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Psoriasis-associated impairment of CCL27/CCR10-derived regulation leads to IL-17A/IL-22-producing skin T cell over-activation

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Capsule summary

Psoriasis-associated suppression of the skin-specific chemokine/receptor CCL27/CCR10 axis leads to enhanced pathogenic IL-17A/IL-22-producing skin T cell activation and inflammation.

Keywords

psoriasis; immune dysregulation; CCL27; CCR10; IL-17A; IL-22; γδT cells; αβT cells; innate lymphoid cells (ILCs); imiquimod; microneedles

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AUTHOR CONTRIBUTION

C.L. performed experiments of IMQ-induced CCR10-KO mice and CCL27 expression analysis, analyzed data and contributed to the manuscript writing, M.X. performed experiments of CCL27-treatment of skin inflammation, analyzed data and contributed to the manuscript writing. J.C. prepared the CCL27 microneedles, performed experiments of CCL27-treatment of skin inflammation, and contributed to the manuscript writing. W.W. initiated experiments on IMQ-treated CCR10 mice. M.D. participated in experiments of the CCL27 expression. Y.W. supervised the study, analyzed data and contributed to the manuscript writing. N.X. designed and supervised the study, analyzed data and wrote the manuscript.

All authors declare no conflict of interest.

To the editor

Skin inflammatory symptoms of psoriasis are predominantly caused by pathogenic cytokines such as IL-17A/IL-22 overproduced by dysregulated T cells. Targeting pathogenic cytokines is effective in treatment of the symptoms but does not treat the T cell dysregulation. Molecular mechanisms responsible for the T cell dysregulation are currently poorly understood. We report here that psoriasis-associated suppression of CCL27/CCR10-derived immune regulatory signals contributes to enhanced IL-17A/IL-22-producing skin T cell over-activation and inflammation.

CCL27 is a skin-specific chemokine expressed by skin epithelial cells, and its receptor, CCR10, is expressed by skin lymphocytes[1]. The CCL27/CCR10 axis has been long suggested to promote skin inflammatory diseases including psoriasis by facilitating skin pathogenic T cell migration[2]. However, clinical studies found that CCL27 is severely suppressed in lesional skin of psoriatic patients[3, 4], inconsistent with a role of the CCL27/CCR10 axis in promoting psoriasis. Using mouse models, we recently discovered that CCR10 is important for homeostatic establishment of resident lymphocytes in healthy skin but dispensable for T cell migration to inflamed skin[5, 6, 7], supporting an opposite hypothesis that loss of CCL27/CCR10-derived regulatory signals might actually lead to enhanced skin T cell over-activation in psoriasis.

We tested this hypothesis first in CCR10-knockout (KO)/EGFP-knockin (KI) mice using an imiquimod (IMQ)-induced model of psoriasis, in which topical IMQ application induces skin inflammation mainly through activation of IL-17A/IL-22-producing γδT (γδT17) cells[8]. In CCR10-KO/EGFP-KI mice, the CCR10 coding sequence was replaced with a coding sequence for EGFP that could be used to report the CCR10 expression[5]. IMQ-treated homozygous CCR10-KO/EGFP-KI ($CCR10^{EGFP/EGFP}$ or $CCR10^{-/-}$ for simplicity) mice had more severe skin inflammation than IMQ-treated heterozygous ($CCR10^{+/EGFP}$ or $CCR10^{+/-}$) littermates while untreated $CCR10^{+/-}$ or $CCR10^{-/-}$ mice had no inflammation (Fig. 1A, Fig. E1A). Associated with this, IMQ-treated $CCR10^{-/-}$ mice had higher expansion of skin $IL\text{-}17A^{+}$, particularly $IL\text{-}17A^{+}IL\text{-}22^{+}$, γδT cells than IMQ-treated $CCR10^{+/-}$ littermates (Fig. 1B-D). Untreated $CCR10^{-/-}$ and $CCR10^{+/-}$ mice had similar skin $IL\text{-}17A^{+}$ γδT cells, few of which expressed IL-22 (Fig. 1B-D). Skin γδT cells of IMQ-treated $CCR10^{-/-}$ and $CCR10^{+/-}$ mice had lower EGFP(CCR10) than their untreated controls (Fig. E1B), suggesting downregulation of CCR10 in activated γδT cells. These results indicate that CCR10-derived signals suppress the IMQ-induced activation of IL-17A/IL-22-producing skin γδT cells and inflammation.

Besides γδT17 cells, IL-17-producing $CD4^{+}$ αβT (Th17) cells and innate lymphoid cells (ILCs) are also involved in psoriasis. IMQ-treated $CCR10^{-/-}$ mice had higher expansion of $IL\text{-}17A^{+}IL\text{-}22^{+}$ $CD4^{+}$ skin αβT cells than IMQ-treated $CCR10^{+/-}$ mice (Fig. E2A-C), suggesting that CCR10 also restricts Th17 cell activation. Associated with this, there were more significantly reduced percentages of $EGFP(CCR10)^{+}$ αβT cells in IMQ-treated skin of $CCR10^{-/-}$ than $CCR10^{+/-}$ mice since activated $IL\text{-}17A^{+}IL\text{-}22^{+}$ skin αβT cells had downregulated CCR10(EGFP) compared to $IL\text{-}17A^{-}IL\text{-}22^{-}$ or $IL\text{-}17A^{+}IL\text{-}22^{-}$ skin αβT cells (Fig. E3A-B). On the other hand, IMQ-treated $CCR10^{-/-}$ and $CCR10^{+/-}$ mice had

similar percentages of IL-17A$^+$ skin ILCs, which did not express IL-22 (Fig. E4A-B). However, IMQ-treated CCR10$^{-/-}$ mice had lower percentages of EGFP(CCR10)$^+$ skin ILCs than IMQ-treated CCR10$^{+/-}$ mice (Fig. E4C). Since CCR10$^+$ ILCs are involved in homeostatic regulation of skin αβT cells[7], these results suggest that CCR10 might support maintenance of regulatory ILCs to restrict the skin T cell activation and inflammation.

To demonstrate further that CCR10 was important in restricting activation of Th17 cells to suppress skin inflammation, we applied IMQ to the skin of Rag1$^{-/-}$ mice that were reconstituted with CCR10$^{-/-}$ or CCR10$^{+/-}$ CD4$^+$ αβT cells (Fig. 1E). In this setting, only αβT cells of the two donor origins had different CCR10-knockout statuses. Indeed, IMQ-treated Rag1$^{-/-}$ mice reconstituted with CCR10$^{-/-}$ αβT cells had more severe skin inflammation than IMQ-treated Rag1$^{-/-}$ mice reconstituted with CCR10$^{+/-}$ αβT cells (Fig. 1F, Fig. E5A). Associated with this, there was higher expansion of IL-17A$^+$IL-22$^+$ skin αβT (CD3$^+$TCRδ$^-$) cells of CCR10$^{-/-}$ than CCR10$^{+/-}$ donor origin (Fig. 1G-H). IL-17A$^+$IL-22$^+$ skin αβT cells expressed lower EGFP(CCR10) than IL-17A$^-$IL-22$^-$ or IL-17A$^+$IL-22$^-$ counterparts (Fig. E5B), again indicating downregulation of CCR10 in activated Th17 cells.

To test further whether CCR10 supported regulatory ILCs to restrict skin Th17 cell activation and inflammation, we applied IMQ to the skin of CCR10$^{+/-}$Rag1$^{-/-}$ or CCR10$^{-/-}$Rag1$^{-/-}$ mice that were reconstituted with same CCR10$^{+/-}$ CD4$^+$ αβT cells (Fig. 1I). In this setting, ILCs of CCR10$^{+/-}$Rag1$^{-/-}$ and CCR10$^{-/-}$Rag1$^{-/-}$ recipients had different CCR10-knockout statuses whereas donor αβT cells had no CCR10 defect. Compared to IMQ-treated CCR10$^{+/-}$Rag1$^{-/-}$ recipients, IMQ-treated CCR10$^{-/-}$Rag1$^{-/-}$ recipients had significantly worse skin inflammation (Fig. 1J, Fig. E6). IMQ-treated CCR10$^{-/-}$Rag1$^{-/-}$ recipients had fewer total and EGFP(CCR10)$^+$ skin ILCs than IMQ-treated CCR10$^{+/-}$Rag1$^{-/-}$ recipients (Fig. 1K-L), confirming that CCR10 is important for maintenance of skin ILCs with regulatory function on helper T cells. Supporting this notion, donor αβT (CD3$^+$TCRδ$^-$) cells in the IMQ-treated skin of CCR10$^{-/-}$Rag1$^{-/-}$ recipients expressed IL-17A and IL-22 more than those of CCR10$^{+/-}$Rag1$^{-/-}$ recipients did (Fig. 1M-N). Together, these results demonstrate that CCR10-dervied signals restrain over-activation of IL-17A/IL-22$^+$ γδT and αβT cells directly and indirectly (through promoting regulatory ILCs) to suppress skin inflammation.

We then tested whether delivery of exogenous CCL27 into the skin could reduce IMQ-induced inflammation in wild-type mice to determine biological importance of psoriasis-associated suppression of CCL27. Like human psoriatic skin[3, 4], IMQ-applied inflamed mouse skin had downregulated CCL27 expression (Fig. 2A), making it an appropriate model to test the effect of exogenous CCL27 delivery.

Microneedle patches made with the biocompatible polymers polyvinylpyrrolidone and polyvinyl alcohol were used to increase efficacy of the CCL27 delivery[9]. After insertion into the skin, tips of microneedles dissolve, allowing for delivery of cargoes evenly across an area of the skin (Fig. 2B, Fig. E7A-B).

Compared to untreated or empty microneedle-treated skin, CCL27-loaded microneedle-treated skin had reduced inflammation (Fig. 2C, Fig. E7C). Correlating with this, CCL27

treatment significantly reduced IMQ-induced expansion of skin γδT cells (Fig. 2D-E, Fig. E7D). Furthermore, CCL27-treated skin contained significantly lower percentages of IL-17A$^+$ γδT cells and IL-17A$^+$ αβT cells than control-treated skin (Fig. 2F,G,H).

In sum, we discovered that impairment of the CCL27/CCR10-mediated immune regulation contributes to increased skin IL-17-producing T cell activation and inflammation in mouse models of psoriasis, suggesting that its restoration might be a new therapeutic strategy against the disease. Clinical relevance and molecular mechanisms of the CCL27/CCR10-mediated inhibition of psoriasis are worth further investigation ("Discussion" in Online Repository).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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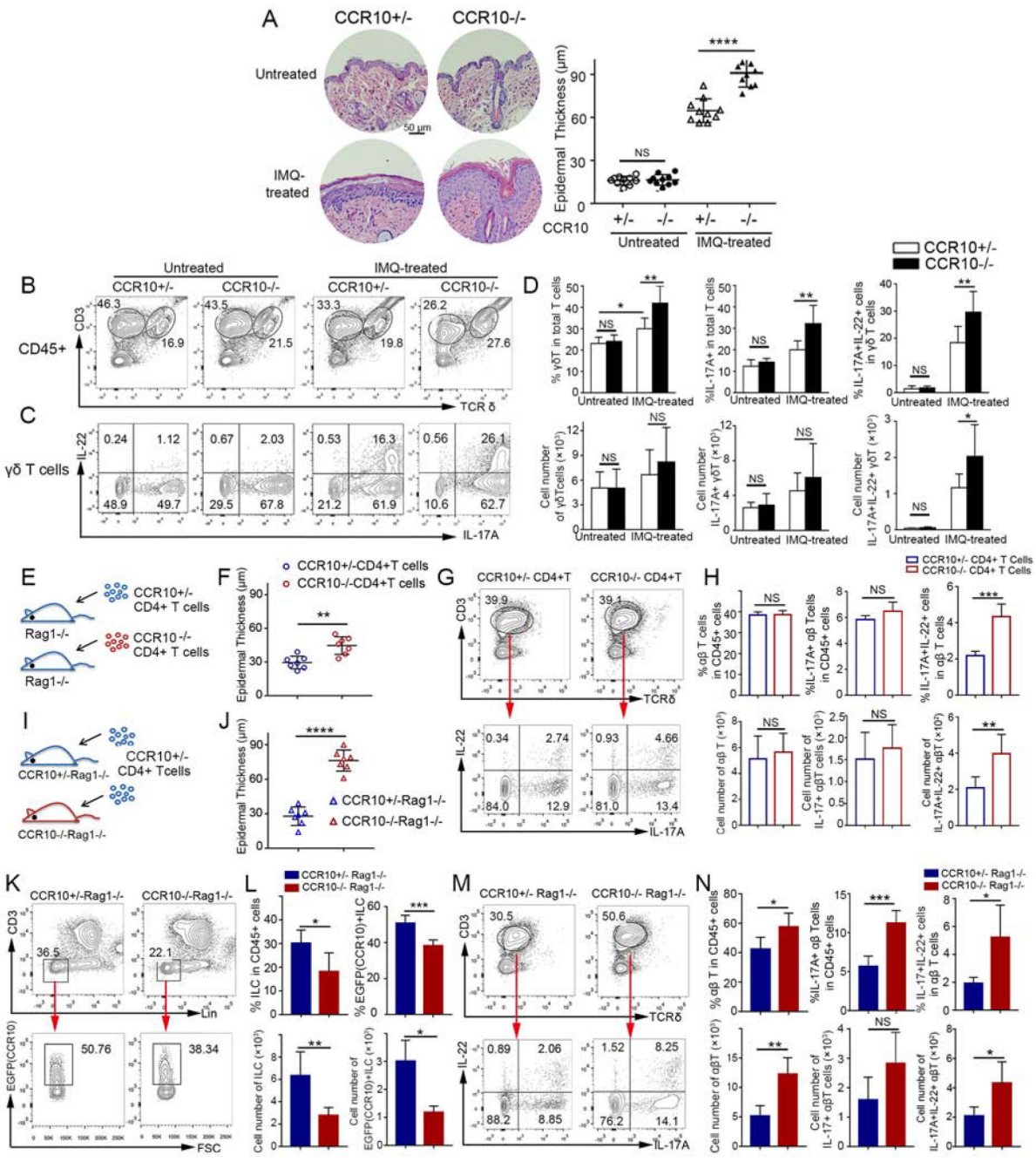

Figure 1.

Enhanced IL-17A/IL-22-producing skin T cell activation and inflammation in IMQ-treated CCR10-knockout mice. **(A,B,C,D)** H&E stained sections and average epidermal thickness (A), flow cytometric (FC) analysis of γδT cells (B) and their IL-17A/IL-22 production (C), and average percentages and numbers of IL-17A/IL-22^{+} γδT cells (D) of untreated and 5 day-IMQ-treated skin of CCR10$^{-/-}$ and CCR10$^{+/-}$ mice. (**E**) Scheme of reconstitution of Rag1$^{-/-}$ mice with CCR10$^{-/-}$ or CCR10$^{+/-}$ CD4^{+} αβT cells. (**F,G,H**) Average epidermal thickness (F), FC analysis of donor αβT cells and their IL-17A/IL-22 production (G), and average percentages and numbers of IL-17A/IL-22^{+} αβT cells (H) of the IMQ-treated skin

of $Rag1^{-/-}$ mice reconstituted with $CCR10^{-/-}$ or $CCR10^{+/-}$ $CD4^+$ αβT cells. **(I)** Scheme of reconstitution of $CCR10^{+/-}Rag1^{-/-}$ or $CCR10^{-/-}Rag1^{-/-}$ mice with same $CCR10^{+/-}$ $CD4^+$ αβT cells. **(J,K,L,M,N)** Average epidermal thickness (J), FC analysis (K) and percentages and numbers (L) of $EGFP(CCR10)^+$ host ILCs, and FC analysis (M) and percentages and numbers (N) of IL-17A/$IL\text{-}22^+$ donor αβT cells of the IMQ-treated skin of $CCR10^{+/-}Rag1^{-/-}$ and $CCR10^{-/-}Rag1^{-/-}$ mice reconstituted with $CCR10^{+/-}$ $CD4^+$ αβT cells. One dot represents one sample (A,F,J). N=5–9 (D) and 5 each (H,L,N). NS: not significant, *P<0.05, **P<0.01, ***P<0.001, ***P<0.0001.

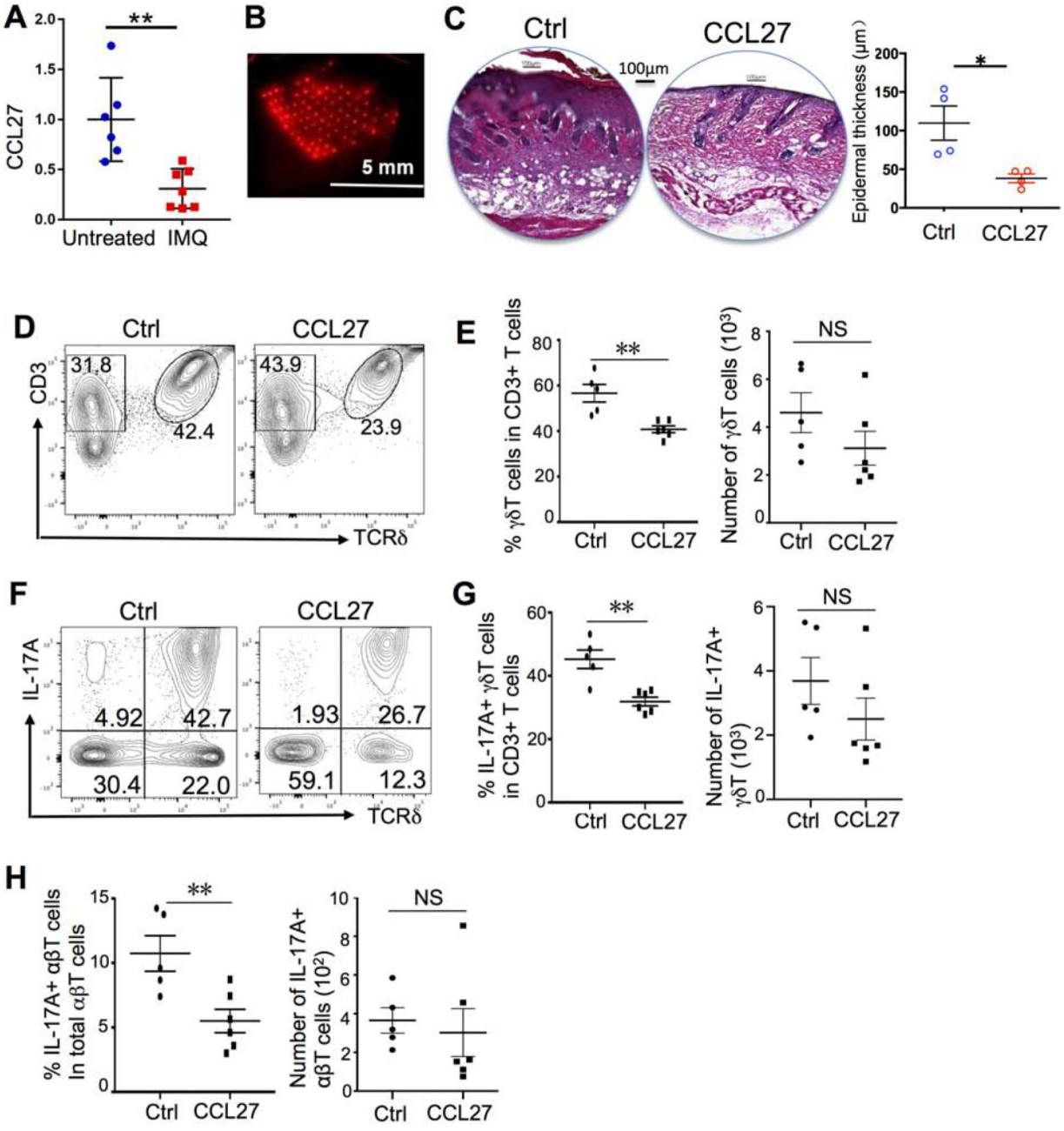

Fig. 2.

Microneedle-mediated trans-epidermal delivery of CCL27 reduces IMQ-induced skin inflammation. **(A)** Real-time RT-PCR analysis of the CCL27 expression in untreated and 5-day IMQ-treated mouse skin. Normalized to GAPDH. **(B)** Image of a whole area of skin injected with arrays of microneedles carrying DyLight 650-labeled protein (Red). (**C**) H&E-stained sections and average epidermal thickness of the skin treated with CCL27- or control (Ctrl) microneedles. Mice were applied with IMQ daily, injected with microneedles on days 0/3/5, analyzed on day 6. (**D,E**) FC analysis of $TCR\delta^{+}CD3^{+}$ γδT and $TCR\delta^{-}CD3^{+}$ (αβ)T cells (D), and percentages and numbers of γδT cells (E) in the CCL27- and control microneedle-treated skin. (**F,G,H**) FC analysis (F), and percentages and numbers of $IL\text{-}17A^{+}$

γδT cells (G) and IL-17A$^+$ αβT cells (H) in the CCL27- and control microneedle-treated skin. In the panels D-H, mice were applied with IMQ daily, treated with microneedles on days 3/5, analyzed on day 6. One dot represents one sample (A,C,E,G,H). *P<0.05, **P<0.01.