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CORRESPONDENCE

Mass cytometry uncovers a distinct peripheral immune profile and upregulated CD38 expression in patients with hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease with a worldwide prevalence of 0.3–4.0%, wherein abscesses and dermal sinus tracts form in intertriginous skin [1]. HS remains poorly treated due to a lack of knowledge regarding its immunopathogenesis [2]. Recent study findings have implicated multiple immune pathways and effector molecules underlying HS pathophysiology [2]. Increased circulating inflammatory proteins, autoantibodies, and extracellular matrix degradation products highlight that systemic inflammation is a feature of the disease [2–4]. Circulating immune cells serve as a good proxy for disease severity and risk in other inflammatory conditions [5]; however, an unbiased characterization of the changes in the peripheral immunome of patients with HS has not been reported and is desperately needed. Cytometry by time-of-flight (CyTOF) enables highly parametric immune profiling, which led to the discovery of disease-associated immunome signatures and cellular effectors in other inflammatory diseases [5].

In this study, we characterized the immune profile of fresh whole-blood samples from a cohort of 18 patients with HS and 11 healthy controls (HCs) (Supplementary Fig. S1A and Supplementary Table S1) using a standardized 30-marker whole-blood immunome profiling panel (Supplementary Table S2) that can identify 33 immune cell populations (Supplementary Table S3). Bivariate gating and marker expression showed good separation of major immune clusters in tSNEs (Supplementary Fig. S1B, C).

Neutrophils are thought to play a key role in HS pathogenesis [2]. We found comparable frequencies of circulating neutrophils between HS and HC samples (Supplementary Fig. S2A). Given that neutrophils constitute a large fraction of total blood leukocytes (40–60%), we excluded them and performed deep immune profiling of $CD45^+$ immune cell subsets. Furthermore, no granulocyte frequencies were significantly different between HS and HC samples (Supplementary Fig. S2A).

Twenty-five $CD45^+$ subpopulations were identified based on their surface marker expression (Fig. 1A). Hierarchical clustering (Supplementary Fig. S3A) and principal component analysis (Supplementary Fig. S3B) of $CD45^+$ immune subsets showed moderate separation between HS and HC cell populations based on $CD45^+$ subset frequency.

Patients with HS had decreased frequencies of total natural killer (NK) cells, while early or late NK cell subsets were not significantly different between patients with HS and HCs (Fig. 1B). The frequencies of total $CD4^+$ T cells and Th1 and Th2 cells were unchanged between patients with HS and HCs, but HS patients had an increased frequency of Th17 cells compared to HCs (Fig. 1C). No significant changes in other memory, effector or innate T-cell populations were observed (Supplementary Fig. S2B–D). To further validate the increase in Th17 cells, we stimulated PBMCs from an additional cohort of patients (Supplementary Table S4) with PMA/ionomycin for 4 h and analyzed the frequency of IL-17-producing T cells by flow cytometry. We found that the frequency of IL-17-producing $CD4^+$ T cells (Th17) was significantly increased in patients with HS compared to HCs (Fig. 1D), while the frequency of IL-17-producing $CD8^+$ T cells (Tc17) was not different between patients with HS and HCs (Supplementary Fig. S2E). Total B-cell and B-cell subset frequencies were not significantly altered between patients with HS and HCs (Supplementary Fig. S2F).

Dendritic cells (DCs) were significantly reduced, but neither myeloid DC nor plasmacytoid DC (pDC) subsets showed a significant reduction (Fig. 1E). Although the total frequency of monocytes (Monos) was unchanged, classical ($CD14^+CD16^-$; C.Monos) and nonclassical ($CD14^-CD16^+$; NC.Monos) monocytes were significantly decreased, whereas intermediate monocytes ($CD14^+CD16^+$; I.Monos) were increased (Fig. 1F).

Machine-learning algorithms can be applied to CyTOF data to perform unbiased clustering and statistical comparisons between conditions [6]. We employed cluster identification, characterization, and regression (CITRUS) analysis to confirm our findings from bivariate gating and to uncover novel disease-associated immune cell signatures in HS. Through CITRUS analysis, we found 11

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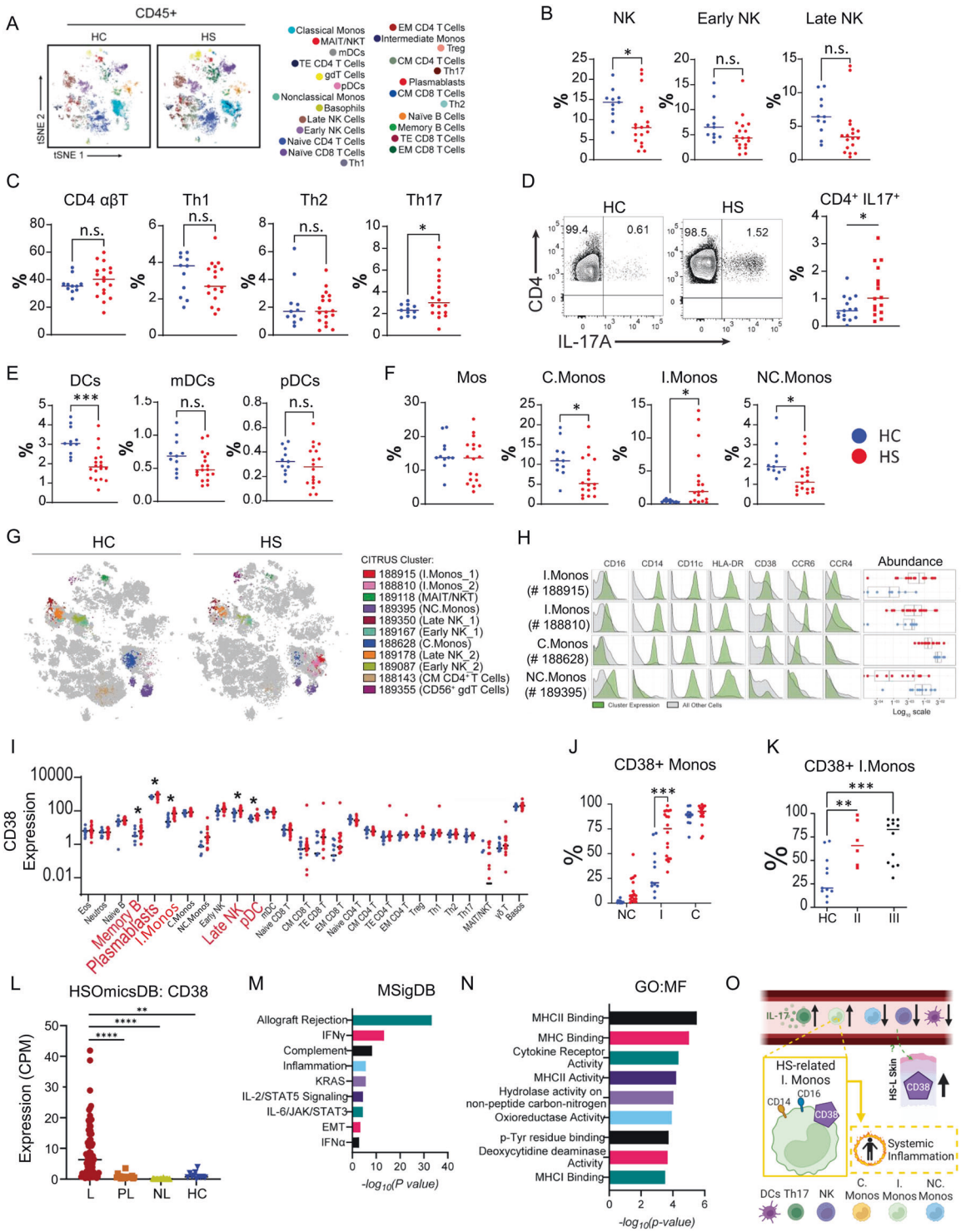

Fig. 1 **A** Representative tSNE plots from patients pregated on CD45$^+$ cells. **B** Frequency of total NK cells and NK cell subsets as the percentage of CD45$^+$ cells. **C** Frequency of total CD4αβ T cells, Th1, Th2, and Th17 cells as the percentage of CD45$^+$ cells. **D** Flow cytometry analysis of Th17 cells in HS samples following 4 h of stimulation with PMA/Ionomycin as the percentage of TCRβ$^+$ cells. **E** Frequency of total DCs and DC subsets as the percentage of CD45$^+$ cells. **F** Frequency of total Monos and Mono subsets as the percentage of CD45$^+$ cells. **G** Localization of significant CITRUS clusters on tSNE plots. **H** Marker expression for Mono CITRUS clusters and their abundance in HS and HC samples. **I** Expression of CD38 in various immune cell subsets. **J** Frequency of CD38$^+$ Monos as a percentage of their respective subset. **K** Frequency of CD38$^+$ I.Monos as a percentage of total I.Monos stratified by Hurley stage. **L** CD38 expression in tissue types from HS-OmicsDB. **M** Top 9 MSigDB gene enrichments for genes significantly correlated with CD38 from HS-OmicsDB. **N** Top 9 GO:MF gene enrichments for genes significantly correlated with CD38 from HS-OmicsDB. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$. **O** Graphical summary of CyTOF-based immune profiling of patients with HS. Statistics for two-way comparison are the result of two-sided Student's *t* tests. Statistics for three- or four-way comparisons are the results of Bonferroni-corrected ANOVA. HC healthy control, HS hidradenitis suppurativa, NK natural killer, Th T-helper, IL interleukin, DC dendritic cell, Monos monocytes, C.Monos classic monocytes, I.Monos intermediate monocytes, NC.Monos nonclassic monocytes

differentially abundant clusters (Supplementary Fig. S4A). We mapped cells back onto tSNE plots (Fig. 1G) and analyzed the expression of lineage markers to identify CITRUS cluster identities. CITRUS recapitulated the dysregulation in monos identified by bivariate gating analysis (Fig. 1H). Other significant CITRUS results are shown in Supplementary Fig. S4B. We next assessed the expression of markers that may indicate a dysregulated function in Monos and found that I.Monos expressed high levels of CD38 (Fig. 1H). CD38, mainly expressed in immune cells, is a molecule that can act as an enzyme with NAD-depleting and intracellular signaling activity or as a receptor with adhesive functions, contributing to autoimmune inflammation [7]. CD38 is induced under inflammatory conditions and has been shown to be required for optimal response to toll-like receptor stimulation, tissue trafficking, antigen presentation, and phagocytosis [7]. C.Monos normally express high levels of CD38 [8]. I.Monos, on the other hand, do not express high levels of CD38 unless stimulated, highlighting that I.Monos in HS are in an activated state [8].

We further confirmed that I.Monos had upregulated CD38 expression in patients with HS compared to HCs using bivariate gating (Fig. 1I). Memory B cells, plasmablasts, late NK cells, and pDCs also exhibited significantly increased CD38 expression in patients with HS, but T cells did not (Fig. 1I).

CD38 is not expressed on NC.Monos, which we leveraged to define the background level of CD38 in Mono subsets (Supplementary Fig. S5) [8]. As expected, C.Monos from HCs were almost entirely $CD38^+$ (Fig. 1J). We found that the frequency of $CD38^+$ I.Monos was dramatically increased in patients with HS (Fig. 1J). Moreover, the frequency of $CD38^+$ I.Monos showed significant elevation in patients with moderate (Hurley stage II) and severe (Hurley stage III) HS (Fig. 1K).

Utilizing HS-OmicsDB (https://shiny.hfhs.org/hsomicsdb/), a database of publicly available bulk RNA sequencing data from HS studies, we sought to determine whether CD38 was upregulated in HS tissues, given that CD38-expressing immune cells might migrate to skin lesions. As expected, CD38 was significantly upregulated in HS lesions (L) compared to HS-perilesional (PL), HS-nonlesional (NL), and HC skin (Fig. 1L). We next performed gene enrichment analyses on genes that had a significant positive correlation with CD38 (FDR < 0.05, $r > 0.5$) (Supplementary Table S5) using MSigDB and gene ontology: molecular function (GO:MF). These genes were enriched for inflammatory pathways, especially those associated with myeloid-mediated inflammation, including allograft rejection, IFNγ signaling, complement activity, IL6 signaling, MHCII activity and cytokine receptor activity (Fig. 1M, N).

Overall, we present the first systematic profiling of the peripheral immunome of patients with HS, which highlights previously unappreciated changes in circulating immune cell subsets in patients with HS. Compared to HCs, we observed a decrease in the frequency of total NK cells, total DCs, C.Monos, and NC.Monos and an increase in Th17 and I.Monos in patients with HS. Most importantly, we found that CD38 was upregulated in specific immune subsets, which likely migrate to the skin to contribute to HS development (Fig. 1O).

Previous studies have shown that Th17 cells were not elevated in the circulation of patients with HS [9]. One explanation for this discrepancy is that our cohort included more patients with severe HS than those of previous studies. A clinical trial showed that IL-17 blockade reduces cutaneous and serum inflammation in HS, highlighting the importance of IL-17 as a key mediator of disease [10]. Although we showed that patients with HS, overall, had an increase in Th17 cells, we noticed that not all patients exhibited increased Th17 cell frequency, which may be one reason why some patients respond better to anti-IL-17 treatment than others. Thus, the frequency of Th17 cells may be worth exploring as a potential predictive marker of the anti-IL-17 response in future translational studies.

Our immune profiling led us to discover a novel disease-associated marker and potential drug target for HS, CD38. CD38 expression on late NK cells, memory B cells, plasmablasts, pDCs and I.Monos was elevated.

CD38 enhances the extravasation of immune cells via interaction with CD31 and CD38 on endothelial cells [7]. The increased expression of CD38 on late NK cells and decreased frequency on NK cells may indicate increased trafficking to the skin, which is supported by increased NK cells in skin lesions (unpublished data from M. Athar). The role of NK cells in HS is poorly studied. NK cells induce antibody-dependent cytotoxicity (ADCC) by binding antibody-coated cells via CD16 and releasing cytolytic molecules [11]. CD38 enhances NK-cell ADCC by interacting with CD16 [12]. The identification of autoantibodies in HS skin suggests an interesting mechanism by which CD38 may enhance autoantibody-mediated inflammation through NK cells in HS lesions.

I.Monos produce high levels of TNFα and IL-1β [8]. In HS, I.Monos express high levels of CD38, indicating an activated status [7]. Adalimumab (anti-TNFα) is the only FDA-approved therapy for patients with HS, despite ~30% of patients failing to respond [13]. Targeting a single cytokine may prove insufficient to treat HS because of multiple inflammatory pathways that are dysregulated in the skin and serum of patients with HS. Targeting multiple dysregulated immune cells may prove more successful. CD38 was reported to be upregulated on peripheral immune cells from patients with systemic lupus erythematous (SLE) [14]. Strikingly, a recent clinical trial showed that anti-CD38 antibodies are effective for treating SLE refractory to anti-TNF [15]. Given the high rate of failure of anti-TNFα therapy and increased CD38 expression on immune cells and in skin lesions of HS, anti-CD38 immunotherapy could be transformative for the management of patients with HS.

Since our study was limited by only assaying whole-blood samples from patients with HS, future studies are needed to determine the cellular effectors in HS lesions with increased CD38 expression and identify the roles of CD38 in these specific immune populations. In conclusion, our study uncovered the distinct peripheral immune profile in HS, and the findings suggest that CD38 may serve as a new therapeutic target for HS. Trials with FDA-approved anti-CD38 immunotherapies may be worth pursuing for HS.

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AUTHOR CONTRIBUTIONS

PD participated in patient recruitment and experimental design; performed research; collected, analyzed, and interpreted data; performed statistical analysis; and drafted and revised the manuscript. IH participated in patient recruitment and experimental design; performed research; and revised the manuscript. CY participated in patient recruitment and data collection. IL analyzed and performed statistical analysis for high-dimensional imaging data and revised the manuscript. JT, KS, and NK collected data and revised the manuscript. AM, RH, GV, and AH participated in patient recruitment. JV participated in data collection, analysis and interpretation and manuscript revisions. SD, MA, WL, HL, and DO participated in manuscript revisions. LZ and Q-SM supervised all aspects of the work; acquired funding and resources; and participated in data analysis and interpretation, manuscript drafting, and manuscript revisions.

COMPETING INTERESTS

IH has served as an investigator (grant to institution) for Pfizer Inc, Bayer, Lenicura, Incyte, Estee Lauder, L'Oreal, Unigen, Avita, Arcutis Biotherapeutics, and Ferndale Laboratories, Inc.; as an Advisory Board member for AbbVie; and as a consultant to Galderma Laboratories, LP, Incyte, Pfizer, UCB, Boehringer Ingelheim, Beiersdorf, and Clarify Medical. RH is a principal investigator for Pfizer, Incyte, Arcutis and the Immune Tolerance Network. WL has received research grant funding from AbbVie, Amgen, Janssen, Leo, Novartis, Pfizer, Regeneron, and TRex Bio. HL is an investigator for Incyte, L'Oreal, Pfizer, and PCORI; has served as a consultant for Pierre Fabre, ISDIN, Ferndale, La Roche-Posay, Cantabria, and Beiersdorf; and has participated as a speaker in general educational sessions for La Roche-Posay and Cantabria Labs. All other authors have no conflicts of interest to report.

ETHICS APPROVAL

These procedures and protocols were approved by the Henry Ford Health Institutional Review Board (IRB# 12826).

ADDITIONAL INFORMATION

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