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Authors

Kim, Jaehwan

Lee, Jongmi

Lee, Jongeun

et al.

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Psoriasis Harbors Multiple Pathogenic Type 17 T-cell Subsets: Selective Modulation by Risankizumab

Authors: Jaehwan Kim, MD, PhD[a,b,c*], Jongmi Lee, MD, PhD[a,b], Jongeun Lee, MD[c], Katherine Kim[a,b], Xuan Li, BS[c], Wei Zhou, PhD[d], Junyue Cao, PhD[d], James G. Krueger, MD, PhD[c*]

Affiliations:
[a]Department of Dermatology, University of California, Davis, Sacramento, California, USA
[b]Dermatology Section, Veterans Affairs Northern California Health Care System, Mather, California, USA
[c]Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, USA
[d]Laboratory of Single-cell Genomics and Population Dynamics, The Rockefeller University, New York, New York, USA

*Corresponding authors:

Jaehwan Kim, MD, PhD
Associate Professor
Department of Dermatology
University of California, Davis
Sacramento, CA 95816
Phone: (916) 843-7000, ext. 15107
Fax: (916) 361-7369
Email: derkim@ucdavis.edu

James G. Krueger, MD PhD
D. Martin Carter Professor in Clinical Investigation
Co-Director of the Center for Clinical and Translational Science
Head of Laboratory for Investigative Dermatology
The Rockefeller University, New York
1230 York Avenue, New York, NY 10065
Phone: (212) 327-8091
Fax: (212) 327-8232
Email: kruegej@mail.rockefeller.edu

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Conflict of interest

J.K. has received research support from AbbVie. J.G.K. has received research support from Pfizer, Amgen, Janssen, Lilly, Merck, Novartis, Kadmon, Dermira, Boehringer, Innovaderm, Kyowa, BMS, Serono, BiogenIdec, Delenex, AbbVie, Sanofi, Baxter, Paraxel, Xenoport, and Kineta. The rest of the authors declare that they have no relevant conflict of interest.

Abstract

Background: Recent single-cell studies indicated that IL-17-producing T-cells (T17) have diverse subsets expressing IL-17A, IL-17F, or a combination of them in human psoriasis skin. However, it is unknown how T17 subsets are differently regulated by IL-23 versus IL-17A blockades.

Objective: We sought to investigate how systemic monoclonal antibody injections blocking IL-23 versus IL-17A differently modify immune cell transcriptomes in human psoriasis skin.

Methods: We analyzed a total of 93 human skin single-cell libraries, including 42 psoriasis pretreatment lesional skin, 25 psoriasis pretreatment non-lesional skin, 12 psoriasis posttreatment after IL-23 inhibition, 4 psoriasis posttreatment after IL-17A inhibition, and 10 control skin samples.

Results: Of the six T17 cell subsets identified, an *IL17A*$^{+}$ *IFNG*$^{+}$ subset and an *IL17F*$^{+}$ *IL10*$^{-}$ subset expressed the IL-23 receptor along with other inflammatory cytokines, and IL-23 inhibition downregulated these potentially pathogenic T17 subsets. In contrast, T17 cells expressing both IL-17A and IL-17F did not express the IL-23 receptor, and the percentage of this potentially non-pathogenic T17 subset increased after IL-23 inhibition. In addition, the expression of the IL-17 negative regulation genes, such as *TNFAIP3*, increased in myeloid cells more after IL-23 inhibition than after IL-17A inhibition.

Conclusions: This study suggests multiple immune mechanisms of how IL-23 inhibition can modify the complex inflammatory environment present in psoriatic skin, highlighting the roles of specific T17 subsets in psoriasis development and background skin protection.

*This study was registered through ClinicalTrials.gov (NCT04630652).

Key messages

- Psoriasis has complex subsets of type 17 T-cells (T17) in active lesions, but pathogenic vs. protective subsets are not known.
- Treatment with risankizumab (anti-IL-23) at its maximal clinical effect selectively reduces or eliminates the likely pathogenic T17 subsets (thus defining them), while other subsets are retained.
- This response differs from secukinumab treatment (anti-IL-17A), highlighting differences in their mechanisms of action.

Capsule Summary: Analysis of single T-cell phenotypes in response to IL-23 inhibition provides new insights into the function of pathogenic T17 subsets in psoriasis and non-pathogenic T17 subsets in background skin protection.

Keywords: psoriasis; IL-17A; IL-17F; IL-23; type 17 T-cells; single-cell RNA sequencing; T-cell; dendritic cell; myeloid cell; keratinocyte

Abbreviations:

cDC1:	Conventional type 1 dendritic cell
DC:	Dendritic cell
DC3:	Type 3 dendritic cell
DEG:	Differentially expressed gene
FCH:	Fold change
FDR:	False discovery rate
FLG:	Filaggrin
GEO:	Gene Expression Omnibus
IL1R1:	IL-1 receptor
IL23R:	IL-23 receptor
IL6R:	IL-6 receptor
KC:	Keratinocyte
LS:	Lesional skin
MHCII:	Major histocompatibility complex class II
mregDC:	Mature dendritic cell enriched in immunoregulatory molecule
NCBI:	National Center for Biotechnology Information
NL:	Non-lesional
PASI:	Psoriasis Area-and-Severity Index
PostTx:	Posttreatment
PreTx:	Pretreatment
scRNA-seq:	Single-cell RNA sequencing
slanDC:	6-sulfo LacNAc^{+} dendritic cell
T17:	Type 17 T-cell
TGFBR1:	TGF-β receptor
TNFAIP3:	TNF-α-induced protein 3
Treg:	Regulatory T-cells
TRM:	Tissue-resident memory T-cell
TRM17:	Tissue-resident memory Type 17 T-cell

Introduction

Psoriasis is a debilitating chronic inflammatory disease that affects over 60 million people worldwide[1]. It is characterized by red and scaly demarcated patches on the skin and gradually becomes a systemic inflammatory condition. Approximately 75% of patients with psoriasis will eventually have at least one comorbid condition, such as psoriatic arthritis, cardiovascular diseases, diabetes, and obesity. Increasing evidence from population-based studies indicates that psoriasis's systemic inflammation shortens the affected individuals' lifespan by at least 3–5 years[2].

In psoriasis skin, IL-23 from inflammatory dendritic cells activate IL-17-producing T-cells (Type 17 T-cells; T17 cells), and the activated T17 cells produce IL-17A and IL-17F[3]. In response to IL-17, keratinocytes produce a range of "feed-forward" inflammatory mediators, such as IL-36γ, amplifying cellular immunity to sustain chronic T-cell activation[4]. The roles of IL-23 and IL-17 as key drivers of psoriasis inflammation have been strongly supported by the high efficacy of biologic agents targeting IL-23 or IL-17 for psoriasis treatment[4], and almost all the phase 3 clinical trials evaluating the efficacy of IL-23 and IL-17 blockades have measured the improvement of psoriasis 3 to 4 months after the first injection. However, IL-23 and IL-17 blockades might have different efficacy for inducing disease remission in the long term or suppressing disease relapses after stopping the injections. In the head-to-head comparison clinical trials, more patients achieved Psoriasis Area-and-Severity Index (PASI) 90 response with an IL-23 inhibitor (guselkumab) (84%) than IL-17A inhibitor (secukinumab) (70%) after 12 months[5], and more patients achieved PASI90 response with an IL-23 inhibitor (risankizuamb) (86.6%) than IL-17A inhibitor (secukinumab) (57.1%) after 13 months[6]. In addition, structured drug wash-out studies identified that many patients maintained PASI90 responses for long, even after receiving a final dose and stopping further injections of IL-23 blockades. In a phase II clinical trial of an IL-23 inhibitor (risankizumab)[7], psoriasis patients received the IL-23 inhibitor injections until month 4 and stopped further injections. Approximately 2/3 of psoriasis patients who attained a PASI90 response in month 3 (76%) still maintained a PASI90 response until 8 months after the last drug dose (47% in month 12).

In our previous studies[8], we have established 5 different IL-17-producing T-cell (T17) subsets with highly differing transcriptomes depending on *IL17A* versus *IL17F* expression and *IFNG* versus *IL10* expression in human psoriasis skin. More recently, it has been demonstrated in psoriasis that a population of IL-26-expressing T-cells that are considered to be T17 intermediates differentiate into pathogenic T17 cells expressing high levels of IL-17 via epithelial crosstalk[9]. Furthermore, we have proposed that systemic monoclonal antibody injections blocking the IL-23/T17 pathway not only downregulate the entire feed-forward immune amplification loop but also promote regulatory gene expression in myeloid cells in human psoriasis skin[10]. In this study, we advanced our single-cell RNA sequencing (scRNA-seq) analysis framework to T17 subsets, T17 intermediates, and regulatory myeloid cells and investigated how systemic monoclonal antibody injections blocking IL-23 versus IL-17A differently modify immune cell transcriptomes in human psoriasis skin. Our study shows that IL-23 inhibition downregulates IL-23 receptor (*IL23R*) expressing T17 subsets, including $IL17A^{+}$ $IFNG^{+}$ and $IL17F^{+}$ $IL10^{-}$ T17 subsets, while $IL23R^{-}$ $IL17A^{+}$ $IL17F^{+}$ T17 cells are retained after IL-23 inhibition. This could potentially explain why the risk of candidiasis is not increased after IL-23 inhibition, unlike IL-17A inhibition. We also report that the expression of the IL-17 negative regulation gene (*TNFAIP3*) increased more after IL-23 inhibition than IL-17A inhibition, indicating that the negative regulation of IL-17 in myeloid cells is more upregulated by IL-23 inhibition than IL-17A inhibition.

Methods

The study was designed to compare single-cell genomic profiles of immune cell subsets in human psoriasis skin before and after blocking IL-23 versus IL-17A. In total, 93 human skin single-cell libraries, including 42 psoriasis pretreatment (PreTx) lesional skin (LS), 25 psoriasis PreTx non-lesional (NL) skin, 12 psoriasis posttreatment (PostTx) IL-23 inhibition, 4 psoriasis PostTx IL-17A inhibition, and 10 control skin samples were analyzed (Figs E1 and E2). Table E1 summarizes age, gender, PASI, biopsy location, and Gene Expression Omnibus (GEO) Series accession number for each single-cell library. The single-cell RNA sequencing data have been deposited in the National Center for Biotechnology Information (NCBI)'s GEO and are publicly accessible through GEO Series accession number GSE278330. Rationale, design, and conduct of clinical trials

(NCT04630652), detailed experiment methods, and statistical analysis are provided in the Supplemental Materials in this article's Online Repository available at www.jacionline.org.

Results

Systemic anti-IL-23 monoclonal antibody injections downregulate but do not eradicate IL-23/T17 cell transcriptome in the posttreatment psoriasis skin

In the anti-IL-23 monoclonal antibody (risankizumab) clinical trial, psoriasis patients received risankizuamb 150 mg injections at baseline, month 1, and month 4 (3 injections). PreTx LS and NL skin biopsy tissues were harvested at baseline, and PostTx LS biopsy tissue was harvested at month 7 (Fig 1, *A*). PostTx LS IL-17A inhibition single-cell libraries were generated from skin biopsy tissues harvested at month 3, after anti-IL-17A monoclonal antibody (secukinumab) 300 mg injections at baseline, week 1, week 2, week 3, week 4, and week 8[8,10,11]. The average PASI score was 16.1 ± 0.9 in psoriasis PreTx, 0.2 ± 0.4 in psoriasis PostTx LS IL-23 inhibition, and 0.7 ± 0.5 in psoriasis PostTx LS IL-17A inhibition (Fig 1, *B*). There was no statistical difference in PASI score in psoriasis PostTx LS between IL-23 inhibition and IL-17A inhibition ($p > 0.05$).

We compared the expression of canonical IL-23/T17 pathway genes at total transcriptome levels (Fig 1, *C*). The T17 cell cytokine (*IL17A* and *IL17F)* transcriptomes were detected in psoriasis PreTx LS and NL, but they were not in control skin. In contrast, the T17 intermediate cytokine (*IL26*[9]*)*, *IL23A*, and *IL23R* transcriptomes were detected not only in psoriasis LS and NL but also in control skin. The expression levels of those cytokines all increased in psoriasis PreTx LS compared to PreTx NL or control skin, and all decreased in psoriasis PostTx LS IL-23 inhibition compared to PreTx LS (False Discovery Rate [FDR] < 0.05). In particular, the expression of *IL23R* in psoriasis PostTx LS IL-23 inhibition was lower than the expression in control skin (FDR < 0.05). Still, *IL17A, IL17F, IL26, IL23A,* and *IL23R* transcriptomes were detected in PostTx LS IL-23 inhibition.

The results showed that systemic IL-23 inhibition substantially downregulated but did not eradicate canonical IL-23/T17 pathway gene expressions even when the systemic IL-23 inhibition had the maximal impact on the skin.

Trajectory of human psoriasis skin type 17 T-cell differentiation is associated with IL-23 receptor, IFNγ, and tissue-resident memory T-cell gene expressions

We identified clusters of T-cells (30,367 cells), dendritic cells (DCs; 25,483 cells), myeloid cells (2,852 cells), melanocytes (4,184 cells), keratinocytes (KCs; 103,910 cells), and fibroblasts (9,149 cells) by dimensionality reduction analysis (Fig 2, *A* and Fig E3, *A*). T-cells were composed of $TRAC^+$ $IL17A^+$ $IL17F^+$ T17 cells (fold change [FCH] > 2 and FDR < 0.05) and $TRAC^+$ $IL17A^-$ $IL17F^-$ non-T17 T-cells at cluster levels. The average expression of the canonical markers for each cluster is presented in Fig 2, *B*.

To identify what genes are regulated in transcriptional re-configuration over the course of cell transition from non-pathogenic T17 cells to pathogenic T17 cells in human psoriasis skin, we constructed the T17 cell differentiation trajectory in the T17 cell cluster, selecting *IL17A*, *IL17F*, and *IL26* as the progress-defining genes. Then, we tested what genes are differentially expressed in association with the trajectory (Fig 2, *C*). Next, we validated if T17 cluster cells expressing genes associated with the trajectory express more *IL17A*, *IL17F*, and *IL26* than T17 cluster cells that do not express those genes (Fig 2, *D*).

Among the cytokines involved in T17 development (IL-1β[12,13], IL-6[14], TGF-β[15,16], IL-23[17], and IFN-γ[18,19]), we observed that *IL23R* and *IFNG* were differentially expressed in association with the T17 cell differentiation trajectory in psoriasis skin T17 cells ($q < 0.05$, Fig 2, *C*). T17 cells expressing *IL23R* and *IFNG* expressed more *IL17A*, *IL17F*, *IL26,* or other T17 cell transcriptomes than T17 cells who did not express those genes (FCH > 2 and FDR < 0.05, Fig 2, *D*). In contrast to *IL23R* and *IFNG*, the IL-1 receptor (*IL1R1*), IL-6 receptor (*IL6R*), and TGF-β receptor (*TGFBR1*) were not differentially expressed in association with the trajectory ($q > 0.05$). T17 cells expressing *IL1R1, IL6R,* or *TGFBR1* did not express more *IL17A*, *IL17F*, and *IL26* than *IL1R1, IL6R,* or *TGFBR1* negative T17 cells (FCH < 2 and FDR > 0.05).

Among human tissue-resident memory T-cell (TRM) markers (*CD103* (*ITGAE*), *CD69*, and *CXCR6*)[20] implicated in psoriasis relapses[21–23], *CD103* and *CXCR6* were differentially expressed in association with the T17 cell differentiation trajectory ($q < 0.05$, Fig 2, *C*). T17 cells expressing *CD103* and *CXCR6* expressed more *IL17A*, *IL17F*, *IL26,* and other T17 cell transcriptomes than T17 cells who did not express those genes (FCH > 2 and FDR < 0.05, Fig 2, *D*). In contrast, *CD69* was not differentially expressed in association with the trajectory ($q > 0.05$). *CD69*$^+$ T17 cells did not express more *IL17A*, *IL17F*, and *IL26* than *CD69*$^-$ T17 cells (FCH < 2 and FDR > 0.05).

The numbers of T17 cluster cells expressing *IL26*, *IL17A*, *IL17F*, *IL23R*, and *IFNG* increased in psoriasis PreTx LS compared to psoriasis PreTx NL. They decreased in psoriasis PostTx LS IL-23 inhibition compared to psoriasis PreTx LS (adjusted $p < 0.05$, Fig 2, *E*). Likewise, the percentages of T17 cluster cells expressing *IL26*, *IL17A*, *IL23R*, and *IFNG* increased in psoriasis PreTx LS compared to psoriasis PreTx NL. They decreased in psoriasis PostTx LS IL-23 inhibition compared to psoriasis PreTx LS (adjusted $p < 0.05$).

The results showed that the expression of IL-23 receptor (*IL23R*) and *IFNG*, together with T17 markers (*CXCL13* and *CD161*) and TRM markers (*CD103* and *CXCR6*), were upregulated in transcriptional re-configuration throughout cell transition from non-pathogenic to pathogenic T17 subsets, and IL-23 inhibition downregulated the expression of *IL23R* and *IFNG* in T17 cells.

Systemic IL-23 inhibition selectively downregulates IL-23 receptor expressing pathogenic T17 subsets, different from IL-17A inhibition

When we performed differential expression testing in the T17 cell cluster, the expression levels of the T17 intermediate marker of *IL26* and psoriasis-specific genes with less established functional roles, such as *ENTPD1*[24], increased in psoriasis PreTx NL compared to control skin (FCH > 2 and FDR < 0.05, Fig 3, *A* and *B*). The expression levels of active T17 cells (*IL17A* and *IL17F*) and T17 cell markers (*CXCL13* and *EBI3*[25]) increased in psoriasis PreTx LS compared to psoriasis PreTx NL or control skin (FCH > 2 and FDR < 0.05). When we compared transcriptome changes induced by IL-23 inhibition versus IL-17A inhibition, the expressions of T17 cell marker (*CD161*[26–30] (*KLRB1*)), T17 cytokine[31] (*CSF2*), and cytotoxic T17 cell transcripts[8] (*GZMB*, *GNLY*, and *PRF1*) decreased (FCH < 0.5 and FDR < 0.05) more in psoriasis PostTx LS IL-23 inhibition than in PostTx LS IL-17A inhibition, indicating that T17 transcriptome expression was more significantly downregulated by IL-23 inhibition than IL-17A inhibition (Fig 3, *B*). The expression level of *IL23R* trended to be decreased after IL-23 inhibition (FCH > 2, $p < 0.05$, and FDR = 0.09) but not after IL-17A inhibition (FCH < 2, $p > 0.05$, and FDR > 0.05) (Fig E3, *B*).

Since we observed IL-23/T17 cell transcriptomes in the clinically improved psoriasis skin after systemic IL-23 inhibition (Fig 1, *C*), we hypothesized that IL-23 inhibition selectively downregulates pathogenic T17 subsets while non-pathogenic T17 subsets are still present in the posttreatment psoriasis skin, and compared single-cell transcriptome profiles of different T17 subsets[8,9] in the T17 cluster - 1) *IL17A*$^+$ *IFNG*$^+$ T17 subset, 2) *IL17A*$^+$ *IFNG*$^-$ T17 subset, 3) *IL17A*$^+$ *IL17F*$^+$ T17 subset, 4) *IL17F*$^+$ *IL10*$^-$ T17 subset, 5) *IL17F*$^+$ *IL10*$^+$ T17 subset, and 6) *IL26*$^+$ *IL17A*$^-$ *IL17F*$^-$ T17 intermediates (Fig 3, *C* and Figs E4 and E5; Table 1).

The *IL17A*$^+$ *IFNG*$^+$ T17 subset supported the hypothesis that IL-23 inhibition selectively downregulates *IL23R*-expressing pathogenic T17 subsets. The *IL17A*$^+$ *IFNG*$^+$ T17 subset expressed high levels of *IL23R*, TRM markers[20] (*CD103* (*ITGAE*), *CD49a* (*ITGA1*), and *CXCR6*), cytotoxic T17 cell transcripts[8] (*CD8A*, *GZMB*, *GNLY*, and *PRF1*), T17 markers (*CXCL13*[24,32–34]), T17 transcription factor (*BATF*[35,36]), T17 cytokines[31] (*IL26*, *IL22*, *IFNG*, *CSF2*, and *TNF*), and psoriasis-specific genes (*ENTPD1*[24]) (FCH > 2 and FDR < 0.05, Fig 3, *C* and Fig E4). The *IL17A*$^+$ *IFNG*$^+$ T17 subset was observed in psoriasis PreTx LS but not in psoriasis PreTx NL or control skin, and this subset expressing *IL23R* was eradicated to 0% after IL-23 inhibition in PostTx LS (Fig 3, *D*). In contrast to the *IL17A*$^+$ *IFNG*$^+$ T17 subset, the *IL17A*$^+$ *IFNG*$^-$ T17 subset was less inflammatory without expressing cytotoxic T17 cell transcripts, but this subset expressed *IL12A* (*p35*), which is known to inhibit T17 cell induction[37–39] (FCH > 2 and FDR < 0.05, Fig 3, *C* and Fig E4). The *IL17A*$^+$ *IFNG*$^-$ T17 subset percentage among T17 subsets did not decrease after IL-23 inhibition (Fig 3, *D*).

The *IL17F*$^{+}$ *IL10*$^{-}$ T17 subset was another *IL23R*-expressing pathogenic T17 subset selectively downregulated by IL-23 inhibition. The *IL17F*$^{+}$ *IL10*$^{-}$ T17 subset expressed high levels of *IL23R*, TRM markers[20] (*CD103* and *CXCR6*), T17 markers (*CXCL13*[24,32–34], *CD161*[26–30], *CCR6*[40], and *EBI3*[25]), T17 cytokines[31] (*IL26*, *IL22*, *IFNG,* and *CSF2*), and inflammatory cytokines (*IL1B* and *IL34*) (FCH > 2 and FDR < 0.05, Fig 3, *C* and Fig E4). The *IL17F*$^{+}$ *IL10*$^{-}$ T17 subset percentage among T17 subsets decreased from 45.91% in PreTx LS to 16.67% in PostTx LS IL-23 inhibition (Fig 3, *D*). The *IL17F*$^{+}$ *IL10*$^{+}$ T17 subset best fit the description of nonpathogenic T17 cells in murine models[8,41–45], but it constituted only 1.17% of T17 cells in psoriasis PreTx LS.

The *IL17A*$^{+}$ *IL17F*$^{+}$ T17 subset expressed no *IL23R* expression but expressed high levels of regulatory cytokines (*IL13*[46–48] and *IL33*[49]), which may reduce T17 inflammation, together with TRM markers[20] (*CD103* and *CXCR6*), T17 markers (*CXCL13*[24,32–34] and *CD161*[26–30]), T17 transcription factor (*BATF*[35,36]), T17 cytokines[31] (*IL26*, *IFNG*, *CSF2*, and *TNF*), and psoriasis-specific genes (*ENTPD1*[24]) (FCH > 2 and FDR < 0.05, Fig 3, *C* and Fig E4). The percentage of *IL17A*$^{+}$ *IL17F*$^{+}$ T17 subset among T17 subsets increased from 15.95% in psoriasis PreTx LS to 50.00% in psoriasis PostTx LS IL-23 inhibition (Fig 3, *D*).

IL26^{+} T17 intermediates (*IL26*$^{+}$ *IL17A*$^{-}$ *IL17F*$^{-}$ T17 cells) expressed high levels of T17 transcriptional regulator (*RORC*[50]) together with *IL23R*, TRM markers[20] (*CD103*, and *CXCR6*), T17 markers (*CXCL13*[24,32–34] and *CD161*[26–30]), T17 cytokines[31] (*IL26*, *IL22*, *IFNG*, *CSF2*, and *IL21*), and psoriasis-specific genes (*ENTPD1*[24]) (FCH > 2 and FDR < 0.05, Fig 3, *C* and Fig E4). Overall, *IL26*$^{+}$ T17 intermediates were present not only in psoriasis PreTx LS but also in psoriasis PreTx NL and control skin, and they were not eliminated by both IL-23 inhibition and IL-17A inhibition (Fig E6).

The results proposed that the *IL17A*$^{+}$ *IFNG*$^{+}$ T17 subset and the *IL17F*$^{+}$ *IL10*$^{-}$ T17 subset were pathogenic T17 subsets expressing IL-23 receptor (*IL23R*), and the *IL17A*$^{+}$ *IL17F*$^{+}$ T17 subset was non-pathogenic or regulatory T17 subset without *IL23R* expression. After systemic IL-23 inhibition, the percentages of pathogenic T17 subsets decreased, the percentage of a non-pathogenic or regulatory T17 subset increased, and T17 intermediates were not eliminated in psoriasis skin.

Systemic IL-23 inhibition downregulates IL-23 expression in dendritic cells, different from IL-17A inhibition

The recent single-cell analysis identified *CD14*$^{+}$ type 3 dendritic cells (DC3s) and *CCR1*$^{+}$ macrophages/ 6-sulfo LacNAc^{+} DCs (slanDCs)/monocyte-derived DCs as the primary cellular sources of *IL23A* in human psoriasis skin[51]. *CD14*$^{+}$ DC3s, marked as *CD5*$^{-}$ *CD1C*$^{+}$ *CD163*$^{+}$ *CD11C* (*ITGAX*)$^{+}$ DCs[52], are reported to co-express *IL23A* and *IL1B*[51]. Supporting the roles of *CD14*$^{+}$ DC3s and *CCR1*$^{+}$ macrophages in psoriasis, the expressions of *CD14*$^{+}$ DC3 markers (*CD14*, *CD1C, CD163*, *CD11C* (*ITGAX*), *CXCL3)* and *CCR1* macrophage marker (*CCR1)* increased together with *IL23A* and *IL1B* in psoriasis PreTx LS DCs compared to psoriasis PreTx NL DCs or control skin DCs (FCH > 2 and FDR < 0.05, Fig 4, *A* and *B*). The expression of other T17-stimulating cytokines (*IL6* and *TGFB1*), the adhesion molecule involved in DC migration (*ITGB2*[53]), DC maturation (*CD80* and *CD209* (*DC-LAMP*)), and a DC scavenger receptor (*CD36*[54]) also increased in psoriasis PreTx LS DCs compared to psoriasis PreTx NL DCs or control skin DCs (FCH > 2 and FDR < 0.05).

We observed decreased expressions of *IL23A, IL1B, CD14*$^{+}$ DC3 markers, and *CCR1* macrophage markers in PostTx LS DCs 7 months after IL-23 inhibition (FCH < 0.5 and FDR < 0.05, Fig 4, *A* and *B*). Like IL-23 inhibition, IL-17A inhibition also decreased expressions of *IL1B, CD14*$^{+}$ DC3 markers, and *CCR1* macrophage markers in PostTx LS DCs (FCH < 0.5 and FDR < 0.05). However, IL-17A inhibition did not significantly decrease *IL23A* in PostTx LS DCs (FCH > 0.5 and FDR > 0.05). When we compared transcriptome changes induced by IL-23 inhibition versus IL-17A inhibition, the expression of *IL23A* was significantly lower after IL-23 inhibition compared to IL-17A inhibition (FCH < 0.5 and FDR < 0.05).

The expression changes of *IL6* and *TGFB1* in DCs differed from the expression changes of *IL23A* in DCs after IL-23 versus IL-17A inhibition. The expression of *IL6* and *TGFB1* in DCs decreased after IL-17A inhibition (FCH < 0.5 and FDR < 0.05), but they did not significantly decrease after IL-23 inhibition (FCH > 0.5 and FDR > 0.05, Fig 4, *B*).

We also observed that the expression of regulatory DC subset and Langerhans cell genes trended to be recovered after IL-23 inhibition (Fig 4, *A* and Fig E7). The expression of marker genes for mature DC enriched in immunoregulatory molecule (mregDCs) (*LAMP3* and *BIRC3*[55]), tolerogenic phenotype of IFN-γ–induced IDO$^+$ DCs (*IDO1*[56–58]), and Langerhans cells (*CD207*, *CD1A*, or *EPCAM*) increased in psoriasis PreTx NL compared to control, decreased in psoriasis PreTx LS compared to PreTx NL, and then increased in PostTx LS IL-23 inhibition compared to PreTx LS (FDR < 0.05).

The results showed that systemic IL-17A inhibition and IL-23 inhibition regulate skin dendritic cell gene expression differently, and dendritic cell IL-23 expression in the skin was more significantly downregulated by IL-23 inhibition than IL-17A inhibition.

Systemic IL-23 inhibition upregulates TNFAIP expressions in myeloid cells, different from IL-17A inhibition

Myeloid cells were reported as 'semimature DCs' in our previous scRNA-seq studies[3,8,59] to emphasize their intermediate expression of major histocompatibility complex class II (MHCII) molecules, and *IL10* expression and regulatory transcriptome profiles consistent with skin-resident *BDCA3*$^+$-regulatory DCs in human normal skin described by Chu *et al.*[60]. The myeloid cell cluster expressed conventional type 1 DC (cDC1) markers (*THBD* (*BDCA3*, *CD141*), regulatory immunoreceptors (*CLEC4A* (*DCIR*[61]), *LILRB1*[62], and *LILRB4*[63]), and inhibitory cytokine (*IL10*) compared to other clusters (FCH > 2 and FDR < 0.05, Fig 5, *A*). Together with *IL10*, *IL1B* was highly expressed in the myeloid cell cluster (FCH > 2 and FDR < 0.05), as previously described in inflammatory skin conditions such as psoriasis and hidradenitis suppurativa[59].

The expression of regulatory immunoreceptor (*CLEC9A* (*DNGR-1*[64]) in psoriasis PostTx LS IL-23 inhibition increased in psoriasis PostTx LS IL-23 inhibition compared to PreTx LS (FCH > 2 and FDR < 0.05, Fig 5, B and C). In addition, the expression changes of *TNFAIP3* and *NFKB1* supported the hypothesis that systemic IL-23 inhibition upregulates negative regulation of IL-17 in myeloid cells. Stimulated DCs/myeloid cells activate the NF-kB to initiate transcription of proinflammatory cytokines such as IL-1, and NF-kB activation is negatively regulated by TNF-α-induced protein 3 (*TNFAIP3* (*A20*))[65–69]. The expressions of *TNFAIP3* in myeloid cells increased in psoriasis PostTx LS IL-23 inhibition compared to PreTx NL (FCH > 2 and FDR < 0.05). At the same time, the expression of *NFKB1* decreased in psoriasis PostTx LS IL-23 inhibition compared to PreTx LS or NL (FCH < 0.5 and FDR < 0.05, Fig 5, *C*).

The results showed systemic IL-23 inhibition upregulated regulatory gene expression in myeloid cells.

Feed-forward inflammatory mediators induced by IL-17 in keratinocytes are more downregulated with IL-23 inhibition than IL-17A inhibition

The single-cell transcriptome of KCs in psoriasis PreTx LS in contrast to PreTx NL or control skin was characterized by 1) increased expressions of IL-17-induced feed-forward inflammatory mediators such as *IL36G* (FCH > 2 and FDR < 0.05, Fig 6, *A* and *B*), 2) increased expression of cytokines stimulating T17 differentiation (*TGFB1*[9], *IL1B*, and *IL6*) (FCH > 2 and FDR < 0.05), 3) increased expression of IL-17-mediated antimicrobial peptides (S100 proteins – *S100A9*, *S100A8*, *S100A12*, and *S100A7A*, and defensins – *DEFB4B*, *DEFB4A*) and a KC hyperproliferation marker (*KRT16*) (FCH > 2 and FDR < 0.05), and 4) decreased expressions of chemotactic ligand for skin-associated memory T lymphocytes binding to CCR10 (*CCL27*[70]), a cytokine that promotes the differentiation of Langerhans cell precursors[71] (*IL34*), a KC stem marker of quiescence (*KRT15*[72]), and filaggrin (*FLG*[8]) (FCH < 0.5 and FDR < 0.05, Fig 6, *A* and *B*).

The expressions of *IL36G, TGFB1, IL1B, IL6, S100A4, S100A7A, DEFB103A,* and *DEFB103B* in KCs decreased (FCH < 0.5 and FDR < 0.05) and the expressions of *CCL27* and *FLG* in KCs increased (FCH > 2 and FDR < 0.05) in psoriasis PostTx LS IL-23 inhibition compared to PreTx LS (Fig 6, *A* and *B*). Among them, the expressions of *IL36G, S100A4, S100A7A, DEFB103A,* and *DEFB103B* in KCs decreased (FCH < 0.5 and FDR < 0.05), and the expressions of *CCL27* in KCs increased (FCH > 2 and FDR < 0.05) more in psoriasis PostTx LS IL-23 inhibition than in PostTx LS IL-17A inhibition.

The results showed that the feed-forward inflammatory mediators in KCs were more significantly downregulated by IL-23 inhibition than by IL-17A inhibition.

Discussion

T17 cells were initially described based on their secretion of IL-17A and IL-17F in the reciprocal relationship with regulatory T-cells (Tregs)[73]. The conditional conversion between pathogenic T17 cells inducing autoimmune inflammation and non-pathogenic IL-17-producing T17 cells controlling excessive inflammation has been demonstrated in animal models of multiple sclerosis and inflammatory bowel diseases[42,74–77]. More recently, scRNA-seq studies of human psoriasis skin identified that IL-26$^+$ T17 intermediates differentiate into IL-17-expressing T17 cells via epithelial crosstalk[9], and discrete T17 subsets express IL-17A or IL-17F, or the combination of IL-17A and IL-17F[8]. However, the diversity and phenotypic differences among T17 subsets are still poorly understood in human skin. It is unknown which T17 subsets are pathogenic T17 cells for the target to treat psoriasis and which T17 subsets are non-pathogenic T17 cells for the target to preserve to defend the skin against extracellular organisms after treatment[78].

Here, we studied the phenotypic diversity of T17 cells in human psoriasis skin and their transcriptomic modifications by systemic IL-23 blockade compared to IL-17A blockade. Our study design was unique for capturing IL-23 blockade transcriptomic modification when the reagent of the highest long-term efficacy (risankizumab) has fully established clinical clearing effects in a cohort of patients whose psoriasis is well-controlled (PASI90 responders) up to 1 year (NCT04630652). A network meta-analysis identified that risankizuamb is currently the reagent with the highest long-term efficacy (estimated PASI90 response rate = 72.5%[79]), and we previously reported that the molecular phenotype of treated psoriasis skin evolves over time after risankizumab injections as gene abundance progressively changed over 3 months[25]. Since transcriptome modification by risankizumab may not be fully developed at an early stage of treatment or soon after the initiation of treatment, we decided to study the effect of risankizumab at a point of stable disease control when clinical effects are near maximum (7 months after the first injection[80,81]).

The analysis of our scRNA-seq data at total transcriptome levels was consistent with previous bulk RNA sequencing studies at different time points[25,82–84] , indicating that systemic IL-23 inhibition downregulates the canonical IL-23/T17 cell pathway gene (*IL26*, *IL17A*, *IL17F*, *IL23A*, and *IL23R*) expressions (FCH > 2 and FDR < 0.05, Fig 1, *C*). Cook *et al.*[85] and Wu *et al.*[86] analyzed scRNA-seq data at least 2 months[85] or 4 months[86] after the first injection of tildrakizumab (estimated PASI90 response rate in a network meta-analysis = 39.7%[79]) and detected a recurring set of recalcitrant, disease-specific transcriptional abnormalities, even in IL-23 inhibition-responsive psoriasis patients. We also observed IL-23/T17 cell transcriptomes in the clinically improved psoriasis skin after systemic IL-23 inhibition at single-cell levels (Fig 2, *E*). In particular, *IL26*$^+$ *IL17A*$^-$ *IL17F*$^-$ T17 intermediates expressed high levels of TRM markers[20] (*CD103* (*ITGAE*) and *CXCR6*), T17 markers (*CXCL13*[24,32–34] and *CD161*[26–30] (*KLRB1*)), T17 cytokines[31] (*IL26*, *IL22*, *IFNG*, *CSF2*, and *IL21*), and *IL23R* (Fig 3, *C* and Fig E4) and were retained in psoriasis PostTx LS IL-23 inhibition (Fig E6). The T17 intermediates may represent a TRM subset that can convert to effector TRM subsets expressing IL-17A and/or IL-17F (TRM17) when exposed to an increasing IL-23 signal after stimulation.

Previous flow cytometry studies with guselkumab reported that IL-23 inhibition partially reduced the frequency of CD8$^+$ CD49a$^+$ CD103$^+$ TRMs, but concluded that the frequencies of TRM17 cells were not decreased after IL-23 inhibition[87]. Our study also showed the overall reduction of TRMs after IL-23 inhibition (Fig 2, *E*), but we further dissected the different effects of IL-23 inhibition on different TRM17 subsets (Fig 3, *C*). Our scRNA-seq study indicated that the *IL17A*$^+$ *IFNG*$^+$ T17 subset and *IL17F*$^+$ *IL10*$^-$ T17 subset are likely pathogenic TRM17 subsets expressing *IL23R* that are selectively downregulated by IL-23 inhibition (Fig 3, *C* and *D* and Fig E4). Those T17 subsets commonly expressed TRM markers[20] (*CD103* and *CXCR6*) and the percentage of the *IL17A*$^+$ *IFNG*$^+$ TRM17 subset decreased from 15.18% in PreTx LS to 0% in PostTx LS IL-23 inhibition, and the percentage of the *IL17F*$^+$ *IL10*$^-$ TRM17 subset decreased from 45.91% in PreTx LS to 16.67% in PostTx LS IL-23 inhibition.

In contrast, the *IL17A*$^+$ *IL17F*$^+$ T17 subset is likely non-pathogenic TRM17 subset expressing TRM markers[20] (*CD103* and *CXCR6*) and regulatory cytokines (*IL13*[46–48] and *IL33*[49]) without expressing *IL23R*. The

percentage of this T17 subset increased from 15.95% in psoriasis PreTx LS to 50.00% in psoriasis PostTx LS IL-23 inhibition (Fig 3, *C* and *D* and Fig E4). Considering that IL-23 inhibition is not associated with an increased risk of candidiasis, unlike IL-17A/IL-17F dual inhibition that is associated with an increased risk of candidiasis[88], the *IL17A*$^{+}$ *IL17F*$^{+}$ T17 subset may represent the non-pathogenic TRM17 cells preserved after IL-23 inhibition due to its lack of *IL23R* expression, defending skin against external surface microbes after treatment[88]. The defending function of the *IL17A*$^{+}$ *IL17F*$^{+}$ T17 subset against microbes is further supported by its dual expressions of *IL17A* and *IL17F* because candidiasis occurred more often with dual blockade of IL-17A and IL-17F (bimekizumab) than with selective blockade of IL-17A alone (secukinumab) in psoriasis patients[89]. In addition, Puel *et al.* reported that genetic etiologies of chronic mucocutaneous candidiasis disease involved autosomal recessive deficiency in the receptor for IL-17A and IL-17F (IL-17RA) or autosomal dominant deficiency of IL-17F, indicating that the protective function of T17 cells may involve the expression of IL-17F[90].

Psoriasis lesions have an expanded number of TRMs, but only a percentage of those TRMs are TRM17 cells (Fig 2, *E*). Furthermore, our study indicated that pathogenic TRM17 cells and non-pathogenic TRM17 cells are differently regulated by IL-23 inhibition (Fig 3, *D*). Therefore, immunologic goal of psoriasis treatment should not be eradicating all TRMs but selectively targeting pathogenic TRM17 subsets (*IL17A*$^{+}$ *IFNG*$^{+}$ and *IL17F*$^{+}$ *IL10*$^{-}$ T17 subsets) while retaining the non-pathogenic TRM17 subset (*IL17A*$^{+}$ *IL17F*$^{+}$ T17 subset) to improve psoriasis and prevent skin infection[91].

Studies in the past decades have established that Tregs are a central element in maintaining peripheral tolerance, including psoriasis skin immune tolerance[3,77]. Surprisingly, our prior studies[3,8,59] have identified higher production of *IL10* in myeloid cells than in Tregs and overwhelming numbers of *IL10*$^{+}$ *BDCA3*$^{+}$ regulatory myeloid cells in psoriasis skin. Because these myeloid cells are less mature (semi-mature) and express other negative regulators, they may contribute to overall immune regulations in human skin[10]. More recently, Frances *et al.*[92] reported that the expression of *TNFAIP3,* a negative regulator of IL-17 and NF-κB signaling, increased in DC3 after 2 weeks of IL-23 inhibition, suggesting that DCs modulate negative feedback loops regulating IL-17/IL-23 signaling[93]. Expanding the previous studies, our current study showed that IL-23 inhibition upregulates regulatory gene expressions in myeloid cells in two different aspects. 1) The expression of regulatory immunoreceptor (*CLEC9A* (*DNGR-1*[64])) increased after IL-23 inhibition (Fig 5, *C*) and 2) The expression of *TNFAIP3* increased, and the expression of *NFKB1* decreased after IL-23 inhibition (Fig 5, *C*).

Our study has limitations. Since it was a single time point study, we could not characterize the kinetics and evolution of transcriptomic changes over different time points. For immune cell-enriched scRNA-seq, we harvested emigrating cells from skin tissues after 48 hours of incubation in a culture medium as previously described[8]. Sato *et al.*[94] reported that our method induces T-cell antigen recovery and upregulation of T-cell activation without antigen cleavages, which are observed when skin immune cells are dissociated by the enzyme tissue digestion method. While our approach enhanced the resolution of T-cell subsets and captured DC/myeloid cells and KCs together, our approach may have enriched the transcriptome of specific immune cell subsets inconsistent with other groups' scRNA-seq data with enzyme tissue digestion and/or immune cell preselection with flow cytometry. To minimize batch-to-batch variability, all the single-cell libraries presented in this study were generated by one investigator using the same experiment protocol. The IL-23 and IL-17 blockers were administered at different doses and dosing intervals, skin biopsies were obtained at different time points, and the PostTx LS IL-17A inhibition group did not have the same statistical power as the PostTx LS IL-23 inhibition group due to the limited sample size. Under these circumstances, it was difficult to determine whether any differences in drug impact can be attributed to different mechanisms of action. To overcome the limitation, we need to conduct a head-to-head clinical trial of IL-23 and IL-17 blockers. In addition, it is unclear whether T17 subsets are unique for psoriasis or if it applies to other skin disorders or other target organs. To further elucidate T17 subsets in human skin, we plan to test the T17 subsets in hidradenitis suppurativa, where T17 cells are stimulated by more diverse cytokines (IL-1β, IL-1α, and IL-6) compared to psoriasis (IL-23)[59].

Unlike diseases of other organs or tissues that involve T17 cells, skin is an organ where T17 cells provide the main mechanism for protection from infection with *Candida albicans* and other environmental pathogens[78]. Conversely, psoriasis is the most common disease driven by excess activation of the IL-23/T17 cell axis, and high levels of disease control possible with IL-23 or IL-17 antagonists suggest overt dependence of this

disease on pathogenic T17 cells[4]. Given the unresolved assignment of distinct T17 subsets to protective versus pathogenic immunity in human skin, analyzing single T-cell phenotypes in response to risankizuamb (IL-23 inhibition) versus secukinumab (IL-17A inhibition) treatments has provided new insights into T-cell functions in psoriasis and background skin protection. Two T17 subsets with the IL-23 receptor expression: an *IL17A*$^{+}$ *IFNG*$^{+}$ T17 subset and an *IL17F*$^{+}$ *IL10*$^{-}$ T17 subset, both synthesizing other inflammatory cytokines, are likely pathogenic for psoriasis (Figs 3, *C* and *D* and Fig E4). In contrast, the *IL17A*$^{+}$ *IL17F*$^{+}$ T17 subset that largely lacks expression of the IL-23 receptor remains as a resident-memory population in resolved psoriasis lesions and might mediate protective immunity as its main function. A population previously identified as non-pathogenic and perhaps regulatory (*IL17F*$^{+}$ *IL10*$^{+}$ T17 subset[8,41–45]) constitutes only 1.17% in untreated psoriasis lesions and does not expand with risankizumab treatment, suggesting treatment outcomes do not depend on T-cell plasticity or switching to this likely regulatory phenotype. Moreover, risankizumab upregulates negative regulation of NF-kB activation by *TNFAIP3* expression in cutaneous myeloid cells (Fig 5, *C*), suggesting multiple immune mechanisms by which risankizumab can modify the complex inflammatory environment present in psoriatic skin. These mechanisms may help restore immune homeostasis that lasts for many months after treatment discontinuation.

Disclosure statement

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Figure Legends

FIG 1. Clinical and molecular responses to IL-23 and IL-17A inhibition in psoriasis. **(A)** Clinical improvement of psoriasis after 7 months of IL-23 inhibition (risankizumab) and skin biopsy sites (LS = Lesional Skin, NL = Non-lesional Skin). **(B)** Psoriasis Area-and-Severity Index (PASI) scores of single-cell libraries. **(C)** Canonical IL-23/Type 17 T-cell pathway gene expression in control, psoriasis pretreatment (PreTx) LS and NL skin, and psoriasis posttreatment after IL-17A inhibition (secukinumab) and IL-23 inhibition (risankizumab) at total transcriptome levels.

FIG 2. Single-cell transcriptome profile of human psoriasis skin. **(A)** The Uniform Manifold Approximation and Projection plot of single-cell clusters. **(B)** Dot plot displaying expression levels of cluster-defining genes. **(C)** Heatmap illustrating pseudotime-dependent genes in the T17 cell differentiation trajectory. **(D)** Volcano plots displaying differentially expressed genes in IL23R, IFNG, CXCL13, CD161 (KLRB1), CD103 (ITGAE) or CXCR6-expressing T17 cluster cells compared to T17 cluster cells who are not expressing those genes. **(E)** Numbers and percentages of IL26, IL17A, IL17F, IL23R, or IFNG-expressing T17 cluster cells per sample in groups of psoriasis pretreatment (PreTx) non-lesional (NL) and lesional skin (LS), and posttreatment (PostTx) LS (after) IL-17A inhibition and IL-23 inhibition.

FIG 3. Single-cell transcriptome changes of T17 cells after IL-17A/IL-23 inhibition. **(A)** Heatmap illustrating different average gene expression between control, psoriasis PreTx NL and LS, and PostTx LS IL-17A and IL-23 inhibition in the T17 cell cluster. **(B)** Volcano plots displaying differentially expressed genes between control, psoriasis PreTx NL and LS, and PostTx LS IL-17A and IL-23 inhibition in the T17 cell cluster. The last panel presents the fold change between psoriasis PostTx LS IL-23 inhibition and IL-17A inhibition, without accounting for transcriptomic changes from psoriasis PreTx LS. **(C)** Heatmap illustrating the average gene expression of T17 cell subsets. **(D)** The proportion changes of T17 subsets between psoriasis PreTx NL and LS, and PostTx LS IL-23 inhibition.

FIG 4. Single-cell transcriptome changes of dendritic cells after IL-17A/IL-23 inhibition. **(A)** Heatmap illustrating different average gene expression between control, psoriasis pretreatment (PreTx) non-lesional (NL) and lesional skin (LS), posttreatment (PostTx) LS (after) IL-17A inhibition and IL-23 inhibition in the dendritic cell (DC) cluster. **(B)** Volcano plots displaying differentially expressed genes between control, PreTx NL, PreTx LS, PostTx LS IL-17A inhibition, and PostTx LS IL-23 inhibition in the DC cluster.

FIG 5. Single-cell transcriptome changes of myeloid cells after IL-17A/IL-23 inhibition. **(A)** Co-expression of myeloid cell cluster-defining genes visualized in low-dimensional space. **(B)** Heatmap illustrating different average gene expression between control, psoriasis pretreatment (PreTx) non-lesional (NL) and lesional skin (LS), posttreatment (PostTx) LS (after) IL-17A inhibition and IL-23 inhibition in the myeloid cell cluster. **(C)** Volcano plots displaying differentially expressed genes between PreTx NL, PreTx LS, and PostTx LS IL-23 inhibition in the myeloid cluster.

FIG 6. Single-cell transcriptome changes of keratinocytes after IL-17A/IL-23 inhibition. **(A)** Heatmap illustrating different average gene expression between control, psoriasis pretreatment (PreTx) non-lesional (NL) and lesional skin (LS), posttreatment (PostTx) LS (after) IL-17A inhibition and IL-23 inhibition in the keratinocyte cluster. **(B)** Volcano plots displaying differentially expressed genes between control, PreTx NL, PreTx LS, PostTx LS IL-17A inhibition, and PostTx LS IL-23 inhibition in the keratinocyte cluster.

Supplementary Figure Legends

Supplementary Fig. 1. Summary of single-cell RNA sequencing data in groups of control, psoriasis pretreatment (PreTx) non-lesional (NL) skin, psoriasis PreTx lesional skin (LS), psoriasis posttreatment (PostTx) LS (after) IL-17A inhibition (secukinumab), and psoriasis PostTx LS IL-23 inhibition (risankizumab): number of cells, number of samples, clinical trial registration number, and publicly accessible National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) accession numbers.

Supplementary Fig. 2. An alluvial diagram visualizes how individual cells are allocated across single-cell libraries, patients, condition (control, psoriasis pretreatment (preTx) non-lesional (NL) and lesional skin (LS), posttreatment (PostTx) IL-17A inhibition (secukinumab) and IL-23 inhibition (risankizumab), and single-cell clusters (T-cell, dendritic cell (DC), myeloid cell, melanocyte, keratinocyte (KC), and fibroblast clusters).

Supplementary Fig. 3. (A) The Uniform Manifold Approximation and Projection plot of single-cell clusters before excluding doublets and merging common immune cell subsets. **(B)** Volcano plots displaying differentially expressed genes between psoriasis PreTx LS, PostTx LS IL-23 inhibition, and IL-17A inhibition in the T17 cell cluster.

Supplementary Fig. 4. Volcano plots displaying differentially expressed genes (DEGs) in T17 subsets compared to other T17 subsets in the T17 cluster.

Supplementary Fig. 5. Co-expression of T17 subset-defining genes visualized in low-dimensional space.

Supplementary Fig. 6. Numbers of T17 intermediates ($IL26^+$ $IL17A^-$ $IL17F^-$ T17 cluster cells) per sample in groups of control, psoriasis pretreatment (PreTx) non-lesional (NL) and lesional skin (LS), and posttreatment (PostTx) LS (after) IL-17A inhibition and IL-23 inhibition.

Supplementary Fig. 7. Volcano plots displaying differentially expressed regulatory dendritic cell (DC) genes between control, psoriasis pretreatment (PreTx) non-lesional (NL) skin, psoriasis PreTx lesional skin (LS), and psoriasis posttreatment (PostTx) LS (after) IL-23 inhibition in the DC cluster.

Table legend

Table 1. Phenotype and characteristics of type 17 T-cell subsets.

Phenotype	IL-23 Receptor expression	Characteristic gene expression	Response after IL-23 inhibition
IL17A^{+} *IFNG*$^{+}$	Yes	*IL26, IL22, IFNG, CSF2*, and *TNF* (T17 cytokines) *CD8A, GZMB, GNLY*, and *PRF1* (cytotoxic T17 cell transcripts)	Eradicated
IL17A^{+} *IFNG*$^{-}$	Yes	*IL12A* (T17 inhibition)	Retained
IL17A^{+} *IL17F*$^{+}$	No	*IL26, IFNG, CSF2*, and *TNF* (T17 cytokines) *IL13* and *IL33* (regulatory cytokines)	Retained
IL17F^{+} *IL10*$^{-}$	Yes	*IL26, IL22, IFNG*, and *CSF2* (T17 cytokines) *IL1B* and *IL34* (inflammatory cytokines)	Reduced
IL17F^{+} *IL10*$^{+}$	No	*IL1RN* (regulatory cytokines)	Not expanded
IL26^{+} *IL17A*$^{-}$ *IL17F*$^{-}$	Yes	*IL26, IL22, IFNG, CSF2*, and *IL21* (T17 cytokines)	Retained