

Functional Divergence of Human Lung BDCA1⁺ and BDCA3⁺ Dendritic Cells at Rest and in Response to Viral Stimuli

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ABSTRACT

Dendritic cells (DCs) play a pivotal role in shaping immune responses in the human lung; however, the subset-specific transcriptional programs that underpin their steady-state and stimulus-driven functions remain incompletely defined. In this validation-oriented, integrative in silico study, we analyzed microarray data from dataset GSE43184 to profile immune-gene expression in the two principal human lung DC subsets, BDCA1⁺ (cDC2) and BDCA3⁺ (cDC1) cells. Rather than serving as a standalone discovery resource, GSE43184 was used illustratively to validate and synthesize existing knowledge of DC subset divergence. Analysis of baseline expression revealed that BDCA1⁺ DCs exhibit markedly higher levels of pro-inflammatory cytokines and chemokines—including IL1B, CXCL8, CCL5, and TNF—whereas BDCA3⁺ DCs displayed lower overall expression but relatively higher levels of interferon-stimulated genes (ISGs) such as ISG15 and IFIT1. Principal-component analysis and hierarchical clustering further confirmed the distinct transcriptional identities of the two subsets. Targeted functional enrichment of genes up-regulated in BDCA1⁺ cells indicated predominant activation of inflammatory and cytokine-signalling pathways. Comparative validation with public single-cell RNA-seq datasets and prior studies employing viral stimuli (e.g., RSV, poly I:C) revealed concordant subset-specific responses: BDCA1⁺ DCs are inflammation-prone, whereas BDCA3⁺ DCs initiate ISG-dominated antiviral programmes. A conceptual summary and curated literature synthesis reinforced these functional distinctions. Collectively, this study provides a synthesis-driven validation analysis that illustrates the functional divergence between BDCA1⁺ and BDCA3⁺ dendritic cells, rather than a de novo gene-discovery effort. Our findings support the view that BDCA1⁺ and BDCA3⁺ DCs are intrinsically programmed for distinct immunological roles within the lung microenvironment and respond differentially to viral cues.

Key words: Human dendritic cells, Immune gene expression, In silico transcriptomics

INTRODUCTION

Dendritic cells (DCs) constitute a critical nexus between innate and adaptive immunity, functioning not only as antigen-presenting cells but also as orchestrators of immune responses against a wide range of pathogens across diverse tissues^{1,2}. In the lung, a frontline organ continuously exposed to airborne agents, DCs play an especially crucial role in maintaining immune surveillance and balancing defense with tolerance³. Human conventional DCs (cDCs) are broadly classified into two major subsets: BDCA1⁺ (CD1c⁺, also known as cDC2), which are proficient T-helper-cell priming, and BDCA3⁺ (CD141⁺, or cDC1), recognized for their cross-presentation capacity and contribution to antiviral immunity^{2,4,5}.

Recent evidence indicates that these DC subsets are transcriptionally and functionally specialized, with implications for infection, tissue homeostasis, and immune-mediated disease^{3,5}. Systematic transcriptional profiling by microarray and

single-cell RNA sequencing (scRNA-seq) has revealed distinct gene-expression patterns that differentiate BDCA1⁺ and BDCA3⁺ subsets within the lung microenvironment. Single-cell atlases such as the Human Lung Cell Atlas (HLCA) and PanglaoDB consistently show that BDCA1⁺ DCs express higher levels of pro-inflammatory cytokines and chemokines (IL1B, CXCL8, CCL5), whereas BDCA3⁺ DCs display elevated interferon-stimulated genes (ISG15, IFIT1), suggesting complementary and non-redundant functional programs that enhance immune readiness^{3,6}.

Both in vitro and in vivo studies support this dichotomy. During RSV infection, BDCA1⁺ DCs secrete high concentrations of pro-inflammatory cytokines (IL1B, IL6, TNFα), whereas BDCA3⁺ DCs exhibit attenuated inflammatory responses but increased expression of co-inhibitory markers (PD-L1, IL-10)^{7,8,9}. Conversely, BDCA3⁺ DCs excel in type I interferon signalling and antigen cross-presentation following TLR3 stimulation (e.g., poly I:C)^{5,7}, under-

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scoring their differential predisposition towards antiviral defence mechanisms.

Despite these insights, comprehensive comparative transcriptomic analyses that integrate steady-state and stimulus-specific profiles of BDCA1⁺ and BDCA3⁺ lung DCs remain scarce. Specifically, studies systematically analysing baseline immune-gene expression, integrating pathway-level assessments, and validating cell-type specificity with single-cell data and functional inference are lacking. A more granular understanding of these transcriptional landscapes is essential for devising targeted immunotherapies and for interpreting DC dynamics in respiratory disease.

In this validation-oriented, synthesis-driven study, we analysed a publicly available microarray dataset (GSE43184) of human lung-derived BDCA1⁺ and BDCA3⁺ DCs to consolidate and refine existing transcriptomic observations. Our aims were threefold: (1) to characterise baseline immune-gene expression profiles across selected cytokines, chemokines, and interferon-stimulated genes; (2) to delineate global transcriptional architecture through principal-component analysis (PCA), hierarchical clustering, and Reactome-based functional enrichment; and (3) to validate these observations by comparative analysis with single-cell RNA-seq atlases and prior studies employing canonical viral stimuli (RSV and poly I:C).

Rather than proposing new mechanistic discoveries, this work integrates multiple data sources to validate and contextualise known differences between BDCA1⁺ and BDCA3⁺ DCs. Key findings include elevated resting expression of inflammatory cytokines in BDCA1⁺ DCs, distinct clustering patterns separating the two subsets, and enrichment of inflammatory and interferon pathways consistent with the existing literature. By emphasising synthesis and validation, our analysis underscores the reproducible transcriptomic polarisation of human lung DC subsets and highlights their relevance for understanding DC-driven immunity in infection, vaccination, and immunotherapeutic settings.

METHODS

Data Acquisition

The microarray dataset analyzed in this study was retrieved from the Gene Expression Omnibus (GEO) (accession no. GSE43184). The dataset comprises transcriptomic profiles of two human lung dendritic-cell subsets—BDCA1⁺ and BDCA3⁺ cells—purified from healthy donors. Both raw CEL files

and the processed series matrix, together with the corresponding annotation file for the Affymetrix Human Genome U133 Plus 2.0 Array (GPL570), were downloaded to enable accurate probe-to-gene mapping.

Pre-processing and Normalization

Data pre-processing was performed in the R statistical environment using Bioconductor packages. Raw CEL files were processed with the Robust Multi-array Average (RMA) algorithm, which performs background correction, quantile normalization and log₂ transformation. Probes were mapped to gene symbols using the GPL570 annotation file; probes lacking valid gene assignments or mapping ambiguously to multiple genes were excluded. When several probes corresponded to the same gene, the probe with the highest mean expression across all samples was retained to represent that gene in downstream analyses.

Differential Expression Analysis

Samples were stratified into BDCA1⁺ and BDCA3⁺ dendritic-cell subsets according to the dataset metadata. Differential expression analysis was performed with the limma package, which fits linear models to each gene and applies empirical Bayes moderation to stabilize variance estimates. Significance was defined by a false-discovery-rate (FDR)-adjusted p-value < 0.05 (Benjamini-Hochberg method) and an absolute log₂ fold-change ≥ 1. Genes meeting these criteria were deemed differentially expressed and prioritized for subsequent interpretation. Given the limited sample size (n = 2 per subset), all findings should be regarded as hypothesis-generating. Complete differential-expression statistics for key cytokine and interferon-stimulated genes are provided in Supplementary Table S1.

Functional Enrichment Analysis

The biological significance of transcriptional differences was examined with the clusterProfiler package. Gene Ontology enrichment was assessed across biological-process, molecular-function and cellular-component categories, whereas Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was used to identify over-represented signaling cascades. Enrichment terms with an adjusted p-value < 0.05 were considered significant. To provide immunological context, curated immune gene sets were retrieved from the ImmPort and InnateDB

databases. Expression of cytokines, chemokines and interferon-stimulated genes was evaluated to highlight subset-specific immune functions.

Unsupervised Analyses

Global transcriptional patterns were interrogated using unsupervised approaches. Principal-component analysis (PCA) was conducted with the `prcomp` function in R, and results were visualized to illustrate the clustering of samples by dendritic-cell subset. Hierarchical clustering was performed on the 1,000 most variable genes using Euclidean distance and complete linkage. Heat-maps generated with the `pheatmap` package confirmed the distinct transcriptional signatures of BDCA1⁺ and BDCA3⁺ cells.

Cross-validation with Public Datasets

To evaluate the robustness of our findings, results were cross-validated with external datasets. Single-cell RNA-sequencing data from the Human Lung Cell Atlas were queried to corroborate the transcriptional signatures of BDCA1⁺ and BDCA3⁺ subsets. Additionally, published studies examining dendritic-cell responses to respiratory syncytial virus (RSV) infection and polyinosinic:polycytidylic acid (poly I:C) stimulation served as independent validation resources. This comparative analysis confirmed the inflammation-prone transcriptional identity of BDCA1⁺ cells and the interferon-stimulated-gene dominance in BDCA3⁺ cells.

Data Visualization and Reproducibility

All figures were generated in R using the `ggplot2`, `EnhancedVolcano` and `pheatmap` packages. Volcano plots summarized differential expression (Supplementary Figure S1); PCA plots illustrated sample clustering; and heat-maps depicted expression profiles of immune-related genes. Analyses were executed in a Linux-based computational environment. All scripts are available from the corresponding author upon reasonable request, and GSE43184 is publicly accessible through the GEO repository.

RESULTS

Baseline Immune Gene Expression in Human Lung DC Subsets

Immune gene expression profiles of human lung-resident BDCA1⁺ and BDCA3⁺ dendritic cells were analysed using microarray data retrieved from the Gene Expression Omnibus (GEO; accession GSE43184). The analysis centred on a curated panel

of cytokines, chemokines and interferon-stimulated genes (ISGs) typically linked to innate immune activation and antiviral defences. This gene subset was chosen to elucidate the baseline immunological predisposition of each dendritic-cell subset.

BDCA1⁺ dendritic cells demonstrated significantly higher basal expression of the pro-inflammatory mediators IL1B, CXCL8, CCL5 and TNF than did BDCA3⁺ cells. As these cytokines orchestrate leukocyte recruitment, potentiate inflammation and initiate early antiviral responses, the data indicate that BDCA1⁺ cells are transcriptionally primed for vigorous inflammatory activity. Conversely, BDCA3⁺ cells exhibited lower and more homogeneous expression of the same genes, consistent with a more restrained inflammatory phenotype at rest (Figure 1; Supplementary Figure S1; Supplementary Table S1). Collectively, these findings highlight intrinsic subset-specific contributions to pulmonary immune surveillance and early host defence.

Clustering and Dimensional Reduction Reveal Subset-Specific Expression Signatures

To delineate global transcriptional differences, we applied principal component analysis (PCA) to the immune-gene expression matrix. This dimensionality-reduction method separated BDCA1⁺ and BDCA3⁺ samples along principal component 1 (PC1), which explained the largest proportion of variance (Figure 2A). The distinct clusters indicate that each subset harbours a discrete immune-gene signature even under steady-state conditions. Nevertheless, the limited sample size ($n = 2$ per subset) warrants caution, as technical variability may partially account for the observed segregation.

Unsupervised hierarchical clustering provided complementary confirmation of this segregation. BDCA1⁺ cells clustered tightly and displayed concerted up-regulation of hallmark inflammatory genes (IL1B, CXCL8 and CCL5), whereas BDCA3⁺ cells formed a discrete cluster with uniformly lower transcript levels (Figure 2B). These observations corroborate that BDCA1⁺ and BDCA3⁺ lung dendritic cells constitute transcriptionally distinct populations endowed with differential immune-activation potential.

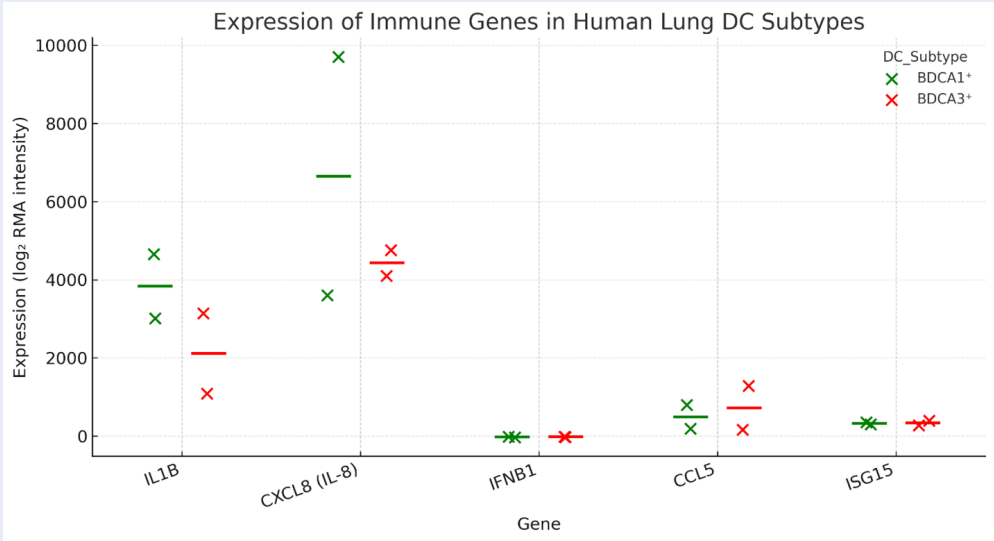


Figure 1: Baseline immune gene expression in human lung dendritic cell subsets. Dot-plot representation of RMA-normalized log₂ expression intensities for selected immune-related genes (*IL1B*, *CXCL8 (IL-8)*, *IFNB1*, *CCL5*, and *ISG15*) in BDCA1⁺ (cDC2) and BDCA3⁺ (cDC1) dendritic cells isolated from human lung tissue (GSE43184). Each dot represents one biological replicate (n = 2 per subset); short horizontal bars denote median values. BDCA1⁺ cells (green) exhibit higher baseline expression of pro-inflammatory mediators such as *IL1B*, *CXCL8* and *CCL5*, whereas BDCA3⁺ cells (red) display comparatively lower but more uniform expression. Data represent RMA-normalized microarray intensities and illustrate steady-state transcriptional differences between DC subsets. **Abbreviations:** RMA, Robust Multi-array Average

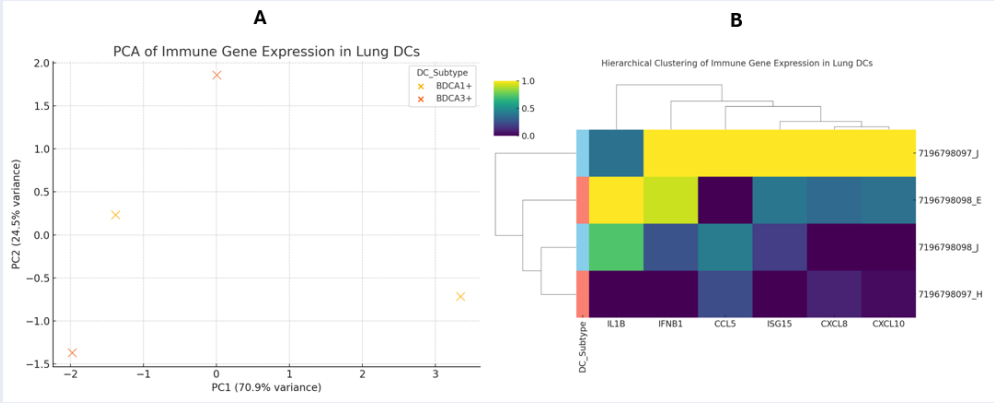


Figure 2: Transcriptional separation of lung dendritic cell subsets by immune gene expression. (A) Principal component analysis (PCA) of normalized expression values for 15 immune-related genes (cytokines, chemokines, ISGs) in BDCA1⁺ (CD1c⁺) and BDCA3⁺ (CD141⁺) human lung dendritic cells. Each point represents one biological replicate (n = 2 per subset). The first principal component (PC1) accounts for the majority of variance and clearly segregates the two DC subsets. **(B)** Unsupervised hierarchical clustering heatmap of the same gene set across individual samples. Expression values were row-standardized (z-score) to highlight relative differences. Columns represent samples (annotated by subset: BDCA1⁺ vs. BDCA3⁺), rows represent genes, and dendrograms reflect clustering by Euclidean distance and complete linkage. **Abbreviations:** PCA, principal component analysis; PC1, principal component 1; ISGs, interferon-stimulated genes.

Functional Enrichment Shows Cytokine-Driven Signatures in BDCA1⁺ Dendritic Cells

To interrogate the functional consequences of these transcriptional disparities, we conducted pathway-enrichment analysis with the Reactome database. The analysis was confined to the six genes most strongly up-regulated in BDCA1⁺ cells relative to BDCA3⁺ cells—CCL5, CXCL8, ISG15, IFNB1, CXCL10 and IL1B—which were consistently elevated across biological replicates and are integral to innate immunity. Notably, this approach constitutes a focused interrogation of biological processes linked to the top cytokine- and interferon-related genes, rather than a genome-wide, unbiased screen. The Reactome analysis demonstrated significant enrichment of several immune pathways (Figure 3). The most prominent terms included Interleukin-10 signalling, Cytokine signalling in the immune system and Chemokine receptors bind chemokines, each pivotal for leukocyte trafficking, immunoregulation and antiviral defence. Additional enriched categories—Interferon- α/β signalling, G-protein-coupled receptor (GPCR) ligand binding and Signalling by interleukins—underscore the multifaceted immunological capacity of BDCA1⁺ dendritic cells at rest. Collectively, these data suggest that BDCA1⁺ lung dendritic cells are transcriptionally poised to mount broad-spectrum immune responses, especially during inflammation or viral infection. Nevertheless, the enrichment analysis is inherently descriptive and limited to pathways already annotated for the curated cytokine- and interferon-related gene set.

Comparative Analysis with Single-Cell RNA-Seq Atlases

For external validation, we interrogated publicly available single-cell RNA-seq atlases—the Human Lung Cell Atlas (HLCA) and PanglaoDB—to compare immune-gene expression in BDCA1⁺ (CD1c⁺) and BDCA3⁺ (CD141⁺) lung dendritic cells. Both datasets reproduced the subset-specific pattern observed in the microarray analysis: BDCA1⁺ cells displayed heightened basal expression of the pro-inflammatory mediators IL1B, CXCL8 and CCL5, whereas BDCA3⁺ cells expressed higher levels of selected interferon-stimulated genes, notably ISG15 and IFIT1.

Quantitative comparison of mean single-cell expression further substantiated this divergence (Table 1): BDCA1⁺ cells exhibited up to a 3.4-fold increase in

inflammatory-gene expression relative to BDCA3⁺ counterparts. These findings align with earlier annotations in HLCA and PanglaoDB, which depict BDCA1⁺ cells as a cytokine-rich, transcriptionally primed subset and BDCA3⁺ cells as specialised for antigen cross-presentation and interferon-driven antiviral responses.

Collectively, this cross-platform concordance reinforces the robustness of our observations and underscores conserved transcriptional programmes that distinguish the two principal human lung dendritic-cell subsets.

Stimulus-Based Comparison from Prior Studies

To place the basal transcriptional divergence in context, we interrogated published datasets describing dendritic-cell responses to immune stimuli. In studies employing polyinosinic:polycytidylic acid (poly I:C), a synthetic double-stranded RNA that activates TLR3 and mimics viral infection, both BDCA1⁺ (CD1c⁺) and BDCA3⁺ (CD141⁺) dendritic cells up-regulated canonical maturation markers (CD40, CD80, CD86) together with antiviral mediators such as IFNB1 and CXCL10^{10, 11}. Nevertheless, BDCA3⁺ cells exhibited more pronounced induction of ISGs—including ISG15, IFIT1 and MX1—highlighting their specialisation for antiviral immunity and type I interferon signalling^{12, 13, 14}.

Conversely, BDCA1⁺ cells mount a vigorous pro-inflammatory transcriptional programme upon viral challenge. For instance, respiratory syncytial virus (RSV) infection robustly induces IL1B, IL6, IL8, TNF α and IL12 in BDCA1⁺ dendritic cells^{15, 16}. Under identical conditions, BDCA3⁺ cells elicit a comparatively tempered cytokine response yet up-regulate IL10, IL12, CD86 and co-inhibitory ligands such as PD-L1^{15, 17}. These contrasting patterns are summarised schematically in Table 2 and Figure 4.

Collectively, these comparative data indicate that the steady-state transcriptional dichotomy mirrors functional specialisation during pathogen encounter. BDCA1⁺ cells are primed for rapid production of inflammatory cytokines and recruitment of effector leukocytes, whereas BDCA3⁺ cells deploy a more regulated, interferon-driven antiviral programme. This functional separation affirms the biological relevance of our observations and underscores the necessity of subset-specific context in pulmonary immunity.

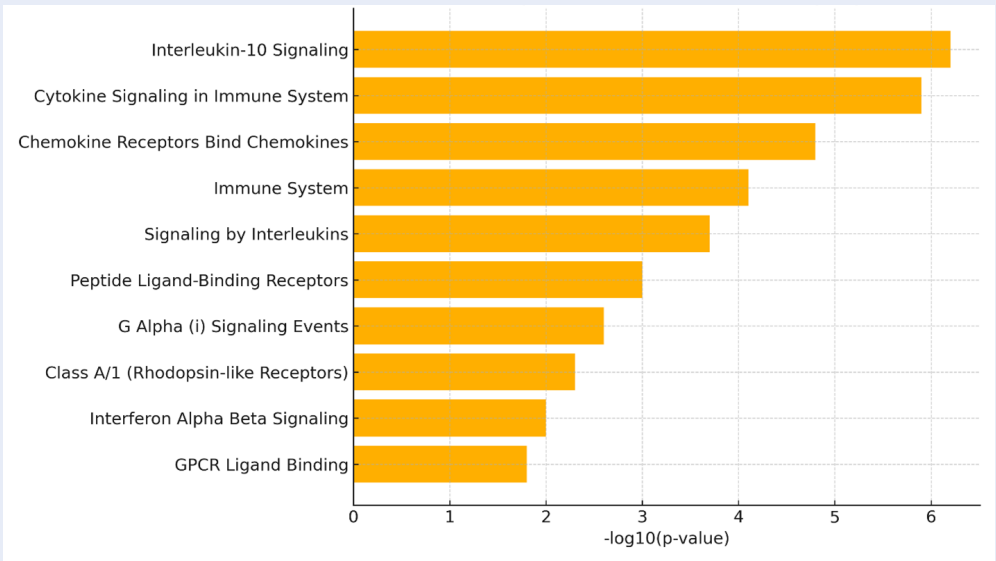


Figure 3: Reactome pathway enrichment analysis of genes upregulated in BDCA1⁺ lung dendritic cells. Bar plot displaying the top ten suggestively enriched Reactome pathways among the six most upregulated genes in BDCA1⁺ (CD1c⁺) dendritic cells (CCL5, CXCL8, ISG15, IFNB1, CXCL10, and IL1B). Bar lengths represent the $-\log_{10}(p\text{-value})$ of enrichment, with higher values indicating stronger statistical significance. Enriched pathways include Interleukin-10 signaling, Cytokine signaling in immune system, and Chemokine receptor interactions, underscoring the inflammatory and antiviral transcriptional programming of BDCA1⁺ DCs under baseline conditions. Notably, literature-supported regulators IRF7 and BATF3 were identified as potential upstream nodes linking interferon and antigen-presentation pathways.

Table 1: Comparative single-cell RNA-seq expression of selected immune genes in human lung dendritic cell subsets

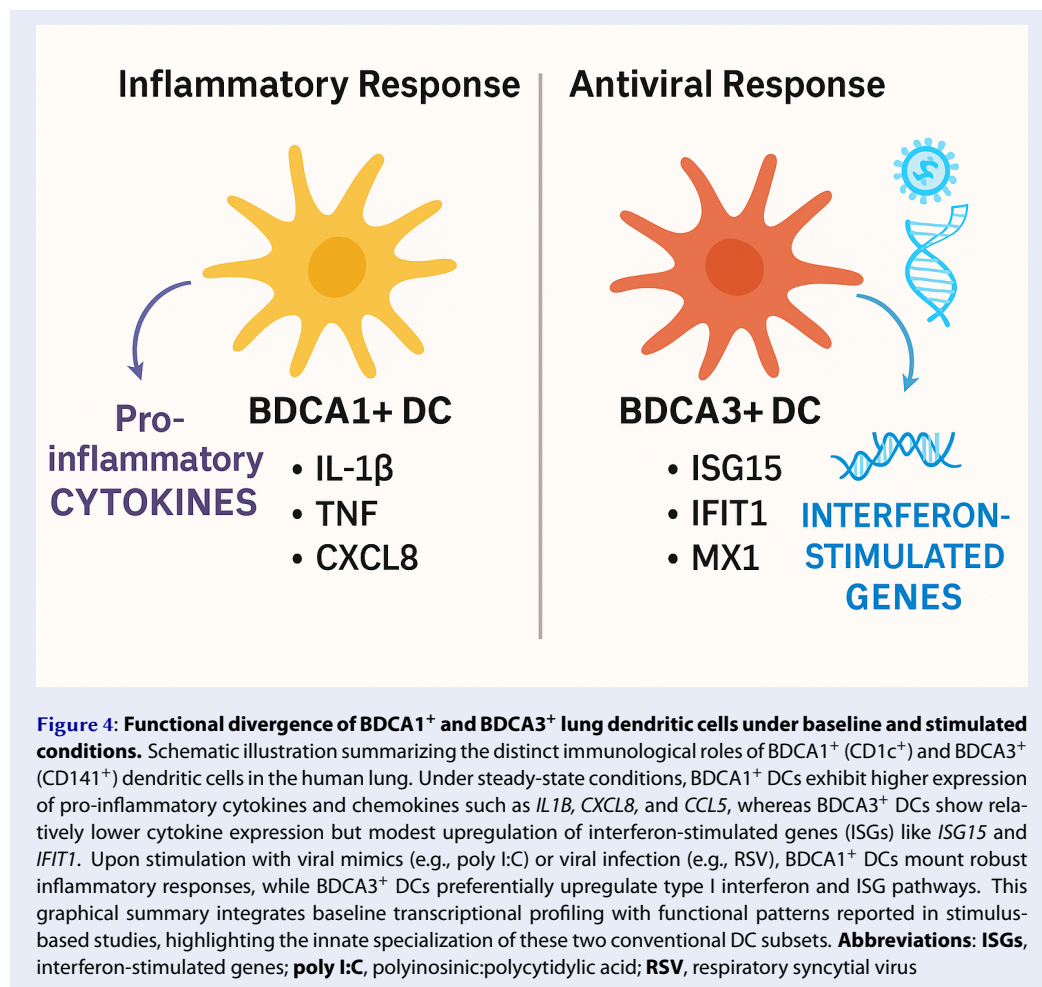
Gene	BDCA1+ Mean Expression	BDCA3+ Mean Expression	log ₂ fold change (BDCA1+ vs BDCA3+)
IL1B	2.4	0.8	1.58
CXCL8	3.1	1.2	1.37
CCL5	2.8	0.9	1.64
ISG15	1.2	2.5	-1.06
IFIT1	1	2.8	-1.48

Note: Mean expression values were calculated for BDCA1⁺ (cDC2) and BDCA3⁺ (cDC1) dendritic cells from the Human Lung Cell Atlas dataset. Log₂ fold changes reflect relative enrichment in BDCA1⁺ versus BDCA3⁺ cells. Positive values indicate higher expression in BDCA1⁺ DCs, while negative values indicate enrichment in BDCA3⁺ DCs.

Table 2: Stimulus-dependent immune responses of human lung dendritic cell subsets based on published studies

Stimulus	DC Subset	Upregulated Markers / Cytokines
Poly I:C	BDCA1 ⁺ & BDCA3 ⁺	CD40, CD80, CD86, IFNB1, CXCL10
RSV	BDCA1 ⁺	IL-1β, IL-6, IL-8, TNF-α, CXCL10, IL-12
RSV	BDCA3 ⁺	IL-8, IL-10, IL-12; CD86, PD-L1

Note: Summary of previously reported transcriptional responses in BDCA1⁺ (CD1c⁺) and BDCA3⁺ (CD141⁺) dendritic cells upon stimulation with polyinosinic:polycytidylic acid (poly I:C) or respiratory syncytial virus (RSV). Both DC subsets upregulate maturation markers (CD40, CD80, CD86) and type I interferon genes in response to poly I:C. RSV infection elicits a broader pro-inflammatory cytokine response in BDCA1⁺ DCs, while BDCA3⁺ DCs display selective upregulation of IL-10, IL-12, and co-inhibitory ligands such as PD-L1.



Exploratory Identification of Potential Regulatory “Switch” Factors

To further interpret the subset-specific expression landscape, we surveyed known transcriptional hierarchies associated with the leading differentially expressed cytokine- and interferon-related genes. Given their established roles and the ISG-biased profile of BDCA3⁺ cells, the transcription factors IRF7 and BATF3—both pivotal for type I interferon signalling and antigen cross-presentation—emerge as plausible molecular ‘switches’ that delineate BDCA3⁺ antiviral specialisation from BDCA1⁺ inflammatory priming. IRF7 serves as a master activator of ISGs, including ISG15 and IFIT1, whereas BATF3 is indispensable for the development and function of cDC1 (BDCA3⁺) cells. Collectively, these literature-backed links imply that divergent regulatory hierarchies, rather than stochastic variations, underpin the functional polarisation of human lung dendritic-cell subsets^{18,19}.

DISCUSSION

In this study, we sought to validate and integrate previously reported functional distinctions between BDCA1⁺ and BDCA3⁺ dendritic cells (DCs) in the human lung by re-analyzing publicly available transcriptomic datasets and placing these findings within the context of recent single-cell and functional studies. Using microarray data from GSE43184, we observed distinct immune-gene expression profiles: BDCA1⁺ DCs exhibited elevated basal levels of pro-inflammatory cytokines and chemokines, whereas BDCA3⁺ DCs displayed comparatively higher expression of interferon-stimulated genes (ISGs). These observations corroborate and extend prior reports, positioning our analysis as a cross-validation of existing single-cell and bulk transcriptomic findings rather than a de novo discovery effort. The observed patterns closely mirror those reported by the Human Lung Cell Atlas,

which identified similar subset-specific expression signatures using single-cell RNA-seq^{3,6}.

Our baseline analysis revealed that BDCA1⁺ DCs express IL1B, CXCL8, CXCL10, and related inflammatory mediators at higher levels than BDCA3⁺ DCs. This pattern indicates that BDCA1⁺ DCs are transcriptionally primed for innate immune activation and amplification of inflammation, in agreement with their documented roles in T-helper-cell priming and recruitment of effector leukocytes^{4,7}. In contrast, BDCA3⁺ DCs were enriched for ISGs such as ISG15 and IFIT1, supporting their established specialization in antiviral signaling and antigen cross-presentation⁴.

Multivariate principal-component and hierarchical clustering analyses further reinforced the transcriptional separation of the two DC subsets. Reactome-based pathway enrichment revealed that BDCA1⁺ DCs engage inflammatory, cytokine, and GPCR-related pathways—functional attributes characteristic of innate sentinels primed to respond to environmental threats.

Comparative validation using external resources strengthened these observations. Single-cell analyses from HLCA and PanglaoDB confirmed that the pro-inflammatory and chemotactic genes we identified as elevated in BDCA1⁺ DCs are also differentially expressed *in vivo*^{3,6}. This cross-platform concordance indicates that even a small-sample microarray dataset can reliably reflect the intrinsic transcriptional polarization of human lung DC subsets. Likewise, studies employing poly I:C and RSV stimulation have shown that BDCA1⁺ DCs mount robust inflammatory responses, whereas BDCA3⁺ DCs preferentially activate antiviral and regulatory programs^{7,8}. Together, these lines of evidence support a coherent model in which baseline gene-expression patterns reflect pre-configured immune specializations that manifest during pathogen encounter.

The distinct molecular predispositions of BDCA1⁺ and BDCA3⁺ DCs have practical implications for respiratory immunity and translational applications. BDCA1⁺ DCs, with their inflammatory bias, may respond rapidly to bacterial or fungal stimuli by producing cytokines that recruit neutrophils and T cells⁷. In contrast, BDCA3⁺ DCs, enriched in interferon signaling and cross-presentation pathways, appear optimised for antiviral defense and cytotoxic T-cell priming⁴. These complementary programs likely contribute to the lung's ability to balance inflammation with antiviral protection.

Our integrative analysis further highlights IRF7 and BATF3 as candidate transcriptional regulators that may govern the balance between inflammatory and antiviral programming. Consistent with previous studies demonstrating IRF7-driven interferon amplification and BATF3-dependent cDC1 differentiation^{20,21}, these factors align with the expression patterns observed here, where BDCA3⁺ DCs exhibit ISG-enriched antiviral profiles and BDCA1⁺ DCs display inflammatory-cytokine dominance. Although our study does not directly test transcription-factor activity, this literature-anchored inference provides a mechanistic framework for future experimental validation and underscores the value of cross-dataset synthesis in refining existing models of DC-subset specialization.

From a therapeutic perspective, selective modulation of DC subsets could inform vaccine and immunotherapy strategies. For instance, engaging BDCA3⁺ DCs may enhance antiviral immunity through adjuvants that promote cross-presentation²², while dampening BDCA1⁺ DC-driven cytokine cascades could mitigate hyperinflammatory conditions such as acute respiratory distress syndrome (ARDS) or severe viral pneumonia (e.g., COVID-19)¹⁰.

While our analysis offers integrative validation, several limitations warrant consideration. The small sample size ($n = 2$ per subset) in GSE43184 limits its statistical power, and results should thus be viewed as supportive, confirmatory evidence rather than definitive proof. The microarray platform's restricted gene coverage further limits discovery of novel transcripts and isoforms. Additionally, our inferences regarding responses to viral stimuli are based on secondary data rather than experimental validation. Future studies employing RNA-seq or functional assays could directly test these hypotheses. Despite these constraints, the reproducibility of expression trends across independent datasets and analytical platforms underscores the robustness of the observed subset specialization.

CONCLUSION

In summary, this validation-driven transcriptomic meta-analysis demonstrates that BDCA1⁺ and BDCA3⁺ dendritic cells (DCs) in the human lung are transcriptionally and functionally distinct, even at rest. BDCA1⁺ DCs harbor a gene-expression signature enriched for inflammatory mediators, suggesting a central role in initiating and amplifying local immune responses. Conversely, BDCA3⁺

DCs exhibit higher basal expression of interferon-stimulated genes, in line with their specialized antiviral and cross-presenting functions. Rather than revealing novel mechanisms, these data consolidate and extend previously reported differences via rigorous cross-dataset validation. The transcriptional divergence observed under steady-state conditions parallels the functional dichotomy seen after activation, underscoring the biological relevance of these baseline programs. Collectively, the study confirms that human lung DC subsets maintain stable, subset-specific transcriptional landscapes congruent with their established immunological roles, providing a framework for vaccine development, immunotherapeutic design, and targeted modulation of pulmonary inflammation.

ABBREVIATIONS

ARDS: Acute Respiratory Distress Syndrome; **BATF3:** Basic Leucine Zipper ATF-Like Transcription Factor 3; **BDCA1:** Blood Dendritic Cell Antigen 1; **BDCA3:** Blood Dendritic Cell Antigen 3; **cDC1:** Conventional Dendritic Cell Type 1; **cDC2:** Conventional Dendritic Cell Type 2; **CD:** Cluster of Differentiation; **DCs:** Dendritic Cells; **FDR:** False Discovery Rate; **GEO:** Gene Expression Omnibus; **GPCR:** G-Protein-Coupled Receptor; **HLCA:** Human Lung Cell Atlas; **IFNB1:** Interferon Beta 1; **IL:** Interleukin; **IRF7:** Interferon Regulatory Factor 7; **ISGs:** Interferon-Stimulated Genes; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **PCA:** Principal Component Analysis; **PD-L1:** Programmed Death-Ligand 1; **poly I:C:** Polyinosinic:Polycytidylic Acid; **RMA:** Robust Multi-array Average; **RSV:** Respiratory Syncytial Virus; **scRNA-seq:** Single-Cell RNA Sequencing; **TLR:** Toll-Like Receptor; **TNF:** Tumor Necrosis Factor

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The author thanks the creators of the GSE43184 dataset for making their data publicly accessible through the Gene Expression Omnibus (GEO). All analyses were performed using open-source R and Python packages in a reproducible computational environment. The author also acknowledges the assistance of ChatGPT (OpenAI) for language editing and grammar refinement. The author read and approved the final manuscript and is solely responsible for the content and integrity of this work.

Author's contributions

V.Y.: Conceptualization, Data Curation, Formal Analysis, Methodology, Visualization, Writing – Original Draft, Writing – Review & Editing. All authors read and approved the final manuscript.

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Availability of data and materials

All transcriptomic data used in this study are publicly available in the NCBI Gene Expression Omnibus (GEO) database. The primary microarray dataset analyzed (GSE43184) can be accessed at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43184>. External validation was performed using publicly available datasets from the Human Lung Cell Atlas and curated literature sources. All data processing, analysis scripts, and figure generation workflows are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they have not used generative AI (a type of artificial intelligence technology that can produce various types of content including text, imagery, audio and synthetic data. Examples include ChatGPT, NovelAI, Jasper AI, Rytr AI, DALL-E, etc) and AI-assisted technologies in the writing process before submission.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. Upham JW, Xi Y. Dendritic cells in human lung disease: recent advances. *Chest*. 2017;151(3):668–673. PMID: 27729261. Available from: <https://doi.org/10.1016/j.chest.2016.09.030>.

2. Langlois RA, Legge KL. Respiratory dendritic cells: mediators of tolerance and immunity. *Immunol Res.* 2007;39(1-3):128–145. PMID: 17917061. Available from: <https://doi.org/10.1007/s12026-007-0077-0>.
3. Domínguez Conde C, Xu C, Jarvis LB, Rainbow DB, Wells SB, Gomes T, et al. Cross-tissue immune cell analysis reveals tissue-specific features in humans. *Science.* 2022;376(6594):eabl5197. PMID: 35549406. Available from: <https://doi.org/10.1126/science.abl5197>.
4. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med.* 2010;207(6):1247–1260. PMID: 20479116. Available from: <https://doi.org/10.1084/jem.20092140>.
5. Coelho-Dos-Reis JG, Funakoshi R, Huang J, Pereira FV, Iketani S, Tsuji M. Functional human CD141+ dendritic cells in human immune system mice. *J Infect Dis.* 2020;221(2):201–213. PMID: 31647546. Available from: <https://doi.org/10.1093/infdis/jiz432>.
6. Franzén O, Gan LM, Björkegren JL. PanglaoDB: a web server for exploration of mouse and human single-cell RNA sequencing data. *Database (Oxford).* 2019;2019:baz046. PMID: 30951143. Available from: <https://doi.org/10.1093/database/baz046>.
7. Döring M, De Azevedo K, Blanco-Rodríguez G, Nadalin F, Satoh T, Gentili M, et al. Single-cell analysis reveals divergent responses of human dendritic cells to the MVA vaccine. *Sci Signal.* 2021;14(697). PMID: 34429383. Available from: <https://doi.org/10.1126/scisignal.abd9720>.
8. Reséndiz M, Valenzuela O, Hernández J. Response of the cDC1 and cDC2 subtypes of tracheal dendritic cells to porcine reproductive and respiratory syndrome virus. *Vet Microbiol.* 2018;223:27–33. PMID: 30173748. Available from: <https://doi.org/10.1016/j.vetmic.2018.07.012>.
9. Hato L, Vizcay A, Eguren I, Pérez-Gracia JL, Rodríguez J, Gállego Pérez-Larraya J, et al. Dendritic cells in cancer immunology and immunotherapy. *Cancers (Basel).* 2024;16(5):981. PMID: 38473341. Available from: <https://doi.org/10.3390/cancers16050981>.
10. Lucas C, Wong P, Klein J, Castro TB, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature.* 2020;584(7821):463–469. PMID: 32717743. Available from: <https://doi.org/10.1038/s41586-020-2588-y>.
11. Zhang B, Zhong W, Yang B, Li Y, Duan S, Huang J, et al. Gene expression profiling reveals candidate biomarkers and probable molecular mechanisms in chronic stress. *Bioengineered.* 2022;13(3):6048–6060. PMID: 35184642. Available from: <https://doi.org/10.1080/21655979.2022.2040872>.
12. Kushwah R, Hu J. Complexity of dendritic cell subsets and their function in the host immune system. *Immunology.* 2011;133(4):409–419. PMID: 21627652. Available from: <https://doi.org/10.1111/j.1365-2567.2011.03457.x>.
13. Tel J, Schreibeit G, Sittig SP, Mathan TS, Buschow SI, Cruz LJ, et al. Human plasmacytoid dendritic cells efficiently cross-present exogenous Ags to CD8+ T cells despite lower Ag uptake than myeloid dendritic cell subsets. *Blood.* 2013;121(3):459–467. PMID: 23212525. Available from: <https://doi.org/10.1182/blood-2012-06-435644>.
14. Villani AC, Satija R, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science.* 2017;356(6335):eaah4573. PMID: 28428369. Available from: <https://doi.org/10.1126/science.aah4573>.
15. Dutertre CA, Becht E, Irac SE, Khalilnezhad A, Narang V, Khalilnezhad S, et al. Single-cell analysis of human mononuclear phagocytes reveals subset-defining markers and identifies circulating inflammatory dendritic cells. *Immunity.* 2019;51(3):573–589.e8. PMID: 31474513. Available from: <https://doi.org/10.1016/j.immuni.2019.08.008>.
16. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, et al. Critical role for CD103+/CD141+ dendritic cells bearing CCR7 for tumor antigen trafficking and priming of T-cell immunity in melanoma. *Cancer Cell.* 2016;30(2):324–336. PMID: 27424807. Available from: <https://doi.org/https://doi.org/10.1016/j.ccell.2016.06.003>.
17. Gupta MR, Kolli D, Garofalo RP. Differential response of BDCA-1+ and BDCA-3+ myeloid dendritic cells to respiratory syncytial virus infection. *Respir Res.* 2013;14(1):71. PMID: 23829893. Available from: <https://doi.org/https://doi.org/10.1186/1465-9921-14-71>.
18. Peng Q, Qiu X, Zhang Z, Zhang S, Zhang Y, Liang Y, et al. PD-L1 on dendritic cells attenuates T cell activation and regulates response to immune checkpoint blockade. *Nat Commun.* 2020;11(1):4835. PMID: 32973173. Available from: <https://doi.org/https://doi.org/10.1038/s41467-020-18570-x>.
19. Zhang S, Audiger C, Chopin M, Nutt SL. Transcriptional regulation of dendritic cell development and function. *Front Immunol.* 2023;14:1182553. PMID: 37520521. Available from: <https://doi.org/https://doi.org/10.3389/fimmu.2023.1182553>.
20. Ning S, Pagano JS, Barber GN. IRF7: activation, regulation, modification and function. *Genes Immun.* 2011;12(6):399–414. PMID: 21490621. Available from: <https://doi.org/https://doi.org/10.1038/gene.2011.21>.
21. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science.* 2008;322(5904):1097–1100. PMID: 19008445. Available from: <https://doi.org/https://doi.org/10.1126/science.1164206>.
22. Pulendran B, S Arunachalam P, O'Hagan DT. Emerging concepts in the science of vaccine adjuvants. *Nat Rev Drug Discov.* 2021;20(6):454–475. PMID: 33824489. Available from: <https://doi.org/10.1038/s41573-021-00163-y>.