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# CD2 expressing innate lymphoid and T cells are critical effectors of immunopathogenesis in hidradenitis suppurativa

Mahendra Pratap Kashyap[a,b,1], Bharat Mishra[c,1,2], Rajesh Sinha[a,b], Lin Jin[a,b], YiFei Gou[c], Nilesh Kumar[c], Kayla F. Goliwas[d], Safiya Haque[b], Jessy Deshane[d], Erik Berglund[e,f,g], David Berglund[e,h], Boni E. Elewski[a,b], Craig A. Elmets[a,b], Mohammad Athar[a,b,3], M. Shahid Mukhtar[c,i,4], and Chander Raman[a,b,4]

Affiliations are included on p. 11.

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**Hidradenitis suppurativa (HS) is a chronic, debilitating inflammatory skin disease with a poorly understood immunopathogenesis. Here, we report that HS lesional skin is characterized by the expansion of innate lymphocytes and T cells expressing CD2, an essential activation receptor and adhesion molecule. Lymphocytes expressing elevated CD2 predominated with unique spatial distribution throughout the epidermis and hypodermis in the HS lesion. $CD2^+$ cells were mainly innate lymphocytes expressing the NK cell marker, CD56, and $CD4^+$ T cells. Importantly, these $CD2^+$ cells interacted with CD58 (LFA3) expressing epidermal keratinocytes and fibroblasts in the hypodermis. Granzyme $A^{bright}$ NKT cells ($CD2^+CD3^+CD56^{bright}$) clustered with α-SMA expressing fibroblasts juxtaposed to epithelialized tunnels and fibrotic regions of the hypodermis. Whereas NK cells ($CD2^+CD56^{dim}$) were perforin$^+$, granzymes $A^+$ and $B^+$, and enriched adjacent to hyperplastic follicular epidermis and tunnels of HS showing presence of apoptotic cells. The cytokines IL-12, IL-15, and IL-18, which enhance NK cell maturation and function were significantly elevated in HS. Ex vivo HS skin explant cultures treated with CD2:CD58 interaction-blocking anti-CD2 monoclonal antibody attenuated secretion of inflammatory cytokines/chemokines and suppressed inflammatory gene signature. Additionally, CD2:CD58 blockade altered miRNAs involved in NK/NKT differentiation and/or function. In summary, we show that a cellular network of heterogenous NKT and NK cell populations drives inflammation and is critical in the pathobiology of HS, including tunnel formation and fibrosis. Finally, CD2 blockade is a viable immunotherapeutic approach for the effective management of HS.**

Hidradenitis suppurativa | NKT cell | CD2 | NK cell | CD4 T cell

## Significance

Hidradenitis suppurativa (HS) is a progressive, debilitating inflammatory disease with poorly defined etiology and incompletely characterized cell populations driving its pathogenesis. Our study identifies that CD2, an activation and adhesion receptor, is expressed at elevated levels on T cells and on innate lymphoid cells [natural killer (NK)/innate lymphoid cell 1 (ILC1), natural killer T (NKT), mucosal-associated invariant T (MAIT)]. We further report that disruption of the cognate interaction between CD2 and its ligand, CD58, is a therapeutic target for the treatment of HS. Importantly, our data suggest that distinct subpopulations of NKT and NK cells are the major drivers of HS disease pathogenesis.

Hidradenitis suppurativa (HS), or acne inversa, is a chronic inflammatory skin disease characterized by deep-seated lesions often associated with obstruction of the hair follicles and hypertrophic scarring in apocrine gland-bearing regions of the body (1, 2). HS is more common among African Americans, with a prevalence of 1 to 4%, female-to-male ratio of 3:1, and mean onset at 21.8 y (3, 4). The disease is marked by painful nodules, malodorous discharge, abscesses, and development of scars and fibrosis (1, 2). These clinical features affect quality of life, leading to depression, social embarrassment, and anxiety in most patients.

Hurley stages I, II, and III, classify the severity of HS. Although inflammation is a key feature, a major gap in knowledge exists regarding the mechanistic underpinning of disease onset, progression, and etiology. The immune cell populations implicated in HS include T cells (CD4 and CD8), B cell populations, neutrophils, and macrophages (5–8). Th1 (and $CD8^+$ T cell) signature cytokines (IFN-γ and TNF-α) and Th17 signature cytokines (IL-17A, IL-17F, IL-22, and IL-6) are elevated in HS (9). Neutrophils within HS lesions promote in situ immune dysregulation and amplify the inflammatory cascade through the activation of the NLRP3-inflammasome pathway and/or suppression of immune regulatory mechanisms (5). The inflammatory milieu of HS recruits monocytes and promotes their differentiation into M1-like macrophages (8, 10). We previously showed that eIF4F protein translation regulatory complex is up-regulated in follicular keratinocytes in HS and contributes to their hyperproliferation (11). We also identified epigenetic mechanisms that regulate the pathogenic inflammatory plasticity of progenitors in HS follicular and interfollicular epithelial cells (12).

The most common treatment for HS involves use of systemic antibiotics and intralesional corticosteroids to reduce inflammation (1). TNF-α blockers and anti-IL-17a (secukinumab) are the biologics used in treating HS patients (6, 13). Targeting IL-17F, IL-1β, and JAKs in HS is currently being investigated in clinical trials (14–17). However, these treatments

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[1]M.P.K. and B.M. contributed equally to this work.

[2]Present address: Department of Genetics at the University of Alabama at Birmingham, Birmingham, AL 35294.

[3]M.A. contributed equally to this work.

[4]To whom correspondence may be addressed. Email: mshahid@clemson.edu or chanderraman@uabmc.edu.

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provide only marginal relief at best, highlighting the critical unmet need for developing new treatment modalities for HS, particularly through the identification of novel druggable targets.

We employed a multiomics approach combining single-cell and bulk transcriptome sequencing, high-definition and multiparameter confocal/immunofluorescence imaging, and miRegulome analysis to clarify deterministic associations among multiple biological factors in HS. We found that lymphocytes expressing high levels of CD2, a costimulation/adhesion molecule, were predominant in HS lesional skin. $CD2^{high}$ cells were primarily innate lymphoid NK cell populations ($CD3^{+}CD56^{bright}$ NKT cells and classical cytolytic $CD56^{dim}$ NK cells) and CD4 T cells. The NKT cells and NK cells were spatially localized in distinct regions across HS lesions. They interacted with CD58 (LFA-3, CD2 ligand) expressing epithelial cells and likely contribute to the various aspects of HS pathogenesis. CD2:CD58 cognate interaction is a central component of the immunological synapse in T cells, NKT, and NK cells and is essential for intracellular activation signals (18). Of significance, blocking the CD2:CD58 cognate interaction in HS microenvironment significantly attenuated the inflammatory gene expression and cytokine profile. Anti-CD2 treatment resulted in $CD2^{+}$ lymphocyte death consistent with a phenomenon previously reported for NK cell fratricide (19).

## Results

**Lymphoid Cell Populations Are Major Pathogenic Immune Cells in HS Skin.** We performed single-cell RNA seq (scRNAseq) analysis on cells isolated from normal (n = 4) and HS (lesional; n = 6) skin. The datasets were log-normalized, integrated using Harmony, and dimensionally reduced with the Uniform Manifold Approximation and Projection for dimension reduction (UMAP) algorithm (*SI Appendix*, Fig. S1*A* and Dataset S1). From an unsupervised analysis of 27,442 cells, 19 clusters were annotated to various populations and subpopulations of adaptive and innate immune cells, keratinocytes, fibroblasts, endothelial cells, and melanocytes, based on the expression of marker genes (Fig. 1*A*, *SI Appendix*, Fig. S1 *B* and *C*, and Dataset S1) (20). The clusters

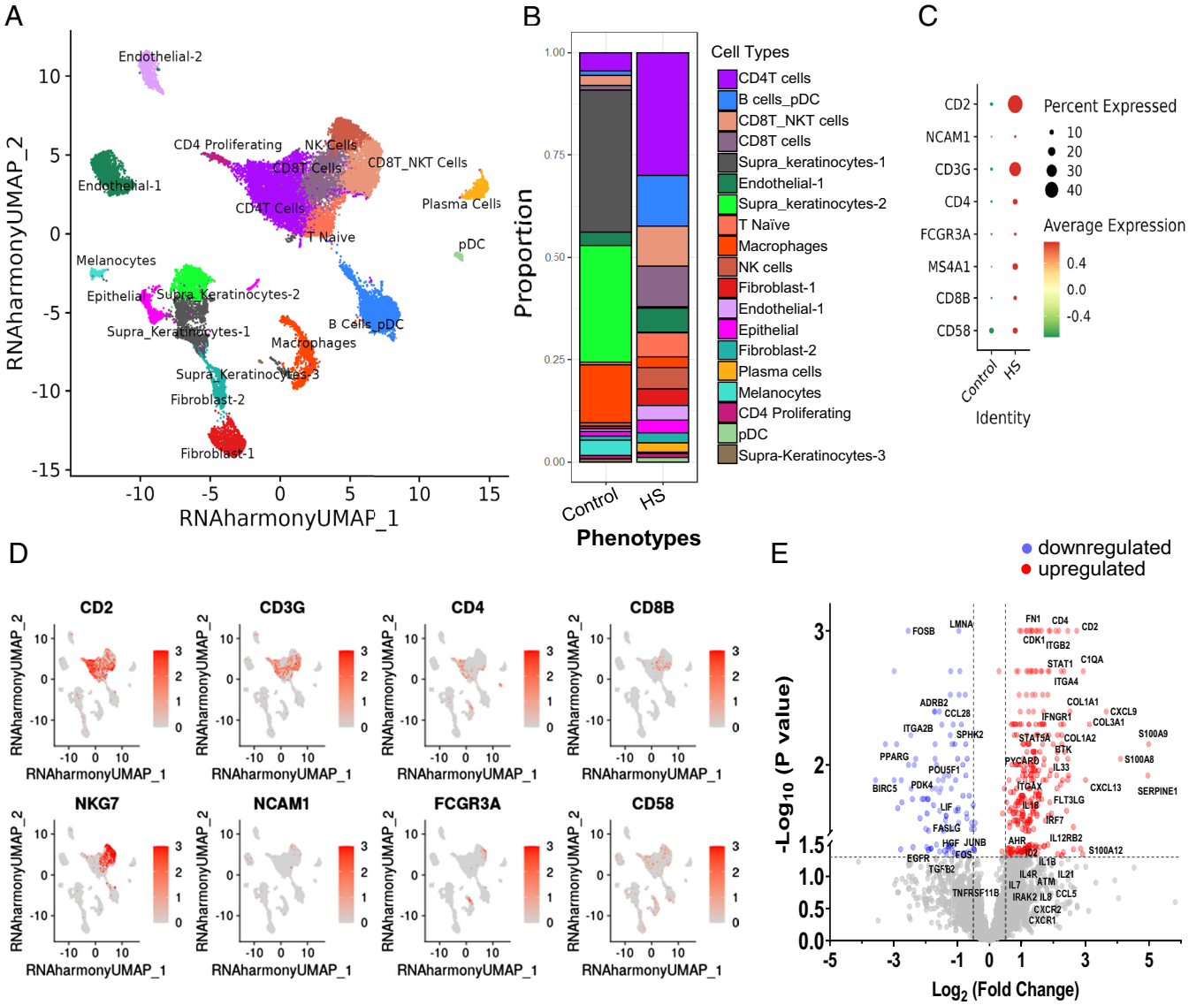


**Fig. 1.** Innate and adaptive immune and nonimmune cells in HS lesion. (*A*) UMAP of cell populations from normal (NS; n = 4) and HS (n = 6) skin. The scRNAseq dataset from 27,442 cells were clustered using Seurat 4.0 and annotated to 19 clusters of immune and nonimmune cell populations. (*B*) Proportion of annotated cell population in each cluster from NS and HS. (*C*) Dot plot depicting normalized expression of genes defining selected cell lineage/function in NS and HS defining genes. (*D*) Feature plot showing cluster localization of selected cell lineage/function-defining genes. (*E*) Volcano plot of differentially expressed genes in HS (n = 8) relative to NS (n = 6 to 8) determined using qPCR arrays.

annotated as CD8T cells and CD8T_NKT cells may contain mixed populations of CD8 T cells and NKT cells, as they share the expression of PRF1, GZMA, GZMB, and NKG7 (*SI Appendix*, Fig. S1*C* and Dataset S1). HS skin had an expanded proportion of CD4 T cells, B cells, CD8 T cells, CD8T_NKT cells, T naïve cells, NK cells, and pDC, compared to normal skin (NS) (Fig. 1*B*, *SI Appendix*, Fig. S1*D*, and Dataset S2). The annotated cell populations in HS from our scRNAseq analysis corroborated the findings reported by others (7, 9, 21, 22). In this study, the cell isolation procedure preferentially dispersed infiltrating immune cell populations, whereas, in our recently published study, we selectively focused on epidermal cell populations representing heterogenous follicular keratinocyte populations in HS (12). Two control samples from public datasets, N3 (GSM6840146) and N4 (GSM6840150), were included in this study that were matched for sex with the HS samples (*SI Appendix*, Fig. S1*D*). H4 (HS_BA) is male (DDX3Y+), and the controls (1, 2) are female (XIST+) (*SI Appendix*, Fig. S1*E*).

The expression levels of several lineage- and function-associated genes were elevated in HS cell populations (Fig. 1*C* and Dataset S1). Among them, *CD2*, a cell surface receptor associated with NKT, NK, and T cell activation (18, 23–25), was highly expressed in a large proportion of immune cell populations in HS skin (Fig.1 *C* and *D* and *SI Appendix*, Fig. S1*F*). Other genes with elevated expression in HS compared to NS included *NCAM1* (CD56), *CD3G* (CD3γ), *CD4*, *MS4A1* (CD20), *CD8B* (CD8β), and *CD58* (LFA3). The monocyte/macrophage cell cluster exhibited a type I and type II IFN inflammatory gene signature, with elevated expression of *STAT1, IFNGR1, IFNGR2, and IRF7* (Dataset S1). Keratinocytes showed high expression of *IL18* (Dataset S1), a cytokine that promotes T, NKT, and NK cell activation and induction of IFN-γ (26–29).

Next, we performed Gene Set Enrichment Analysis to identify the enrichment of hallmarks and canonical pathways in all scRNAseq clusters. The most enriched pathways in CD8T, NK, and CD8T_NKT cell populations were TNFR1/2, TCRA, CTLA4, TCR, T helper, NKT signaling, type I IFN (IFN-α, IFN-β), and type II IFN (IFN-γ) response, PI3K-AKT-mTOR signaling, and unfolded protein response signaling (*SI Appendix*, Fig. S2 and Dataset S1). Additionally, we analyzed bulk transcriptome profile from four independent public datasets: GSE151243, GSE154773, GSE79150, and GSE128637. The analysis showed that 1,614, 2,080, 434, and 359 differentially expressed genes (DEGs) were significantly up-regulated, and 1,354, 1,747, 309, and 300, were significantly downmodulated in HS relative to NS, respectively (*SI Appendix*, Fig. S3*A*). Kyoto Encyclopedia of Genes and Genomes analysis revealed that many DEGs were involved in modulating immune responses, cell activation, stress response, inflammatory responses, IL17 signaling, cell differentiation, lipid metabolism, and ion transport (*SI Appendix*, Fig. S3*A*). Ingenuity Pathway Analysis (IPA) revealed several signaling pathways, including NK cell signaling, that were significantly activated in HS (*SI Appendix*, Fig. S3*B*).

To validate the scRNA-seq data, we performed a TaqMan-based qRT-PCR gene expression array using skin from a cohort of HS patients (n = 8) and controls (n = 6 to 8) (*SI Appendix*, Fig. S4*A*). The qRT-PCR OpenArrays, representing 2,429 gene targets, showed that 170 genes were significantly up-regulated (Log2FC ≥ 1; $P < 0.05$) and 66 genes were significantly down-regulated (Log2FC ≤ −1; $P < 0.05$) in HS compared to controls (Fig. 1*E*, *SI Appendix*, Fig. S4 *B* and *C*, and Dataset S3). *CD2*, *S100* gene family, *BTK* (kinase), *ITGAX* (integrin), *C1QA* (complement protein), several cytokines/chemokines and their receptors, phosphatases, kinases, and other molecules associated with an inflammatory signature were among the genes whose expression was significantly elevated in HS compared to controls (Fig. 1*E*, *SI Appendix*, Fig. S4*C*, and Dataset S3). We recently identified a progenitor keratinocyte population specific to HS, expressing *S100A7/8/9* and *KRT6* gene family members, which is associated with the triggering of IL1, IL10, and complement cascades (12). This population likely contributes to the recruitment of immune cells during disease progression. It remains to be demonstrated whether this epithelial cell population specifically requires interaction with CD2 to elicit IL-1, IL-10, and complement cascade responses.

**Elevated CD2 Expression and Distinct Distribution of CD56 Expressing Innate Lymphoid Cells in HS Lesions.** $CD2^+$ lymphocytes have been implicated in psoriasis and other skin conditions; however, their elevated expression on lymphocyte populations in HS has not been previously reported (30). To validate this finding and to characterize the phenotypes and location of $CD2^{hi}$ T cells and innate lymphocytes in HS lesions, we stained serial sections of skin from HS patients (n = 5 to 7) and controls (n = 3) for CD2 and the phenotypic markers CD3, CD4, CD8, CD56, and CD20, using immunohistochemistry (IHC). In HS, $CD2^+$ lymphocytes were present in large numbers within both the epidermal and hypodermal compartments, scattered as individual cells and/or organized in clusters throughout the entire HS section (Fig. 2*A* and *SI Appendix*, Figs. S5*A* and S6). This feature was consistent across all seven HS sections, with exponentially higher numbers of $CD2^+$ cells in HS than in NS (Fig. 2 *A* violin plot and *Inset* scatter graph). Many of the $CD2^+$ cells coexpressed CD3, indicating that they were T cells and/or NKT cells (Fig. 2*B* and *SI Appendix*, Figs. S5*A* and S6). The $CD3^-CD2^+$ cells likely represent classical NK cells and/or T cells/NKT cells in which CD3 is downmodulated. Greater numbers of $CD4^+$ T cells were present in HS than in NS, with a higher frequency within the hypodermis (Fig. 2*C* and *SI Appendix*, Fig. S4*A*). Although HS contained greater numbers of $CD8\beta^+$ T cells than NS, overall their numbers were very low (<1.0% of $CD2^+$ cells) (Fig. 2*D* and *SI Appendix*, Figs. S5*A* and S6). Remarkably, HS skin contained large numbers of $CD56^+$ (NKT/NK) lymphocytes, which were present adjacent to the hyperproliferative regions of epidermis and in the hypodermal area, including tunnel regions (Fig. 2*E* and *SI Appendix*, Figs. S5*A* and S6). Notably, the numbers of $CD56^+$ cells were significantly greater than those of $CD4^+$ T cells in HS (*SI Appendix*, Fig. S5*B*). The $CD56^+$ cells in HS were mostly $CD56^{bright}CD3^+$ and therefore likely are NKT cells. A few $CD56^{dim}CD3^-$, representing mature NK cells, were also present, which likely reflects the fact that CD56 staining in IHC primarily captures $CD56^{bright}$ cells (Fig. 2*E* and *SI Appendix*, Fig. S5*A*). As previously reported in HS, B cells ($CD20^+$) were present in significantly greater numbers than in NS, but they were primarily localized within tertiary follicle-like structures (Fig. 2*F* and *SI Appendix*, Figs. S5*A* and S6) (31). Notably, there were significantly fewer $CD20^+$ B cells than $CD56^+$ cells in HS (*SI Appendix*, Fig. S5*B*). These lymphocytes were relatively uncommon in NS (Fig. 2 *A*–*F* graphs, *SI Appendix*, Figs. S5*A* and S6).

While NK cell and NKT cell populations have been reported in HS lesions (8, 9, 22), their potential importance in HS pathogenesis was not fully appreciated. NKT cells and NK cells are heterogenous and have functions determined by their spatial distribution in tissues and/or organs (32). We, therefore, employed high-resolution immunofluorescent microscopy to investigate whether the spatial localization of NKT cells and NK cells in HS skin relates to the distinct features of HS pathogenesis. Skin sections were stained with Hematoxylin and Eosin (H&E) to localize features of HS pathology, and the cells within these regions were interrogated for the expression of CD56, CD3, and CD2 by immunofluorescence microscopy. Unlike the morphology of healthy skin, HS skin was characterized by epidermal/hypodermal hyperproliferation and tunnels protruding

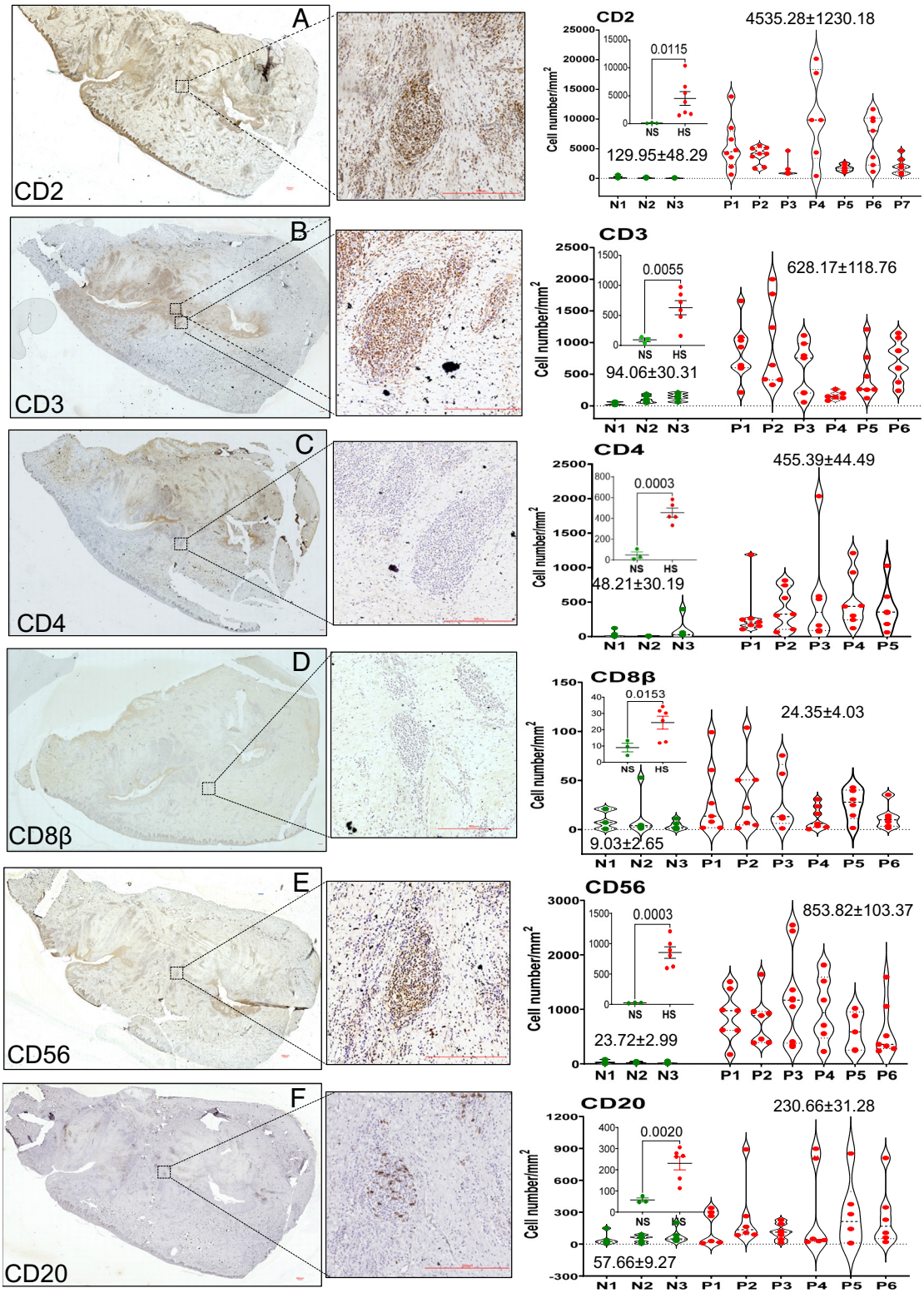


**Fig. 2.** CD2-expressing lymphocytes predominate in HS lesional skin. IHC of serial sections of HS skin stained with antibodies to CD2 (*A*), CD3 (*B*), CD4 (*C*), CD8β (*D*), CD56 (*E*), and CD20 (*F*). The red scale bar in each micrograph is 300 μm. Micrographs for controls are shown in *SI Appendix*, Fig. S6. Additional details in *SI Appendix*.

deep into hypodermal areas with extensive immune cell infiltration (Fig. 3*A* and *SI Appendix*, Fig. S7). As expected, NS contained very few T cells ($CD3^+CD56^-$), NKT ($CD3^+CD56^{bright}$), or classical NK cells ($CD56^{dim}$) (Fig. 3 *A* and *B* and *SI Appendix*, Fig. S8). In contrast, HS skin contained large numbers of $CD2^{bright}$ $CD56^{dim}$ NK cells, $CD3^+CD56^{bright}$ NKT cells, and T cells ($CD3^+CD56^-$). Classical NK cells were predominant in the hyperproliferative epidermis and also present in the hypodermal regions of HS skin (Fig. 3*A* and *SI Appendix*, Fig. S8). NKT cells were enriched adjacent to the tunnels and hair follicular regions (Fig. 3*A* and *SI Appendix*, Fig. S8). Quantification showed that the epidermal and hypodermal regions of HS contain significantly fewer NKT cells than near the tunnel areas (Fig. 3*B*). Consistent with the IHC analysis (Fig. 2), 62 to 71% of $CD2^+$ cells expressed CD56 in HS (*SI Appendix*, Fig. S8 *B* and *C*).

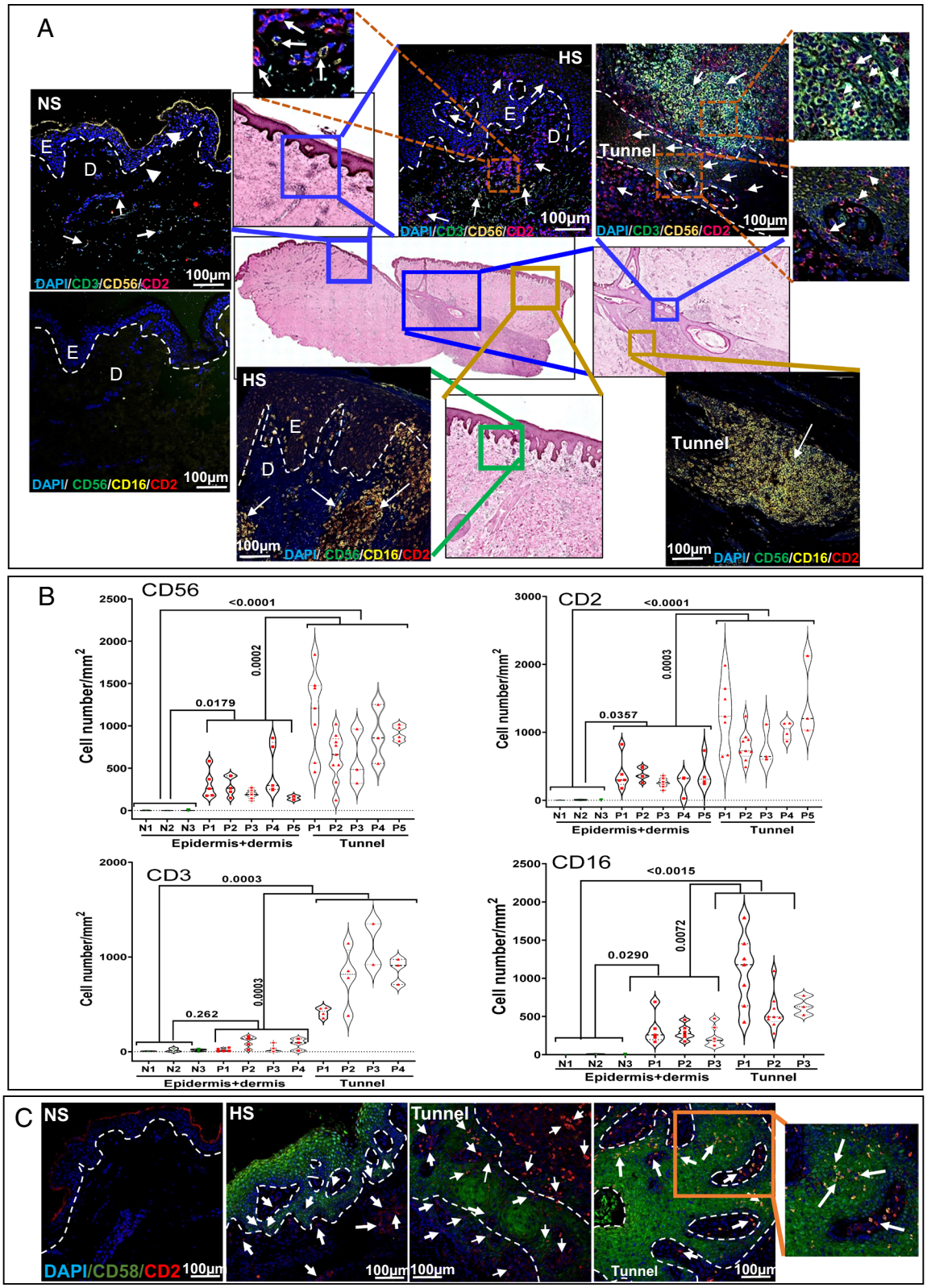


**Fig. 3.** NKT and NK cells are spatially distributed in different regions of HS Skin. (*A*) Immunofluorescence staining for CD3, CD56, and CD2 (*Upper*) and CD2, CD3, and CD16 expressing cells (*Lower*) in NS and HS. Boxes in H&E "stitched" image (*Center*) indicate areas of epidermis/hypodermis or tunnel region in HS as depicted in the immunofluorescent images. (Scale bar, 100 μm.) Arrows point to $CD2^+CD3^+CD56^{bright}$ (NKT cells) and $CD2^+CD3^-CD56^{dim}$ (mature NK cells) in the epidermal/hypodermal and tunnel regions. T cells in the images are $CD2^+CD3^{bright}CD56^-$. Tunnels show spatially separated NK cells from clusters of NKT cells. *E*, epidermis and *D*, dermis/hypodermis (*B*) Violin plots of absolute numbers of $CD56^+$, $CD2^+$, $CD3^+$, and $CD16^+$ cells in epidermal/hypodermal and tunnel regions. 20× resolution confocal images (minimum 20/section) were acquired and numbers of cells determined in each image using CellSense software. Each dot in each violin plot represents numbers of cells in an image; *P* values as indicated are from one-way ANOVA nonparametric analysis. (*C*) Lower panels show $CD2^+$ cells interacting with $CD58^+$ (keratinocyte) cells. Data are representative of HS (n = 3) and NS (n = 3).

To further distinguish between T cells and NK populations, we stained for the expression of CD16 (FcγRIII), an activation receptor known to be induced on NKT and NK cells but not T cells. Most cells in HS were $CD56^{+}CD16^{+}$ establishing that these were indeed NK cell populations (Fig. 3 *A* and *B* and *SI Appendix*, Fig. S9).

CD58 (LFA3) is the cognate ligand for CD2, and their interaction is critical for T cell and NKT/NK cell activation and function (18, 23, 33). We observed that epithelial cells in HS compared to NS, expressed greatly elevated levels of CD58 and interacted with $CD2^{+}$ cells (Fig. 3*C* and *SI Appendix*, Fig. S10*A*). Western blot analysis confirmed that CD58 expression was greatly elevated in HS compared to NS (*SI Appendix*, Fig. S10 *B* and *C*). Our data show that NKT cells and NK cells are major lymphocyte populations within HS skin, and they are spatially localized in distinct regions of HS lesions, to perform independent roles in orchestrating distinct key features of disease pathogenesis.

**B Lineage, CD8 T Cells, and Myeloid Cells in HS Skin.** Our IHC analysis recapitulated the findings reported in other studies, showing that HS skin contained expanded B cell populations organized within tertiary follicle-like structures (Fig. 2*F* and *SI Appendix*, Fig S5*A*) (31), and very few $CD8^{+}$ T cells (Fig. 2*D* and *SI Appendix*, Fig. S5*A*) (6, 7). Immunofluorescence staining for B cells ($CD20^{+}$) and plasma cells ($IgG^{+}$) confirmed the presence of B cell populations in HS (*SI Appendix*, Figs. S11–S15). HS skin contained very few $CD8\beta^{+}$ T cells (*SI Appendix*, Figs. S14 and S16). As previously reported, HS skin also contained an expanded proportion of macrophages ($CD68^{+}$, $EMR1^{+}$) and neutrophils ($MPO^{+}$) (*SI Appendix*, Figs. S11 and S12) (5, 8, 34). $MPO^{+}$ cells were mostly localized in areas of abscess formation in HS skin (*SI Appendix*, Figs. S17 and S18).

**Distinct Populations of NKT and NK Cells in HS Lesions.** The spatial separation of NKT and NK cell populations within HS lesions is intriguing, leading us to predict existence of subpopulations with potentially distinct roles in HS pathogenesis. Perforin (PRF1) and granzymes (GZMA and GZMB) are essential for the cytotoxic activity of NKT and NK on target cells (32, 35). In contrast to NS, HS skin contains a large number of $PRF1^{+}GZMA^{+}GZMB^{+}$ classical NK cells ($CD3^{-}CD56^{dim}$) located adjacent to the epidermis and in the hypodermal region, including areas juxtaposed to tunnels (Fig. 4 *A*–*C* and *SI Appendix*, Figs. S19–S21). The tunnels in HS are enriched in $TUNEL^{+}$ cells (detailed below), suggesting a mechanistic role for NK cells in tissue remodeling and tunnel formation through the promotion of follicular epithelial cell death. While NKT cells and NK cells exhibit similar expression levels of GZMA, the expression of PRF1 and GZMB is distinctly lower in NKT cells compared to NK cells. A significant proportion of the $CD56^{dim}CD2^{hi}$ $GZMB^{hi}$ cells also express CD11b (MAC1/ITGAM/CR3) (Fig. 4*C* and *SI Appendix*, Fig. S21). The regions adjacent to tunnels also contain $CD2^{+}CD3^{+}CD56^{-}$ cells, most likely $CD4^{+}$ T cells (*SI Appendix*, Figs. S12 and S19). $CD8\beta^{+}$ cells do not express CD56 and were rarely observed in tunnel regions (*SI Appendix*, Fig. S16). Overall, the differential expression of these proteins in NK populations from different regions of HS skin reveals a heterogeneity in NKT and NK cell populations. Next, we performed subclustering analysis by extracting the scRNAseq data of the lymphocyte cluster (Fig. 4*D*). From reclustering and unsupervised annotation, we identified 11 lymphocyte populations: three naïve T, two CD4, and one each of CD8, NKT, Treg, NK, MAIT, and γδ-T cell populations (Fig. 4*D*). Gene expression of NKT cells (*NCAM1*/CD56, *FCGR3A*/CD16, *PRF1*, *IL32*, *GZMK*, *CCL5*, *CD3D*) indicates that they are likely adaptive NK cells (Fig. 4*E* and Dataset S1) (36). The NK cells exhibit gene expression (*NCAM1, XCL1, XCL2, PRF1, GZMB*) characteristic of cytokine-induced memory-like NK cells (36). *TRDC* (TCR delta constant gene) was expressed in γδ T cells, NK, and NKT cells (Fig. 4*E*). While its expression in γδ T cells is expected, its presence in NK and NKT seems unusual. However, *TRDC* transcripts, but not protein, have been reported in NK and NKT cells by others, though the significance of these data remains unknown (37). Additionally, we performed the pseudotime analysis of the immune subclusters to identify the overall trajectory of transcriptome changes. We found two distinct pseudotime patterns: one for CD4 T cells trajectory and another for NKT, NK, and MAIT cells, along the pseudotemporal space starting with the T Naïve-1 cluster (Fig. 4*F*). We also observed pseudotime DEGs in module 2, which expresses NK and NKT cell markers (Fig. 4 *G* and *H* and Dataset S4). Furthermore, the gene ontology of module 2 genes demonstrates that the enriched pathways are myeloid cell differentiation, regulation of cell–cell adhesion, positive regulation of lymphocyte activation, hormone metabolic process, leukocyte mediated immunity, leukocyte proliferation, lymphocyte-mediated immunity, wound healing, tissue remodeling, and additional skin behavior and immune system-related pathways (Dataset S4). Taken together, these findings indicate that the NKT cells and NK cells have distinct roles in HS pathogenesis.

**Functionally Distinct Roles for NK and NKT in HS Pathogenesis.** The mechanism underlying hair follicle remodeling leading to tunnel formation may involve mechanotransduction associated with keratinocyte cell death and immune cells' infiltration. Therefore, the presence of NK cell populations adjacent to $TUNEL^{+}$ cells in the hypodermis, especially along the peripheral regions and deep tunnels, could indicate their role in tunnel formation (Fig. 5*A* and *SI Appendix*, Figs. S22 and S23). $CD3^{+}CD56^{bright}$ NKT cells are distinctly localized separately from NK ($CD56^{dim}$) cells and predominantly within the outer region of tunnels (Fig. 3*A*). $CD2^{+}$ lymphocytes located in the peripheral regions of the tunnels express PAR2, a protein that counteracts apoptosis (38). CD2 was not expressed on apoptotic cells, suggesting that NKT cells and NK cells may up-regulate PAR2 as a mechanism to prolong the survival of interacting cells needed for driving the complex pathogenic events in HS. Consistently, it has been demonstrated that HS tunnels are immunologically active and manifest proinflammatory association with a high expression of inflammatory gene profiles (22, 39).

A key feature of HS disease progression is tissue remodeling and associated fibrosis (7, 22). Several genes associated with this process—*COL1A1, COL1A2, COL3A1, FN1, MMP9, SFRP2,* and *CXCL12*—are significantly increased in HS (Dataset S3) (40). In HS, we found NK cell populations ($CD2^{+}$) interacting with αSMA+ cells (activated fibroblasts) (41) in the hypodermis (direct interaction observed in z-projection images—Fig. 5*B*, *SI Appendix*, Figs. S24 and S25, and Movie S1). Similar interaction has been previously observed in the participation of NKT subpopulations in liver fibrosis (42). Very few B cells ($CD19^{+}$) associate with $\alpha SMA^{+}$ cells, indicating that they are not directly involved in fibrosis, unlike in systemic sclerosis (Fig. 5*B*) (43). Hippo signaling likely drives fibrosis in HS, as earlier proposed (22); however, the role of NK cells is likely significant. It remains to be demonstrated whether Hippos signaling plays a role in this NKT/NK pathophysiology of fibrosis.

**NKT and NK Cell Recruitment and Activation: Cytokine and Chemokine Expression in HS Tissue.** We assayed the expression of cytokines, chemokines, and growth factors in skin tissue from both controls and HS patients. The cytokines IFN-γ, IL-1β,

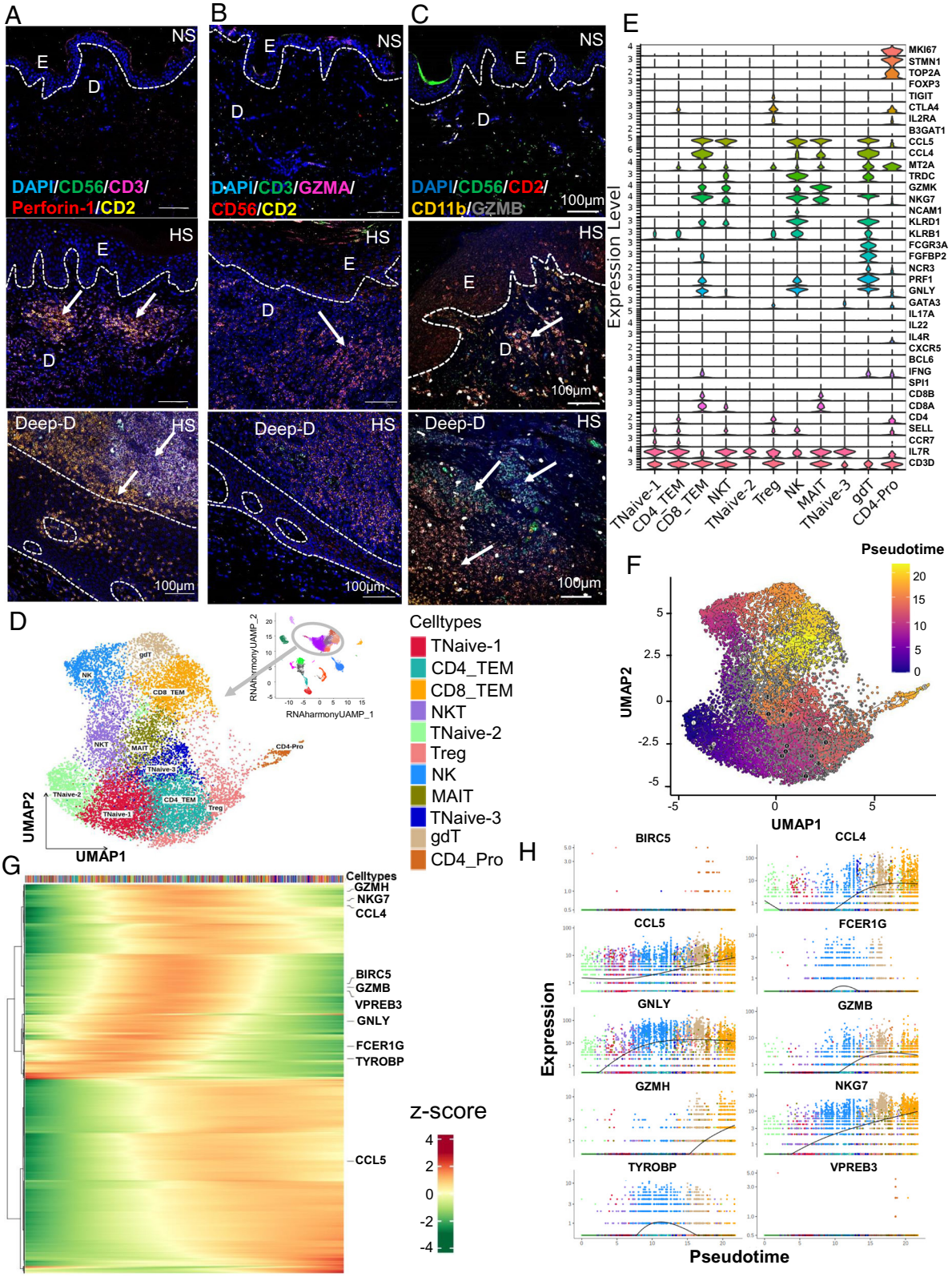


**Fig. 4.** Heterogenous populations of NKT and NK cells in HS. Perforin (*A*), Granzyme A (*B*), and CD11b and granzyme B expression (*C*), in NKT ($CD3^+CD2^+CD56^{bright}$) and NK ($CD2^+CD56^{dim}$) cells in NS (*Top* row) and HS (*Middle* and *Lower*), bar is 100 μm. (*A*) $Perforin^+$ NKT and NK cells are prominent in the epidermis/dermis (E/D), tunnel region (deep-D). (*B*) Granzyme A is expressed in NKT and NK cells. (*C*) Some NK cells ($CD56^{dim}$), but not NKT cells in HS express CD11b and expression of granzyme B is primarily in NK cells. (*D*) UMAP of 11 immune cell subclusters extracted from scRNA-seq analysis shown in Fig. 1*A*. (*E*) Expression of NKT, NK, MAIT, CD4, CD8 associated phenotypic markers and functional genes in each subcluster. (*F*) Pseudotime trajectory analysis of subclusters with T Naïve-2 as the starting point. Dark purple is initial time and orange is the farthest time in pseudotime scale. (*G*) The pseudotime trajectory involved gene expression heatmap. (*H*) The expression pattern of top 10 trajectory-determining genes.

IL-4, IL-6, IL-8, IL-12p70, IL-15, IL-17a, IL-18, IL-22, IL-27, and TNF-α were significantly elevated in HS compared to controls (*SI Appendix*, Figs. S26 and S27). IFN-γ and TNF-α are major effector cytokines expressed by NK cells and NKT cells. Additionally, the elevated levels of IL-15 and IL-18 in HS, likely produced by keratinocytes (Dataset S1) (44), would induce

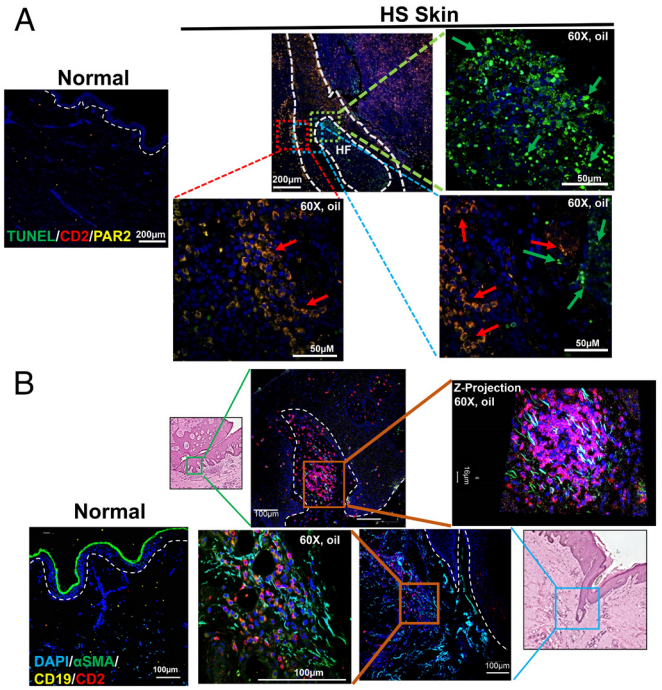


**Fig. 5.** NKT and NK cells are associated with pathological processes in HS. (*A*) Localization of NKT/NK cell populations ($CD2^+$) in regions of ongoing apoptosis ($TUNEL^+$). Immunofluorescence staining shows PAR2 expressing $CD2^+$ lymphoid cells adjacent to cells undergoing apoptosis ($TUNEL^+$) within sinus tracts/modified hair follicles of HS. (*B*) NKT cells interacting with fibroblasts in areas of ongoing fibrosis in HS. Z-projected micrographs show CD2-expressing NKT cells physically interacting with αSMA expressing fibroblasts within modified hair follicles. Very few $CD19^+$ cells are observed in regions of ongoing fibrosis.

CD2 as well as enhance the cytotoxic activity of NKT cells and NK cells (28, 29, 45). IL-12 was also elevated in HS; signaling from this cytokine promotes survival and expansion of NK cells, independent of IL-15 and IL-18 (46). The levels of IFN-α, IL-1α, IL-2, IL-7, IL-9, IL-31, and TNF-β were similar in HS and control skin (*SI Appendix*, Fig. S27*B*). Although IL-13 was elevated in HS skin, its levels were not statistically different from those in NS. The chemokines and growth factors CCL2 (MCP1), CCL3 (MIP-1α), CCL4, (MIP-1β), CCL5 [Regulated on Activation, Normal T Expressed and Secreted (RANTES)], CCL11 (Eotaxin), CXCL1 (GROα/KC), CXCL9, CXCL10 (IP10), CXCL12 (SDF-1), NGFβ, EGF, FGF-2, HGF, and PIGF1 were elevated in HS skin compared to controls (*SI Appendix*, Figs. S26 and S27*A*). IL-8 (CXCL8), the IL-1β induced cytokine/chemokine produced by keratinocytes with a crucial role in recruiting neutrophils, was significantly enhanced in HS (47). Although BDNF, PDGF-BB, PIGF-1, VEGF, and SCF, were elevated in HS, their levels were not significantly different from NS (*SI Appendix*, Fig. S27).

**CD2 Blockade in HS Skin Attenuates the Production of Cytokines, Chemokines, and Pathogenic Gene Signature When Tested in Organotypic Cultures.** Based on our findings that CD2 is up-regulated on all NK cell populations and T cells, and that its cognate receptor, CD58, is enhanced on skin epidermal and resident cells, we hypothesized that the CD2:CD58 interaction is central to HS immunopathogenic processes. Therefore, its blockade is likely to reverse the expression of disease-associated inflammatory and tissue remodeling cytokines, chemokines, and growth factors, as well as the underlying gene expression profile. We tested this hypothesis using our recently developed HS skin organotypic cultures (48) with two experimental paradigms: CD2 blockade by itself and following challenge with Lipopolysaccharide (LPS). Bacterial colonization is a key feature of HS, and LPS (TLR2 and TLR4 agonist) is a major bacterial cell wall component that acts on keratinocytes, fibroblasts, and innate immune cells to activate the inflammasome pathway, inducing the expression of inflammatory cytokines and chemokines, including IL-15 and IL-18 (49). We found that HS skin cultures (n = 3) treated with anti-CD2 monoclonal antibody (mAb), compared to those treated with IgG (control), showed significantly reduced levels of cytokines, chemokines, and growth factors associated with inflammatory pathogenesis, including those responsible for NK cell activation and/or maturation (Fig. 6 *A* and *D* and *SI Appendix*, Fig. S27*A*). IFN-γ (essential NK effector), IL-15, IL-18, BDNF (NK and T cell activation and/or differentiation), RANTES, IP-10, and SDF-1α (chemokines associated with NK and T cell recruitment) were among those reduced by anti-CD2 treatment. We also examined changes in gene expression using inflammatory gene expression arrays and found that genes associated with NK and T cell activation and effector function were attenuated following treatment with anti-CD2 (Fig. 6*B*, *SI Appendix*, Fig. S28*B*, and Dataset S5). The explants treated with anti-CD2 had fragile tissue architecture with significantly enhanced numbers of apoptotic cells (TUNEL+) as well as a disrupted epidermal tissue (Fig. 6*C* and *SI Appendix*, Figs. S28*E* and S29). Additionally, immunofluorescence staining identified CD2-expressing clumps of enucleated cells which could probably be an outcome of nuclear condensation (*SI Appendix*, Fig. S29*B*). The mechanism of $CD2^+$ cell death is consistent with fratricide described previously (19). In absence of an appropriate and acceptable animal model of HS pathogenesis, our organotypic disease skin culture system is key to providing mechanistic insights into therapeutic agents.

Anti-CD2 treatment also reversed the pathogenic cytokine production and gene expression signature in LPS-treated HS skin cultures (Fig. 6 *D* and *E*, *SI Appendix*, Fig. S28 *C* and *D*, and Dataset S6). Specifically, we found that anti-CD2 treatment led to the decrease in LPS-induced cytokines/chemokines/growth factors and downregulation of gene signatures associated with TLR2/4 signaling. These include IL-10, IL-15, IL-18, CXCL1, BDNF, FGF-2, *IRAK2*, *MAPK*, *IL4R*, and others (Fig. 6 *D* and *E* and *SI Appendix*, Fig. S28*C*). Intracellular signals in NK cells following engagement of CD2 by CD58 (LFA-3) induces expression and secretion of IFN-γ and TNF-α, which reciprocally act on keratinocytes to enhance expression of IL-15 and IL-18 (30, 50). Thus, a blockade of CD2:CD58 interaction by anti-CD2 would interrupt this intercellular amplification cascade and predictably decrease IL-15, IL-18, and other keratinocyte-produced cytokines/chemokines.

To identify the main signaling pathways in HS that are down-regulated by CD2 blockade, we performed IPA using data from qRT-PCR HS vs. NS array (Fig. 1*F*) and anti-CD2 vs. IgG array (Fig. 6*B*). IPA revealed that several signaling pathways up-regulated in HS were down-regulated by anti-CD2 treatment (*SI Appendix*, Fig. S30 *A* and *B*). For example, the four highest-ranking upstream regulatory drivers in HS, LPS-signal, *TNF*, *IFNG*, and *TGFB1*, were down modulated by anti-CD2 treatment (*SI Appendix*, Fig. S31 *A* and *B*). Bacterial colonization in tunnels/abscesses may be the source of LPS signals. Conceptually, these data reveal a central role for CD2 signaling/costimulation in modulating the immunopathogenesis of HS.

**MicroRNA (miR) Landscape Confers NK Cell Differentiation and/or Activation.** To interrogate the transcriptional regulome, we performed a global microRNA (miRs) analysis with tissue from nine HS and six healthy controls. We identified 36 differentially expressed miRs in HS compared to NS, of which 27 miRs were significantly up-regulated and nine miRs were significantly down-regulated

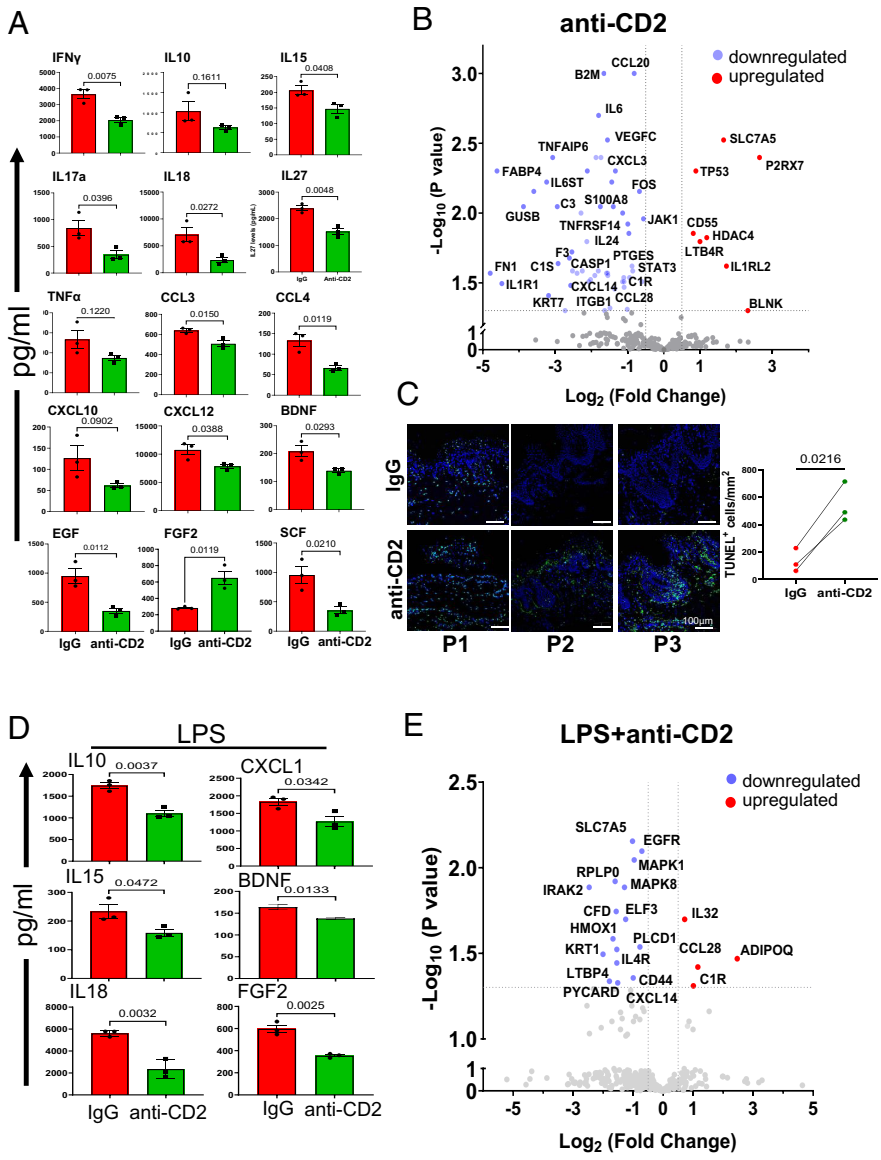


**Fig. 6.** CD2 blockade in HS organotypic skin culture attenuates proinflammatory and fibrotic signaling pathways. Skin tissue from HS skin (n = 3) was cultured in transwell plates with anti-CD2 or control IgG antibodies (*A*, *B*, and *C*) or LPS with anti-CD2 or control IgG (*D* and *E*) for 72 h. Cytokine, chemokine, and growth factor levels in supernates of explant cultures (*A* and *D*). Student's *t* test of IgG vs. anti-CD2 (*$P \le 0.05$, **$P \le 0.01$). Volcano plots (*B* and *E*) depicting changes in the expression of inflammatory gene signatures in anti-CD2 treated explants. Disruption of CD2:CD5 interaction in HS skin explant cultures down-modulates the expression of several inflammatory and fibrotic marker genes at the transcriptional level. TUNEL staining of explant tissue cultured with IgG or anti-CD2 from three HS patients (*C*). Graph is quantification of apoptotic cells (TUNEL+) in explants of each HS patient and change in numbers between IgG and anti-CD2 treatment. Unpaired Student's *t* test with Welch's correction was used to identify statistical significance ($P \le 0.05$) between IgG vs. anti-CD2.

($\log_2$FC > |1|, *P*-value < 0.05) (*SI Appendix*, Figs. S32 and S33*A* and Dataset S7). Eleven of the 36 differentially expressed miRs (miR-150, miR-27a5p, miR-155, miR-21, miR-142-3P, miR-126, miR-29b, Let7, and miR-200a) play a role in NK cell differentiation and/or function (*SI Appendix*, Fig. S33 *B* and *C*) (51, 52). Particularly, miRNAs (mir-150, miR-155, miR-21, miR-200a, miR29a-5p, Let7) that enhance NKT or NK cell differentiation and/or activity were elevated in expression, while miRNAs that regulate NK activity (miR-27a-5p, miR-181a-2-3p) were decreased in expression in HS relative to NS (*SI Appendix*, Figs. S32*A* and S33*C*) (52).

Epigenetic modifications to chromosomal DNA regulate the expression of miRNAs, which are altered by metabolic changes and other mechanisms (53). Metformin, which targets Adenosine Monophosphate activated protein Kinase (AMPK) signals, showed some benefit in reducing HS pathogenesis (54). The engagement of CD2 by CD58 modulates AMPK, a kinase involved in different aspects of metabolism (55). We therefore assessed whether the antagonism of CD2:CD58 interaction had any effect on miRNA expression pattern. We found significantly elevated expression of miR-27a-5p, miR-99b-5p, and mir-30c in explant cultures treated with anti-CD2 mAb (*SI Appendix*, Fig. S32*B* and Dataset S8). MiR-27a-5p, which targets the expression of perforin and granzyme B in NK cells (52), is down modulated in HS compared to NS (*SI Appendix*, Fig. S32*A*). miR-99b-5p regulates the expression of cathepsin L, an enzyme required for the cleavage and activation of perforin and granzyme B within NK cell lytic vesicles, and miR-30c is associated with modulating NK cell effector function (56, 57). Overall, the miR profile in HS reflects increased NK/NKT cell effector function, which is modulated by anti-CD2-treatment in ex vivo HS skin explants.

## Discussion

Multiple adaptive and innate immune cell populations and their molecular mediators are associated with HS pathogenesis; however, therapeutic interventions based on these findings have not been highly impressive (1). In this study, we demonstrate that the expression of CD2, a T cell and NK cell activation/adhesion molecule, is highly elevated in HS lesional skin. This finding holds substantial translational potential, which we explored in HS skin explant cultures using antagonist anti-CD2 mAb to interfere with the CD2:CD58 interaction. We report that phenotypically heterogenous populations of NKT and NK cell populations are widespread in HS lesions and are distributed in distinct spatial

locations. Thus, NKT and NK cells may contribute to various features of HS pathogenesis. We have identified an immune cell–keratinocyte–fibroblast interactome in HS, with CD2:CD58 being a central component. We also reveal a miRNA regulome that facilitates the activation, functional regulation, and/or differentiation of NKT and NK cell populations.

Our scRNAseq dataset revealed that CD2 was a highly expressed gene in all non-B lineage lymphocyte populations in HS patients and IHC analysis established that $CD2^{hi}$ cells were the predominant lymphocyte populations distributed across multiple areas of HS lesional skin. The majority of these $CD2^{+}$ cells expressed CD56, a phenotype consistent with NKT and/or NK cells (32). It is known that cognate CD2:CD58 engagement in NK cells and T cells promotes the formation of an immunological synapse, cytoskeletal reorganization, and is essential for activation, differentiation, and immune response (18, 24, 30, 33). The elevated levels of IFN-γ levels in HS observed in this study, and by others (7) reflect cognate CD2–CD58 interaction, recruitment of CD16 (activating Fc-γ receptor) to the immunological synapse, and integration of intracellular signals leading to enhanced NK cell cytolytic activity (7, 18, 33, 55). We recently reported CD2 as one of the hub regulators in skin diseases including HS, atopic dermatitis (AD), and rosacea (58). Notably, CD8 T cells ($CD8\alpha^{+}CD8\beta^{+}$) were underrepresented in HS.

The distinct spatial distribution of NKT and NK cells supports the notion that these populations have discrete roles in HS pathogenesis. Using CD56 (NCAM1) expression in the absence of CD3 as a defining marker for NK cells, we report that $CD56^{dim}$ NK cells were localized in the hypodermal regions and around the periphery of tunnels/sinus tracts. These NK cells expressed high levels of perforin, granzyme A, and granzyme B, presenting a highly cytolytic cell population (32). Consistently we found $TUNEL^{+}$ epithelial cells in this region. In contrast, $CD56^{bright}$ NKT ($CD3^{+}$) cells expressed high levels of granzyme A but low levels of granzyme B and perforin, resulting in a less cytolytic functional phenotype. Importantly, these NKT cells were spatially distributed in locations distinct from those of cytolytic NK cells and physically interacted with fibroblasts in areas of progressive fibrosis. All NKT cells, NK cells, and $CD4^{+}$ T cells expressed CD2, and they interacted with cells expressing elevated levels of CD58 (LFA3). The majority of the $CD58^{hi}$ cells were keratinocytes, based on morphology and spatial localization, as reported previously (12). In corroboration, the elevated expression of CD58 in keratinocytes is also evident in scRNAseq data. Since CD58 is widely expressed in macrophages, neutrophils, fibroblasts, and mesenchymal stem cells, it is tempting to speculate that their interaction with subsets of NK cell populations contributes to HS pathogenesis. Overall, our observations reveal that the NKT/NK cell populations in HS are heterogenous and differ in their contributions to disease pathogenesis. Similarly, these innate lymphoid cells showed distinct spatial distribution and functional interactions with keratinocyte and fibroblasts, providing support for their role in pathogenesis.

HS skin contained elevated levels of CXCL10 (IP10), CXCL12 (SDF-1), CCL1 (MCP1), CCL2 (MIP1-α), and CCL5 (RANTES) chemokines. These chemokines are produced by keratinocytes when activated by the inflammasome pathway (34, 59). Additionally, we observed increased levels of IL-12, IL-15, and IL-18, the cytokines that individually or in combination activate NK cells and enhance their proliferation and functional activity (29, 46). These cytokines are produced by keratinocytes following activation of TLR-inflammasome pathway. In HS, we found elevated levels of IL-8 (CXCL8), which can be produced by $CD4^{+}$ T cells, NK cells, keratinocytes, and macrophages, and recruits neutrophils to sites of inflammation (60).

In skin, NK cell populations are present in increased numbers in psoriasis; however, their role in driving the disease is unclear (9, 61). While skin-resident NKT cells and local expression of CXCL12 may drive AD, blood NK cells recruited to the skin may play an important role in regulating type 2 inflammation and attenuating AD (62). Skin infections recruit NK cells and provide an important effector function to some secondary bacterial and viral infections (63). In HS, infections may provide the initial signals to recruit NKT and NK cells, which later establish as skin-resident cells to drive pathogenesis. In support of this, our earlier data showed a decrease in NK cell populations in the peripheral blood of HS patients (64).

Blockade of CD2:CD58 (LFA3) engagement is immunoregulatory and crucial for suppressing immune responses, particularly in the transplantation setting (19, 23, 65). Siplizumab is a high-affinity humanized anti-CD2 IgG1 mAb currently undergoing clinical trials broadly within transplantation and autoimmunity (19, 66). Although immune modulatory treatments can enhance susceptibility to infections, particularly viral reactivations, to date, there is no evidence for high levels of such complications. Alefacept, a CD58-IgG fusion protein that targets CD2:CD58 interaction, has been generally safe and efficacious in psoriasis (67). However, alefacept was withdrawn from the market largely because of challenges with the manufacturing process rather than due to safety concerns. Considering the possibility that CD2:CD58 cognate interaction has a critical function in HS pathogenesis, we tested the therapeutic potential of disrupting CD2:CD58 interaction using anti-CD2 mAb in the organotypic culture system that we developed (48). Treatment of HS explants in culture with anti-CD2 mAb led to a dramatic reduction in the expression of multiple genes associated with immune and nonimmune cell activation, inflammation, and proliferation. Among these genes are *CD55* and *TP53* (p53), whose protein products are known to have important functions in enhancing immune tolerance, suppression of NK cell function, and/or attenuating inflammation (68). In addition to reducing the secretion of cytokines, chemokines, and growth factors, disruption of CD2:CD58 interaction by anti-CD2 induces apoptosis in epidermal and hypodermal areas of the tissue. Interestingly, the treated tissue was highly fragile, and we observed sloughing of certain regions including the epidermal region. Thus, our data suggest a critical role for CD2:CD58 engagement in the inflammatory pathogenesis of HS. We observed an identical outcome for anti-CD2 mAb treatment of HS tissues stimulated with LPS. Since TLR2 and/or TLR4 (LPS receptor) are not expressed on NKT and NK cells (69), the inhibitory effect of anti-CD2 treatment on the expression of cytokines (IL-15, IL-18, IL-12) produced by keratinocytes, fibroblasts, and innate immune cells likely represents an essential requirement of CD2:CD58 interaction in this process. Siplizumab, which blocks CD2:CD58 interaction, has demonstrated effect on promoting tolerance by enhancing regulatory T cell populations and controlling memory T cells (65). Thus, blocking CD2:CD58 interaction may be more efficacious for HS treatment as it also has an inhibitory effect on the production of cytokines such as TNF-α and IL-17a (this study) that are currently considered as HS therapeutic targets.

The spatial localization and cellular interactions between NKT cells and fibroblasts support a prominent role for NKT cells in fibrosis. Furthermore, NKT cells have been shown to promote fibrosis in the liver and lung previously (70). A recent study by Van Straalen et al. (22) reported that fibrosis in HS is promoted by multiple cell populations (T cells, B cells, and myeloid cells);

however, NKT cells were not included among them. Thus, our study expands this list. However, the difference between the studies may reflect patient populations, while our study mostly represents female African American individuals with late-stage II and III HS requiring surgical excision of the involved skin. Additionally, expression of *NCAM1* (CD56) is not readily detected in scRNAseq datasets of mixed cell populations from tissues, necessitating direct assay for $CD56^+$ cells by immunohistology to identify NK cell populations, as presented in our study.

Epigenetic data from the miRNA regulome underscore the importance of the pathobiology of NKT and NK cells (51, 52). MiR-155, induced by IL-12 and IL-18, enhances the expression of IFN-γ in NK cells (71) and is highly elevated in HS. Some MiRNAs, such as miR-150, promote differentiation to mature NK cells while repressing generation of NKT cells, thereby opposing biological effects in NKT and NK cells (51). Previous studies have shown that miR-27a-5p and miR-181a attenuate NKT or NK cell differentiation (72). In HS, these miRs were expressed at lower levels than in controls. In sum, these data support a regulatory interactome of NKT/NK cell expansion/activation for their interaction with keratinocytes and fibroblasts in HS pathogenesis.

In summary, we identified a striking heterogeneity in NKT and NK cell populations in HS skin, with each subpopulation playing distinct roles in disease pathogenesis. We observed elevated expression of CD2 and its interaction with CD58 on keratinocytes and fibroblasts with a critical in cytokine/chemokine production. Furthermore, we found that disrupting the CD2:CD58 cognate interaction attenuates immunopathogenesis of HS. Our data reveal a previously undescribed translational cellular and molecular underpinning of HS pathogenesis.

## Materials and Methods

**Human Subjects.** Institutional Review Board of the University of Alabama at Birmingham approved the collection and use of surgically discarded, deidentified skin samples from HS patients and controls (IRB-300005214). All samples were surgically discarded skin tissues that were stripped from all identifying information prior to use in the study (*SI Appendix*, Table S1). Freshly resected skin tissues were used for scRNA-seq and ex vivo skin explant cultures.

This study is mostly from African American female patients who were refractory to all previous treatments. However, CD2 blockade may be beneficial to all HS patients irrespective of race and gender.

**Data, Materials, and Software Availability.** The processed data of this study have been deposited in the GEO database under accession code GSE274880 (73). All other data are included in the article and/or supporting information.

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Author affiliations: [a]Center for Epigenomics and Translational Research in Inflammatory Skin Diseases, University of Alabama at Birmingham, Birmingham, AL 35294; [b]Department of Dermatology at the University of Alabama at Birmingham, Birmingham, AL 35294; [c]Department of Biology at the University of Alabama at Birmingham, Birmingham, AL 35294; [d]Department of Medicine (Division of Pulmonary, Allergy and Critical Care Medicine) at the University of Alabama at Birmingham, Birmingham, AL 35294; [e]Department of Research and Development, ITB-MED AB, Stockholm 113 68, Sweden; [f]Endocrine and Sarcoma Surgery Unit, Department of Molecular Medicine and Surgery, and Division of Transplantation Surgery, Karolinska Institute, Stockholm 171 76, Sweden; [g]Department of Clinical Science, Intervention and Technology, Karolinska Institute, Stockholm 171 77, Sweden; [h]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala 751 85, Sweden; and [i]Department of Genetics & Biochemistry at Clemson University, Clemson, SC 29634

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