c) Expression of *Cxcl2, Cxcl3, Cxcl5,* and *Cxcr2* mRNA measured by qPCR in back skin tissues of WT or CD30LKO mice treated with IMQ for 5 days. The figure shows fold changes of the indicated genes normalized for *Rps17* mRNA versus skin tissue of WT mice.

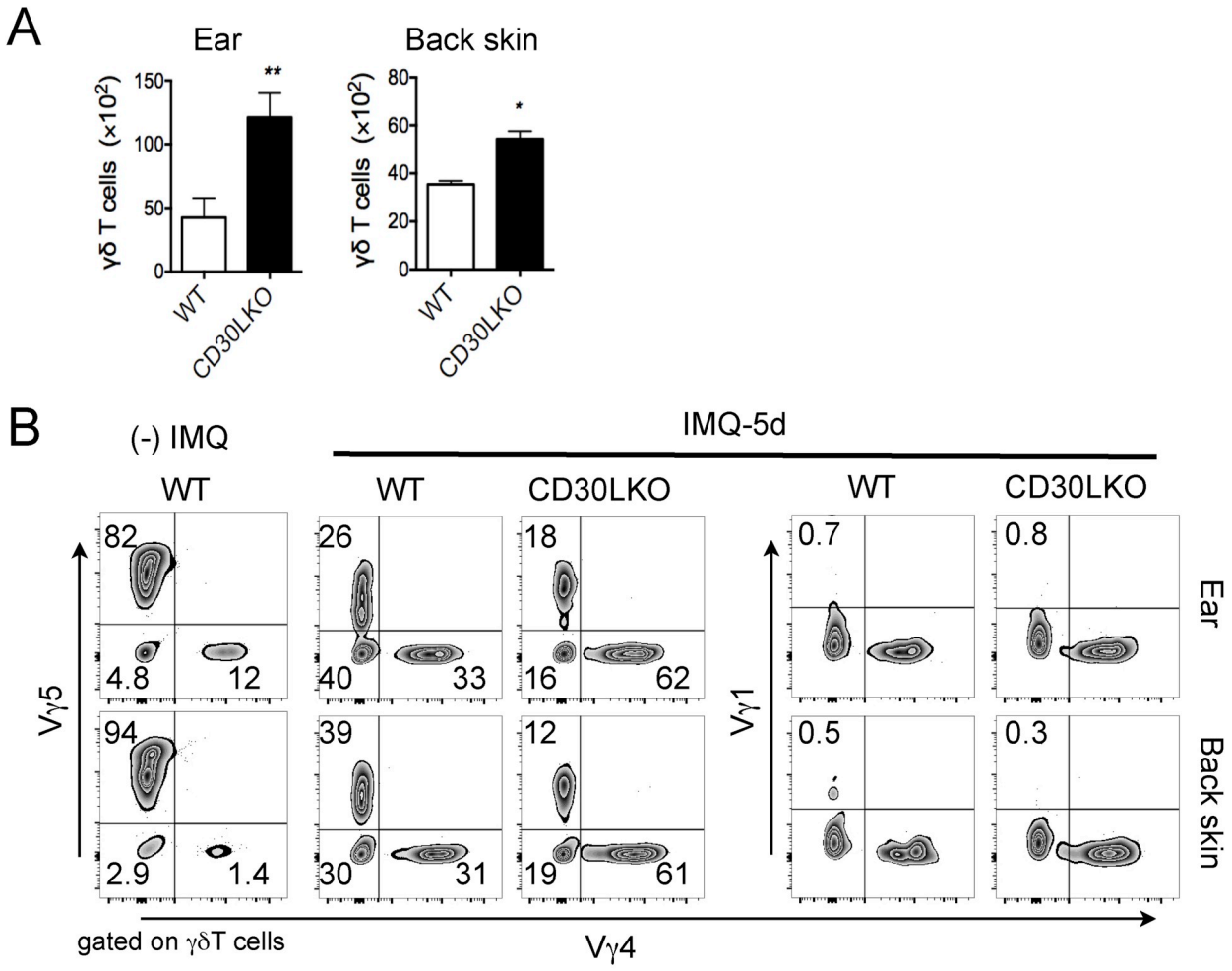

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Fig. 3. The skin-homing of γδ T cell subsets in CD30LKO mice treated with IMQ. Single cells were isolated from skin lesions from WT or CD30LKO mice at day 5 after IMQ- or vehicle-treatment, then were analyzed by flow cytometry. (**a**) The absolute number of total γδ T cells in ear or back skin of WT or CD30LKO mice treated with IMQ for 5 days. (**b-c**) The frequency and the cell number of γδ T cell subsets (including $V\gamma1^+$, $V\gamma4^+$, $V\gamma5^+$, and $V\gamma1^-V\gamma4^-V\gamma5^-$ subsets) in skin lesions of ear and back skin of WT or CD30LKO mice treated with IMQ or vehicle are indicated. Data indicate the mean ± s.d. (n = 3 mice per group) from a representative of three separate experiments. Statistically significant differences between WT and CD30LKO mice are shown (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

2.4. Histological analysis

Before and/or after treatment with IMQ, the mouse back skin or ear was fixed in formalin and embedded in paraffin. Sections (6 μm) were stained with hematoxylin and eosin (H&E).

2.5. RNA reverse transcription and qPCR

Total RNA was extracted from tissues of back skin on day 5 after IMQ-induced psoriasis using TRIzol™ reagent (TakaRa, Japan); a NanoDrop spectrophotometer (ND-2000c) was used for RNA quality control. Complementary DNA was synthesized using PrimeScript™ RT regent Kit with gDNA Eraser (TakaRa, AK4501). qPCR was carried out with the SYBR®Premix Ex Taq™ II (TakaRa, AHX1024 N) in a Light Cycler 480 II (Roche). Relative quantities (Δ cycle threshold (Ct) value) were obtained by subtracting the Ct value of *Cxcl2, Cxcl3, Cxcl5, Cxcr2, Ccr6, Ccl20, Il-17a, Il-17f,* or *Il-22* from Rps17. The fold change was calculated according to formula $2^{-\Delta\Delta Ct}$. Each reaction was performed in triplicate. Primers sequences were used as described in Supplementary Table 1.

2.6. Cell preparation

dLN samples were minced through a 70-μm mesh to obtain single-cell suspensions. To isolate single cells from mouse whole-back or ear skin before and/or after IMQ-treatment, back or ear skin tissue was excised and the subcutaneous fat or the middle cartilage layer, respectively, was removed. The skin tissues were incubated in 3.5 ml 10% RPMI (Wako, Japan) containing 1 ml type IV collagenase (10 mg/ml, Sigma-Aldrich) and 500 μl Dnase I (1 mg/ml, Sigma-Aldrich) for 75 min at 37 °C. Then, digestion was terminated by adding an equal amount of RPMI containing 10% FCS (Wako, Japan) and 1% penicillin/streptomycin (Wako, Japan). Finally, the single cells were filtered through a 70-μm filter (Corning).

2.7. Flow cytometric analysis

Single-cell suspensions were extracted from skin lesions and dLNs as described above. The cells were preincubated with an Fcγ receptor-blocking mAb (CD16/32; 2.4G2) for 15 min at 4 °C to prevent non-specific staining. After washing, cells were stained with combinations of mAbs against various surface antigens. Stained cells were washed twice and re-suspended. In some experiments, propidium iodide (PI, 1 μg/ml) was added before analysis using a FACSCelesta Flow Cytometer (BD Biosciences) to detect and exclude dead cells for the analysis of surface staining.

For intracellular cytokine staining, cells were stimulated with phorbol myristate acetate (PMA, 25 ng/ml; Sigma-Aldrich) and ionomycin (1 μg/ml, Sigma-Aldrich) for 5 h at 37 °C. Brefeldin A (10 μg/ml; Sigma-Aldrich) was added for the last 4 h of incubation. After culture, cells were harvested and surface stained with various mAbs for 30 min at 4 °C. Then, the intracellular expression of IL-17A, IL-17F and IL-22 was analyzed using a BD Cytofix/Cytoperm™ (BD Biosciences) or Transcription Factor Buffer Set (BD Pharmingen) according to the manufacturer's instructions. Finally, cells were washed, resuspended, and analyzed with a FACSCelesta Flow Cytometer. The data were analyzed using BD FACSDiva 8 (BD Biosciences).

2.8. Enzyme-linked immunosorbent assay

To measure cytokine production by T cells from skin lesions or dLNs, single cells were isolated and cultured with 96-well flat-bottom plates (Falcon; BD Biosciences) coated with anti-CD3 (10 μg/ml) and soluble anti-CD28 mAbs (1 μg/ml) for 48 h at 37 °C under 5% CO_2. IL-17A, IL-17F, IL-22, IL-6, or TNF-α secretion in the culture supernatant was measured using an appropriate ELISA Kit (R&D Systems) according to the manufacturer's protocols. In some experiments, the skin lesions of mice treated with IMQ for 5 days were isolated and homogenized, then the level of various cytokines in the supernatants was measured by ELISA.

2.9. In vivo treatment of mice with agonistic anti-CD30 mAb

Agonistic anti-CD30 mAb (clone mCD30.1) was obtained as previously described [23]. The mAbs, diluted to 1 mg/ml in PBS, were stored at −80 °C until use. For *in vivo* activation, 100 μg/head of agonistic anti-CD30 mAb or isotype control antibody (hamster IgG1, BD Biosciences) was injected i.p. into WT mice from day 0 to day 5 after IMQ-induced psoriasis.

2.10. Statistical analysis

The data were analyzed with GraphPad Prism 7 and are presented as the mean ± s.d. A Student's *t*-test was used when two conditions were compared, while analysis of variance (ANOVA) with Bonferroni or Newman-Keuls correction was used for multiple comparisons. Probability values of < 0.05 were considered significant; two-sided Student's *t*-tests or ANOVA were performed. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

3. Results

3.1. Cd30l gene deletion leads to a marked increase in susceptibility to psoriasis induced by IMQ

First, vehicle cream (control group) or Aldara Ointment containing 5% imiquimod (experimental group) was administered at a dose of 62.5 mg/day/head to the skin on the back of each wildtype (WT) mouse to induce psoriasis. The treated skin tissue was collected on days 0, 3, and 5 after IMQ-treatment. Total protein was extracted from skin tissue and changes in CD30L expression were measured. We found that the expression of CD30L protein in the skin lesions gradually increased with the development of skin inflammation (Supplementary Fig. 1). Subsequently, we examined different cell types for the expression of CD30L and CD30 in mouse skin prior to and following IMQ-treatment. It was found that γδ T and $CD4^+$ T cells, especially the $V\gamma4^+\gamma\delta$ T cell subset, mainly express CD30; however, the other cell types in the inflammatory skin lesions have extremely low expression. The expression of CD30 in $V\gamma4^+\gamma\delta$ T cells was markedly increased following IMQ-treatment (Supplementary Fig. 2). We also found that $V\gamma4^+\gamma\delta$ T, $CD4^+$ T, and $CD11b^+F4/80^+Gr\text{-}1^-$ cells express CD30L. In addition to CD30, the expression of CD30L in $V\gamma4^+\gamma\delta$ T cells in the inflammatory skin lesions was significantly higher than that in naïve mice (Supplementary Fig. 2). Moreover, there was no significant difference in CD30L expression in skin macrophages ($CD11b^+F4/80^+Gr\text{-}1^-$) prior to and following IMQ-treatment (Supplementary Fig. 2b). Thus, we hypothesized that the *Cd30l* gene might play an important role in the formation of psoriatic

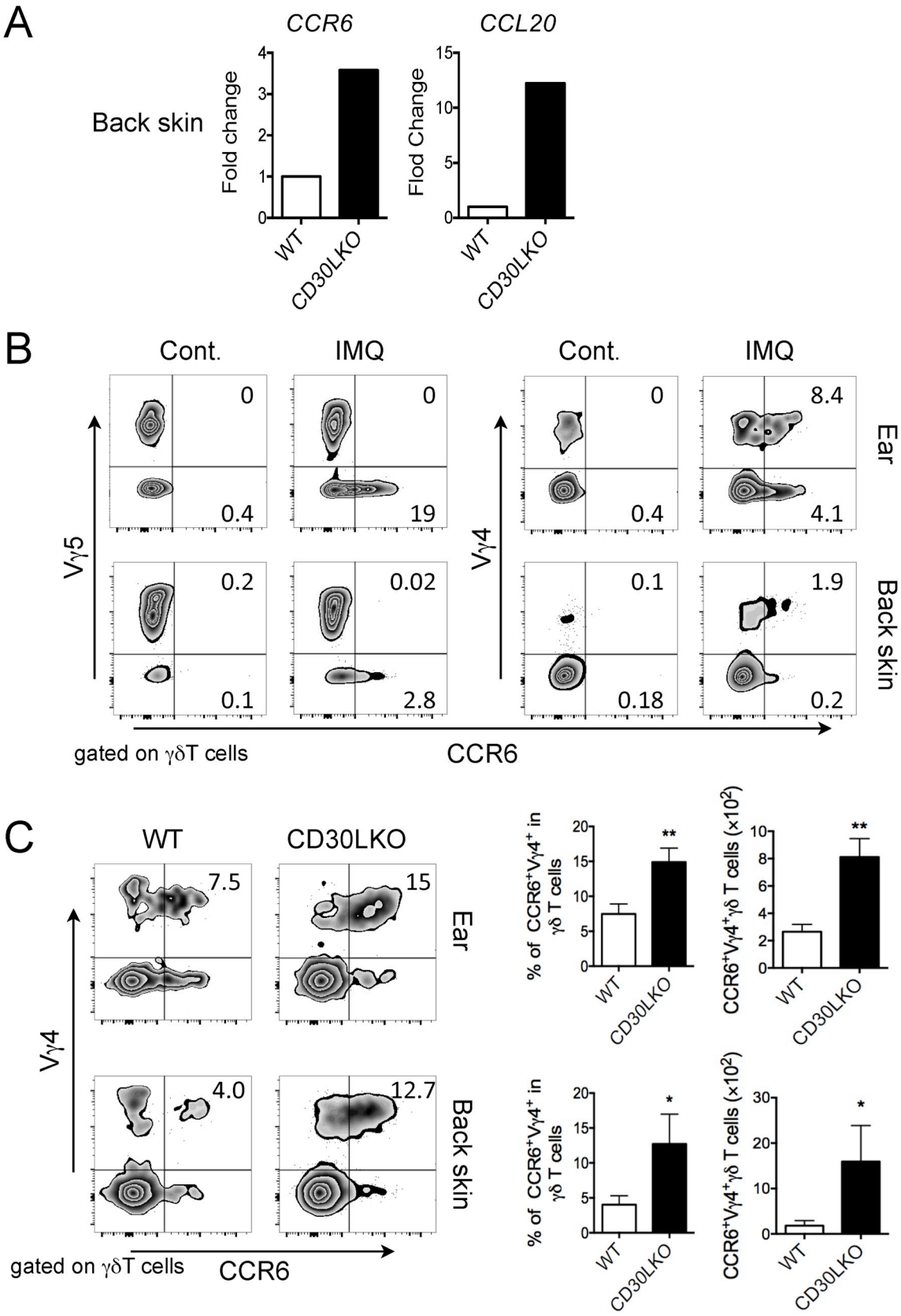


Fig. 4. The expression of CCR6 in γδ T cell subsets increased in CD30LKO mice after IMQ-treatment. (**a**) Expression of *Ccr6 and Ccl2*0 mRNA measured by qPCR in back skin tissues of WT or CD30LKO mice treated with IMQ for 5 days. The figure shows fold changes of the indicated genes normalized for *Rps17* mRNA versus skin tissue of WT mice. (**b**) The expression of CCR6 in the $Vg5^+$ or $Vg4^+$ γδ T cell subsets in ear or back skin of WT mice treated with IMQ or vehicle for 5 days. Data indicate the mean (n = 3–4 mice per group) from a representative of three separate experiments. (**c**) The percentage and the absolute number of $CCR6^+V\gamma4^+$ γδ T cells in ear and back skin of WT or CD30LKO mice treated with IMQ are indicated. Data indicate the mean ± s.d. (n = 3 mice per group) from a representative of three separate experiments. Statistically significant differences between WT and CD30LKO mice are shown (*$P < 0.05$; **$P < 0.01$).

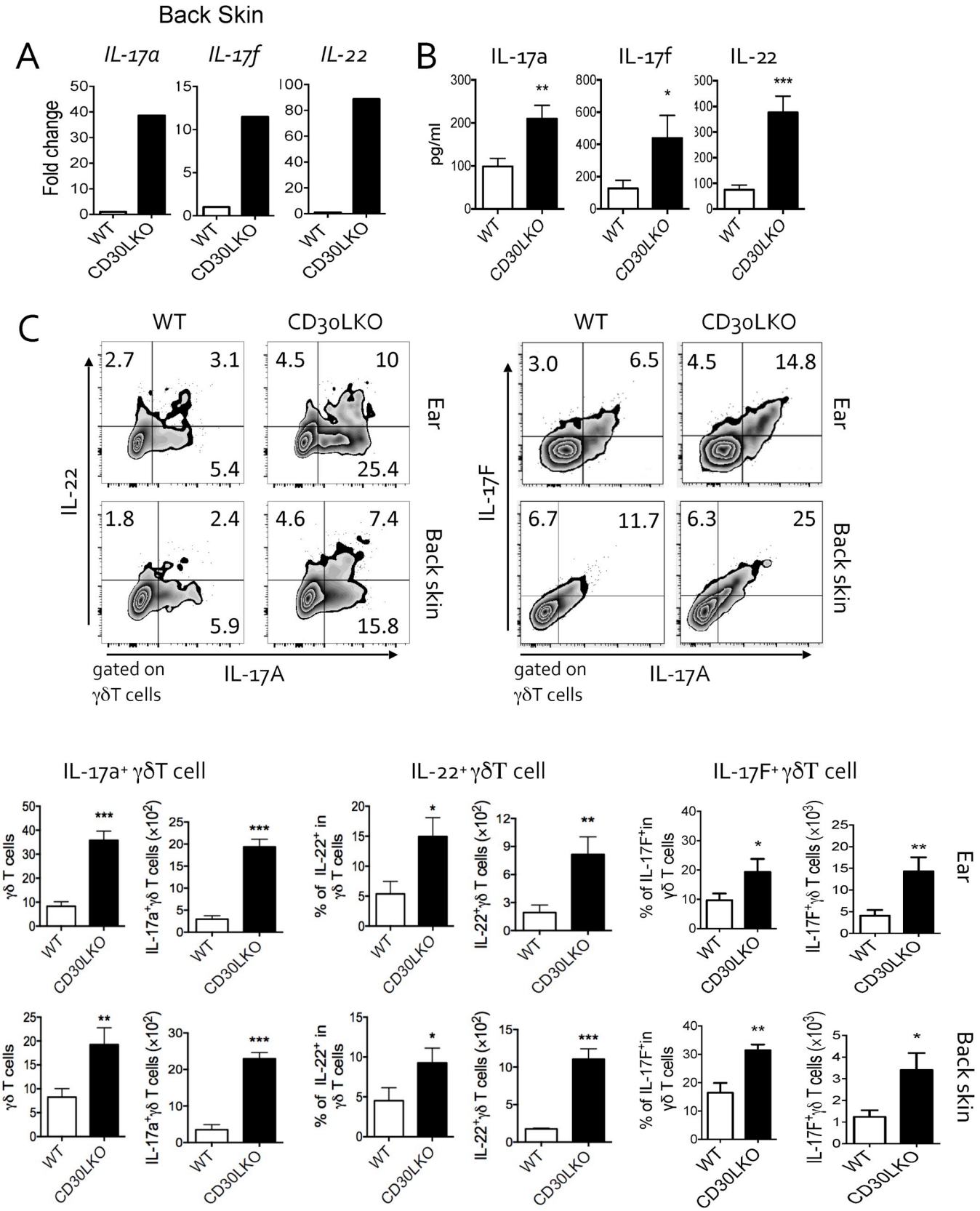


Fig. 5. CD30L deficiency enhances Th17-related cytokine production from V$\gamma4^+$ γδ T cells. The levels of IL-17A, IL-17F and IL-22 mRNA (**a**) or protein (**b**) in the skin lesions of WT or CD30LKO mice were measured at day 5 after IMQ-treatment. Data indicate the mean ± s.d. (n = 5 mice per group) from a representative of two separate experiments. The frequency and the absolute number of IL-17A-, IL-22-, or IL-17F-producing γδ T cells (**c**) or V$\gamma4^+$ γδ T cells (**d**) in ear or back skin from WT or CD30LKO mice treated with IMQ. Data indicate the mean ± s.d. (n = 3–4 mice per group) from a representative of three separate experiments. Statistically significant differences between WT and CD30LKO mice are shown (*$P < 0.05$; **$P < 0.01$; ***$P < 0.01$).

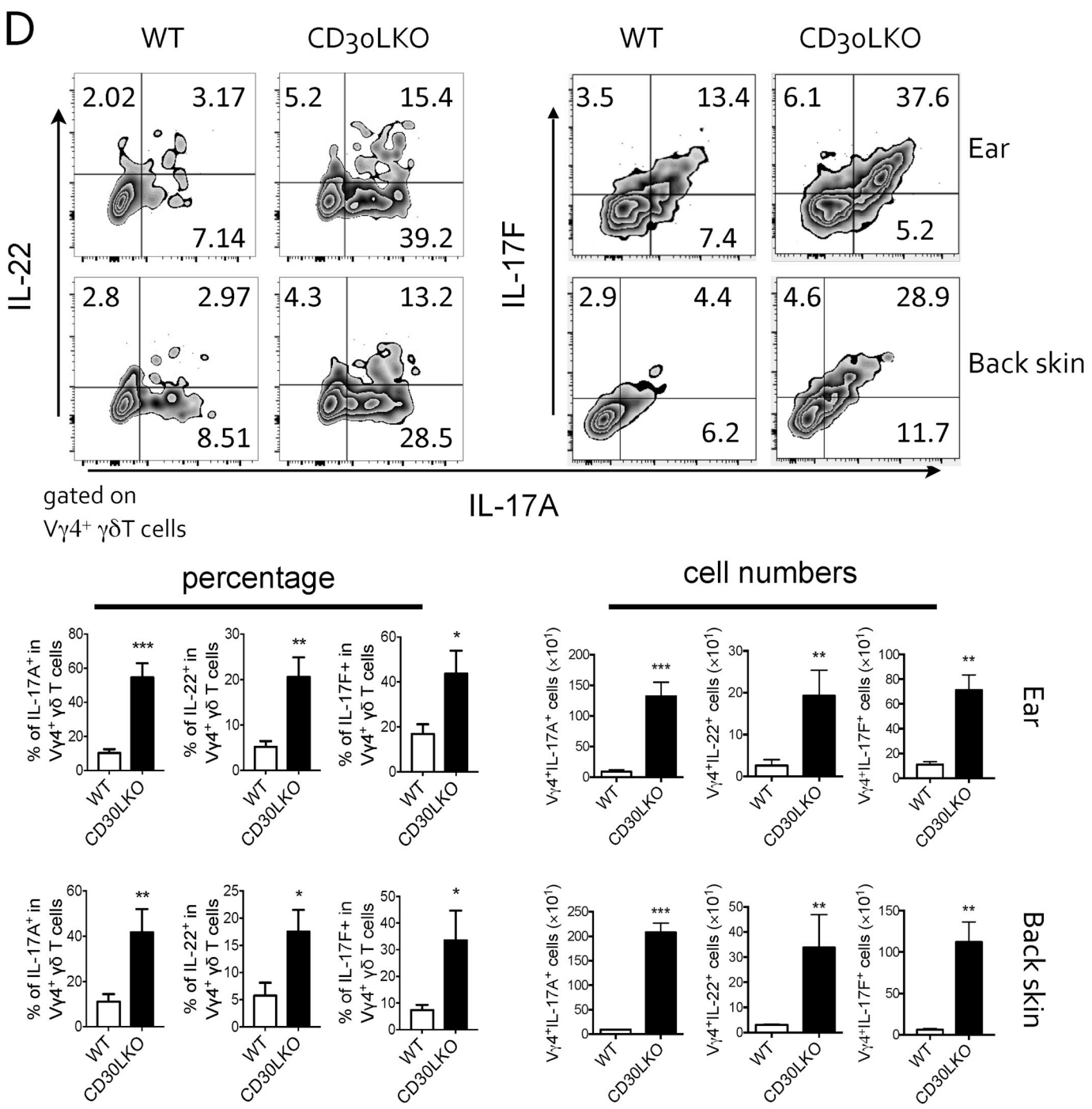
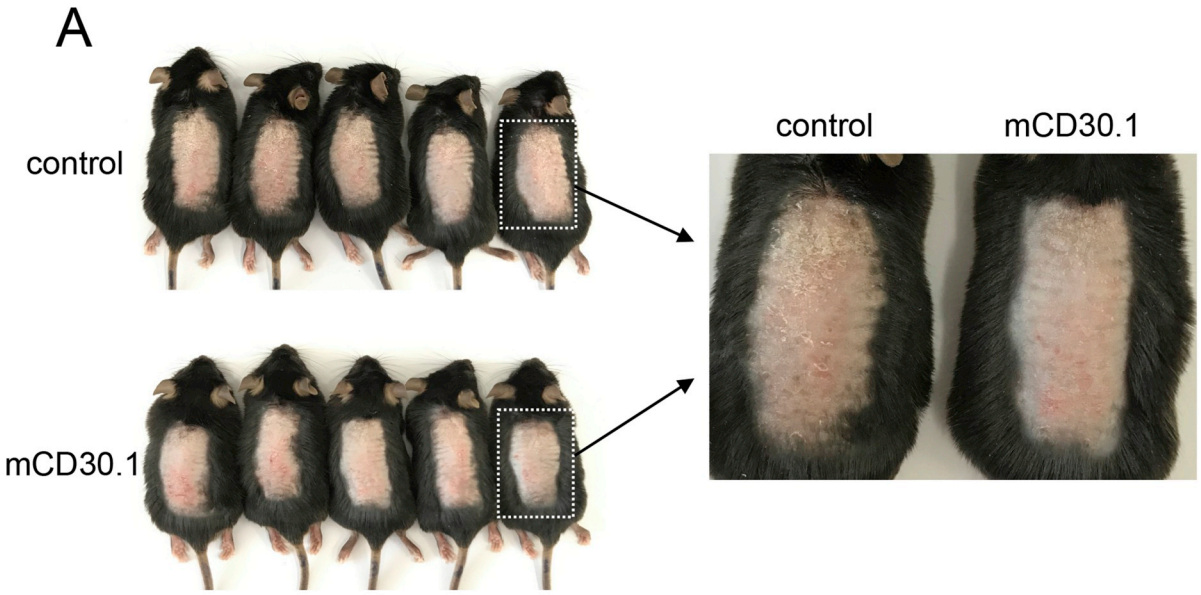


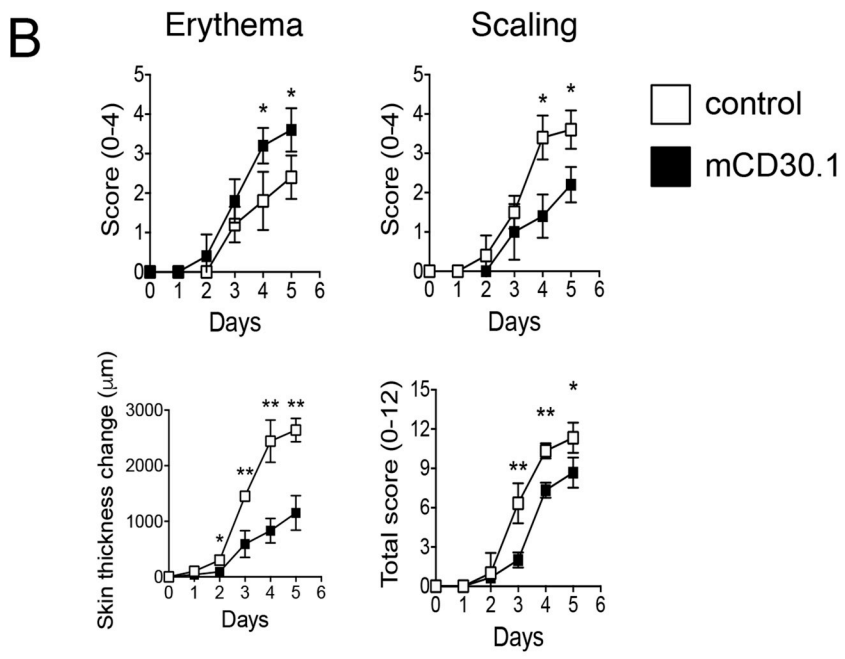
Fig. 5. (*continued*)

lesions. In order to further explore the role of *Cd30l* gene in the pathogenesis of psoriasis, we used CD30LKO mice to establish the IMQ-induced psoriasis model and dynamically compared the effects of the *Cd30l* gene deletion on the occurrence and development of psoriasis skin inflammation in mice. As shown in Fig. 1a–d, the scaling, erythema, skin thickness on the back or ear, and the total score of back skin were increased significantly in CD30LKO mice on day 5 after IMQ-treatment as compared with WT mice. Moreover, histopathological examination showed that the epidermis of the back and ear skin of CD30LKO mice was markedly thickened and the infiltration of inflammatory cells into the skin tissue was significantly increased (Fig. 1b). These results demonstrate that the deletion of *Cd30l* gene leads to the aggravation of psoriasis-like skin inflammation in mice induced by IMQ, suggesting that CD30L/CD30 signal transduction may play an important protective role in the pathogenesis of psoriasis.

3.2. *Cd30l* gene deletion results in increased inflammatory cell infiltration in skin lesions of mice

The inflammatory cells infiltrating in the inflammatory skin tissues, such as neutrophils and macrophages, play an important role in promoting the formation of the “malignant loop” for psoriasis [1,26,27,28]. Therefore, we investigated the effects of *Cd30l* gene deletion on the infiltration of neutrophils and macrophages into the skin lesions. The results showed that *Cd30l*-deficiency resulted in a significant increase in the percentage of and absolute numbers of





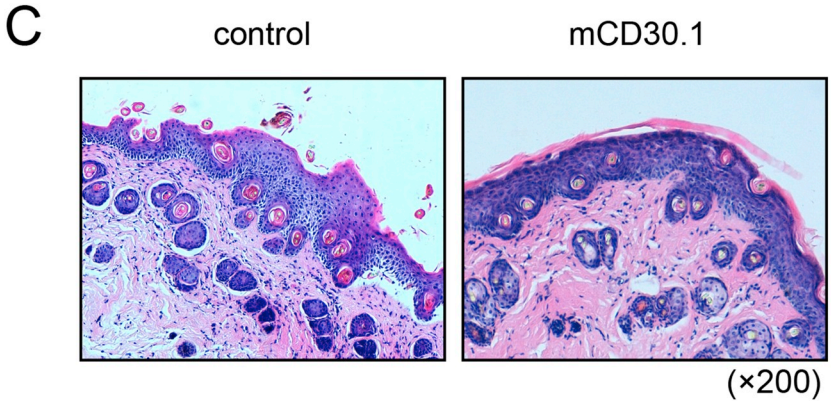


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Fig. 6. mCD30.1 mA b treatment significantly improved psoriatic inflammatory symptoms in WT mice. (**a**) Phenotypic presentation of mouse back skin lesions in WT mice after mCD30.1 mA b treatment (n = 5 per group). (**b**) WT mice were treated with IMQ and mCD30.1 mA b for 5 days. Erythema, scaling, and thickness of the back skin were scored on a scale from 0 to 4; scores were obtained from 5 mice per group. Data are presented as the mean ± s.d. obtained from a representative of two separate experiments. Statistically significant differences between control- and mCD30.1 mA b-treated mice are shown (*$P < 0.05$; **$P < 0.01$). (**c**) Histological presentation of WT mice back skin lesions treated with control or mCD30.1 mA b at day 5 (× 200).

neutrophils ($CD11b^+Gr\text{-}1^+$) and macrophages ($CD11b^+F4/80^+$) in inflamed tissue at day 5 after IMQ-administration (Fig. 2a and b). It has been reported that neutrophils or macrophages expressing chemokine receptor CXCR2 can migrate to skin lesions under the induction of chemokines CXCL2, CXCL3, or CXCL5 and participate in the formation of skin inflammation [7,29]. As shown in Fig. 2c, the expression of *Cxcl2, Cxcl3, Cxcl5,* and *Cxcr2* mRNA in the skin lesions of CD30LKO mice were significantly higher than those in WT mice. These results suggest that these neutrophils or macrophages may migrate from the draining lymph node (dLN) to the inflamed skin tissue through the interaction of CXCR2 and its ligands. Next, we further detected CXCR2 expression on the neutrophils or macrophages found in the dLN before and after the induction of psoriasis. As shown in Supplementary Fig. 3a, in normal WT mice, low levels of CXCR2 were expressed in $CD11b^+Gr\text{-}1^+$ cells or $CD11b^+F4/80^+$ cells from the dLN. However, with the development of psoriatic skin inflammation, the expression of CXCR2 in $CD11b^+Gr\text{-}1^+$ cells increased gradually, while the expression of CXCR2 in $CD11b^+F4/80^+$ cells did not change significantly. Thus, IMQ-induced skin inflammation can upregulate CXCR2 expression on $CD11b^+Gr\text{-}1^+$ neutrophils, then promote the migration of these neutrophils to the skin lesions. Therefore, we analyzed whether *Cd30l* gene deletion affects CXCR2 expression in inflammatory cells, such as $CD11b^+Gr\text{-}1^+$, $CD11b^+F4/80^+$, or γδT cells in the dLN. We found that CXCR2 expression on $CD11b^+Gr\text{-}1^+$ cells in the dLN from CD30LKO mice was significantly upregulated on day 1 after IMQ-induced psoriasis as compared with WT mice, but there was no significant change in CXCR2 expression in $CD11b^+F4/80^+$ cells or γδT cells (Supplementary Fig. 3b). Therefore, these results suggest that *Cd30l* gene deletion leads to enhanced infiltration of $CXCR2^+CD11b^+Gr\text{-}1^+$ neutrophils into the skin lesions from the dLN, and thus accelerates the formation of the "malignant loop" of psoriasis.

3.3. *Cd30l gene deletion enhances the ability of $V\gamma4^+$ γδ T cell homing to sites of skin inflammation*

It has been reported that T17 cells, including Th17 ($IL\text{-}17A^+CD4^+$ T cells), Tc17 ($IL\text{-}17A^+CD8^+$ T cells), and γδT17 ($IL\text{-}17A^+$ γδT cells), have the ability to promote inflammatory cells (neutrophils, macrophages, *etc.*) to infiltrate inflamed skin tissue by secreting IL-17A, IL-22, IL-17F, and other inflammatory cytokines, thus promoting the development of psoriasis [13,30,31,32]. Therefore, we continued to investigate whether the increase in $CXCR2^+CD11b^+Gr\text{-}1^+$ neutrophils in the skin lesions of mice caused by *Cd30l* gene deletion is related to T17 cells and their associated cytokines. First, we detected the skin infiltration of $CD4^+$ or $CD8^+$ αβT cells, but there were no significant differences between WT and CD30LKO mice (Supplementary Fig. 4). The results revealed that *Cd30l* gene deletion did not affect the homing of cutaneous αβ T cells. Subsequently, we detected the proportion and quantity of γδ T cells and their subsets in skin lesions. We found that compared with WT mice, the total number of γδ T cells in the ear or back skin lesions of CD30LKO mice increased significantly (Fig. 3a). Further analysis of γδ T cell subsets showed that the major γδ T cell subsets in the normal skin tissue of mice was "skin-resident γδ T cells", i.e., $V\gamma5^+$ γδ T cells, and after inducing skin inflammation, the percentage of $V\gamma5^+\gamma\delta$ T cells was markedly decreased, while that of $V\gamma4^+$ γδ T cells was significantly increased (Fig. 3b). These results indicate that increased cutaneous $V\gamma4^+$ γδ T cells in the skin lesions may migrate from dLNs; we refer to these cells as "skin-homing γδ T cells". Compared with WT mice, there was no significant increase in the number of "skin-resident γδ T cells" in CD30LKO mice, but both the proportion and absolute cell number of $V\gamma4^+$ "skin-homing γδ T cells" were increased significantly. Meanwhile, there was a relative decrease in the percentage of $V\gamma1^-V\gamma4^-V\gamma5^-$ γδ T cells and almost no Vγ1 expression in γδ T cells within the skin lesions (Fig. 3b and c). These results demonstrate that *Cd30l* gene deletion selectively promotes the skin-homing of $V\gamma4^+$ γδ T cells, rather than affecting $CD4^+$ Th17 or $CD8^+$ Tc17 cells, suggesting that CD30L/CD30 signaling may play an important protective role in the skin inflammatory responses mediated by $V\gamma4^+$ γδ T cells.

3.4. *Cd30l gene deletion selectively enhances the expression of CCR6 and Ki-67 in $CD27^-V\gamma4^+\gamma\delta$ T cell subsets*

A large number of studies have shown that CCR6 expression is essential for skin homing of T17 cells, including γδ T17 cells [13,16,33,34]. Therefore, we first discussed the effect of *Cd30l* gene deletion on CCR6 expression in γδ T cells in inflammatory skin lesions. We found levels of *Ccr6* and *Ccl20* mRNA expression in the skin lesions of CD30LKO mice were markedly higher than those in WT mice (Fig. 4a). Compared with normal mice, the expression of CCR6 on γδ T cells markedly increased in skin lesions after IMQ-induced psoriasis (Fig. 4b). Of note, CCR6 was primarily expressed on homing $V\gamma4^+$ γδ T cells, but not on resident $V\gamma5^+$ γδ T cells (Fig. 4b). Furthermore, the frequency and absolute cell number of $CCR6^+$ $V\gamma4^+$ γδ T cells in skin lesions in CD30LKO mice were significantly higher than that in WT mice (Fig. 4c).

The skin homing γδ T cells are mainly derived from either the dLN or circulation, so we further analyzed the γδ T cells and their subsets within the dLN. As shown in Supplementary Fig. 5a, the percentage and cell number of the $V\gamma4^+$ γδ T cell subset in the dLN of CD30LKO mice markedly increased at day 5 after IMQ-administration, while $V\gamma1^+$ γδ T cells had no significant change, as compared with those in WT mice. Furthermore, the expression of CCR6 in γδ T cells was significantly higher than that in WT mice (Supplementary Fig. 5b). Moreover, in γδ T cell subsets of CD30LKO mice, only $V\gamma4^+$ γδ T cells expressing CCR6 were significantly increased when compared with those in WT mice, yet $V\gamma1^+\gamma\delta$ T cells from the dLN from WT and CD30LKO mice hardly expressed CCR6 (Supplementary Fig. 5b–d). We noted that compared with WT mice, there was a significant increase in the percentage and cell number of the $CD27^-V\gamma4^+\gamma\delta$ T cell subset in dLN of CD30LKO mice, but no evident change in the cell number in the $CD27^+V\gamma4^+\gamma\delta$ T cell subset (Supplementary Fig. 5e). In addition, we measured expression of Ki-67, a proliferation marker, and found that Ki-67 expression in γδ T cells and particularly $V\gamma4^+$ γδ T cell subset in dLN of CD30LKO mice was significantly increased (Supplementary Fig. 5f). Therefore, these results suggest that *Cd30l* gene deletion promotes the proliferation of the $CCR6^+$ $CD27^-$ $V\gamma4^+$ γδ T cell subset in the dLN of mice with IMQ-induced psoriasis. Furthermore, an increase in the number of skin-homing $V\gamma4^+$ γδ T cells were observed, thus contributing to the formation of "malignant loop" in psoriasis and aggravating the symptoms and progression of psoriasis.

3.5. *CD30L/CD30 signaling transduction down-regulates CCR6 expression in the $V\gamma4^+$ γδ T cell subset*

The experimental results described above suggest that CD30L/CD30

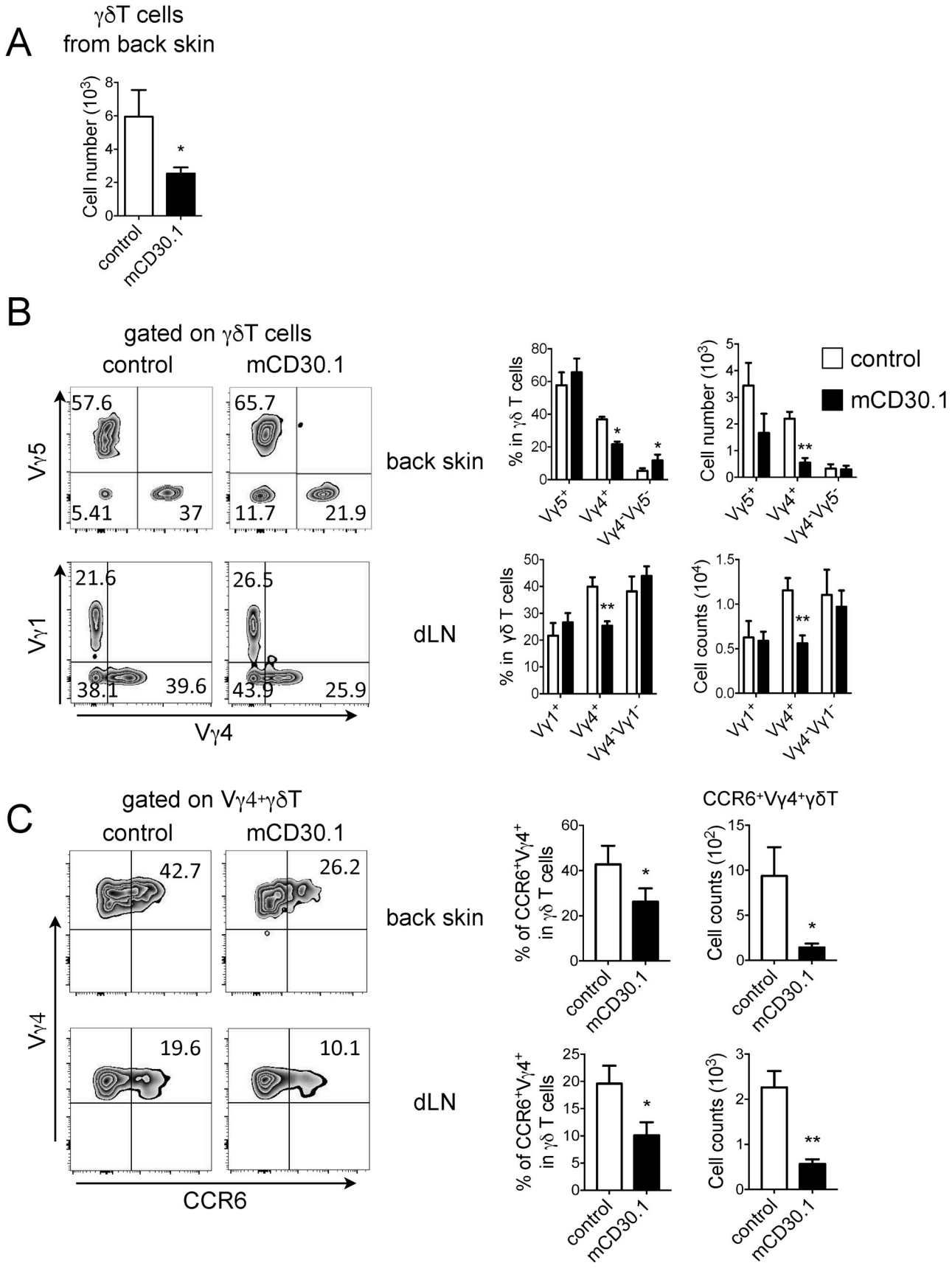

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Fig. 7. mCD30.1 mA b treatment significantly inhibited the function of Vγ4$^+$ γδ T17 cells. WT mice were treated with mCD30.1 mA b or control mAb during psoriasis-induction by IMQ; single cells from back skin or the dLN were analyzed by flow cytometry at day 5 after IMQ/mAb treatment. (**a**) The total cell number of γδ T cells in back skin was enumerated. (b) The cell number and percentage of γδ T cell subsets (Vγ1$^+$, Vγ4$^+$, Vγ5$^+$ or Vγ1$^-$Vγ4$^-$) from back skin or dLNs were measured. (c) The expression of CCR6 in Vγ4$^+$ γδ T cells and the absolute number of CCR6$^+$Vγ4$^+$ γδ T cells in back skin or dLN are shown. The cell number and percentage of IL-17A- or IL-17F-producing γδ T cells (d) and IL-17A- or IL-17F-producing Vγ4$^+$ γδ T cells (e) in back skin or dLN were measured. Data indicate the mean ± s.d. (n = 5 mice per group) from a representative of three separate experiments. Statistically significant differences between control and mCD30.1 mA b treated mice are shown (*P < 0.05; **P < 0.01).

signaling may selectively contribute to migration of Vγ4$^+$ γδ T cell subset into the inflamed skin tissue. In order to explore this possibility, we dynamically and comparatively analyzed the expression correlation between CD30L and CCR6 in γδ T cell subsets. *In vitro*, after the culture of dLN cells from WT mice with or without anti-CD3 mAb stimulation, we detected the expression changes of CCR6 or CD30L in various γδ T cell subsets. The CCR6-expressing γδ T cell subset was primarily a Vγ4$^+$ subset (Supplementary Fig. 6a and b). After activation via TCR stimulation, CD30L$^{+/+}$Vγ4$^+$ γδ T cells expressed high level of CD30L (Supplementary Fig. 6c), but CCR6 was simultaneously gradually downregulated. This result is consistent with a recent report, that γδ T17 cells downregulate CCR6 upon activation [35]. However, CD30L$^{-/-}$Vγ4$^+$ γδ T cells continued to express high level of CCR6 (Supplementary Fig. 6d). In addition, CD30 expression on Vγ4$^+$ γδ T cells in skin lesions from CD30LKO mice was significantly increased, as compared with that in WT mice (Supplementary Fig. 6e). These findings suggest that CCR6 was primarily expressed on the Vγ4$^+$ γδ T cell subset and up-regulation of CD30L expression appeared to inhibit CCR6 expression in Vγ4$^+$ γδ T cells. These data suggest that the absence of CD30L/CD30 signal transduction may cause activated Vγ4$^+$ γδ T cells in the dLN to continuously express a high level of CCR6, further promoting their migration into inflammatory skin tissue and thus

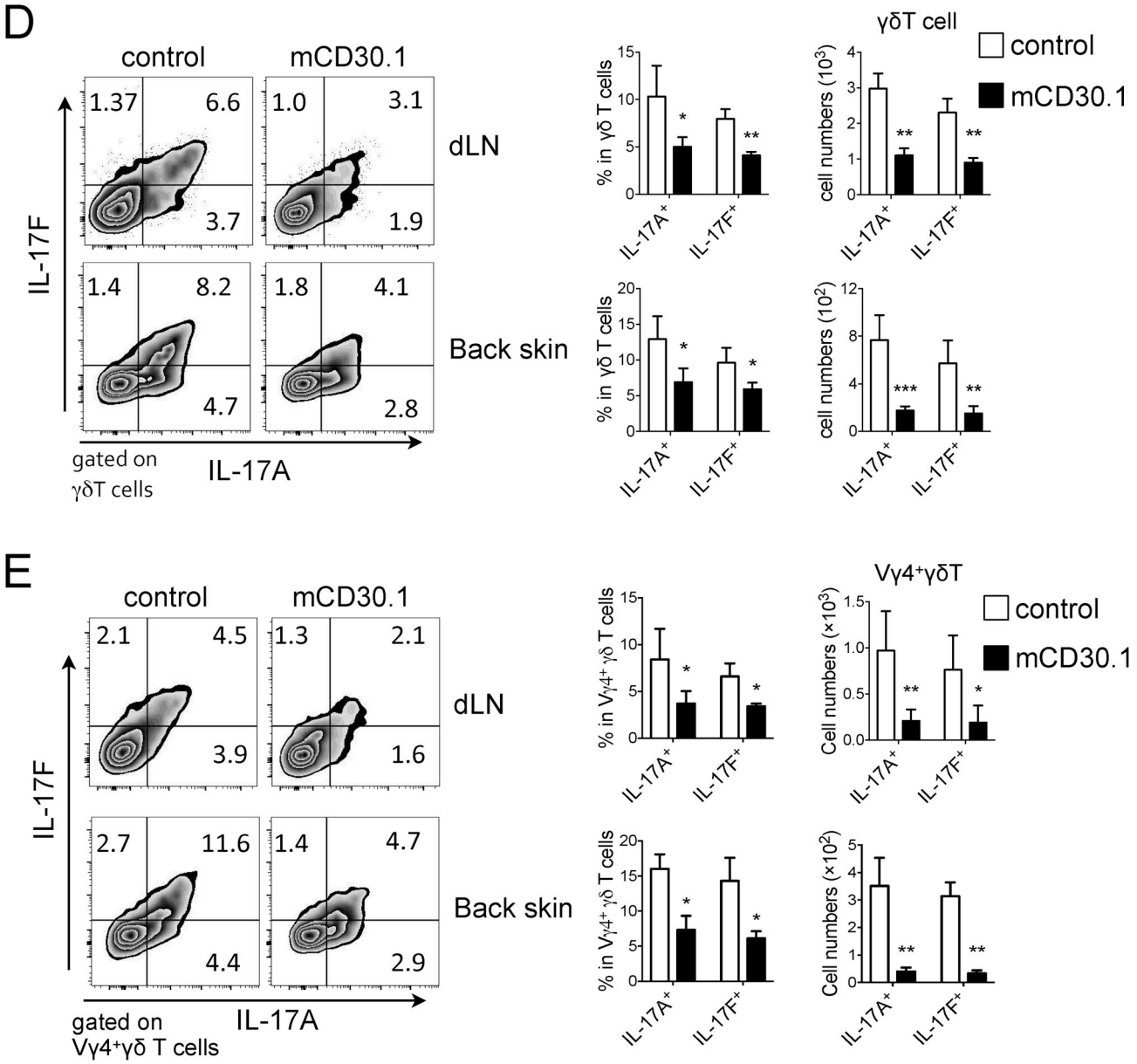

Fig. 7. (*continued*)

mediating the skin inflammatory response.

3.6. Cd30l gene deletion enhances cytokine production by CCR6$^+$Vγ4$^+$ γδ T17 cells

CCR6$^+$Vγ4$^+$ γδ T cells in the dLN migrated to the psoriatic skin lesions and contributed to the excessive proliferation of KCs and the release of bioactive factors through the secretion several cytokines, thus accelerating the formation of the "malignant loop" in psoriasis [36]. Therefore, we further investigated the function of activated γδ T cells, especially Vγ4$^+$ γδ T cell subset in the skin lesions and dLNs. The expressions of IL-17A, IL-22 and IL-17F in the skin lesions of CD30LKO mice were significantly higher than those in WT mice at mRNA and protein levels (Fig. 5a and b). Compared with WT mice, the frequency and the number of IL-17A-, IL-22-, or IL-17F-producing γδ T cells in the skin lesions of CD30LKO mice were increased (Fig. 5c), while no significant change was observed in IFN-γ-producing γδ T cells (data not shown). In particular, the expression of IL-17A, IL-22, and IL-17F in the Vγ4$^+$ γδ T cell subset in skin lesions was significantly increased (Fig. 5d). Furthermore, IL-17A$^+$ or IL-22$^+$ γδ T cells from the dLN also expressed high level of CCR6 in CD30LKO mice as compared with those in WT mice (Supplementary Fig. 7a and b). In γδ T cell subsets, the percentage and absolute cell number of IL-17A$^+$, IL-22$^+$, or IL-17A$^+$ IL-22$^+$ Vγ4$^+$ γδ T cells in the dLN were markedly increased in CD30LKO mice (Supplementary Fig. 7c). In addition, the secretion of inflammatory cytokines such as IL-22, IL-17A, IL-6, and TNFα in the dLN were also significantly higher in CD30LKO mice than those in WT mice (Supplementary Fig. 7d). Thus, *Cd30l* gene deletion selectively induces Vγ4$^+$ γδ T cells in the dLN to continuously express high level of CCR6 under the chemotactic action of CCL20; then CCR6$^+$CD27$^-$Vγ4$^+$ γδ T cells migrate into the inflammatory skin tissues and induce the infiltration of neutrophils to the skin lesions by producing a large quantity of γδT17-associated cytokines including IL-17A, IL-22, and IL-17F, thus promoting the progression of psoriasis in mice. Therefore, CD30L/CD30 signal transduction plays an important and novel protective role in the pathogenesis of IMQ-induced psoriasis in mice.

3.7. Evaluation of the therapeutic efficacy of a CD30L/CD30 signaling-targeted agonistic mAb on psoriasis in WT mice

Here, we have demonstrated that CD30L/CD30 signal transduction plays a protective role in the pathogenesis of IMQ-induced psoriasis via *in vivo* and *in vitro* experiments. Thus, we evaluated the therapeutic efficacy of a CD30L/CD30 signaling targeted agonistic mAb on psoriasis in WT mice. First, the agonistic anti-mouse CD30 antibody (clone: mCD30.1) was prepared with hybridoma cells and then purified; we have previously demonstrated that the mCD30.1 mA b can specifically bind to CD30 molecules and stimulate CD30 signal transduction [23]. WT mice were injected intraperitoneally with mCD30.1 mA b or isotype antibody (hamster IgG1, as a control) at a dose of 100 μg/head/day from 0 d to 5 d during psoriasis induction with IMQ. Thereafter, the development of psoriasis in mice was observed. As shown in Fig. 6, after treatment with agonistic mCD30.1 mA b, the scaling, erythema, skin thickness and related disease activity index scores of the mice were markedly improved compared with those in the control group. Second, the number of γδ T cells in the skin lesions was markedly reduced after treatment with agonistic mCD30.1 mA b (Fig. 7a); the decrease was primarily observed in the percentage and absolute number of Vγ4$^+$γδ T cells (Fig. 7b). Furthermore, the expression CCR6 in the Vγ4$^+$γδ T cell subset also significantly down-regulated (Fig. 7c). Meanwhile, there was a marked reduction in the expression of IL-17A, IL-22, and IL-17F in γδ T cells, particularly in the Vγ4$^+$γδ T cell subset in both the dLN and the skin of mice after agonistic mCD30.1 mA b treatment as compared with control group (Fig. 7d and e). Finally, we also investigated the effects of antibody-regulated CD30L/CD30 signaling on the expression of IL-17A and CCR6 in Vγ4$^+$γδ T cells from dLN *in vitro*. In accordance with the *in vivo* experimental results, blocking CD30L/CD30 signaling with a soluble anti-CD30L mAb significantly promoted the expression of IL-17A and CCR6 in activated Vγ4$^+$γδ T cells, and stimulating this signaling with an agonistic mCD30.1 mA b reversed these results (Supplementary Fig. 8). These results demonstrate that stimulation of CD30L/CD30 signal transduction with the agonistic mCD30.1 mA b can partially inhibit migration to the skin by the Vγ4$^+$ γδ T cell subset and can downregulate the secretion of Th17-related cytokines, thus partially inhibiting the formation of "malignant loop" of psoriasis. This treatment had a significant therapeutic effect on psoriasis in mice. Therefore, the CD30L/CD30 signal may be a potential candidate target for immunological therapy of psoriasis.

4. Discussion

In this study, we investigated the role of CD30L/CD30 signal transduction in the inflammatory responses mediated by skin γδT17 cells, using CD30LKO mice to establish an IMQ-induced skin psoriasis animal model. Our results show that *CD30l* gene deletion causes γδ T cells, especially the Vγ4$^+$γδ T cell subset, to express high level of CCR6, promotes skin-homing of the Vγ4$^+$γδ T cell subset, and secretion of the cytokines IL-17A, IL-22, and IL-17F, thus accelerating formation of "malignant loop" of psoriasis and aggravating skin inflammation in CD30LKO mice. In addition, we confirmed that agonistic antibody therapy targeting CD30 signaling has a significant effect on psoriasis in WT mice, suggesting that the CD30L/CD30 signaling may be a potential candidate target for immunological therapy in psoriasis.

Previously, it was widely believed that the IFN-γ/TNFα-Th1 axis and the IL-23/IL-17A-Th17 axis played a pivotal role in the pathogenesis of psoriasis in both humans and mice. Th1 cells produce TNFα and IFN-γ, whereas Th17 cells secrete IL-17A, IL-17F, and IL-22. When delivered to the skin, these proinflammatory cytokines can act on KCs and endothelial cells, leading to activation, proliferation and production of antimicrobial peptides [2,37]. In our previous studies, we demonstrated that CD30L/CD30 signaling plays an important role in enhancing Th17 and Th1 cells responses in the intestinal mucosa and nervous system [24,25,38,39]. Here, we unexpectedly found that *Cd30l* gene deletion caused a significant increase in susceptibility to psoriasis induced by IMQ. Although the accumulation of CD4$^+$ or CD8$^+$ αβ T cells in the skin lesions of both WT and CD30LKO mice after inducing skin inflammation was significantly higher than that in normal skin tissues, there was no significant difference in the recruitment of αβ T cells to the inflamed lesions between WT and CD30LKO mice. Thus, it appears that the enhanced inflammatory response in skin lesions of CD30LKO mice is not related to immune responses mediated by αβ T cells. This result is not consistent with the role of CD30L/CD30 signaling in the intestinal mucosal immune response mediated by Th1 or Th17 cells; however, it suggests that CD30L/CD30 signaling outcomes differ depending on the "regional immunity" of the tissue type and thus may play different regulatory roles in the skin and intestinal mucosa tissues.

Recently, a large number of studies showed that the primary source of IL-17A was not the expected Th17 cells, but a distinct population of skin-invading immune cells called γδ T cells, which are widely involved in the occurrence and development of psoriasis [12,13,14,40]. Further, γδ T cells ablation results in drastically reduced disease [12,40]. In addition, γδ T cells also contribute to the production of IL-22 and IL-17F and expressed the chemokine receptor CCR6 [40], which allows them to be recruited into skin [41]. At the same time, conventional αβ T cells, including Th17 cells, do not contribute to the development of psoriasis lesions in this model [42,43]. The relevance of these findings was underlined in human studies where a subset of IL-17A-producting γδ T cells was found in the skin of patients with psoriasis [11]. Taken together, all this evidence suggests that skin-homing γδ T cells are the predominant source of so-called Th17 cytokines, such as IL-17A, IL-17F, and IL-22. According to our results in this study, skin-homing γδ T cells mainly include the Vγ4$^+$ and Vγ6$^+$ γδ T cell subsets, but not the Vγ1$^+$

subset. Noticeably, *Cd30l* gene deletion only resulted in enhanced skin-homing of the Vγ4$^+$ subset, but not the Vγ6$^+$ subset. In contrast, the migration of Vγ6$^+$ γδ T cell subset into skin lesions in CD30LKO mice decreased as compared with that in WT mice. This finding is consistent with our previous study in intestinal mucosa and mucosa-related tissues [23]. Moreover, Vγ4$^+$ γδ T cells, but not other subsets of γδ T cells, in both the dLNs and skin lesions of CD30LKO mice expressed high levels of CCR6 after psoriasis induction by IMQ. In accordance with our *in vitro* results, *Cd30l* gene deficiency may upregulate CCR6 expression on Vγ4$^+$ γδ T cells. Hence, CD30/CD30L signal transduction may inhibit skin-homing by downregulating the expression of CCR6 in Vγ4$^+$γδ T cells. However, CD30L was also found to be highly expressed in skin macrophages, even though the expression level was not significantly different prior to and following the induction of skin inflammation. Therefore, the cellular interaction patterns through CD30L and CD30 and the specific intracellular signal regulation mechanisms remain uncertain, at least in the present study, and require further investigation.

Based on CD27 expression, γδ T cell subsets can be further divided into two subsets: CD27$^+$ and CD27$^-$. The CD27$^-$ subset primarily expresses T17-associated cytokines like IL-17A, while the CD27$^+$ subset are the primary source of IFN-γ [44]. We showed that increased Vγ4$^+$ γδ T cells in the dLN of CD30LKO mice are part of the CD27$^-$ population, but not the CD27$^+$ population. Therefore, the production of IL-17A, IL-17F, or IL-22 by CD30L$^{-/-}$CCR6$^+$Vγ4$^+$ γδ T cells was significantly increased while the production of INF-γ. Indeed, these findings were verified in subsequent treatment experiments, where it was found that stimulation of CD30L/CD30 signaling by an agonistic anti-CD30 mAb effectively alleviated psoriasis skin inflammation symptoms in WT mice, through down-regulation of CCR6 expression in Vγ4$^+$ γδ T cells and inhibition of secretion of T17-related cytokines.

5. Conclusion

The present study has clarified the regulatory effects of CD30L/CD30 signaling on the skin-homing, proliferation, and cytokine production of the Vγ4$^+$ γδ T cell subset during the skin inflammation associated with psoriasis. In addition, we found that targeting the CD30L/CD30 signal via agonistic antibody therapy had a significant effect on psoriasis in mice. Our findings reveal a previously unknown CD30L/CD30 signal-mediated protective mechanism for psoriasis and highlight a critical role for Vγ4$^+$ γδ T cells in the formation of an immune “malignant loop” during psoriasis. Therefore, modulation of CD30L/CD30 signaling by an agonistic anti-CD30 mAb may become a novel biological therapy for psoriasis.

Author contributions

D.Y., Y.Y., and X.Z. performed experiments, analyzed data, and wrote the manuscript; X.W., R.Q., and F.Y. established and evaluated IMQ-induced psoriasis models; X.M. and B.W. reviewed and wrote the manuscript. Y.Y. provided the CD30LKO mice. Y.W. and X.S. designed the project, supervised experiments, and wrote the manuscript.

Conflicts of interest

The authors have no financial conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.04.009.

References

[1] N. Ayala-Fontanez, D.C. Soler, T.S. McCormick, Current knowledge on psoriasis and autoimmune diseases, Psoriasis Targets Ther. 6 (2016) 7–32, https://doi.org/10.2147/PTT.S64950.
[2] G.K. Perera, P. Di Meglio, F.O. Nestle, Psoriasis, Annu. Rev. Pathol. Mech. Dis. 7 (2012) 385–422, https://doi.org/10.1146/annurev-pathol-011811-132448.
[3] M.A. Lowes, M. Suárez-Fariñas, J.G. Krueger, Immunology of psoriasis, Annu. Rev. Immunol. 32 (2014) 227–255, https://doi.org/10.1146/annurev-immunol-032713-120225.
[4] E.F. Wagner, H.B. Schonthaler, J. Guinea-Viniegra, E. Tschachler, Psoriasis: what we have learned from mouse models, 6 Nature Publishing Group, 2010, pp. 704–714, https://doi.org/10.1038/nrrheum.2010.157.
[5] R.A. Clark, Skin-resident T cells: the ups and downs of on site immunity, J. Investig. Dermatol. 130 (2010) 362–370, https://doi.org/10.1038/jid.2009.247.
[6] J. Kim, J.G. Krueger, Highly effective new treatments for psoriasis target the IL-23/type 17 T cell autoimmune Axis, Annu. Rev. Med. 68 (2017) 255–269, https://doi.org/10.1146/annurev-med-042915-103905.
[7] T.P. Singh, C.H. Lee, J.M. Farber, Chemokine receptors in psoriasis, Expert Opin. Ther. Targets 17 (2013) 1405–1422, https://doi.org/10.1517/14728222.2013.838220.
[8] K.E. Nograles, L.C. Zaba, E. Guttman-Yassky, J. Fuentes-Duculan, M. Suárez-Fariñas, I. Cardinale, et al., Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways, Br. J. Dermatol. 159 (2008) 1092–1102, https://doi.org/10.1111/j.1365-2133.2008.08769.x.
[9] S. Eyerich, K. Eyerich, D. Pennino, T. Carbone, F. Nasorri, S. Pallotta, et al., Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling, J. Clin. Investig. 119 (2009) 3573–3585, https://doi.org/10.1172/JCI40202.
[10] M. Kennedy-Crispin, E. Billick, H. Mitsui, N. Gulati, H. Fujita, P. Gilleaudeau, et al., Human keratinocytes' response to injury upregulates CCL20 and other genes linking innate and adaptive immunity, J. Investig. Dermatol. 132 (2012) 105–113, https://doi.org/10.1038/jid.2011.262.
[11] U. Laggner, P. Di Meglio, G.K. Perera, C. Hundhausen, K.E. Lacy, N. Ali, et al., Identification of a novel proinflammatory human skin-homing Vγ9Vδ2 T cell subset with a potential role in psoriasis, J. Immunol. 187 (2011) 2783–2793, https://doi.org/10.4049/jimmunol.1100804.
[12] F. Ramírez-Valle, E.E. Gray, J.G. Cyster, Inflammation induces dermal Vγ4+ γδT17 memory-like cells that travel to distant skin and accelerate secondary IL-17-driven responses, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 8046–8051, https://doi.org/10.1073/pnas.1508990112.
[13] Y. Cai, X. Shen, C. Ding, C. Qi, K. Li, X. Li, et al., Pivotal role of dermal IL-17-producing γδ T cells in skin inflammation, Immunity 35 (2011) 596–610, https://doi.org/10.1016/j.immuni.2011.08.001.
[14] R. Yoshiki, K. Kabashima, T. Honda, S. Nakamizo, Y. Sawada, K. Sugita, et al., IL-23 from langerhans cells is required for the development of imiquimod-induced psoriasis-like dermatitis by induction of IL-17a-producing γδ T cells, J. Investig. Dermatol. 134 (2014) 1912–1921, https://doi.org/10.1038/jid.2014.98.
[15] S. Shibata, C.S. Hau, A. Mitsui, M. Kamata, Y. Asano, M. Sugaya, et al., Adiponectin regulates psoriasiform skin inflammation by suppressing IL-17 production from γδ-T cells, Nat. Commun. 6 (2015) 1–14, https://doi.org/10.1038/ncomms8687.
[16] T. Mabuchi, T.P. Singh, T. Takekoshi, G.-F. Jia, X. Wu, M.C. Kao, et al., CCR6 is required for epidermal trafficking of γδ-T cells in an IL-23-induced model of psoriasiform dermatitis, J. Investig. Dermatol. 133 (2013) 164–171, https://doi.org/10.1038/jid.2012.260.
[17] Y. Imai, N. Ayithan, X. Wu, Y. Yuan, L. Wang, S.T. Hwang, Cutting edge: PD-1 regulates imiquimod-induced psoriasiform dermatitis through inhibition of IL-17a expression by innate γδ-low T cells, J. Immunol. 195 (2015) 421–425, https://doi.org/10.4049/jimmunol.1500448.
[18] T.H. Watts, TNF/TNFR family members in costimulation of T cell responses, Annu. Rev. Immunol. 23 (2005) 23–68, https://doi.org/10.1146/annurev.immunol.23.021704.115839.
[19] L.K. Ward-Kavanagh, W.W. Lin, J.R. Šedý, C.F. Ware, The TNF receptor superfamily in Co-stimulating and Co-inhibitory responses, Immunity 44 (2016) 1005–1019, https://doi.org/10.1016/j.immuni.2016.04.019.
[20] M. Fischer, I.T. Harvima, R.F.S. Carvalho, C. Möller, A. Naukkarinen, G. Enblad, et al., Mast cell CD30 ligand is upregulated in cutaneous inflammation and mediates degranulation-independent chemokine secretion, J. Clin. Investig. 116 (2006) 2748–2756, https://doi.org/10.1172/JCI24274.
[21] B.R. Blazar, R.B. Levy, T.W. Mak, A. Panoskaltsis-Mortari, H. Muta, M. Jones, et al., CD30/CD30 ligand (CD153) interaction regulates CD4+ T cell-mediated graft-versus-host disease, J. Immunol. 173 (2004) 2933–2941.
[22] J.S. Heilig, S. Tonegawa, Diversity of murine gamma genes and expression in fetal and adult T lymphocytes, Nature 322 (1986) 836–840, https://doi.org/10.1038/322836a0.
[23] X. Sun, K. Shibata, H. Yamada, Y. Guo, H. Muta, E.R. Podack, et al., CD30L/CD30 is critical for maintenance of IL-17A-producing γδ T cells bearing Vγ6 in mucosa-associated tissues in mice, Mucosal Immunol. 6 (2013) 1191–1201, https://doi.org/10.1038/mi.2013.18.
[24] X. Sun, H. Yamada, K. Shibata, H. Muta, K. Tani, E.R. Podack, et al., CD30 ligand/CD30 plays a critical role in Th17 differentiation in mice, J. Immunol. 185 (2010) 2222–2230, https://doi.org/10.4049/jimmunol.1000024.
[25] X. Sun, H. Yamada, K. Shibata, H. Muta, K. Tani, E.R. Podack, et al., CD30 ligand is

a target for a novel biological therapy against colitis associated with Th17 responses, J. Immunol. 185 (2010) 7671–7680, https://doi.org/10.4049/jimmunol.1002229.
[26] H. Sumida, K. Yanagida, Y. Kita, J. Abe, K. Matsushima, M. Nakamura, et al., Interplay between CXCR2 and BLT1 facilitates neutrophil infiltration and resultant keratinocyte activation in a murine model of imiquimod-induced psoriasis, J. Immunol. 192 (2014) 4361–4369, https://doi.org/10.4049/jimmunol.1302959.
[27] J.A. Diaz-Perez, M.E. Killeen, Y. Yang, C.D. Carey, L.D. Falo, A.R. Mathers, Extracellular ATP and IL-23 form a local inflammatory circuit leading to the development of a neutrophil-dependent psoriasiform dermatitis, J. Investig. Dermatol. 138 (2018) 2595–2605, https://doi.org/10.1016/j.jid.2018.05.018.
[28] H.J. Kim, J.Y. Roh, Y. Jung, Eosinophils accelerate pathogenesis of psoriasis by supporting an inflammatory milieu that promotes neutrophil infiltration, J. Investig. Dermatol. 138 (2018) 2185–2194, https://doi.org/10.1016/j.jid.2018.03.1509.
[29] Y. Natsuaki, G. Egawa, S. Nakamizo, S. Ono, S. Hanakawa, T. Okada, et al., Perivascular leukocyte clusters are essential for efficient activation of effector T cells in the skin, Nat. Immunol. 15 (2014) 1064–1069, https://doi.org/10.1038/ni.2992.
[30] Y. Zheng, D.M. Danilenko, P. Valdez, I. Kasman, J. Eastham-Anderson, J. Wu, et al., Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis, Nature 445 (2007) 648–651, https://doi.org/10.1038/nature05505.
[31] C. Ortega, S. Fernández-A, J.M. Carrillo, P. Romero, I.J. Molina, J.C. Moreno, et al., IL-17-producing CD8+ T lymphocytes from psoriasis skin plaques are cytotoxic effector cells that secrete Th17-related cytokines, J. Leukoc. Biol. 86 (2009) 435–443, https://doi.org/10.1189/JLB.0109046.
[32] Y. Cai, C. Fleming, J. Yan, New insights of T cells in the pathogenesis of psoriasis, Cell. Mol. Immunol. 9 (2012) 302–309, https://doi.org/10.1038/cmi.2012.15.
[33] M.N. Hedrick, A.S. Lonsdorf, A.-K. Shirakawa, C.-C. Richard Lee, F. Liao, S.P. Singh, et al., CCR6 is required for IL-23-induced psoriasis-like inflammation in mice, J. Clin. Investig. 119 (2009) 2317–2329, https://doi.org/10.1172/JCI37378.
[34] T. Mabuchi, T. Takekoshi, S.T. Hwang, Epidermal CCR6 +γδ T cells are major producers of IL-22 and IL-17 in a murine model of psoriasiform dermatitis, J. Immunol. 187 (2011) 5026–5031, https://doi.org/10.4049/jimmunol.1101817.
[35] D.R. McKenzie, E.E. Kara, C.R. Bastow, T.S. Tyllis, K.A. Fenix, C.E. Gregor, et al., IL-17-producing γδ T cells switch migratory patterns between resting and activated states, Nat. Commun. 8 (2017) 15632, https://doi.org/10.1038/ncomms15632.
[36] E.E. Gray, F. Ramírez-Valle, Y. Xu, S. Wu, Z. Wu, K.E. Karjalainen, et al., Deficiency in IL-17-committed Vγ4+ γδ T cells in a spontaneous Sox13-mutant CD45.1+ congenic mouse substrain provides protection from dermatitis, Nat. Immunol. 14 (2013) 584–592, https://doi.org/10.1038/ni.2585.
[37] B. Becher, S. Pantelyushin, Interleukin-17–producing γδ T cells go under the skin? Nat. Med. 18 (2012) 1748–1750, https://doi.org/10.1038/nm.3016.
[38] X. Sun, S. Somada, K. Shibata, H. Muta, H. Yamada, H. Yoshihara, et al., A critical role of CD30 ligand/CD30 in controlling inflammatory bowel diseases in mice, Gastroenterology 134 (2008) 447–458, https://doi.org/10.1053/j.gastro.2007.11.004.
[39] K. Shinoda, X. Sun, A. Oyamada, H. Yamada, H. Muta, E.R. Podack, et al., CD30 ligand is a new therapeutic target for central nervous system autoimmunity, J. Autoimmun. 57 (2015) 14–23, https://doi.org/10.1016/j.jaut.2014.11.005.
[40] S. Pantelyushin, S. Haak, B. Ingold, P. Kulig, F.L. Heppner, A.A. Navarini, et al., Rorγt+ innate lymphocytes and γδ T cells initiate psoriasiform plaque formation in mice, J. Clin. Investig. 122 (2012) 2252–2256, https://doi.org/10.1172/JCI61862.
[41] J.J. Campbell, K. Ebsworth, L.S. Ertl, J.P. McMahon, D. Newland, Y. Wang, et al., IL-17-Secreting γδ T cells are completely dependent upon CCR6 for homing to inflamed skin, J. Immunol. 199 (2017) 3129–3136, https://doi.org/10.4049/jimmunol.1700826.
[42] M. Gilliet, C. Conrad, M. Geiges, A. Cozzio, W. Thürlimann, G. Burg, et al., Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors, Arch. Dermatol. 140 (2004) 1490–1495, https://doi.org/10.1001/archderm.140.12.1490.
[43] R. Bissonnette, K. Papp, C. Maari, Y. Yao, G. Robbie, W.I. White, et al., A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti-interferon-alfa monoclonal antibody, in subjects with chronic psoriasis, J. Am. Acad. Dermatol. 62 (2010) 427–436, https://doi.org/10.1016/j.jaad.2009.05.042.
[44] J.C. Ribot, A. deBarros, D.J. Pang, J.F. Neves, V. Peperzak, S.J. Roberts, et al., CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets, Nat. Immunol. 10 (2009) 427–436, https://doi.org/10.1038/ni.1717.