HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2024 April 29.

Published in final edited form as:

J Allergy Clin Immunol. 2023 September ; 152(3): 656–666. doi:10.1016/j.jaci.2023.05.012.

Single-cell transcriptomics suggest distinct upstream drivers of IL-17A/F in hidradenitis versus psoriasis

Jaehwan Kim, MD, PhD[1,2,3,*], **Jongmi Lee, MD, PhD**[2], **Xuan Li, BS**[3], **Hyun Soo Lee, MD**[3], **Katherine Kim**[2], **Vasuma Chaparala**[2], **William Murphy, PhD**[1], **Wei Zhou, PhD**[4], **Junyue Cao, PhD**[4], **Michelle A. Lowes, MBBS, PhD**[3], **James G. Krueger, MD, PhD**[3,*]

[1]Department of Dermatology, University of California, Davis, Sacramento, California, USA

[2]Dermatology Section, Veterans Affairs Northern California Health Care System, Mather, California, USA

[3]Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, USA

[4]Laboratory of Single-cell Genomics and Population Dynamics, The Rockefeller University, New York, New York, USA

Abstract

Background: Based on the mounting evidence that Type 17 T-cells (T17 cells) and increased IL-17 play a key role in driving hidradenitis suppurativa (HS) lesion development, biologics used previously in psoriasis that block signaling of IL-17A and/or IL-17F isoforms have been repurposed to treat HS.

Objective: Our research aimed to characterize the transcriptome of HS T17 cells compared to the transcriptome of psoriasis T17 cells, along with their ligand-receptor interactions with neighborhood immune cell subsets.

Methods: Single-cell data of 12,300 cutaneous immune cells from 8 de-roofing surgical HS skin samples including dermal tunnels were compared with single-cell data of psoriasis skin (19,525 cells from 11 samples) and control skin (11,920 cells from 10 samples). All of the single-cell data were generated by the same protocol.

Results: HS T17 cells expressed lower levels of *IL23R* and higher levels of *IL1R1* and *IL17F* compared to psoriasis T17 cells ($p < 0.05$). HS regulatory T-cells (Tregs) expressed higher levels of *IL1R1* and *IL17F* compared to psoriasis Tregs ($p < 0.05$). Semimature dendritic cells (DCs) were the major immune cell subsets expressing *IL1B* in HS, and IL-1B ligand-receptor interactions between semimature DCs and T17 cells were increased in HS compared to psoriasis ($p < 0.05$). HS dermal tunnel keratinocytes (KCs) expressed inflammatory cytokines (*IL17C*, *IL1A*, *IL1B, and IL6*) which differed from the HS epidermis KCs (*IL36G*) ($p < 0.05$). *IL6*, which synergizes with *IL1B* to maintain cytokine expression in T17 cells, was mainly expressed by

*Corresponding authors: **Jaehwan Kim, MD, PhD**, Assistant Professor, Department of Dermatology, University of California, Davis, Sacramento, CA 95816, ext. 15107, Fax: (916) 361-7369, Jaehwan.Kim@va.gov, **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, Fax: (212) 327-8232, kruegej@mail.rockefeller.edu.

fibroblasts in HS, which also expressed *IL11*+ inflammatory fibroblast genes (*IL11*, *IL24*, *IL6*, and *POSTN*) involved in paracrine IL-1/IL-6 loop.

Conclusion: The IL-1B-T17 cell cytokine axis is likely a dominant pathway in HS with HS T17 cells activated by IL-1B signaling, unlike psoriasis T17 cells which are activated by IL-23 signaling.

Clinical Implication: Biologics targeting IL-17 isoforms and IL-1B may be effective for HS but biologics targeting IL-23 may be less effective for HS.

Capsule Summary:

Unlike psoriasis, type 17 T-cells in hidradenitis suppurativa may be activated by IL-1B signaling interacting with neighboring semimature dendritic cells, dermal tunnel keratinocytes, and fibroblasts.

Keywords

Hidradenitis suppurativa; psoriasis; IL-17A; IL-17F; IL-23; IL-1; IL-1R1; type 17 T-cells; single-cell RNA sequencing; T-cell; dendritic cell; keratinocyte; fibroblast

Introduction

Hidradenitis suppurativa (HS) is a painful debilitating complex cutaneous inflammatory disease with evolving morphologies from superficial nodules and deep abscesses to draining dermal tunnels[1]. Once formed, HS dermal tunnels do not usually resolve spontaneously or with medical treatments, and traditional surgical management results in high rates of recurrence and complications[2]. HS is also associated with comorbidities including diabetes and Crohn's disease. However, no cure exists for this devastating condition. As of this writing, there is only one FDA-approved medication for HS, adalimumab, but remission on therapy is uncommon[3].

The HS microenvironment has been characterized by activation of the IL-17-producing T-cells (Type 17 T-cell, T17 cell)[4–8] and IL-1 pathway[9,10], functional modification of dendritic cells and macrophages[11,12], and an influx of B-cells and plasma cells[13]. Based on the mounting evidence that the activation of T17 cells plays a key role in driving HS lesion development[4–8], psoriasis biologics targeting the IL-23/T17 cell autoimmune axis are being currently tested to repurpose them for HS treatment in multiple clinical trials[14].

In psoriasis, IL-23 from dendritic cells activates T17 cells, and activated T17 cells produce IL-17[15]. Both monoclonal antibodies targeting IL-23 and monoclonal antibodies targeting IL-17 are highly effective for psoriasis[15]. In HS, however, recent clinical trials showed contradictory results between monoclonal antibodies targeting IL-23 and monoclonal antibodies targeting IL-17: anti-IL-23 monoclonal antibodies did not meet their primary endpoint in HS compared to the placebo in phase II trials (NCT03926169 for risankizumab and NCT03628924 for guselkumab). In contrast, anti-IL-17 monoclonal antibody demonstrated statistically significant improvement in HS compared to the placebo in a phase II trial (NCT03248531 for bimekizumab)[4], and phase III trials (NCT03713632

and NCT03713619 for secukinumab)[16]. A small pilot study with an anti-IL-17R antibody was very successful (NCT03960268)[6].

Although psoriasis biologics targeting the IL-23/T17 cell autoimmune axis are currently being tested to repurpose them for HS treatment[14], head-to-head genomic comparison to understand different immunity between HS and psoriasis has been challenging due to the heterogeneous morphologies of HS. Depending on the morphologies and the size of HS tissues obtained for the analysis, gene expression profiles with bulk RNA sequencing are highly variant at total skin levels. Single-cell RNA sequencing may overcome the limitation of bulk RNA sequencing by segregating gene expression profiles of different immune cell subsets, and recent single-cell analyses that compared HS and control skin characterized immune cells infiltrated in the HS at single-cell levels[11,13]. However, until now, immune cells involved in the IL-23/T17 cell autoimmune axis were not directly compared between HS and psoriasis at single-cell levels[11,13].

We compared human skin single-cell transcriptome of T17 cells, regulatory T-cells (Tregs), dendritic cells (DCs), keratinocytes (KCs), and fibroblasts between HS and psoriasis[17,18] to gain insights into the contradictory clinical trial results of monoclonal antibodies targeting IL-23 versus (vs.) IL-17 in HS. We found distinct single-cell profiles and ligand-receptor interactions of T17 cells in HS different from psoriasis, suggesting the alternative T17 cell activation pathways in HS. Overall, in HS, there was a predominant IL-1B-T17 cell interaction, compared to psoriasis skin which was characterized by an IL-23-T17 cell pathway.

Methods

The study was designed to compare single-cell genomic profiles of immune cell subsets between HS, psoriasis, and control skin. 12,300 cells from 8 HS samples, 19,525 cells from 11 psoriasis pre-treatment lesional skin samples, and 11,920 cells from 10 control skin samples were compared for the downstream analyses (Supplemental Table 1). For the detailed experiment methods, please see the Supplemental methods section in the article's Online Repository.

Harvesting immune cells from discarded skin specimens collected during HS surgeries

Discarded and de-identified HS skin specimens were collected during HS dermal tunnel de-roofing surgical procedures. To label epidermal KCs and dermal tunnel KCs separately, the epidermis and dermis of collected skin samples were separated after incubation in 0.2% Dispase II (Sigma-Aldrich) for 3 hours (Supplemental Figure 1). Then, the epidermis and dermis were separately incubated in RPMI-1640 medium with L-glutamine (Cytiva) supplemented with 10% human albumin serum (Sigma-Aldrich) in a humidified incubator at 37°C and 5% CO_2. Cells that had emigrated out of the epidermis and dermis were separately harvested after 48 hours.

Single-cell library construction

Single-cell libraries of the epidermis and single-cell libraries of the dermis were separately constructed from 2 HS skin specimens with dermal tunnels to compare KC transcriptome

between HS epidermis and HS dermal tunnels (Supplemental Figure 1). It was assumed that the KC transcriptome in the single-cell libraries of the dermis represents HS dermal tunnel KCs, while the KC transcriptome in the single-cell libraries of the epidermis represents HS epidermis KCs.

Single-cell library integration and harmonization

We used the Seurat R package (version 4.0.3) installed in R (version 4.1.0) for the downstream single-cell RNA sequencing (scRNA-seq) data analysis[19]. First, we merged single-cell libraries which were prepared by the same reagent kit versions and sequenced by the same sequencer. Then, the merged single-cell libraries were integrated into a single dataset with harmonization. To harmonize the merged single-cell libraries into a single dataset reducing batch effects, correspondences between cells in the merged datasets were identified, and the correspondences were used for data integration[20]. All the single-cell libraries have been deposited in NCBI's Gene Expression Omnibus and are publicly accessible through GEO Series accession number GSE220116.

Single-cell data analyses

Principal component analysis and graph-based clustering analysis were performed, and then the differentially expressed genes (DEGs) and the average gene expression were found between HS, psoriasis, and control. For the receptor-ligand interaction analysis, the fold changes of statistically significant DEGs ($p < 0.05$) were entered to calculate receptor-ligand interaction between different cell clusters.

Statistical analysis

Differentially expressed genes between HS, psoriasis, and control cells were identified by a Wilcoxon Rank Sum test. A value of $p < 0.05$ was considered significant. p-value adjustment was performed using Bonferroni correction for comparisons at total cluster levels. A fold change (FCH) > 1.2 and $p < 0.05$ were used as cut-offs to define differentially expressed genes at the levels of target gene expressing cells within a cluster. Fisher's test was used to determine whether the comparison groups differ in the proportion of cells within a cluster.

Results

Hidradenitis suppurativa (HS) single-cells are composed of higher proportions of T-cell subset, B-cell, plasma cell, mast cell, and fibroblast cluster cells compared to psoriasis single-cells

Dimensionality reduction analysis of 43,745 single-cells of HS, psoriasis, and control samples (29 in total) identified clusters of NK cells, $CD161^+$ T-cells, $CD8^+$ T-cells, $CD4^+$ T-cells, Tregs, B cells, plasma cells, mast cells, mature DCs, semimature DCs, melanocytes, and KCs in different layers of Stratum (S.) corneum, S. granulosum, S. spinosum, S. basale, and fibroblasts without subclustering (Supplemental Figure 2A–B). The percentages of cells in $CD161^+$ T-cell (6.8% in HS vs. 4.0% in psoriasis), $CD4^+$ T-cell (18.0% in HS vs. 12.5% in psoriasis), B-cell (22.3% in HS vs. 0.2% in psoriasis), plasma cell (3.0% in HS vs. 0.1% in psoriasis), mast cell (1.5% in HS vs. 0.5% in psoriasis), and fibroblast (2.1% in HS vs. 0.5% in psoriasis) clusters were higher in HS compared to psoriasis ($p < 0.05$)

(Supplemental Figure 2C). In contrast, the percentages of cells in mature DC (2.7% in HS vs. 13.9% in psoriasis), semimature DC (2.7% in HS vs. 4.0% in psoriasis), melanocyte (1.2% in HS vs. 2.4% in psoriasis), KC in S. corneum (18.5% in HS vs. 39.6% in psoriasis), and KC in S. granulosum (0.5% in KC vs. 3.6% in psoriasis) clusters were lower in HS compared to psoriasis ($p < 0.05$).

HS Type 17 T-cells (T17 cells) express lower levels of IL-23 receptor and higher levels of IL-1 receptor and IL17F compared to psoriasis T17 cells

The expression of T17 cell cytokine *IL17A* and *IL17F* was higher, and the expression of IL-23 receptor (*IL23R*) was lower in HS compared to psoriasis at total immune cell levels (Figure 1A, $p < 0.05$). When the average gene expression of pathogenic T-cell subset clusters ($CD161^+$ T-cell, $CD8^+$ T-cell, and $CD4^+$ T-cell clusters) was compared between HS, psoriasis, and control, HS $CD161^+$ T-cell cluster cells expressed high levels of *IL17A*, *IL17F* and IL-1 receptor (*IL1R1)* (Supplemental Figure 3). When T17 cells were defined as T17 cell lineage marker[8,21] (*CD161*(*KLRB1*) & *IL17A* or *IL17F*) expressing T-cells ($CD3D^+$ cells in the T-cell clusters), HS T17 cells expressed high levels of *IL17A*, *IL17F*, *IL1R1*, and *RORC* while psoriasis T17 cells expressed high levels of IL-23 receptor (*IL23R*), *IFNG*, *IL22* and *IL26* (Figure 1B).

When HS T17 cell subsets were subdivided by *IL17A* vs. *IL17F* expression, 76.4% of HS T17 cells expressed either *IL17A* (13.9%) or *IL17F* (62.4%), and only 23.6% of HS T17 cells expressed *IL17A* and *IL17F* together (Figure 1C). HS $IL17A^+$ ($IL17F^+$ or $IL17F^-$) T17 cell subsets (37.5%) expressed high levels of inflammatory cytokines, such as *IL26*, *TNF*, and *LTA*, which have been reported in psoriasis $IL17A^+$ T17 cells[17]. Within the HS $IL17A^+$ T17 cell subsets, HS $IL17A^+$ $IL17F^-$ T17 cells (13.9%) expressed high levels of inflammatory cytokine (*IFNG*), transcription factor (*RORC*), and resident memory T-cell marker of *CD69*[22], which have been reported in psoriasis $IL17A^+$ $IFNG^+$ T17 cell subsets[17]. HS $IL17A^+$ $IL17F^+$ T17 cells (23.6%) expressed different resident memory T-cell markers of *CD103*(*ITGAE)*[22] and *IL1R1*.

HS $IL17F^+$ ($IL17A^-$) T17 cell subsets (62.4%) expressed high levels of proinflammatory cytokine *IL32*, which has been reported to be increased in both HS[23] and psoriasis[24]. When *IL17A* or *IL17F* expressing T17 cell subsets were compared between HS and psoriasis (Figure 1D and Supplemental Figure 4), HS $IL17F^+$ ($IL17A^-$) T17 cell subsets expressed lower levels of *IL23R* and higher levels of *IL1R1* and *IL17F* compared to psoriasis $IL17F^+$ ($IL17A^-$) T17 cell subsets (FCH > 1.2 and $p < 0.05$)

HS regulatory T-cells (Tregs) express higher levels of IL-1 receptor and IL17F compared to psoriasis Tregs

When the average gene expression of cells in the Treg cluster was compared between HS, psoriasis, and control, HS Treg cluster cells expressed high levels of T17 cell cytokines (*IL17A* and *IL17F*), IL-1 receptor (*IL1R1*), IL-6 signaling that prevents immune suppression by Tregs[25] (*IL6*), and Treg receptors (*CD25*(*IL2RA*), *CTLA4*, and *CCR7*[26]) (Figure 2A). In contrast, HS Treg cluster cells expressed low levels of functional Treg cytokines (*IL10* and *TGFB1*).

When Tregs were defined as *CD3D*$^{+}$ *CD4*$^{+}$ *CD25*(*IL2RA*)$^{+}$ *FOXP3*$^{+}$ cells in the T-cell clusters, HS Tregs expressed higher levels of *IL1R1*, *IL17F*, *CD25*(*IL2RA*), *CTLA4*, and *CCR7* compared to psoriasis Tregs (FCH > 1.2 and $p < 0.05$) (Figure 2B). When HS Tregs with *IL17A* or *IL17F* expression and HS Tregs without *IL17A* or *IL17F* expression were compared, 4.5% of HS Tregs expressed *IL17A* or *IL17F* (*IL17A/F*$^{+}$). HS *IL17A/F*$^{+}$ Tregs expressed high levels of *IL1R1*, *IL17A*, and *IL17F*, and low levels of *IL2RA*, *CTLA4*, and *CCR7* compared to HS *IL17A/F*$^{-}$ Tregs (Figure 2C).

Semimature dendritic cells are the major immune cell subsets expressing IL1B in HS

When the average gene expression of mature and semimature DC clusters was compared between HS, psoriasis, and control, HS mature DC cluster cells expressed high levels of MHC class II molecules (*HLA-DRA*), and skin DC markers of *DC-LAMP*(*LAMP3*), *CIITA*, *CD86*, *CD40* and *PD1-L1*(*CD274*), which have been described in psoriasis mature DCs[17] (Figure 3B). When mature DCs were defined as *HLA-DRA*$^{+}$ *DC-LAMP(LAMP3)*$^{+}$ cells in the mature DC cluster, HS mature DCs expressed higher level of *IL6R, CCR7, DC-LAMP(LAMP3),* and *CIITA,* and lower levels of *LCN2* and *LYZ* compared to psoriasis mature DCs (FCH > 1.2 and $p < 0.05$) (Supplemental Figure 5).

HS semimature DC cluster cells expressed high levels of skin DC markers (*CD11c*(*ITGAX*) and *CD14*) and regulatory DC markers (*BDCA3*(*THBD*), *LILRB2*, and *IL10*) as described in psoriasis semimature DCs[17], but they expressed low levels of *IL23A* in contrast to psoriasis semimature DC cluster cells (Figure 3B). Importantly, semimature DCs were the major immune cell subsets expressing *IL1B* in HS[9,27]. The expression of *IL1B* was higher in HS compared to psoriasis at total immune cell levels ($p < 0.05$), and the expression of *IL1B* was the highest in the HS semimature DC cluster among all clusters (Figure 3A). When semimature DCs were defined as *HLA-DRA*$^{+}$ *THBD(BDCA3)*$^{+}$ cells in the semimature DC cluster, HS semimature DCs expressed higher levels of *IL1B, IL1A,* and *IL6* compared to psoriasis semimature DCs (FCH > 1.2 and $p < 0.05$) (Figure 3C and Supplemental Figure 6).

HS dermal tunnel keratinocytes (KCs) express inflammatory cytokines different from HS epidermis KCs

When the average gene expression of KC clusters was compared between HS dermal tunnel, HS epidermis, psoriasis, and control, the HS dermal tunnel KC cluster expressed high levels of *IL17C* and *IL1A* in S. Corneum, *IL6* in S. Spinosum, and *IL1B* in S. Basale (Figure 4B).

When KCs were defined as cells in the KC-S. Corneum, KC-S. Granulosum, KC-S. Spinosum, and KC-S. Basale clusters, the HS dermal tunnel KCs expressed higher levels of *IL1B* and *IL1A* compared to psoriasis KCs (Figure 4C, FCH > 1.2 and $p < 0.05$). HS epidermis KCs expressed higher levels of *IL36G* compared to the HS dermal tunnel KCs (Figure 4A and 4D, FCH > 1.2 and $p < 0.05$). Both the HS epidermis KCs and the HS dermal tunnel KCs expressed higher levels of *IL1RL1*(*ST2*) compared to psoriasis KCs. The expression levels of *IL1RL1*(*ST2*) were higher in HS epidermis KCs compared to HS dermal tunnel KCs (Figure 4A–D and Supplemental Figure 7, FCH > 1.2 and $p < 0.05$).

HS fibroblasts are IL11$^+$ inflammatory fibroblasts expressing paracrine IL-1/IL-6 loop genes

HS fibroblast cluster expressed the highest levels of *IL11*$^+$ inflammatory fibroblast genes (*IL11* and *IL24*)[28–30] and fibroblast paracrine IL-1/IL-6 loop genes (*IL6* and *POSTN*)[31–33] among all clusters (Figure 5A). When fibroblasts were defined as *COL1A1*$^+$ cells in the fibroblast cluster, HS fibroblasts expressed higher levels of *IL11*, *IL24*, *IL6*, *POSTN* together with *IL1B*, *IL33*, and *CCL20* compared to psoriasis fibroblasts (Figure 5B and 5C, FCH > 1.2 and $p < 0.05$).

IL1B ligand-receptor interactions between semimature DCs and T17 cells are increased in HS compared to psoriasis

To elucidate the interactions of T17 cells with neighboring immune cells that were more activated in HS compared to psoriasis, we analyzed ligand-receptor interactions using differentially expressed genes between HS and psoriasis of T17 cells, mature DCs, semimature DCs, KCs in dermal tunnels, and KCs in the epidermis. The ligand-receptor interactions of IL-17F (ligand: *IL17F*, receptor: *IL17RA* or *IL17RC*), IL-1B (ligand: *IL1B*, receptor: *IL1R1*), IL-6 (ligand: *IL6*, receptor: *IL6R*), and IL-33 (ligand: *IL33*, receptor: *IL1RL1(ST2)*) were increased in HS compared to psoriasis since the expression of both ligands and receptors in the immune cell subsets was increased in HS compared to psoriasis (Figure 6, $p < 0.05$).

When we selected the 10 most enriched ligand-receptor interactions in HS compared to psoriasis, *IL1B* (semimature DCs) – *IL1R1* (T17 cells) was the most enriched interaction with T17 cell receptor expression (Figure 6). The interactions with T17 cell ligand expression included *IL17F* (T17 cells) – *IL17RC* (dermal tunnel KCs and epidermis KCs) or *IL17RA* (semimature DCs)[34]. The interactions with fibroblast ligand expression included *IL6* (fibroblasts) – *IL6R* (mature DCs and dermal tunnel KCs, and epidermis KCs) and *IL33* (fibroblasts) – *IL1RL1* (dermal tunnel KCs and epidermis KCs).

Discussion

In this study, we have analyzed single-cell transcriptomic data from HS skin obtained during de-roofing dermal tunnels and compared it to psoriasis and healthy skin processed in the same manner. Prior single-cell transcriptomic studies of HS focused on increased B cell and plasma cells[13], functional modification of dendritic cells and macrophages[11,12], and relative reduction of Tregs[11]. This study extends those findings to characterize the transcriptome of *IL17A*$^+$ or *IL17F*$^+$ T17 cells and their ligand-receptor interactions with neighborhood immune cell subsets in HS compared to psoriasis by utilizing our immune cell-enriched single-cell transcriptomic approach[17]. In HS, there was a predominant IL-1B-T17 cell cytokine axis (Figure 6), compared to psoriasis skin which was characterized by an IL-23-Th17 pathway[15]. This data has important implications for the treatment of HS, suggesting that agents that target IL-1B and IL-17 should be more successful than those that target IL-23 (Supplemental Figure 8), and this has been largely borne out in HS clinical studies conducted to date.

IL-17 is a critical pro-inflammatory cytokine, with a key role in host defense against microbes. Aberrant IL-17 expression is linked to chronic inflammatory skin diseases such as psoriasis, where it can be targeted for effective treatment[15]. In recent studies, IL-17 mRNA and protein were shown to be expressed in HS lesions, IL-17 was found in suppurative discharge, and serum IL-17 was elevated[35–40]. Our data showed cellular expression of both *IL17A* and *IL17F* in HS T17 cells, providing support for the concept that agents targeting both of these cytokines, and/or blocking the IL-17 receptor, could be a successful approach to reducing the effects of IL-17 in HS. Early small studies of anti-IL-17R (brodalumab) in HS were promising (NCT03960268)[6], and two anti-IL-17 agents have recently completed phase III trials for HS supporting this observation (NCT04242446 & NCT04242498 for bimekizumab and NCT03713632 & NCT03713619 for secukinumab)[41]. Furthermore, our data implies that the effects of IL-17A blockade and IL-17F blockade might be different in HS patients as 76.4% of HS T17 cells expressed either *IL17A* or *IL17F* and only 23.6% of HS T17 cells expressed *IL17A* and *IL17F* together (Figure 1C). HS T17 cell transcriptome was different depending on the expression of *IL17A* or *IL17F* as observed in psoriasis T17 cells[17]. HS Tregs may be more dysfunctional than psoriasis Tregs as 4.5% of HS Tregs expressed *IL17A* or *IL17F*, and HS Tregs expressed higher levels of *IL17F* compared to psoriasis Tregs (Figure 2B–C, $p < 0.05$).

Another important innate pro-inflammatory cytokine is IL-1, and IL-1B is higher in HS suppurative discharge than IL-1A[39] (Figure 3 and Supplemental Figure 6). Cytokine expression pattern of HS lesional skin is marked by a dominant IL-1B presence[9,10], and semimature DCs were the major immune cell subsets expressing *IL1B* in HS[9,27]. Considering that semimature DCs are *BDCA3*(*THBD*)$^{+}$ *IL10*$^{+}$ regulatory DCs in human skin[17], regulatory DCs in HS may be dysfunctional expressing high levels of *IL1B* (Figure 3C). In HS, a small anti-IL-1R antibody study (anakinra)[42,43] and a small anti-IL-1A study (bermekimab)[44–47] have shown promising results. However, given the more widespread expression of *IL1B*, our data suggest anti-IL-1B agents may be more effective than anti-IL-1A in HS (Supplemental Figure 6).

IL-23 is a heterodimer composed of a unique IL-23p19 and shared IL-23p40 chain, and is predominantly produced by dendritic cells to drive Th17 cell activation. IL-23 has shown to be elevated in HS lesional skin[35]. A biologic targeting this shared IL-23p40 chain (ustekinumab) has been evaluated in a phase II HS study with some benefit[47,48]. Recently, however, two phase II studies of anti-IL-23p19 agents (NCT03926169 for risankizumab and NCT03628924 for guselkumab) failed to meet their primary clinical endpoint compared to the placebo[49]. Our data showing low IL-23R expression on T17 cells may explain why these agents were not successful in HS (Figure 1).

Fibroblasts, whose gene expression profiles showed phenotypes of *IL11*$^{+}$ inflammatory fibroblasts (*IL11* and *IL24*)[28–30] and fibroblasts involved in paracrine IL-1/IL-6 loop (*IL6* and *POSTN*)[31–33], were the major immune cell subsets expressing *IL6* in HS. IL-6 has many systemic roles, including increasing acute phase reactants, Th17 cell activation[50], and Treg dysfunction[25]. IL-6 has shown to be elevated in HS skin, suppurative discharge, and serum[39,51]. Hence, IL-6 may play a role in the systemic effects of HS such as comorbidities including anemia.

IL-32 is a pro-inflammatory cytokine that has not been well studied in skin diseases. It was specifically elevated in HS at the cutaneous mRNA, protein, and serum level compared to psoriasis and atopic dermatitis[23]. IL-32 protein was expressed in HS lesions in T cells, dermal natural killer cells, macrophages, and dendritic cells. IL-32 may amplify inflammation in HS as it can be produced by cytokines including IL-1B and in turn increase the production of cytokines such as IL-1B, IL-6, and TNF. In this study, *IL32* appears to be produced by *IL17F*$^{+}$ T17 cells. This cytokine may also be involved in fibrosis, a prominent feature of HS lesions[52]. IL-32 may offer a possible upstream therapeutic target that is specific to HS.

These data also support the potential contribution of dermal tunnel KCs to the pathogenesis of HS lesions. Recent studies have implicated dermal tunnel KCs as active participants in HS inflammation, rather than simply bystanders[53]. In our study, KCs lining dermal tunnels expressed high levels of *IL17C, IL1A, IL1B,* and *IL6* (Figure 4B), and they expressed higher levels of *IL1B* and *IL6* compared to KCs lining the epidermis (Figure 4D, $p < 0.05$).

There are several limitations to our study. Since the study analyzed discarded and de-identified HS skin specimens collected during surgical procedures, patient demographics and current treatment information were not available. The biological specimens were limited in number, but they were all collected from the same lesion type in patients with moderate-severe HS. The transcriptome data and in silico ligand-receptor interaction analysis were not validated by functional studies at protein levels.

Overall, our data show that the predominant IL-1B-Th17 cytokine axis in HS offers a rationale for exciting therapeutic targets, hopefully offering new biological treatment options for patients with HS. Future studies will continue to explore this cytokine axis across greater numbers and types of HS surgical and biopsy specimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

This work was supported, in part, by grant no. UL1TR001866 from the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH) Clinical and Translational Science Award (CTSA) program. This work was supported, in part, by the Hidradenitis Suppurativa Foundation Dandy Research Grant and the University of California, Davis Department of Dermatology Seed Grant programs.

Disclosure of potential conflict of interest:

J.K. was supported by NIAMS K23 Career Development Award (K23AR080043). J.K. has received research support from Novartis and AbbVie. MAL has served on the advisory boards for Abbvie, InflaRx, Janssen, and UCB, Viela Bio, and consulted for Almirall, BSN medical, Incyte, Janssen, Kymera, Phoenicis, and XBiotech. MAL is on the medical board of the Hidradenitis Suppurativa Foundation. 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.

Abbreviations:

DC	Dendritic cell
HS	Hidreadnitis suppurativa
KC	Keratinocyte
NK	Natural Killer
S.	Stratum
scRNA-seq	Single-cell RNA sequencing
T17	Type 17 T-cell
Treg	Regulatory T-cell
UMAP	Uniform Manifold Approximation and Projection

References

1. Dunstan RW, Salte KM, Todorović V, Lowe M, Wetter JB, Harms PW, et al. Histologic progression of acne inversa/hidradenitis suppurativa: Implications for future investigations and therapeutic intervention. Exp Dermatol. 2021;30:820–30. [PubMed: 33377546]
2. Saylor DK, Brownstone ND, Naik HB. Office-Based Surgical Intervention for Hidradenitis Suppurativa (HS): A Focused Review for Dermatologists. Dermatol Ther . 2020;10:529–49.
3. Kimball AB, Okun MM, Williams DA, Gottlieb AB, Papp KA, Zouboulis CC, et al. Two phase 3 trials of adalimumab for hidradenitis suppurativa. N Engl J Med. 2016;375:422–34. [PubMed: 27518661]
4. Glatt S, Jemec GBE, Forman S, Sayed C, Schmieder G, Weisman J, et al. Efficacy and safety of bimekizumab in moderate to severe hidradenitis suppurativa: A phase 2, double-blind, placebo-controlled randomized clinical trial. JAMA Dermatol. 2021;157:1279–88. [PubMed: 34406364]
5. Navrazhina K, Garcet S, Frew JW, Zheng X, Coats I, Guttman-Yassky E, et al. The inflammatory proteome of hidradenitis suppurativa skin is more expansive than that of psoriasis vulgaris. J Am Acad Dermatol [Internet]. 2021; Available from: 10.1016/j.jaad.2021.07.035
6. Frew JW, Navrazhina K, Grand D, Sullivan-Whalen M, Gilleaudeau P, Garcet S, et al. The effect of subcutaneous brodalumab on clinical disease activity in hidradenitis suppurativa: An open-label cohort study. J Am Acad Dermatol. 2020;83:1341–8. [PubMed: 32416208]
7. Casseres RG, Prussick L, Zancanaro P, Rothstein B, Joshipura D, Saraiya A, et al. Secukinumab in the treatment of moderate to severe hidradenitis suppurativa: Results of an open-label trial. J Am Acad Dermatol. 2020;82:1524–6. [PubMed: 32044410]
8. Moran B, Sweeney CM, Hughes R, Malara A, Kirthi S, Tobin AM, et al. Hidradenitis Suppurativa Is Characterized by Dysregulation of the Th17:Treg Cell Axis, Which Is Corrected by Anti-TNF Therapy. J Invest Dermatol. 2017;137:2389–95. [PubMed: 28652108]
9. Witte-Händel E, Wolk K, Tsaousi A, Irmer ML, Mößner R, Shomroni O, et al. The IL-1 pathway is hyperactive in hidradenitis suppurativa and contributes to skin infiltration and destruction. J Invest Dermatol. 2019;139:1294–305. [PubMed: 30528824]
10. Vossen ARJV, van Straalen KR, Florencia EF, Prens EP. Lesional inflammatory profile in hidradenitis suppurativa is not solely driven by IL-1. J Invest Dermatol. 2020;140:1463–1466.e2. [PubMed: 32081612]
11. Lowe MM, Naik HB, Clancy S, Pauli M, Smith KM, Bi Y, et al. Immunopathogenesis of hidradenitis suppurativa and response to anti-TNF-α therapy. JCI Insight [Internet]. 2020;5. Available from: 10.1172/jci.insight.139932

12. Mariottoni P, Jiang SW, Prestwood CA, Jain V, Suwanpradid J, Whitley MJ, et al. Single-Cell RNA Sequencing Reveals Cellular and Transcriptional Changes Associated With M1 Macrophage Polarization in Hidradenitis Suppurativa. Front Med. 2021;8:665873.
13. Gudjonsson JE, Tsoi LC, Ma F, Billi AC, van Straalen KR, Vossen ARJV, et al. Contribution of plasma cells and B cells to hidradenitis suppurativa pathogenesis. JCI Insight [Internet]. 2020;5. Available from: 10.1172/jci.insight.139930
14. Flood KS, Porter ML, Kimball AB. Biologic Treatment for Hidradenitis Suppurativa. Am J Clin Dermatol. 2019;20:625–38. [PubMed: 31140067]
15. Kim J, Krueger JG. Highly Effective New Treatments for Psoriasis Target the IL-23/Type 17 T Cell Autoimmune Axis. Annu Rev Med. 2017;68:255–69. [PubMed: 27686018]
16. East M, Sports US, League BP, League BC, Family BR, Live TV, et al. Novartis Cosentyx® shows clinically meaningful symptom improvements in patients with hidradenitis suppurativa in pivotal Phase III trials. itbusinessnet.com [Internet]. Available from: https://itbusinessnet.com/2022/09/novartis-cosentyx-shows-clinically-meaningful-symptom-improvements-in-patients-with-hidradenitis-suppurativa-in-pivotal-phase-iii-trials/
17. Kim J, Lee J, Kim HJ, Kameyama N, Nazarian R, Der E, et al. Single-cell transcriptomics applied to emigrating cells from psoriasis elucidate pathogenic vs. regulatory immune cell subsets. J Allergy Clin Immunol [Internet]. 2021; Available from: 10.1016/j.jaci.2021.04.021
18. The editors' choice. J Allergy Clin Immunol. 2021;148:1140–5.
19. Satija R, Farrell JA, Gennert D, Schier AF, Regev A. Spatial reconstruction of single-cell gene expression data. Nat Biotechnol. 2015;33:495–502. [PubMed: 25867923]
20. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM III, et al. Comprehensive Integration of Single-Cell Data. Cell. 2019;
21. Cosmi L, De Palma R, Santarlasci V, Maggi L, Capone M, Frosali F, et al. Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. J Exp Med. 2008;205:1903–16. [PubMed: 18663128]
22. Watanabe R, Gehad A, Yang C, Scott LL, Teague JE, Schlapbach C, et al. Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. Sci Transl Med. 2015;7:279ra39.
23. Thomi R, Yerly D, Yawalkar N, Simon D, Schlapbach C, Hunger RE. Interleukin-32 is highly expressed in lesions of hidradenitis suppurativa. Br J Dermatol. 2017;177:1358–66. [PubMed: 28301691]
24. Kempuraj D, Conti P, Vasiadi M, Alysandratos KD, Tagen M, Kalogeromitros D, et al. IL-32 is increased along with tryptase in lesional psoriatic skin and is up-regulated by substance P in human mast cells. Eur J Dermatol. 2010;20:865–7. [PubMed: 21047723]
25. Goodman WA, Levine AD, Massari JV, Sugiyama H, McCormick TS, Cooper KD. IL-6 signaling in psoriasis prevents immune suppression by regulatory T cells. The Journal of Immunology. 2009;183:3170–6. [PubMed: 19648274]
26. Schneider MA, Meingassner JG, Lipp M, Moore HD, Rot A. CCR7 is required for the in vivo function of CD4+ CD25+ regulatory T cells. J Exp Med. 2007;204:735–45. [PubMed: 17371928]
27. van der Zee HH, de Ruiter L, van den Broecke DG, Dik WA, Laman JD, Prens EP. Elevated levels of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-α and IL-1β. Br J Dermatol. 2011;164:1292–8. [PubMed: 21332464]
28. Nishina T, Deguchi Y, Ohshima D, Takeda W, Ohtsuka M, Shichino S, et al. Interleukin-11-expressing fibroblasts have a unique gene signature correlated with poor prognosis of colorectal cancer. Nat Commun. 2021;12:2281. [PubMed: 33863879]
29. Widjaja AA, Chothani S, Viswanathan S, Goh JWT, Lim WW, Cook SA. IL11 stimulates IL33 expression and proinflammatory fibroblast activation across tissues. Int J Mol Sci. 2022;23:8900. [PubMed: 36012165]
30. Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, et al. Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis. Cell. 2019;178:714–730.e22. [PubMed: 31348891]

31. Yamato H, Kimura K, Fukui E, Kanou T, Ose N, Funaki S, et al. Periostin secreted by activated fibroblasts in idiopathic pulmonary fibrosis promotes tumorigenesis of non-small cell lung cancer. Sci Rep. 2021;11:21114. [PubMed: 34702952]
32. Hortells L, Valiente-Alandi I, Thomas ZM, Agnew EJ, Schnell DJ, York AJ, et al. A specialized population of Periostin-expressing cardiac fibroblasts contributes to postnatal cardiomyocyte maturation and innervation. Proc Natl Acad Sci U S A. 2020;117:21469–79. [PubMed: 32817558]
33. Taniguchi K, Arima K, Masuoka M, Ohta S, Shiraishi H, Ontsuka K, et al. Periostin controls keratinocyte proliferation and differentiation by interacting with the paracrine IL-1α/IL-6 loop. J Invest Dermatol. 2014;134:1295–304. [PubMed: 24352037]
34. Tucci M, Stucci S, Savonarola A, Ciavarella S, Cafforio P, Dammacco F, et al. Immature dendritic cells in multiple myeloma are prone to osteoclast-like differentiation through interleukin-17A stimulation. Br J Haematol. 2013;161:821–31. [PubMed: 23594390]
35. Schlapbach C, Hänni T, Yawalkar N, Hunger RE. Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. J Am Acad Dermatol. 2011;65:790–8. [PubMed: 21641076]
36. Lima AL, Karl I, Giner T, Poppe H, Schmidt M, Presser D, et al. Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. Br J Dermatol. 2016;174:514–21. [PubMed: 26436522]
37. Kelly G, Hughes R, McGarry T, van den Born M, Adamzik K, Fitzgerald R, et al. Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa. Br J Dermatol. 2015;173:1431–9. [PubMed: 26282467]
38. Navrazhina K, Frew JW, Krueger JG. Interleukin 17C is elevated in lesional tissue of hidradenitis suppurativa. Br J Dermatol. 2020;182:1045–7. [PubMed: 31556100]
39. Kanni T, Tzanetakou V, Savva A, Kersten B, Pistiki A, van de Veerdonk FL, et al. Compartmentalized cytokine responses in hidradenitis suppurativa. PLoS One. 2015;10:e0130522. [PubMed: 26091259]
40. Matusiak Ł, Szczęch J, Bieniek A, Nowicka-Suszko D, Szepietowski JC. Increased interleukin (IL)-17 serum levels in patients with hidradenitis suppurativa: Implications for treatment with anti-IL-17 agents. J Am Acad Dermatol. 2017;76:670–5. [PubMed: 28041632]
41. Kimball AB, Jemec GBE, Alavi A, Reguiai Z, Gottlieb AB, Bechara FG, et al. Secukinumab in moderate-to-severe hidradenitis suppurativa (SUNSHINE and SUNRISE): week 16 and week 52 results of two identical, multicentre, randomised, placebo-controlled, double-blind phase 3 trials. Lancet. 2023;401:747–61. [PubMed: 36746171]
42. Tzanetakou V, Kanni T, Giatrakou S, Katoulis A, Papadavid E, Netea MG, et al. Safety and efficacy of anakinra in severe hidradenitis suppurativa: A randomized clinical trial. JAMA Dermatol. 2016;152:52–9. [PubMed: 26579854]
43. André R, Marescassier H, Gabay C, Pittet B, Laffitte E. Long-term therapy with anakinra in hidradenitis suppurativa in three patients. Int J Dermatol. 2019;58:e208–9. [PubMed: 31353451]
44. Kernel Networks Inc. A study to evaluate the efficacy, safety and tolerability of bermekimab in patients with hidradenitis suppurativa. Case Medical Research [Internet]. 2019; Available from: 10.31525/ct1-nct04019041
45. Gottlieb A, Natsis NE, Kerdel F, Forman S, Gonzalez E, Jimenez G, et al. A phase II open-label study of bermekimab in patients with hidradenitis suppurativa shows resolution of inflammatory lesions and pain. J Invest Dermatol. 2020;140:1538–1545.e2. [PubMed: 32004568]
46. Cavalli G, Colafrancesco S, Emmi G, Imazio M, Lopalco G, Maggio MC, et al. Interleukin 1α: a comprehensive review on the role of IL-1α in the pathogenesis and treatment of autoimmune and inflammatory diseases. Autoimmun Rev. 2021;20:102763. [PubMed: 33482337]
47. Blok JL, Li K, Brodmerkel C, Jonkman MF, Horváth B. Gene expression profiling of skin and blood in hidradenitis suppurativa. Br J Dermatol. 2016;174:1392–4. [PubMed: 26707687]
48. Blok JL, Li K, Brodmerkel C, Horvátovich P, Jonkman MF, Horváth B. Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. Br J Dermatol. 2016;174:839–46. [PubMed: 26641739]
49. Kimball AB, Prens EP, Passeron T, Maverakis E, Turchin I, Beeck S, et al. Efficacy and Safety of Risankizumab for the Treatment of Hidradenitis Suppurativa: A Phase 2, Randomized, Placebo-Controlled Trial. Dermatol Ther . 2023;1–13.

50. Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A, et al. Induction and molecular signature of pathogenic TH17 cells. Nat Immunol. 2012;13:991–9. [PubMed: 22961052]

51. Jiménez-Gallo D, de la Varga-Martínez R, Ossorio-García L, Albarrán-Planelles C, Rodríguez C, Linares-Barrios M. The clinical significance of increased serum proinflammatory cytokines, C-reactive protein, and erythrocyte sedimentation rate in patients with Hidradenitis Suppurativa. Mediators Inflamm. 2017;2017:2450401.

52. Moschen AR, Fritz T, Clouston AD, Rebhan I, Bauhofer O, Barrie HD, et al. Interleukin-32: a new proinflammatory cytokine involved in hepatitis C virus-related liver inflammation and fibrosis. Hepatology. 2011;53:1819–29. [PubMed: 21381070]

53. Navrazhina K, Frew JW, Gilleaudeau P, Sullivan-Whalen M, Garcet S, Krueger JG. Epithelialized tunnels are a source of inflammation in hidradenitis suppurativa. J Allergy Clin Immunol. 2021;147:2213–24. [PubMed: 33548397]

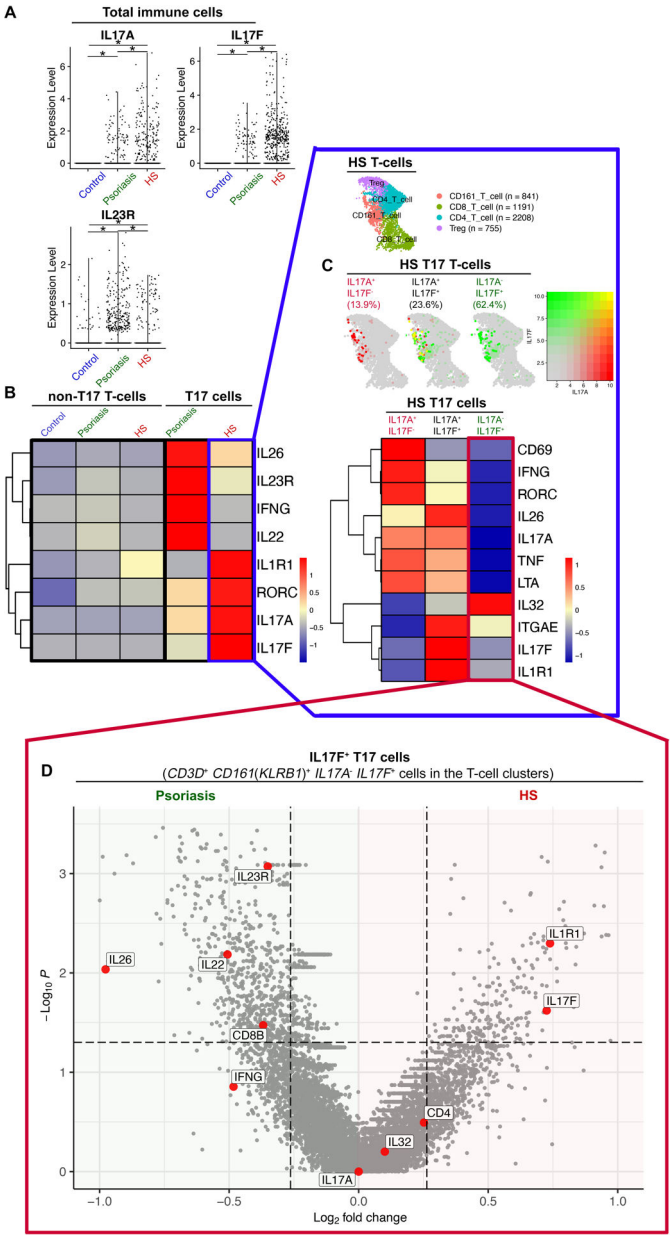

Figure 1.

T17 cell gene expression comparison between HS and psoriasis. **A**, *IL17A*, *IL17F*, and *IL23R* expression comparison between HS, psoriasis, and control in total immune cells (*$p < 0.05$). **B**, The average gene expression of T17 cells and non-T17 T-cells, split by HS, psoriasis, and control. **C**, The average gene expression of HS T17 cells, split by *IL17A* or *IL17F* expression. *IL17A* (*red*), *IL17F* (*green*), and *IL17A* and *IL17F* coexpression (*yellow*) within the T-cell subset clusters visualized in low-dimensional space. **D**, Volcano plot displaying differentially expressed genes of *IL17F*$^+$ T17 cells between HS and psoriasis (vertical dotted line = fold change of 1.2, horizontal dotted line = *p*-value of 0.05).

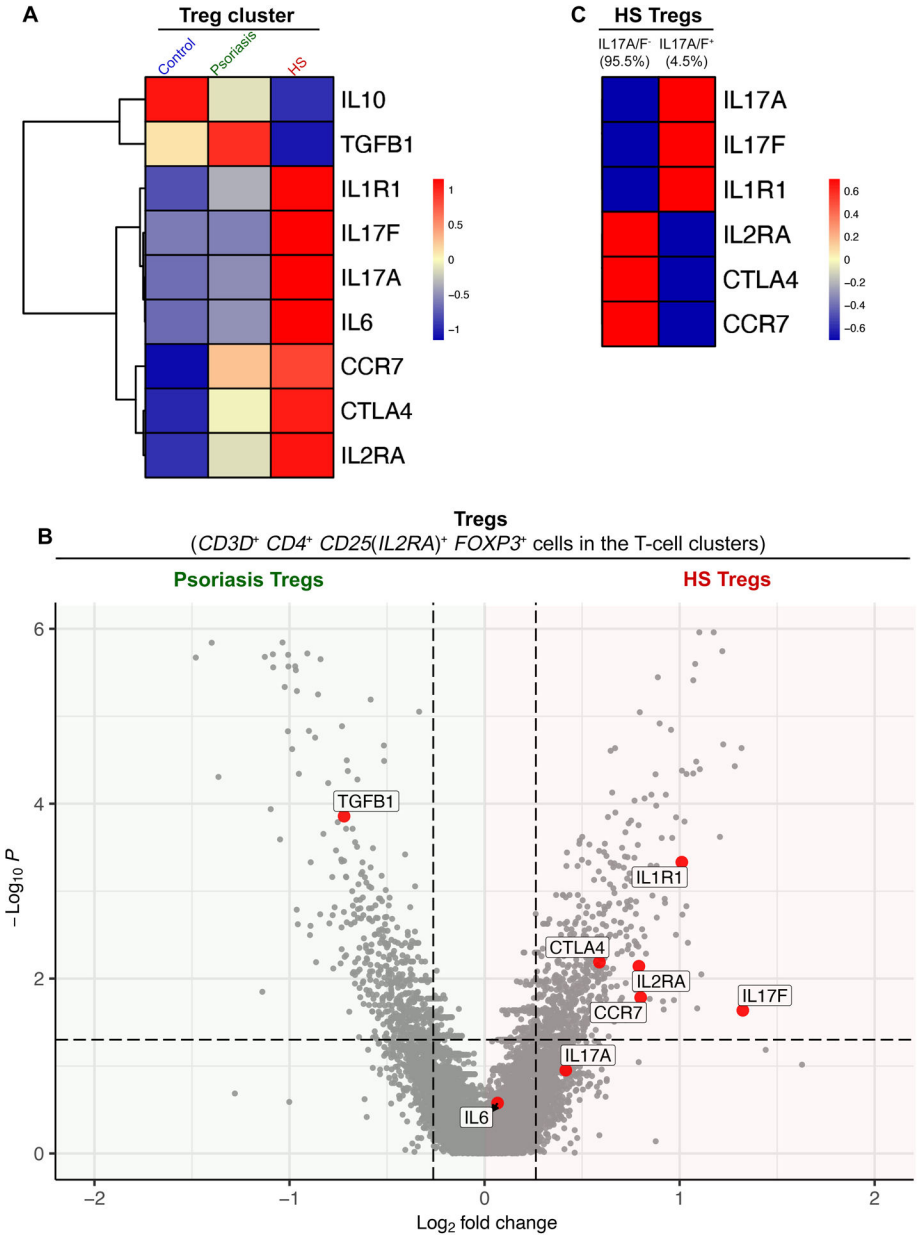

Figure 2.

Treg gene expression comparison between HS, psoriasis, and control. **A**, The average gene expression of the Treg cluster, split by HS, psoriasis, and control. **B**, Volcano plot displaying differentially expressed genes of Tregs between HS and psoriasis (vertical dotted line = fold change of 1.2, horizontal dotted line = *p*-value of 0.05). **C**, The average gene expression of HS Tregs, split by Tregs with *IL17A/F* expression vs. Tregs without *IL17A/F* expression.

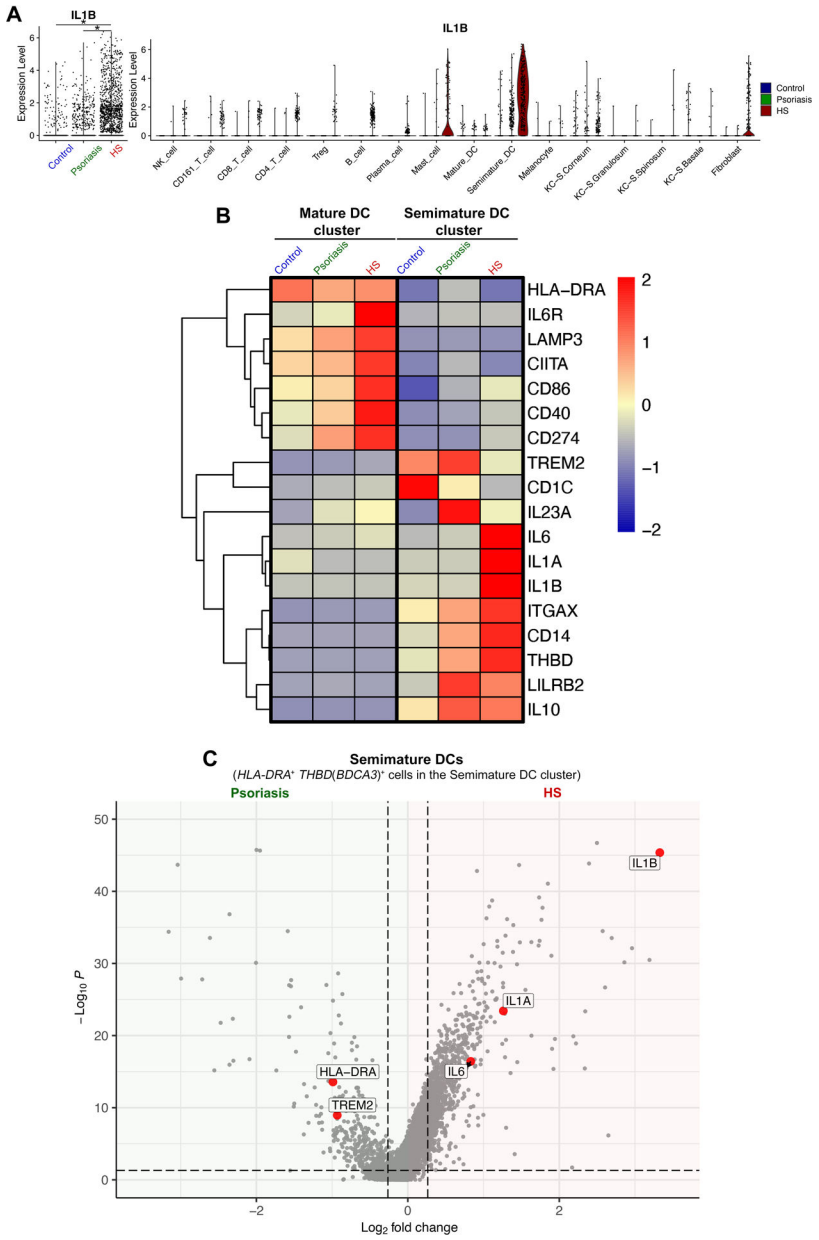

Figure 3.

DC gene expression comparison between HS, psoriasis, and control. **A**, *IL1B* expression comparison between HS, psoriasis, and control in total immune cells (*$p < 0.05$) and immune cell subset clusters (violin plot). **B**, The average gene expression of the mature and semimature DC clusters, split by HS, psoriasis, and control. **C**, Volcano plot displaying differentially expressed genes of semimature DCs between HS and psoriasis (vertical dotted line = fold change of 1.2, horizontal dotted line = *p*-value of 0.05).

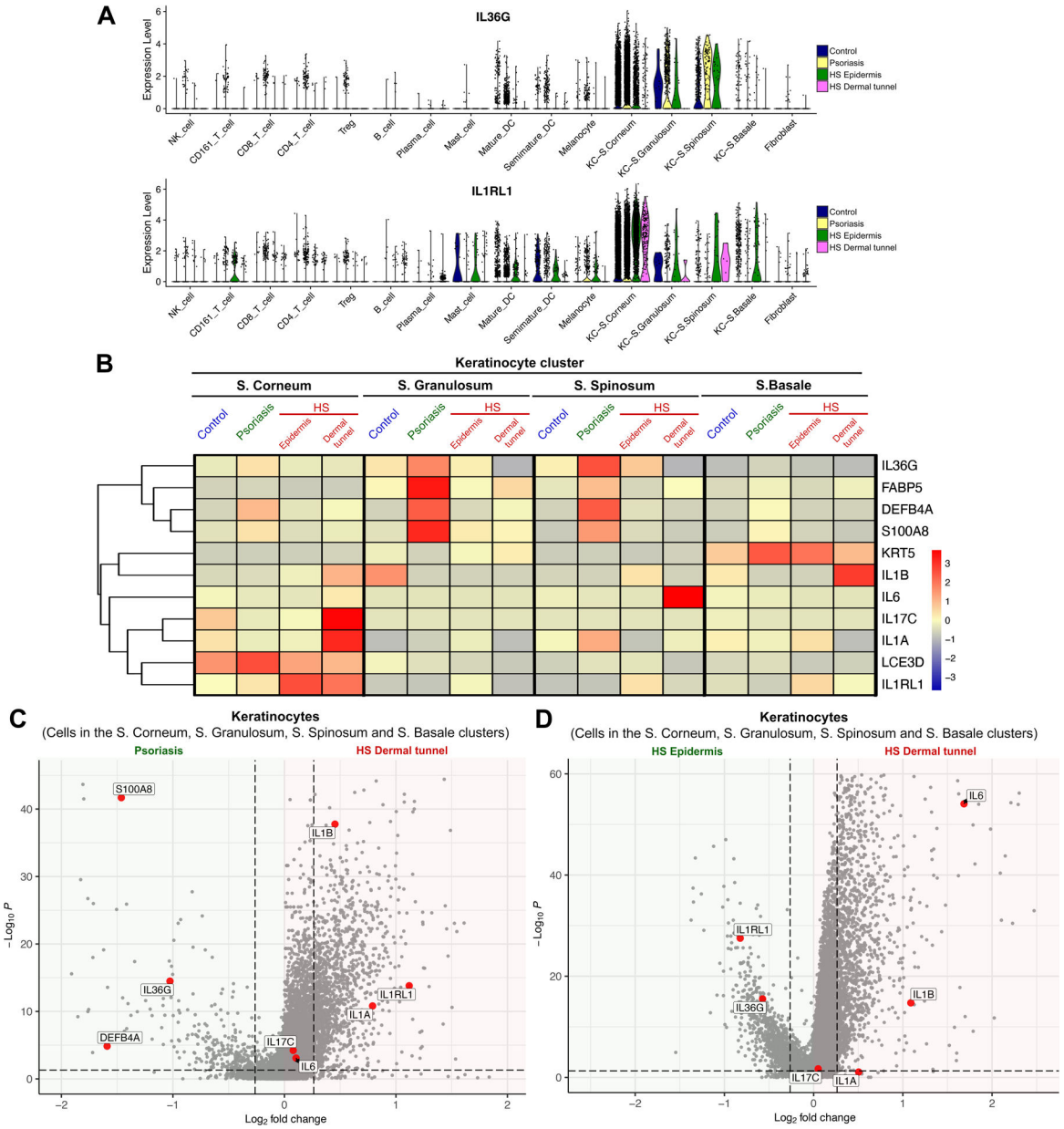

Figure 4.

Keratinocyte gene expression comparison between HS, psoriasis, and control. **A**, *IL36G* and *IL1RL1(ST2)* expression comparison between HS epidermis, HS dermal tunnel, psoriasis, and control in immune cell subset clusters (violin plot). **B**, The average gene expression of the keratinocyte clusters, split by HS dermal tunnel, HS epidermis, psoriasis, and control. **C, D**, Volcano plots displaying differentially expressed genes of keratinocytes between (**C**) HS dermal tunnel and psoriasis and (**D**) HS dermal tunnel and HS epidermis (vertical dotted line = fold change of 1.2, horizontal dotted line = *p*-value of 0.05).

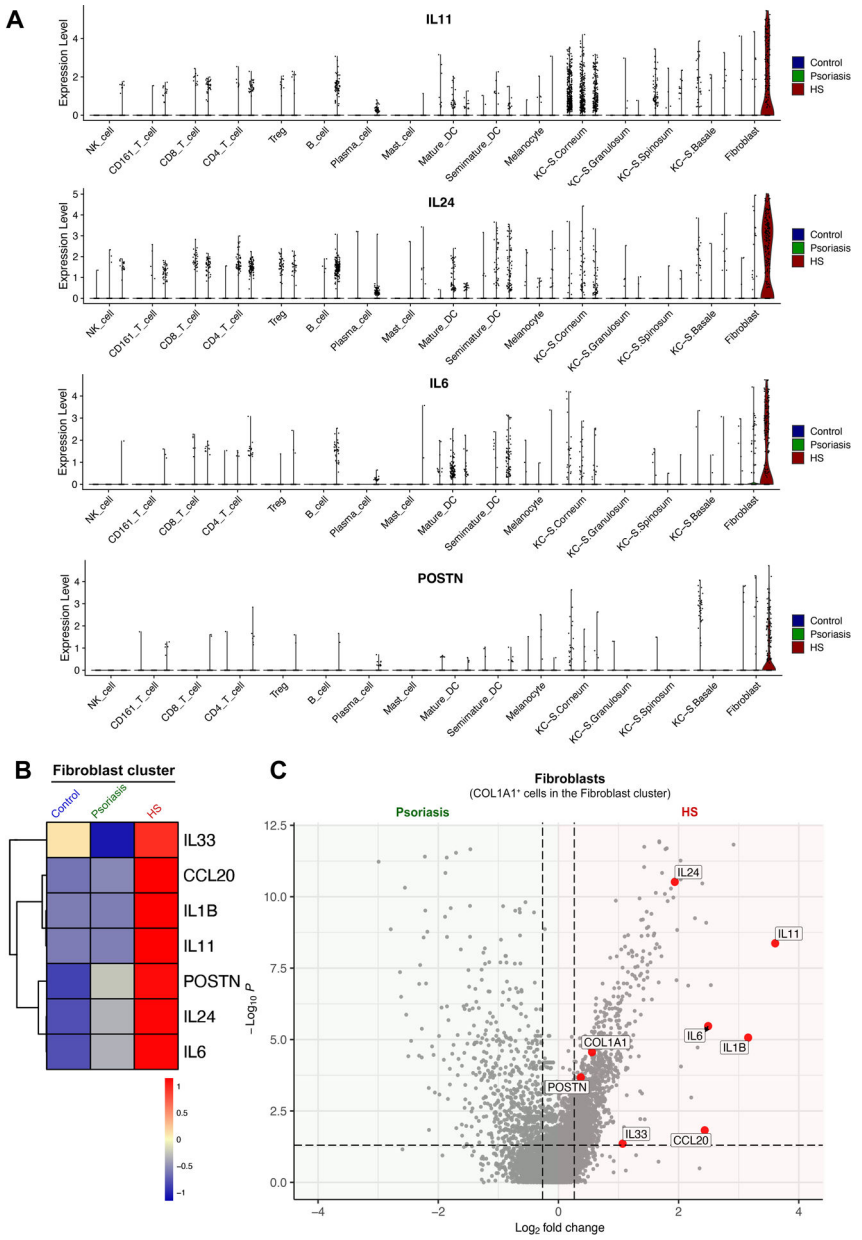

Figure 5.

Fibroblast gene expression comparison between HS, psoriasis, and control. **A**, *IL11*, *IL24*, *IL6*, *and POSTN* expression comparison between HS, psoriasis, and control in immune cell subset clusters (violin plot). **B**, The average gene expression of the fibroblast cluster, split by HS, psoriasis, and control. **C**, Volcano plot displaying differentially expressed genes of fibroblasts between HS and psoriasis (vertical dotted line = fold change of 1.2, horizontal dotted line = *p*-value of 0.05).

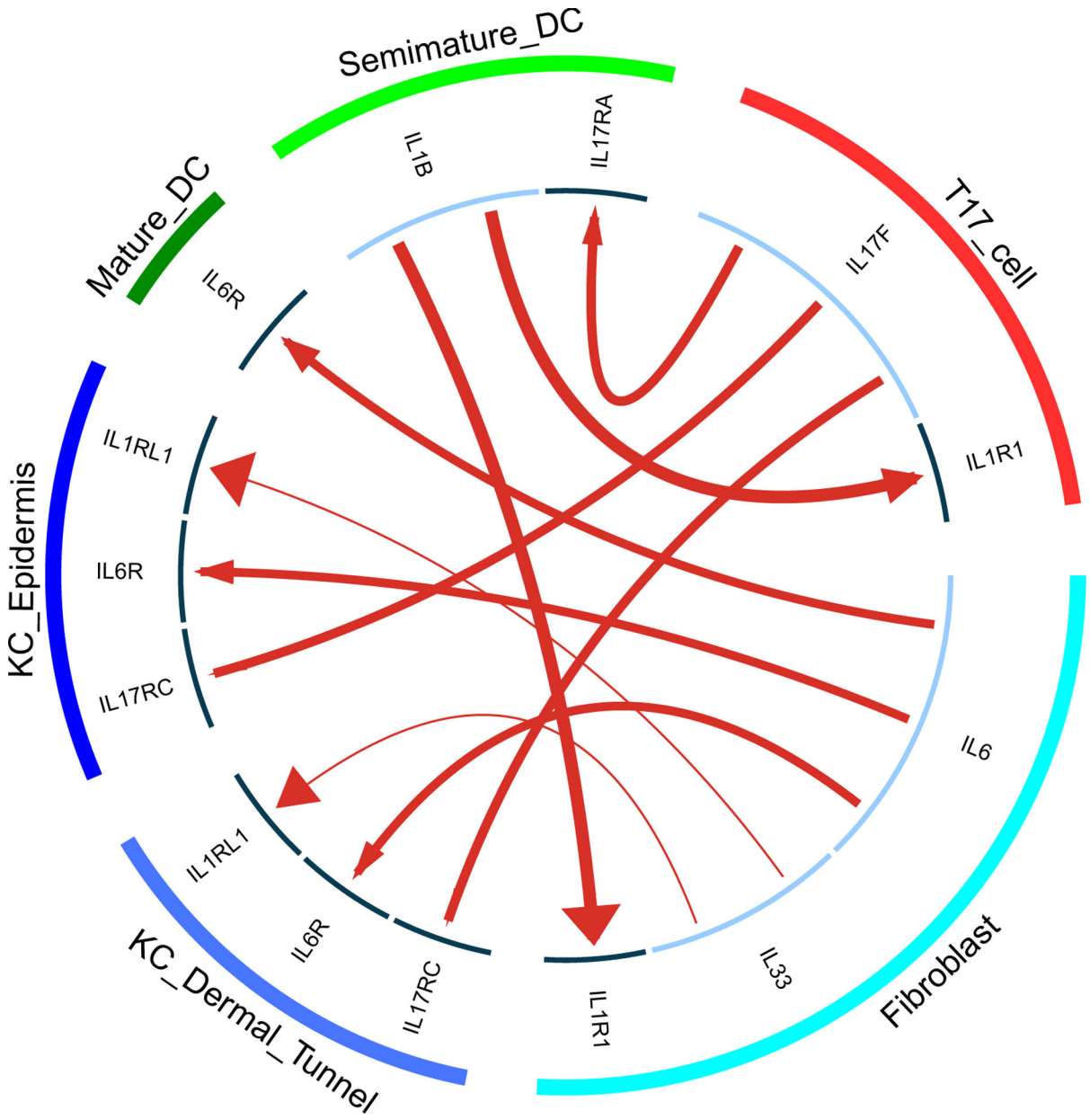

Figure 6.

Top 10 ligand-receptor interactions of IL-17F, IL-1B, IL-6, and IL-33 that are increased in HS compared to psoriasis