

# Late inflammatory monocytes define circulatory immune dysregulation observed in skin microbiome-stratified atopic dermatitis

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May 23, 2023

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To the Editor,

Atopic dermatitis (AD) is a skin inflammatory disorder well described for significant disease heterogeneity. (1) Previously, we defined steady-state microbial configurations dermatotypes A and B that robustly reflected heterogeneity in AD clinical severity, cutaneous barrier properties and skin microbiome composition. (2) Here, we explored circulating immune dysregulation underlying dermatotype-stratified AD.

We performed single-cell RNA sequencing (scRNA-seq) of PBMCs from healthy subjects with dermatotype A (n = 4) and AD patients with dermatotypes A (n = 4) and B (n = 6). UMAP reduction distinguished 13 major immune clusters using transcriptional profile differences, identified using DISCO CELLiD (3) and top differentially expressed genes (DEGs; **Supplementary Figure 1A**). Major clusters corroborated well across all groups as a testimony to limited sample distortion by inter-individual or batch effects (**Supplementary Figure 1B**). Critically, AD patients harboured a marked enrichment in monocyte population cluster 1, whereby dermatotype stratification saw a further enrichment in dermatotype B (**Supplementary Figure 1B**).

To reveal finer expression details, we sub-clustered myeloid cells, obtaining 11 sub-populations whose identities were similarly assigned following DISCO CELLiD and top DEGs (**Supplementary Figure 1C**). Clusters with low cell counts and not of myeloid origin were removed prior to down sampling for normalization, yielding 7 myeloid clusters of interest. Majority of clusters represent CD14+ monocytes except for clusters 5 and 7 corresponding to CD16+ monocytes and conventional dendritic cells (cDC) respectively (**Figure 1A**). CD14+ monocytes segregated into 4 transcriptionally distinct states (**Figure 1A and B**): early activation monocytes expressing proinflammatory alarmins (*S100A8* and *S100A12*; clusters 0 and 1), transitional monocytes concomitantly expressing alarmins and chemokines (*S100A12* and *CCL3*; cluster 2), intermediate monocytes expressing antigen presentation genes (*HLA-DPB1* and *HLA-DPA1*; cluster 3), and late inflammatory monocytes expressing proinflammatory cytokines (*IL1B*, *CCL3* and *TNF*; cluster 4, **Supplementary Figure 1D**) (4, 5). Late inflammatory monocytes (cluster 4) were notably enriched in AD dermatotype B (**Figure 1A and C**) and exhibited upregulation in MHC class II (*HLA-DQA2*, *HLA-DQB1*), monocyte-related (*ITGAM*, *FGD2*) and proinflammatory genes (*CCL4L2*, *CCL3L1* and *CCL20*).

), suggestive of inflammatory dysregulation (**Figure 1D and Supplementary Table 1**). Taken together, dermatotype stratification addressed part of the cellular heterogeneity observed in circulating immune profiles, characterised particularly by late inflammatory monocytes enrichment in dermatotype B.

Complimenting scRNA-seq findings with CytoFLEX (**Supplementary Figure 2A**), unstimulated PBMCs were stained and secretion of inflammatory cytokines CCL3, CCL4, TNF- $\alpha$ , IL-1 $\beta$ , and IL-8 was monitored (**Supplementary Table 2**). Cytokine secretion by classical (CD14+ CD16-) monocytes were significantly elevated in AD compared to healthy controls (**Figures 2A, B and Supplementary Figure 2B**). Atopy-relevant cell types were examined, revealing similar levels between AD dermatotypes A and B but elevated presence of Th2 and Th2a in AD dermatotype B as opposed to healthy controls (**Figures 2C and D**). The reverse was observed for NK and ILC3s, where AD subjects with dermatotype B registered cell deficiencies (**Figures 2E and F**). Comparing between AD dermatotypes, ILC3s were significantly diminished in dermatotype B (**Figure 2F**). Healthy and AD subjects harbouring dermatotype A shared similar expression of aforementioned cells, suggesting that disease alone could not differentiate immune dysregulation observed. Next, we quantified 92 inflammatory biomarkers in plasma using Olink Proteomics Proximity Extension Assay (PEA) Target 96 Inflammation Panel. AD subjects recorded significantly higher expression of TNF-associated cytokines TRANCE and TRAIL, monocyte chemoattractant MCP-4, IL-18, and IL-2 than healthy controls (**Figures 2G-J and Supplementary Figure 2C**). Noticeably, higher basal abundance of TRANCE and MCP-4 was observed in AD dermatotype B than dermatotype A (**Figures 2G and I**), lending additional support to circulatory immune dysregulation previously observed in inflammatory monocytes.

Our findings provide unique insights into immune heterogeneity underlying AD patients stratified by defined skin microbiome configurations. Definingly, dermatotype B is characterised by microbial dysbiosis with reduced species richness, fewer commensal species and increased presence of *S. aureus* virulence genes. (2) Rather than atopy-relevant Type 2 cells, late inflammatory monocytes demonstrated a striking enrichment in AD dermatotype B, particularly in the upregulation of proinflammatory cytokine transcripts. Coupled with skin microbial dysbiosis that increases cutaneous exposure to potent allergic inflammation inducers such as  $\delta$ -toxin (2, 6), immune dysregulation directed by monocytes could predispose AD skin towards flare predisposition and clinical severity as observed in dermatotype B. Basal secretion levels of proinflammatory cytokines by classical monocytes are elevated in AD regardless of dermatotype, suggesting higher baseline inflammation driven by disease. While no stimulation was conducted here, earlier reports have demonstrated that LPS activation of monocytes upregulates transcriptional expression of proinflammatory cytokines IL-1 $\beta$ , CCL3 and IL-8. (4) Similarly, heightened proinflammatory cytokines and monocyte-related proteins were detected in plasma of AD dermatotype B compared to dermatotype A subjects, validating scRNA-seq findings of monocyte-driven immune dysregulation and inflammation.

Collectively, circulatory immune profiling of dermatotypes, defined by alterations in skin microbiota compositions, suggest a pathophysiological role for inflammatory monocytes. Future studies are needed to ascertain the functional and mechanistic roles of late inflammatory monocytes in dermatotype-stratified AD. Importantly, our study highlights the potential clinical utility of dermatotype stratification to indicate the cellular basis for increased inflammation and exacerbated immune dysregulation, setting precedence for better understanding of disease prognosis.

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**Funding source:** Supported by grants from the Singapore Immunology Network (grants SIgN-06-006, SIgN-08-020, and SIgN-10-029), the National Medical Research Council (grant NMRC/1150/2008) Singapore, and

the Agency for Science, Technology and Research, Singapore. The SIgN Immunomonitoring platform supported by a BMRC (Biomedical Research Council) IAF (Industry Alignment Fund) 311006 grant and BMRC transition funds (No. H16/99/b0/011). A.K. Andiappan was supported by an NMRC (National Medical Research Council) YIRG (Young Individual Research Grant) grant (OFYIRG17nov065), an Agency for Science, Technology and Research CDA (Career Development Award) grant (202D800012), and fellowships from the European Academy of Allergy and Clinical Immunology and European Respiratory Society. National Healthcare Group/A\*STAR/Nanyang Technological University Skin Research Grant (SRG/14032) funding to A.T., Y-W.Y and J.E.A.C. A\*STAR BMRC Central Research Fund (ATR) to J.E.A.C. The funding agencies had no role in the study design, data collection and analysis, decision to publish, or preparation of this article.

## Main Figure Legends

**Figure 1: scRNA-seq of myeloid cells.** (A) UMAP dimensionality reduction and clustering of myeloid cells based on transcriptional profiles segregated by AD subjects with dermatotype A (AD\_A; n = 4) and B (AD\_B; n = 5) and healthy controls with dermatotype A (Healthy\_A; n = 4). Cell subsets were delineated based on KNN (k-nearest neighbours) clustering and identified using DISCO CELLiD and differentially expressed genes (DEGs). Clusters not of myeloid origin and low cell counts were removed before downsampling for normalisation. (B) Heatmap showing the relative expression of DEGs from each cluster represented by logFC values. (C) Proportion of cells represented by each cluster. (D) Heatmap of relative expression of top DEGs from cluster 4 between AD\_A and AD\_B (min.pct = 0.25 and logFC.threshold = 0.25). Expression is shown in terms of Z-scores.

**Figure 2: Differences in circulatory inflammation in PBMCs and plasma.** (A-F) Cytotflex analysis of (A) CCL3+ CCL4+ and (B) TNF-a+ IL-1b+ proportions secreted by classical monocytes from PBMCs across dermatotype A healthy (Healthy\_A; n = 8) and AD subjects (AD\_A; n = 5) and dermatotype B AD subjects (AD\_B; n = 6). Proportions of (C) Th2, (D) Th2a, (E) NK cells and (F) ILC3 in PBMCs measured by flow cytometry. (G-J) Scatter plots depicting inflammatory protein biomarkers statistically significant across Healthy\_A (n = 13), AD\_A (n = 16) and AD\_B (n = 10) subjects: (G) TRANCE, (H) TRAIL, (I) MCP-4 (J) IL-18. Expression reported as normalised protein expression (NPX) values. For all graphs, topmost line depicts statistical significance by Kruskal-Wallis across all 3 groups while brackets depict P-values of paired Mann-Whitney U (\* P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001).

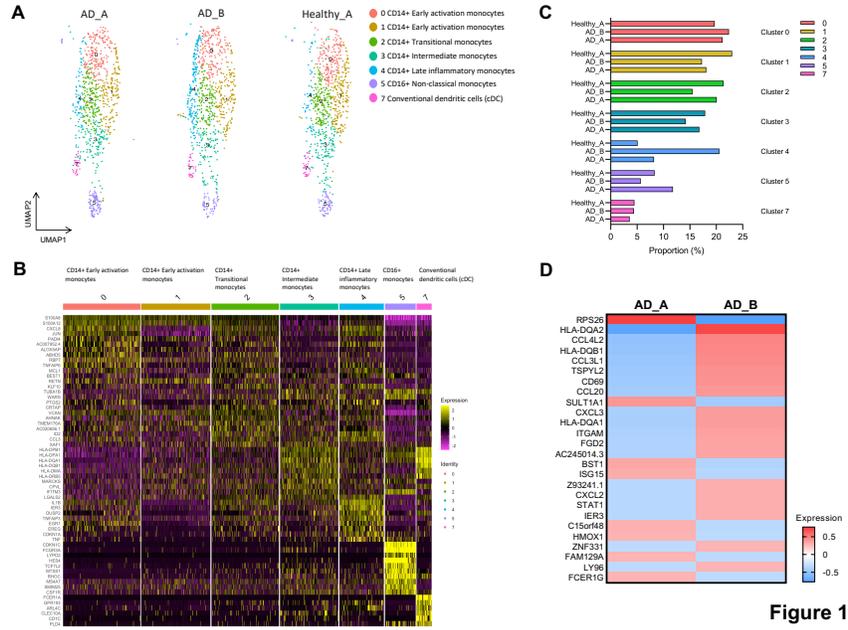


Figure 1

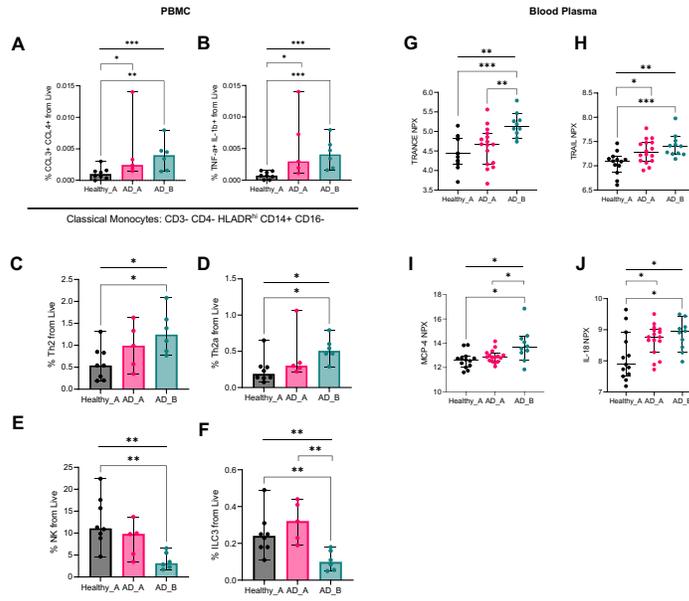


Figure 2