

Comparison of Keratinocyte and Myeloid Transcriptional Phenotypes and Shared Candidate Features Between Acute Irritant and Allergic Contact Dermatitis Based on Single-Cell RNA Sequencing

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Abstract | This study compared the transcriptional phenotypes of keratinocytes (KCs) and the myeloid compartment after exposure in acute irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD), and identified candidate shared features. This study is a secondary analysis of public single-cell RNA sequencing data. We analyzed GEO dataset GSE267929, focusing on healthy individuals and squaric acid dibutyl ester (SADBE)-sensitized volunteers; in the latter group, sodium lauryl sulfate (SLS) induced ICD and SADBE induced ACD. Samples included vehicle, non-lesional, and healthy control groups. After quality control (including doublet removal and ambient-RNA correction) and annotation with Seurat v5, we performed pseudobulk differential expression, subject-blocked enrichment, program scoring, CellChat, and Milo analyses. A total of 43,303 cells were assigned to 14 populations/states, and 1,727 cells were recovered from ICD samples. KC stress/injury markers were consistently increased in ICD donors (an ICD-predominant phenotype rather than a shared feature): stress keratins were upregulated in all 5 paired donors, and MIF was upregulated in all 6 evaluable donors; KRT6A, MIF, and S100A7 had nominal $P < 0.05$ (nominal P refers to the raw P value without multiple-comparison correction and is exploratory). Here, MIF is produced by KCs and does not originate solely from macrophages. The IL-8/neutrophil program was upregulated and enriched in both KCs and myeloid cells on Day 2 after patch application in ICD and ACD, but did not reach significance on Day 4 in ACD (Day 2 refers to 2 days after patch application; Day 4 refers to patch application for 2 days, removal, and observation for another 2 days); no formal time-interaction test was performed, so this cannot be described as a decrease over time. CXCL8 and MMP12 were identified as hypothesis-generating shared candidate molecules for further validation. In this study, “shared” operationally means that both diseases show upregulation relative to non-lesional skin, rather than formal equivalence or interaction testing. The molecular initiation, the temporal order of the KC → myeloid axis, and common downstream effects remain undetermined, and the results cannot be extrapolated to unsensitized populations or chronic dermatitis.

Keywords | Irritant contact dermatitis; Allergic contact dermatitis; Single-cell RNA sequencing; Keratinocytes; Myeloid transcriptional phenotype

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1 Introduction

Contact dermatitis is one of the most common diseases in dermatology and occupational health [1]. It includes irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). ACD is a T cell-mediated type IV delayed hypersensitivity reaction that occurs after contact with a hapten and requires prior sensitization. ICD is caused by direct irritant damage, does not require sensitization, and is dose-dependent [2,3]. ICD accounts for more than 80% of occupational skin diseases. Long-term hand contact with detergents and solvents can cause persistent irritant hand eczema [1,3,4]. Clinical management still relies mainly on irritant avoidance, barrier repair, and topical corticosteroids for symptom control [5].

The skin contains an immune microenvironment composed of keratinocytes (KCs), Langerhans cells, dendritic cells (DCs), macrophages, T cells, and other cells. KCs serve as both a barrier and the first line of innate immunity. Chemical irritation can cause barrier disruption, expression of stress keratins (KRT6A, KRT16, KRT17), heat shock and oxidative stress responses, and release of alarmins (also termed danger signals), that is, damage-associated molecular patterns (DAMPs), which recruit and activate myeloid cells [6,7]. On this basis, a working model of ICD has been proposed in which chemical injury to KCs initiates and then amplifies myeloid inflammation. This differs from ACD initiation, which involves hapten sensitization, antigen presentation, and specific T-cell activation [8].

Previous transcriptomic studies of skin tissue have compared inflammation induced by chemical allergens and irritants. These studies found distinguishable molecular features (disease-type differences) and overlap in epidermal barrier, substance metabolism, and cell proliferation, suggesting both differences and commonalities [7]. The present study reanalyzed the original single-cell dataset underlying that work. In that work, ACD and ICD were induced in parallel at different body sites in the same subjects, and the composition and state atlas of cells recovered by suction blisters were systematically characterized. Specific differences between the two reaction types in KCs and immune cells were revealed, and some findings were orthogonally validated using blister fluid proteomics [9]. A single-cell study of nickel allergy in humans described dynamic changes in different cell populations during the reaction and provided a

reference for identifying the roles of various cells in human contact allergic reactions [10]. In our previous work, we used the same dataset to perform a preliminary characterization of the immune microenvironment of ACD lesions, identified 14 cell types, and observed Th1-type immune activation and KC stress. However, we had not directly compared ICD and ACD within the same subjects [11]. This study is a secondary reanalysis of GSE267929; reference [11] is a preliminary characterization of the immune microenvironment based on the same dataset, and the incremental contributions of this study are the compartment-resolved analysis of KCs/myeloid cells, ACD time stratification, and donor-level MIF analysis, which do not duplicate that reference.

Although ICD is caused by direct irritant injury and ACD is driven by specific immunity after hapten sensitization, their initiation steps differ. In the early phase of reaction formation, damaged KCs may initiate stress/injury programs and recruit myeloid cells through chemokines such as IL-8. To test this hypothesis, we focused on the KC and broad myeloid compartments, which have not been specifically characterized in public data. We stratified ACD into Day 2 and Day 4 (Day 4: patch removed after 2 days and observed for another 2 days). Accordingly, this study proposed two mutually independent hypotheses: (i) ICD manifests a stronger KC injury/stress phenotype because of direct irritant injury; and (ii) ICD and ACD share an IL-8/neutrophil-related transcriptional direction in KCs and myeloid cells in the early phase of reaction formation (Day 2). In this study, “shared” operationally means that both diseases show upregulation relative to non-lesional skin, rather than formal equivalence or interaction testing. We integrated within-subject pairing, subject-blocked enrichment, paired Milo, donor-level MIF testing, and ligand/transcription factor footprints to characterize post-exposure transcriptional phenotypes and identify candidate shared features. The objectives of this study were: (i) to compare the KC and myeloid transcriptional phenotypes of ICD and ACD within the same subjects; (ii) to identify shared candidate features using prespecified criteria; and (iii) to generate hypotheses for future validation rather than to provide diagnostic biomarkers. This study is an exploratory secondary analysis and was not designed to test causality or diagnostic accuracy. This study does not provide diagnostic performance data and therefore does

not aim to directly achieve early discrimination between ICD and ACD.

2 Materials and Methods

2.1 Data source, study subjects, and ethics statement

Data were obtained from the Gene Expression Omnibus (GEO; accession GSE267929) [9]. This dataset is an experimental contact dermatitis model in healthy adult volunteers. Volunteers were first sensitized with SADBE. Then, SADBE, SLS (to induce ICD), and acetone vehicle patches were applied to different body sites in the same volunteers, and non-lesional skin areas were also retained. Non-lesional skin from 12 independent healthy controls was also included. Samples from each group were collected by suction blister, captured using inDrop, and sequenced on Illumina NextSeq 550 (BioProject PRJNA1113649).

Day 2 refers to sampling 2 days after SADBE patch application. Day 4 refers to patch application for 2 days, removal, and sampling after another 2 days. SLS and vehicle samples were collected approximately 2 days after patch application. ACD Day 2 and Day 4 samples came from the same cohort of SADBE-sensitized volunteers (with highly overlapping donor sets), but they were independent suction blister samples collected at different time points and different sites, rather than repeated sampling from the same site; therefore, Day 2 and Day 4 were modeled separately and were not treated as paired time points. The specific concentrations of SLS and SADBE and the anatomical sites of patch application were not reported on the GEO public page or in the sample metadata. A total of 21 volunteers were included: 9 who completed sensitization and challenge (P1–P9) and 12 independent healthy controls. There were 9 men and 12 women, all healthy adults. The sequencing data for P9 included only vehicle, Day 2/Day 4 SADBE, and non-lesional samples, but no SLS sample. GEO did not specify whether SLS was not applied or whether no qualified cells were obtained. Therefore, only P1–P8 were included in the ICD analysis.

For each analysis, minimum cell numbers were prespecified according to compartment recovery: global pseudobulk ≥ 40 ; KC and myeloid pseudobulk differential expression and enrichment ≥ 20 ; transcription factor/pathway footprints ≥ 30 ; KC injury

program ≥ 20 ; myeloid activation profile ≥ 15 . Analyses that did not meet these thresholds were excluded. The original study obtained ethical approval and informed consent from all participants (see reference [9]); this study used only de-identified public data and involved no new human procedures, so no additional ethical approval was required for this secondary analysis. The authors declare no conflicts of interest.

2.2 Data preprocessing and quality control

Raw matrices were merged using Seurat v5 [12,13] and quality-controlled based on 200–6,000 detected genes per cell and mitochondrial transcript proportion $< 20\%$. A total of 21,492 genes were retained, and 45,942 cells passed the initial quality control (from 50,820 cells before quality control). Doublets were then detected within each of the 21 capture batches with scDblFinder (v1.26.9^[14]); the doublet rate was automatically estimated by artificial doublet simulation, `set.seed(42)`; samples with fewer than 100 cells were skipped, and ambient RNA was corrected with DecontX (celda v1.28.0^[15]); after clustering by cell type, a Bayesian contamination model was used to estimate and remove contamination from singlet cells. Clustering by cell type before correction was performed to use cell-type-specific expression distributions to estimate contamination; this correction preceded downstream differential analysis to avoid circular reasoning introduced by clustering before correction. This removed 2,639 putative doublets (5.74%) and yielded 43,303 cells for the main analysis. Because GEO provides processed matrices without empty-droplet profiles, empty-droplet-based methods such as SoupX and CellBender could not be applied. We performed LogNormalize, principal component analysis (PCA), uniform manifold approximation and projection (UMAP) dimensionality reduction, and shared nearest neighbor (SNN) clustering (resolution = 0.5). We then carried out global annotation, followed by reclustering of KCs and myeloid cells.

Cell recovery was highly unbalanced among groups (Table 1): only 1,727 cells were recovered from 8 ICD donors, with a median of 183.5 cells per donor (range 11–627), whereas 26,010 cells were recovered from the 12 independent healthy controls. Batch and condition were also partially confounded: each of the 12 independent healthy controls occupied a separate capture batch, whereas the 9 subjects were included in

their respective batches in parallel with vehicle, non-lesional, ICD, and ACD samples. Within-subject pairing can offset batch effects within a batch, but healthy controls and reaction samples belonged to different batches. Therefore, healthy controls were used only as a background reference and were not included in the main inference. Because all samples used the same library preparation and sequencing platform and all downstream inferences used subjects as blocks, no further cross-sample batch integration was performed.

Except for per-sample cell numbers (Table 1), comparisons used subjects as the unit, and the number of pairable donors was reported. Random seeds were fixed with `set.seed` in all analysis modules. For KC and myeloid reclustering, the first 20 principal components were calculated, and two-dimensional UMAP was generated from the first 15 PCs for visualization. Myeloid resolution was 0.4, and global resolution was 0.5, yielding 15 clusters.

2.3 Cell type annotation and subclustering

The 15 original clusters obtained at global resolution = 0.5 were each annotated with a single label. Two clusters (clusters 7 and 11) were both melanocytes and were merged into one reported label, so the 15 clusters were grouped into 14 labels. Global annotation integrated lineage markers and the literature and yielded 14 labels: KCs (basal, differentiated, activated, stressed, periadnexal, other), myeloid cells (Langerhans/DC, macrophages, M2-like, other DC), melanocytes, and T cells. There were 6 global KC labels (including periadnexal KCs; no proliferative state was present). KC reclustering yielded another 6 states (basal, differentiated, activated, stressed, proliferative, other). Periadnexal KCs were merged into the other state, and a proliferative state was added. Therefore, Figures 1 and 2A use the global labels (including periadnexal KCs), while Figures 2D–F use the reclustering nomenclature (including proliferative KCs). Figure 2E shows the combined proportion of the activated, stressed, and proliferative (activation-related) states among all KCs. Figure 2F shows the composition of each KC state. The denominators and merging methods differ between Figures 2E and 2F, so absolute values cannot be directly compared.

Proportions were calculated as within-sample proportions. Paired Wilcoxon signed-rank tests were used for lesions and non-lesional skin from the same

subject. Unpaired Wilcoxon rank-sum tests were used for comparisons involving independent healthy controls. All tests were two-sided, with $P < 0.05$ as the significance threshold. When fewer than 6 pairable donors were available, the two-sided signed-rank test could not mathematically reach $P < 0.05$. When all 5 pairs were in the same direction, the exact two-sided P value was 0.0625. The software normal approximation (exact = FALSE, with continuity correction) was approximately 0.059. Neither was considered significant. Such comparisons only report donor-level direction consistency and P values. Donor-level paired differences in single-gene pseudobulk logCPM were also tested using paired t tests (two-sided, paired $n \geq 3$), and mean differences with 95% confidence intervals (CIs) were reported. As a prespecified sensitivity analysis, the same paired differences were tested using the exact two-sided paired Wilcoxon signed-rank test. When $n < 3$, only descriptive results were reported. KRT6A and KRT16 (stress keratins) and S100A7 (alarmin module) were prespecified as a limited set of candidate genes. Only nominal P values were reported, and no further multiple-comparison correction was performed for single genes. Module- and pathway-level enrichment was still determined using BH-corrected FDR.

2.4 Differential expression and pathway enrichment analysis

Differential expression was performed using sample-level pseudobulk combined with edgeR [16]. Raw counts were aggregated by subject and experimental condition. Merged samples with at least 40 broad-lineage cells and at least 20 KCs and at least 20 myeloid cells were included. After filtering low-expression genes and TMM normalization, a design matrix was constructed with subjects as blocks (design formula: $\sim$ subject + condition) to estimate the effect of each condition relative to non-lesional skin within the same subject, thereby adjusting for individual baseline differences; ACD Day 2 and Day 4 were included as separate condition levels. The Benjamini-Hochberg (BH) method was used to control the false discovery rate (FDR), with thresholds of $FDR < 0.05$ and $|\log_2 FC| > 1$. The number of donors actually included in each analysis and the number of ICD–non-lesional pairs are detailed in Table 1. In the myeloid pseudobulk analysis, only 1 pair was available (P1).

Pathway enrichment was performed using the fry function of limma^[17] (FRY, fast rotation, a self-contained gene-set test based on rotation testing) under subject blocking. ACD samples were modeled separately for Day 2 and Day 4. In addition to MSigDB Hallmark gene sets^[18], 10 custom gene sets were used (IL-1, TNF/NF- κ B, inflammasome, IL-8/neutrophil, IFN, Th2, antigen presentation, barrier, oxidative/NRF2, and matrix remodeling). Hallmark enrichment and gene annotation were supplemented with gene set enrichment analysis (GSEA)^[19] and clusterProfiler^[20]. The IL-8/neutrophil program included CXCL8, CXCL1, CXCL2, CXCL3, CXCL5, S100A8, and S100A9. The direction of ICD and ACD was assessed by Spearman correlation of log2FC relative to the shared non-lesional reference. Because the reference was shared, genes were not independent, and the broad myeloid compartment was a mixed subpopulation, this analysis was used only for descriptive aggregate direction. Ligand-receptor and CellChat^[21] analyses are detailed in Section 2.5.

We also performed direct paired comparisons between ICD and ACD within the same subjects. Subjects with both ICD and ACD samples were selected, and samples had to meet the following cell-number criteria: broad-lineage and KC pseudosamples ≥ 40 cells and myeloid ≥ 20 cells. These samples were used to compare gene expression differences between ACD and ICD. The numbers of paired samples available for analysis were 6 pairs for broad-lineage cells, 6 pairs for KCs, and 4 pairs for myeloid cells.

2.5 Cell communication analysis

For each reaction group, cells were first downsampled by cell type, and CellChat objects were constructed using the cell-level normalized expression matrix. CellChat was used to model each reaction group separately, and probabilities were calculated using the truncated-mean mass-action model. Both ligand- and receptor-expressing groups had at least 5 cells. CellChat group summaries provide only a deterministic metric and no donor-level estimate or uncertainty. Probability changes are group-level descriptions and not paired increments. In sparse inDrop data, a probability of 0 only indicates that the group did not meet the cell-number threshold or that the interaction was not detected in the database; it does not indicate pathway absence. For MIF, donor-level

pseudobulk logCPM expression of KC MIF (6 pairable donors) and myeloid CD74 and CD44 (7 donors each) was tested using two-sided paired Wilcoxon signed-rank tests.

2.6 Program scoring

Program scoring used AddModuleScore to calculate KC stress keratin, alarmin, and immediate early programs, as well as myeloid M1, M2, DC maturation, Langerhans cell (LC) migration, and phagolysosomal programs. The M2/MRC1 repair score was composed of repair-related genes such as the M2-like macrophage markers MRC1 (CD206) and CD163 and was used to describe a repair-like phenotype, rather than to make a formal M2 polarization determination. Each module used its own controls, and absolute scores were not compared across modules. Some genes served as both state markers and module genes. Therefore, only state composition and marker expression changes were described. Candidate features were judged by combining shared upregulation, enrichment, and donor direction. KRT6A, KRT16, S100A7, HSPA1A, and other genes were used for both state annotation and injury modules, resulting in marker overlap. Results are therefore expressed as state composition and marker expression changes, and program hierarchy was not determined on this basis. No composite score was set for candidate features. Candidate features were discussed independently from multiple perspectives, including shared upregulation statistics, subject-blocked enrichment, and donor direction consistency.

Shared candidate features were defined a priori by simultaneously meeting the following quantitative criteria: (i) upregulation in both ICD and ACD Day 2, either at the pseudobulk level (BH-FDR < 0.05 and $|\log_2\text{FC}| > 1$) or at the subject-blocked pathway level (fry enrichment FDR < 0.05 in both reactions); (ii) a consistent direction in the majority of evaluable donors (at least 4 of 5 donors for module scores); and (iii) an adequate detection rate (expressed in at least 10% of cells in the relevant compartment for ligand-receptor and candidate-gene analyses). Genes that met the criteria in only one reaction, were expressed in fewer than 10% of cells, or showed an inconsistent donor direction were not advanced as shared candidates. CXCL8 and MMP12 were retained because they recurred across pseudobulk differential expression and fry enrichment. Although CXCL8 did not show a fully

consistent donor direction and was sparse in the inDrop data (expressed in only a few cells), it recurred across myeloid pseudobulk differential expression and fry enrichment and, together with MMP12, constituted the IL-8/neutrophil direction; it was therefore explicitly demoted to a “secondary hypothesis-generating candidate molecule” rather than a validated biomarker and was retained together with MMP12.

2.7 Neighborhood-level differential abundance testing

Neighborhood-level differential abundance analysis was performed using miloR [22], based only on ICD cells and paired autologous non-lesional cells to construct a KNN neighborhood graph. Before downsampling, there were 3,990 cells. Downsampling was performed when a single sample had more than 600 cells, and 3,331 cells were retained after downsampling. The KNN graph was constructed based on the first 20 principal components from PCA, followed by neighborhood sampling and filtering, yielding 290 valid neighborhoods. Paired testing was performed with subjects as block variables (7 paired donors). Multiple testing was corrected using spatial false discovery rate (SpatialFDR), with $\text{SpatialFDR} < 0.05$ as the main threshold and $\text{SpatialFDR} < 0.10$ as the exploratory threshold. The KNN construction in this analysis was based on PCA dimensionality reduction and was independent of the two-dimensional UMAP used for visualization in Section 2.2. This method only assesses relative changes in cell neighborhoods and cannot reflect absolute cell infiltration. The comparison between ICD and independent healthy controls was unpaired and was confounded by donor, sampling site, and sensitization status. Those results are for reference only.

2.8 Prior-network inference of transcription factor and pathway activity

PROGENy [23] was used to infer the activity of 14 pathways. DoRothEA [24] (VIPER, via the decoupleR framework [25]) was used to infer transcription factor activity. NicheNet [26] was used to infer upstream ligands using myeloid differentially expressed genes in ICD relative to autologous non-lesional skin as the response set. NicheNet analysis based on pseudobulk sample-level results identified 9 candidate ligands: ligands expressed in >10% of KCs in ICD and their receptors expressed in >10% of myeloid cells. The

predictive performance of candidate ligands was close to random, and no ligand with stable predictive ability was identified; therefore, the NicheNet results are explicitly reported as a negative exploratory result and were not included in the main conclusions. This analysis was performed only for the myeloid ICD vs. non-lesional comparison. All three methods depend on prior databases and filtering thresholds. PROGENy/DoRothEA did not undergo donor-level statistical testing, so these analyses are exploratory analyses based on prior networks and are not cross-validation or direct evidence of pathway functional activation.

2.9 Confirmatory versus exploratory analyses

To avoid placing analyses with different levels of evidence on the same footing, this study prespecified a distinction between confirmatory and exploratory analyses. Analyses using subjects as the unit and including multiple-comparison correction or exact paired testing (pseudobulk differential expression, fry enrichment, paired composition, and donor-level MIF testing) were considered primary confirmatory evidence; analyses that did not provide donor-level uncertainty (CellChat, PROGENy/DoRothEA, NicheNet, Milo, and module scoring) were all exploratory results. Among these, NicheNet performed close to random and is reported as a negative exploratory result, and the myeloid pseudobulk result containing only one pair of donors is presented descriptively only.

3 Results

3.1 Single-cell transcriptomic atlas of ICD/ACD

After quality control, 43,303 cells were annotated into 14 cell populations/states, including KCs (basal, differentiated, activated, stressed, periadnexal/other), myeloid cells (Langerhans/DC, macrophages, M2-like, other DC), melanocytes, and CD4/CD8 T cells. No fibroblasts, endothelial cells, B cells, or NK cells were detected, consistent with the fact that suction blisters mainly recover the epidermis and infiltrating cells in tissue fluid. Broad-lineage annotation was reliable. Some labels represented KC states or poorly resolved repair-related programs and had lower resolution. Cell recovery was highly uneven (Table 1, Table 2, Figure 1): the median number of cells per ICD donor was only 183.5 (range 11–627), far lower than in healthy

controls. Subsequent comparisons used subjects as the unit and reported the number of included donors.

Table 1 Subject inclusion, post-QC cell numbers, and sample sizes for each analysis

Part 1 Cell recovery by condition (post-QC)			
Condition	Donors (n)	Post-QC cells	Cells per donor, median (range)
Control (independent healthy)	12	26,010	1,539.5 (108–10,007)
Non-lesional (sensitized, P_NL)	8*	2,263	187 (2–1,232)
Vehicle (acetone)	5	565	46 (1–287)
ICD (SLS)	8†	1,727	183.5 (11–627)
ACD, Day 2 (SADBE)	8‡	3,565	252.5 (16–1,945)
ACD, Day 4 (SADBE)	9	9,173	223 (5–6,805)

Part 2 Donors included per analysis (minimum-cell threshold)						
Analysis (threshold)	Control	Non-lesional	Vehicle	ICD	ACD	Paired (ICD vs non-lesional)
Pseudobulk DE, global (≥40)	12	7	3	6	8	5
KC pseudobulk / fry (≥20)	12	6	2	6	8 (D2 6 / D4 7)	5
Myeloid pseudobulk / fry (≥20)	11	4	1	4	8 (D2 6 / D4 6)	1
KC TF footprint (≥30)	—	—	—	—	—	5
Myeloid TF footprint (≥30)	—	—	—	—	—	1
Milo paired abundance test	—	—	—	7	—	7
MIF donor-level test	—	—	—	KC 6	Myeloid 7	6 / 7

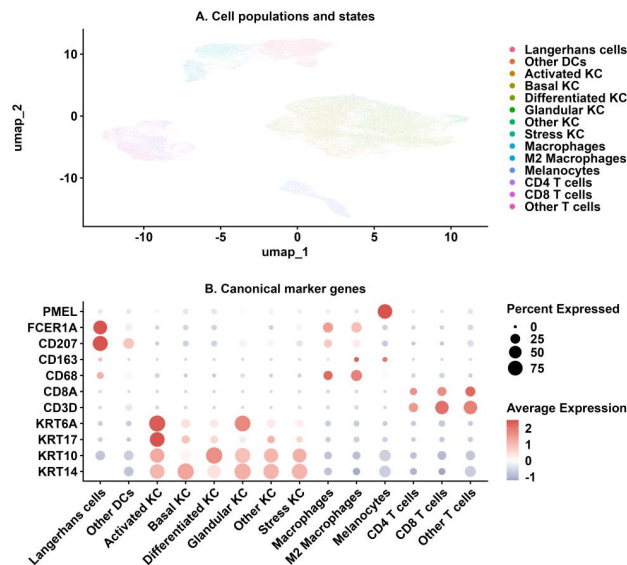
Notes: 50,820 cells were present before initial QC and 45,942 after initial QC; scDblFinder removed 2,639 detected doublets (5.74%) and DeconX corrected ambient RNA, yielding 43,303 cells for the main analysis. Observed post-QC ranges were 201–5,447 genes/cell, 301–31,419 UMI/cell and mitochondrial fraction 0–4.5%, with 21,492 genes retained.
ICD = irritant contact dermatitis induced by sodium lauryl sulfate (SLS); ACD = allergic contact dermatitis induced by squaric acid dibutyl ester (SADBE).
Day 2 = sampled after 2 days of patch application; Day 4 = 2 days of application plus 2 days after removal; median and range are across donors with recovered cells.
*P4 yielded no non-lesional cells; †P9 had no SLS sample (GEO does not state whether the SLS patch was omitted or no viable cells were recovered); ‡P5 yielded no Day 2 cells.
Paired analyses use the within-subject non-lesional site; tests with fewer than 3 paired donors are descriptive only.

Table 2 Donor-level sample types, post-QC cell numbers, and pairing status for the 9 subjects who completed challenge (P1–P9)

Donor-level sample types, post-QC cell numbers, and pairing availability for the 9 challenged subjects (P1–P9)							
Subject	Non-lesional	Vehicle	ICD	ACD	Broad pair	KC pair	Myeloid pair
P1	235	0	229	570	Yes	Yes	Yes
P2	69	0	253	171	Yes	Yes	No
P3	1,232	1	138	8,750	Yes	Yes	No
P4	—	—	385	573	No	No	No
P5	2	—	18	12	No	No	No
P6	139	287	66	186	Yes	Yes	No
P7	40	11	11	570	No	No	No
P8	261	220	627	1,059	Yes	Yes	No
P9	285	46	—	847	No	No	No

Values are post-QC cell counts; em dash (—) indicates no sample or zero recovered cells. ACD counts combine Day 2 and Day 4 samples.
Broad pair = both ICD and autologous non-lesional samples met the global pseudobulk threshold (≥40 cells); KC pair and myeloid pair used the ≥20-cell threshold.
Five subjects (P1, P2, P3, P6, P8) were available for broad-lineage and KC paired comparisons; only P1 met the myeloid paired threshold.

Figure 1 Single-cell transcriptomic atlas. A: UMAP of 14 cell populations/states; B: expression proportions of lineage marker genes across populations.

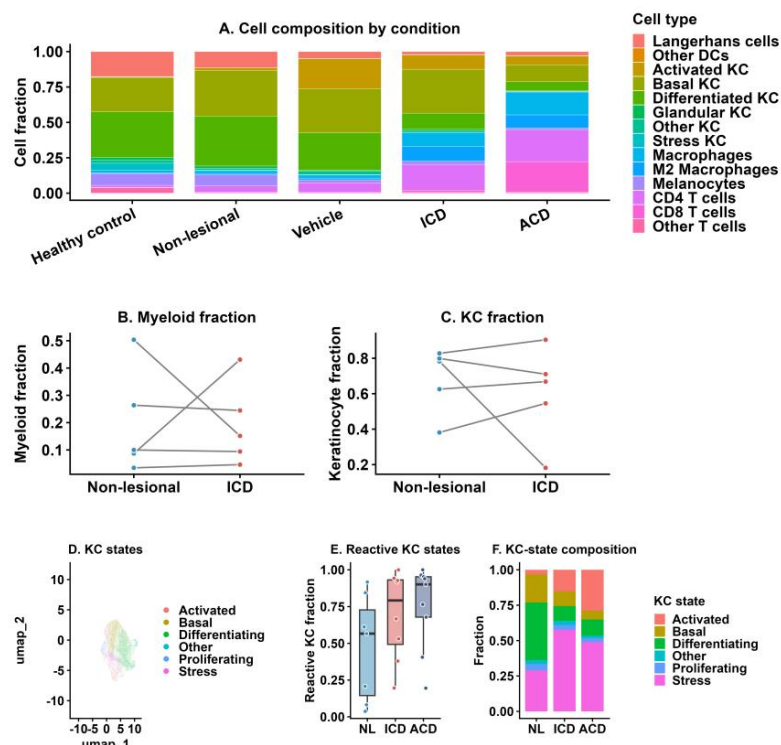


3.2 Cell composition and KC states

Using within-sample proportions as the unit and subjects as replicates (Figures 2A–D and 2F), two-sided paired signed-rank tests were performed for 5 paired donors (P1, P2, P3, P6, and P8) with at least 40 total cells in both ICD and autologous non-lesional samples. There were no significant differences in the proportions of broad-lineage myeloid cells (two-sided paired signed-rank test, exact $P = 0.81$; the proportion increased in 2 of 5 donors) or KCs (exact $P = 1.0$; the proportion increased in 3 of 5 donors; that is, the ranks of increases and decreases were symmetric, with no consistent direction of change, which does not mean that the two groups had equal proportions). Subpopulations showed only directional changes.

Macrophages increased in all 5 donors, activated KCs increased in 4 donors, and differentiated KCs and Langerhans/DC decreased in approximately 4 donors. However, because there were only 5 pairs, the lowest achievable P value for the two-sided signed-rank test was 0.0625, and none reached $P < 0.05$ (macrophages: software normal approximation $P = 0.059$, exact $P = 0.0625$). Therefore, these findings are described only as directional. The direction in ICD and independent healthy controls was similar, but donors, sites, and sensitization backgrounds were mixed, so these results are for reference only. KC reclustering yielded 6 states. The combined activation-related states (activated, stressed, and proliferative) were increased in lesional areas of ICD and ACD relative to autologous non-lesional areas (Figure 2E).

Figure 2 Cell composition and KC states. A: Stacked bar plot of cell type proportions across conditions; B: Paired myeloid cell proportions ($n = 5$ donors: P1/P2/P3/P6/P8; two-sided paired Wilcoxon signed-rank test, exact $P = 0.81$, not significant); C: Paired KC proportions ($n = 5$, same 5 donors and same denominator as B; two-sided paired Wilcoxon signed-rank test, $P = 1.0$, not significant); D: UMAP of KC states; E: Donor-level proportions of the three activation-related states combined (activated, stressed, and proliferative) among all KCs; F: Composition of each KC state. Lines in B and C connect the same subject; blue labels indicate donor IDs.



3.3 KC injury, stress, and danger signals

KC module scores were calculated for barrier, oxidative/NRF2, stress keratin, alarmin, and immediate early programs (Figure 3A). Among the 5 donors with

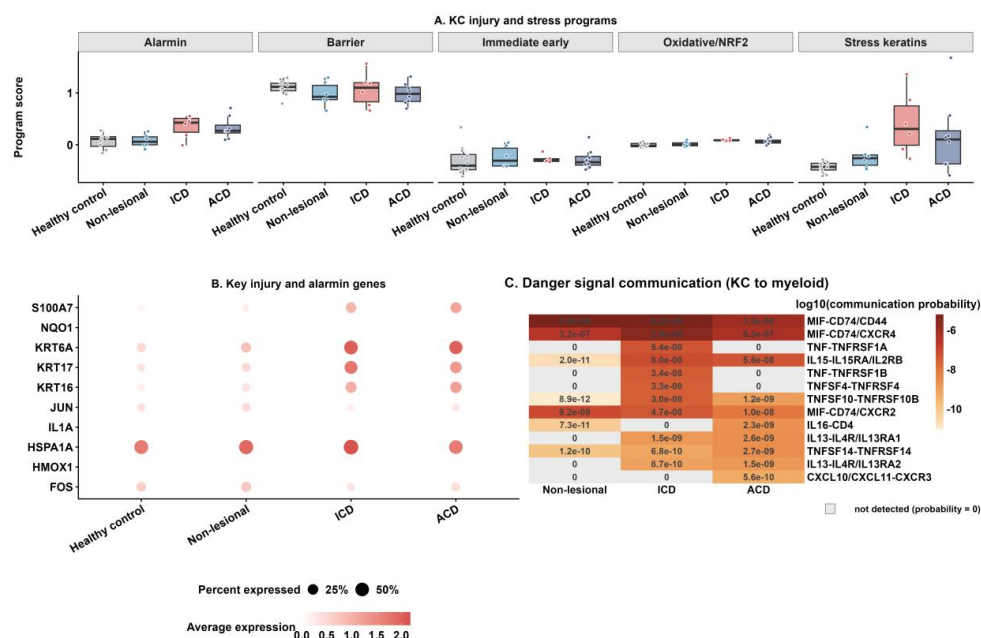
paired ICD and autologous non-lesional samples, stress keratins were upregulated in all 5 donors, alarmin and NRF2 target modules were upregulated in 4 donors each, the barrier module was upregulated in 3 donors, and the immediate early module showed inconsistent

direction. Donor-level logCPM paired t tests were performed for prespecified candidate genes ($n = 5$; the mean difference is not equivalent to the log2FC output by edgeR; only nominal P values are reported, without single-gene multiple-comparison correction). KRT6A (paired t test $P = 0.0075$), MIF ($P = 0.009$), and S100A7 ($P = 0.046$) had nominal $P < 0.05$, whereas KRT16 did not reach nominal significance ($P = 0.23$) and is reported only as a member of the stress keratin module. In the prespecified exact paired Wilcoxon signed-rank sensitivity test, because the minimum achievable P value for this test with $n = 5$ is 0.0625, KRT6A, MIF, S100A7, and KRT17 all had $P = 0.0625$ and KRT16 had $P = 0.3125$, none of which could reach $P < 0.05$. This indicates that the single-gene results with $n = 5$ are sensitive to the choice of test method; they are therefore reported only as exploratory nominal results and interpreted based on donor direction consistency, not as validated findings. Although the

NRF2 target module showed an overall upward trend, its classic target genes HMOX1 and NQO1 were sparsely expressed at this time point and were not stably detected. This result cannot exclude a biological role for NRF2. The expression proportions and mean expression levels of key injury and alarmin genes in KCs in each group are shown in Figure 3B.

To explore the possibility that KCs transmit danger signals to myeloid cells, ligand-receptor communication probabilities were extracted with KCs as ligand senders and myeloid cells as receivers (Figure 3C). TNF–TNFRSF1A/B was detected only in the ICD group. The communication probabilities of MIF–CD74/CD44 and MIF–CD74/CXCR4 were highest in the ICD group, whereas MIF–CD74/CXCR2 was highest in the non-lesional group. These group-level results suggest that KCs from ICD highly express stress/injury markers and have the potential to release danger signals.

Figure 3 KC injury, stress, and danger signals. A: Donor-level scores for barrier, oxidative NRF2, stress keratin, alarmin, and immediate early modules (points represent the same donor; ICD vs. autologous non-lesional, $n = 5$; described by donor consistency counts; scores are only compared between groups within the same module and absolute values are not compared across modules); B: Expression proportions and mean expression of key injury/alarmin genes in KCs in each group; C: Communication probabilities of danger-signal ligand-receptor axes with KCs as senders and myeloid cells as receivers (color indicates log10 communication probability; numbers in cells are raw probabilities; 0/not detected is gray; these are group-level deterministic metrics without uncertainty).



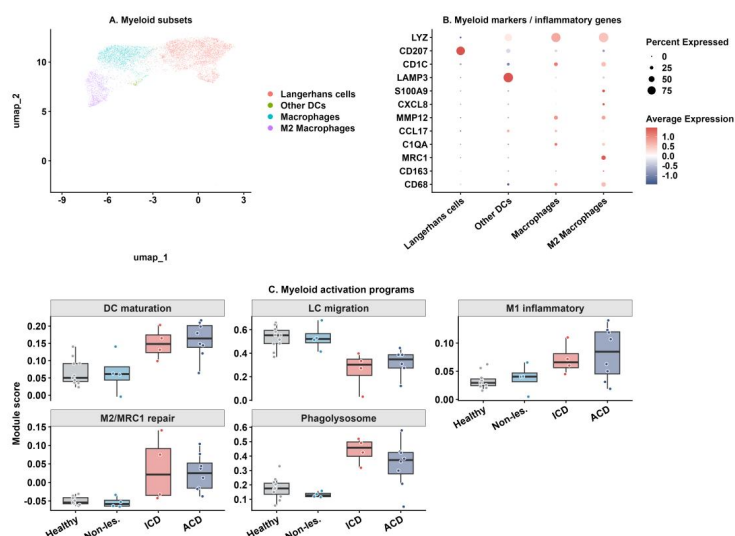
3.4 Myeloid subpopulation composition and activation programs

Reclustering of myeloid cells identified Langerhans/DC, macrophage, M2-like, and other DC subpopulations (Figure 4A). On average across

samples, the proportion of macrophages was higher in ICD lesions, whereas the proportions of M2-like cells and Langerhans/DC were lower. Donor-level scoring was further performed for 5 myeloid modules: M1, M2, DC maturation, LC migration, and phagolysosomal function (Figure 4C). Only samples with at least 15 myeloid cells were included. Four ICD donors (P1, P2, P4, and P8) entered the analysis. P3, P5, P6, and P7 were excluded because they had fewer than 15 myeloid cells (13, 9, 10, and 5 cells, respectively). Although myeloid cells were detected in 8 ICD donors, only P1

could be paired with an autologous non-lesional sample. In this single pair, M1, DC maturation, and phagolysosomal module scores were synchronously upregulated, and LC migration scores decreased. The M2/MRC1 repair score was heterogeneous among donors. Because there was only 1 paired sample, these findings are only exploratory transcriptional trends and cannot represent the actual phagocytic, migratory, or maturation functions of cells (Figure 4).

Figure 4 Myeloid subpopulations and activation programs. A: UMAP of myeloid subpopulations; B: Expression proportions and mean expression of myeloid marker/inflammatory genes; C: Donor-level scores for M1, M2, DC maturation, LC migration, and phagolysosomal modules (only samples with ≥ 15 myeloid cells were included; ICD donors P1/P2/P4/P8; P3/P5/P6/P7 had fewer than 15 myeloid cells (13/9/10/5) and were not included; only P1 had paired ICD and autologous non-lesional samples; because $n < 5$, no statistical testing was performed and only direction is described).



3.5 Cell communication

Ligand-receptor co-expression and group-level CellChat analysis showed that, at the whole-cell level, the probability of IL-1 was higher in autologous non-lesional samples, and CXCL10/CXCL11–CXCR3 was detected only in ACD. In donor-level testing, KC MIF was consistently upregulated in all 6 donors (paired signed-rank $P = 0.031$), whereas paired increments in myeloid receptors CD74 and CD44 were not significant in 7 donors each ($P > 0.05$). Therefore, KC MIF upregulation can be described as an independent donor-level finding, but pooled group probabilities cannot be used to establish it as a validated dominant axis. Probabilities of 0 for CXCL1/2/3, IL-33, TSLP, and others only indicate that

they were not detected under the 10% threshold and database conditions; they do not indicate pathway absence.

3.6 Differential expression, candidate shared features, and pathway enrichment

The edgeR analysis comparing ICD with autologous non-lesional samples showed that S100A9 and MMP12 were significantly upregulated, whereas S100A8 and CXCL8 did not reach statistical significance. Subject-blocked fry enrichment showed that the IL-8/neutrophil program was significantly upregulated and enriched in KCs and myeloid cells on Day 2 in both ICD and ACD (FDR < 0.05 for both, Figure 5C), but did not reach significance on Day 4 in ACD (FDR > 0.05 for both). Because no donor-aware

time interaction test was performed, the results can only be stated as significant on Day 2 and not significant on Day 4; they cannot be described as decreasing over time. In the stratified analysis, 13 myeloid genes were commonly upregulated in ICD and ACD (Table 3). The core genes were MMP12 and CXCL8, and the others included ARL8B, ATF5, BIRC3, CDKN1A, COTL1, CCL17, PSAP, LAMP3, CLEC10A, LYZ, and PLEK. S100A8/A9 in the myeloid compartment did not reach the stratified shared threshold. The log₂FC direction of myeloid cells in ICD and combined ACD relative to the shared non-lesional reference was positively correlated (Spearman $r = 0.84$, $n = 1,388$ shared genes). Because the two axes shared a reference, genes were not independent, and the broad myeloid compartment was a mixed subpopulation, this result is only a descriptive aggregate direction. CXCL8 was sparse in inDrop data and highly expressed in only a few cells, so it should be interpreted together with detection rate (Figure 5). Direct ICD–ACD paired testing in the same patients (6 pairs for broad-lineage cells, 6 pairs for KCs, and 4 pairs for myeloid cells) showed no significant genes at

the broad-lineage or KC scale (smallest FDR 0.29 and 0.93, respectively). At the myeloid scale, four highly expressed housekeeping/heat-shock transcripts—HSP90AA1, CRIP1, HSPH1, and the lncRNA MALAT1—reached FDR < 0.05 (smallest FDR 0.005), all higher in ACD. Because there were only 4 pairs, residual degrees of freedom were very low, and these transcripts were ubiquitous housekeeping/heat-shock genes rather than inflammatory effectors, they were not interpreted as stable disease-type differences. The candidate inflammatory genes MMP12, CXCL8, CCL17, and S100A9 were all non-significant in this direct comparison. In summary, this important negative result from the direct ICD–ACD comparison can be summarized as follows: no significant inflammatory genes were detected at the broad-lineage or KC level; at the myeloid level, only four housekeeping/heat-shock transcripts were significant; and none of the candidate inflammatory genes (MMP12, CXCL8, CCL17, and S100A9) was significant.

Figure 5 Differential expression, candidate shared features, and enrichment. A: Global ICD vs. non-lesional volcano plot (BH-FDR<0.05 and |log₂FC| > 1); B: Counts of upregulated/downregulated genes in ICD/ACD relative to autologous non-lesional samples (thresholds: BH-FDR<0.05 and |log₂FC| > 1; reference group was autologous non-lesional samples); C: Subject-blocked fry enrichment (D2 and D4 indicate ACD Day 2 and Day 4; * indicates FDR<0.05); D: Scatter plot of log₂FC in myeloid cells for ICD and combined ACD relative to the shared non-lesional reference (Spearman $r = 0.84$, $n = 1,388$).

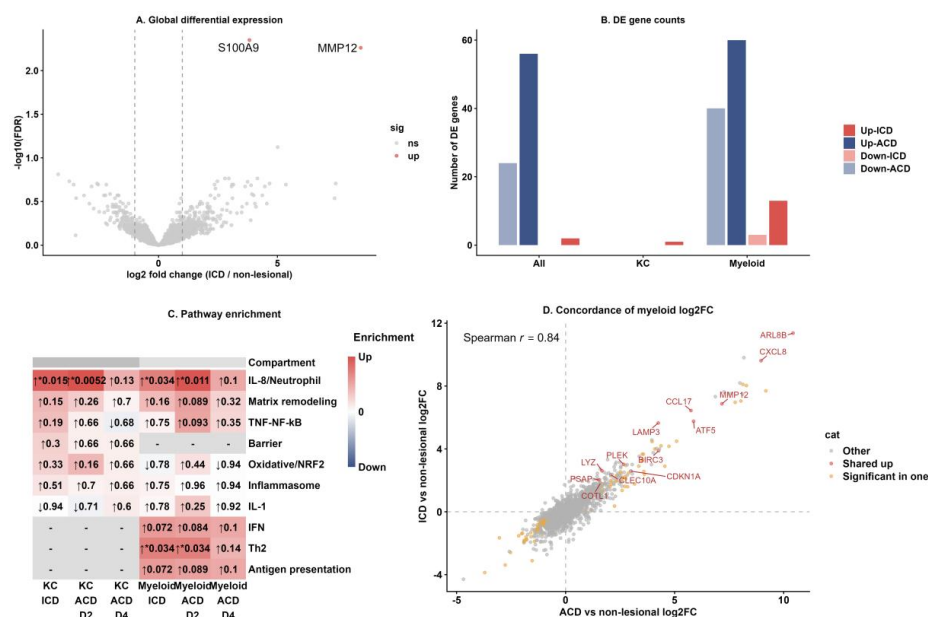


Table 3 Effect sizes and detection rates of the 13 myeloid genes commonly upregulated in ICD and ACD

Effect sizes and detection rates of the 13 myeloid genes commonly upregulated in ICD and ACD

Gene	ICD log2FC	ICD FDR	ACD log2FC	ACD FDR	pct NL	pct ICD	pct ACD
ARL8B	11.37	0.0015	10.43	0.0004	1.2%	14.9%	11.2%
MMP12	6.87	0.0029	7.17	0.0001	0.6%	12.1%	30.8%
ATF5	5.76	0.0065	5.87	0.0005	1.2%	24.1%	32.7%
BIRC3	3.88	0.0086	4.27	0.0001	5.3%	9.7%	11.4%
CDKN1A	2.59	0.0086	2.99	0.0001	7.4%	33.3%	43.1%
COTL1	1.83	0.0090	1.60	0.0018	32.8%	81.3%	80.6%
CCL17	6.44	0.0169	5.75	0.0085	0.6%	22.6%	11.1%
CXCL8	9.61	0.0191	8.96	0.0122	0.3%	6.9%	5.7%
PSAP	2.03	0.0197	1.52	0.0159	13.9%	53.6%	38.6%
LAMP3	5.65	0.0233	4.25	0.0132	2.8%	6.2%	8.4%
CLEC10A	2.35	0.0262	2.07	0.0105	9.0%	35.1%	59.3%
LYZ	2.61	0.0360	1.67	0.0369	18.9%	78.2%	80.9%
PLEK	3.02	0.0474	2.66	0.0159	4.3%	23.3%	27.6%

log2FC and FDR are from myeloid pseudobulk differential expression (ICD vs. non-lesional; combined ACD vs. non-lesional).

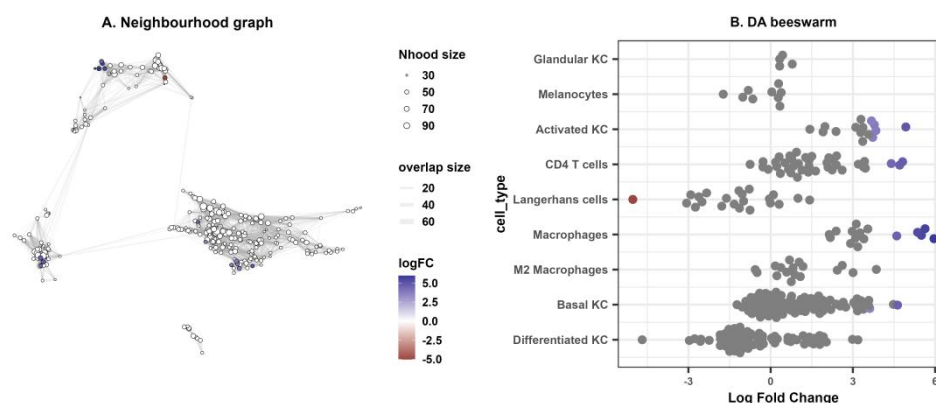
pct NL, pct ICD and pct ACD are the fractions of myeloid cells with non-zero expression in non-lesional, ICD and ACD samples, respectively.

3.7 Neighborhood-level differential abundance

Paired Milo analysis identified 16 of 290 neighborhoods at the exploratory threshold SpatialFDR < 0.10, of which 6 reached the main threshold SpatialFDR < 0.05. Enriched neighborhoods were predominantly macrophages (5, minimum q = 0.008), activated KCs (5, minimum q = 0.050), and CD4 T cells (3, minimum q = 0.049); basal KCs appeared only at the exploratory threshold (2, minimum q = 0.054), and one Langerhans-cell neighborhood was relatively depleted (q = 0.062) (Figure 6). These local

relative-abundance signals were concentrated in activated KCs, macrophages, and CD4 T cells, with a relative reduction in Langerhans cells. There were only 7 pairs, recovery was highly unbalanced, and stability was limited. The analysis only reflects local relative abundance of recovered cells and does not infer absolute infiltration. Although many neighborhoods reached significance in the unpaired ICD vs. control comparison, donors, sites, and sensitization backgrounds were confounded, so this was not used for main inference.

Figure 6 Milo neighborhood differential abundance. A: Neighborhood graph; point size represents the number of cells in the neighborhood, and color represents neighborhood abundance log2FC; B: Swarm plot of differential abundance by cell type; the x-axis is neighborhood abundance log2FC.



3.8 Upstream regulation and ligand activity footprints

Group-level feature analysis based on PROGENy and DoRothEA showed that myeloid NF- κ B/TNF- α ,

MAPK, and CEBPB scores were shifted upward in ICD lesions relative to non-lesional skin, whereas these signal changes were not obvious in KCs. ACD was relatively more prominent in the JAK-STAT/STAT1

direction.

NicheNet was used to infer upstream ligands acting on myeloid cells. Myeloid differentially expressed genes from ICD vs. autologous non-lesional samples were used as the response set, and genes expressed in this cell population were used as background. Nine candidate ligands were identified, requiring ligand expression in KCs in ICD and corresponding receptor expression in myeloid cells. The predictive performance of candidate ligands was similar to that expected by chance, and no upstream ligand with stable predictive ability was identified.

Combining donor-level MIF and fry results, it can be reasonably inferred that acute ICD and ACD show partially overlapping inflammatory transcriptional trends in KCs and myeloid cells.

4 Discussion

In this study, we performed a compartmentalized, time-stratified single-cell reanalysis of KCs and broad myeloid cells recovered by suction blister in the acute SLS/SADBE model in the same healthy, SADBE-sensitized volunteers. The original study constructed the atlas of this model, confirmed cell-level differences between ACD and ICD, and completed blister fluid protein validation [9]. Tissue transcriptomics also suggested both commonalities and differences between the two reaction types [7]. The main additions of this study are KC–myeloid compartment resolution, ACD time stratification, donor-level MIF testing, and exploratory ligand–transcription factor analysis. This study is a cross-sectional observation. Although ACD included Day 2 and Day 4 sampling, it lacked continuous baseline samples from the same site. KC stress, MIF, and myeloid activation were coexisting phenotypes, and event timing cannot yet be confirmed.

4.1 Post-exposure injury phenotype of KCs in ICD

At the KC level, stress keratins showed high consistency in ICD donors, with the stress-keratin module upregulated in all 5 paired donors, and the alarmin module was shifted upward in most ICD donors. Among the prespecified single genes, KRT6A, MIF, and S100A7 reached nominal $P < 0.05$, whereas KRT16 was directionally consistent but did not reach nominal significance ($P = 0.23$). IL-1 and HSPA1A

were used only as module components and for illustrative expression display, and no donor-by-donor single-gene testing was performed. These findings are consistent with the understanding of barrier/stress responses after direct chemical irritation by SLS and with studies of barrier biomarkers in patch testing and clinical contact dermatitis [27]. However, these markers also participate in state definition, so they should be interpreted as changes in the state composition and marker expression of recovered KCs. The lack of robust detection of HMOX1 and NQO1 only indicates that they were not stably captured at the acute time point in sparse data; it cannot exclude a role for NRF2 at the protein level or at other time points. The ICD myeloid NF- κ B/TNF- α direction suggested by PROGENy/DoRothEA depends on priors and was not tested donor by donor, so it is only a hypothesis.

4.2 MIF danger signals and myeloid inflammatory phenotypes

Consistent upregulation of KC MIF in all 6 donors ($P = 0.031$) is a relatively rare example of a consistent donor-level signal. However, paired increments in myeloid receptors CD74/CD44 were not significant. High CellChat probabilities are pooled group-level metrics without donor uncertainty and can only suggest that MIF is a candidate KC-released signal; they cannot establish functional transmission. The cross-sectional design cannot determine whether KC signals precede myeloid activation. Recruitment and amplification of myeloid inflammation by KCs through alarmins have been reported in diseases such as psoriasis [28], and experimental evidence supports targeting the inflammasome to alleviate ICD [29]. However, the present data did not directly validate these steps.

4.3 Shared candidate transcriptional features of the two reaction types

The most notable shared feature between the two reaction types is the IL-8/neutrophil program. It was significantly enriched by fry in both KCs and myeloid cells on Day 2 in ICD and ACD, but not on Day 4 in ACD. Because no time interaction test was performed, the results can only be stated as significant on Day 2 and not significant on Day 4 in ACD, rather than declining over time. This neutrophil direction is consistent with reports that surfactants recruit neutrophils through IL-1 β /CCL2 [30]. The log2FC of myeloid cells in ICD and combined ACD relative to

the shared non-lesional reference was correlated ($r = 0.84$). The two reaction types shared the IL-8/neutrophil transcriptional direction on Day 2, which may partly explain the overlap in early clinical manifestations and the difficulty of differential diagnosis.

Under this framework, CXCL8 and MMP12 repeatedly appeared in observations such as myeloid pseudobulk differences ($FDR < 0.05$ for both ICD and ACD) and fry enrichment. MMP12 showed increased cell-level mean expression in all 6 evaluable donors (ICD vs. autologous non-lesional), whereas CXCL8 was expressed in only a few cells and showed less consistent donor-by-donor direction (4 of 6 evaluable donors). Both can serve as candidates for shared post-exposure inflammatory direction. Although S100A9 was significantly upregulated in global combined pseudobulk ($FDR = 0.0045$), it did not reach FDR significance in the myeloid pseudobulk ($FDR \approx 0.49$) owing to high between-donor variability; S100A8/A9 [31] did not reach thresholds in the myeloid compartment for either comparison, suggesting that its global signal may be affected by an increased proportion of myeloid cells and that its evidence level as a shared candidate is lower. In addition, CXCL8 was sparse in inDrop data and highly expressed in only a few cells. Its receptors CXCR1/2 did not reach the detection threshold in $KC \rightarrow$ myeloid communication (probability 0), and neutrophils were rarely recovered in this suction blister compartment. Therefore, CXCL8 and MMP12 are currently only transcript-level directional findings and cannot be used as protein or functional markers. Whether they are true common downstream factors requires validation with blister fluid proteins and functional experiments.

4.4 Similarities and differences between ICD and ACD

The relatively ACD-specific MHC-I and IFN- γ features and the prominent barrier injury phenotype of ICD both come from the original study [9]. In this study, direct ICD-ACD paired testing (6 pairs for broad-lineage cells, 6 pairs for KCs, and 4 pairs for myeloid cells) showed no significant genes at the broad-lineage or KC scale (smallest FDR 0.29 and 0.93). At the myeloid scale, four highly expressed housekeeping/heat-shock genes (HSP90AA1, CRIP1, HSPH1, and the lncRNA MALAT1) reached $FDR < 0.05$, all higher in ACD. Because there were only 4

pairs, residual degrees of freedom were very low, and these genes were housekeeping rather than inflammatory, they were not interpreted as stable disease-type differences, and the candidate inflammatory genes were non-significant. Therefore, the MHC-I, IFN- γ , and protein validation findings from the original study are not presented as independent results of this article. The ACD STAT1 signal suggested by PROGENy/DoRothEA is only a group-mean transcriptional footprint. Th2/IL-20 cytokine features can help distinguish atopic dermatitis from various types of contact dermatitis [32]. It should be noted that although both ICD and ACD showed upregulation relative to non-lesional skin, no inflammatory difference was detected in their direct comparison. Possible reasons include the small number of paired samples, low statistical power, highly unbalanced cell recovery among groups, the myeloid compartment being a mixed subpopulation, and the two comparison axes sharing the same non-lesional reference (which makes genes non-independent). Therefore, a negative result does not mean that the two diseases are completely identical; rather, no stable difference could be detected with the current samples and power.

4.5 Translational prospects of candidate directions

The CXCL8-CXCR1/2 axis, MMP12, S100A8/A9, and CCL17-CCR4 are mechanistic candidates, and none has an approved indication for ICD/ACD. These findings only suggest testable translational directions and do not constitute treatment recommendations. We propose a staged validation strategy: first, confirm protein abundance and localization in suction blister fluid and skin tissue (for example, by ELISA, immunohistochemistry, or spatial transcriptomics); second, define cellular origin and KC-myeloid proximity using spatial transcriptomics; and third, establish function and causality through KC-myeloid co-culture, blocking antibodies or receptor antagonists targeting the CXCL8-CXCR1/2 axis and MMP12, and in vivo irritant/allergen models. Evidence- and consensus-based guidelines are available for the diagnosis, prevention, and treatment of hand eczema (the most common clinical phenotype of ICD/ACD) [33]; translating candidate molecules into clinically usable biomarkers still requires staged validation within this framework.

4.6 Limitations

This study has several limitations. (1) Each sample is a cross-sectional snapshot at a single time point after reaction formation. ACD included Day 2 and Day 4, but there was no continuous baseline from the same site, so the order or causality of KC and myeloid events cannot be determined. (2) Only 1,727 cells were recovered in the ICD group, with a median of 183.5 cells per donor. Only 1 patient had a pairable myeloid pseudobulk sample. Doublets and ambient RNA were computationally addressed with scDblFinder and DecontX, but GEO provided no empty-droplet profiles, so empty-droplet-based methods such as SoupX or CellBender could not be applied; cross-sample batch integration was not performed. (3) Program scores are affected by subpopulation composition, and modules overlap with state markers. Transcriptional modules are not equivalent to function. (4) Communication and prior-network analyses are group-level/group-mean method-dependent results, and NicheNet was near random. The conclusions are limited to acute SLS/SADBE reactions in healthy, already sensitized volunteers and to the suction blister compartment, and cannot be extrapolated to unsensitized populations or chronic, cumulative dermatitis. (5) Suction blisters mainly recover the epidermis and infiltrating cells in tissue fluid, resulting in selection bias regarding sampling site and cell type; this dataset did not recover neutrophils, fibroblasts, endothelial cells, B cells, or NK cells, so the roles of these cells could not be evaluated, and absolute cell infiltration numbers could not be inferred from the single-cell data. (6) No formal donor-level time-interaction test was performed, so differences between Day 2 and Day 4 cannot be described as a temporal trend. Future studies should use larger and balanced cohorts, serial sampling from the same site, inclusion of unsensitized and chronic dermatitis controls, and validation combining blister fluid proteins, spatial transcriptomics, and KC–myeloid co-culture/blocking functional experiments.

4.7 Conclusion

In summary, in the human acute SLS/SADBE contact dermatitis model in healthy volunteers sensitized with SADBE, KCs and broad myeloid cells obtained by suction blister showed multiple coexisting stress and IL-8/neutrophil-related transcriptional features. Some features showed consistent

transcriptional trends on Day 2 after exposure in both ICD and ACD. CXCL8 and MMP12 can serve as candidate molecules for further validation. Transcriptional trends related to myeloid phagocytosis and DC maturation came from only 1 paired donor, and MRC1/CD163-mediated repair-related features were not consistent among donors. Due to the cross-sectional design, unbalanced sample distribution, and small sample size, this study cannot clarify the molecular initiation differences between the two reaction types, the temporal relationship between KC and myeloid activation, or the common downstream mechanisms. The model "KC injury—myeloid inflammation amplification—shared downstream effects" is only a working model that requires experimental validation.

Author contributions

Yu Deng: Writing–review & editing, Writing–original draft, Visualization, Investigation. Pei Liu: Writing–review & editing, Writing–original draft, Visualization, Investigation. Xiao Shan: Writing–review & editing, Investigation. Zhihao Zhao: Writing–review & editing, Investigation. Chao Cheng: Writing–review & editing, Supervision, Project administration. Xingqi Wang: Writing–review & editing, Supervision, Conceptualization, Funding acquisition, Project administration.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

The single-cell RNA-sequencing data analyzed in

this study are publicly available in the Gene Expression Omnibus (GEO) under accession GSE267929 (BioProject PRJNA1113649; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE267929>), accessed in September 2026. GEO provides processed count matrices but no empty-droplet (raw) profiles. Analyses were performed in R 4.6.0 using Seurat 5.5.1, edgeR 4.99.4, limma 3.68.5, miloR 2.8.1, CellChat 1.6.1, decoupleR 2.17.0, scDblFinder 1.26.9, and celda (DecontX) 1.28.0; random seeds were fixed with set.seed in each analysis module. The complete analysis code and parameter settings are available from the corresponding author upon reasonable request.

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