Rheumatology, 2025, **64**, SI37–SI46
https://doi.org/10.1093/rheumatology/keae572
Advance access publication 16 October 2024
Original Article

British Society for Rheumatology RHEUMATOLOGY OXFORD

Clinical science

Unique transcriptomic profile of peripheral blood monocytes in rheumatoid arthritis-associated interstitial lung disease

Jill A. Poole[1,*], Aaron Schwab[1], Geoffrey M. Thiele[1,2], Bryant R. England[1,2], Amy J. Nelson[1], Angela Gleason[1], Michael J. Duryee[1,2], Kristina L. Bailey[1,2], Debra J. Romberger[1,2], Daniel Hershberger[1], Joel Van De Graaff[1,2], Sara M. May[1,2], Rhonda Walenz[1], Bridget Kramer[1], Ted R. Mikuls[1,2]

[1]Department of Internal Medicine, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA
[2]Medicine and Research, Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, NE, USA

*Correspondence to: Jill A. Poole, Department of Internal Medicine, College of Medicine, University of Nebraska Medical Center, 985900 Nebraska Medical Center, Omaha, NE 68198-5900, USA. E-mail: japoole@unmc.edu

Abstract

Objectives: Though interstitial lung disease (ILD) contributes to excess morbidity and mortality in rheumatoid arthritis (RA), RA-ILD pathogenesis remains incompletely defined. As intermediate, non-classical and suppressed CD14$^+$ monocytes are expanded in RA-ILD, this study sought to characterize gene expression profiles of circulating monocytes in RA-ILD.

Methods: Peripheral blood mononuclear cells were collected from patients with RA without lung disease ($n=5$), RA-ILD ($n=5$), idiopathic pulmonary fibrosis (IPF; $n=5$), and controls without lung and autoimmune disease ($n=4$). RNA was extracted from CD14$^+$ isolated monocytes and subjected to transcriptional analysis of 1365 genes. Gene enrichment and pathway analyses were performed.

Results: Unsupervised clustering grouped patients with RA-ILD together with IPF for myeloid innate genes. For fibrosis genes, patients with RA-ILD clustered independent of comparator groups. There were 103, 66 and 64 upregulated and 66, 14 and 25 downregulated genes for RA-ILD, RA, and IPF, *vs* controls, respectively. For RA-ILD, there was increased expression of genes involved in regulating inflammation and fibrosis (*SOCS3*, *CECAM1*, *LTB4R2*, *CLEC7A*, *IRF7*, *PHYKPL*, *GBP5*, *RAPGEF*), epigenetic modification (*KDM5D*, *KMT2D*, *OGT*) and macrophage activation. Top canonical pathways included macrophage differentiation-activation, IL-12, neuroinflammatory, glucocorticoid receptor and IL-27 signalling.

Conclusions: Circulating monocytes in RA-ILD patients demonstrate unique gene expression profiles, with innate immune gene features more aligned with IPF as opposed to RA in the absence of clinical lung disease, and with fibrosis gene expression that was distinct from RA and IPF. These studies are important for understanding disease pathogenesis and may provide information for future therapeutic targets in RA-ILD.

Keywords: interstitial lung disease, rheumatoid arthritis, monocyte, circulating, blood, gene, pathway.

Rheumatology key messages

- Circulating monocytes in RA-ILD patients demonstrate unique gene expression profiles.
- Innate immune genes of RA-ILD aligned with IPF as opposed to RA without lung disease.
- Fibrosis genes were distinct from RA and IPF, all to information future targeted approaches.

Introduction

Interstitial lung disease (ILD) in rheumatoid arthritis (RA) clinically impacts ∼10% of patients with RA and subclinically affects an additional 20–30%, contributing to significant morbidity and mortality [1, 2]. ILD in RA includes usual interstitial pneumonia (UIP), non-specific interstitial pneumonia, obliterative bronchiolitis and organizing pneumonia [3]. In comparison, idiopathic pulmonary fibrosis (IPF) is characterized by the UIP pattern. Whereas therapeutic options for ILD and pulmonary fibrosis have expanded with the availability of anti-fibrotic drugs, these therapies have limited efficacy and tolerability [4, 5], and respiratory disease remains among the most frequent causes of death in RA [6, 7]. The complexity of RA-ILD pathogenesis includes the interplay among autoimmunity, inflammatory and fibrotic pathway dysregulation, oxidative stress, and exposure to various environmental and occupational factors in genetically susceptible persons [6, 8]. In addition, the immune microenvironment of

Received: 24 June 2024. **Accepted:** 27 September 2024

peripheral blood and other cellular mediators likely serves an important pathogenic role in RA-ILD with recent evidence supporting expansion of several circulating myeloid cell subpopulations [9].

Peripheral blood monocytes are important immune cells as they present antigen, activate self-reactive T cells, migrate to sites of inflammation, differentiate into macrophages, and secrete mediators to orchestrate tissue inflammation, autoimmunity and wound repair processes. In both IPF and RA-ILD, high monocyte counts have been associated with poor prognosis [10, 11]. In patients with early RA and particularly RA characterized by active joint disease, non-classical or intermediate monocytes are also increased [12–14]. Monocytic myeloid-derived suppressor cells (mMDSCs) represent a subpopulation of $CD14^+$ monocytic cells ($HLA\text{-}DR^{lo}CD33^{hi}$) that are typically associated with poor prognosis in cancer [15], with evolving evidence suggesting an association of these cells with inflammatory and fibrotic diseases including both RA and IPF [9, 16–18]. Recently, we identified a significant expansion of circulating $CD14^+$ monocytes in RA-ILD, demonstrating an increase in subpopulations of alternatively activated intermediate monocytes (i.e. $HLA\text{-}DR^{hi}$, $CD16^{hi}$ expression) as well as expansion of monocytes with suppressive features (i.e. $HLA\text{-}DR^{lo}$, $CD16^{lo}CD15^{lo}CD33^{hi}$ expression, mMDSCs) [9]. Collectively, the phenotypical profile of these circulating $CD14^+$ monocytes in patients with RA-ILD was more aligned with IPF than with RA in the absence of clinically apparent lung disease [9]. However, the precise contribution of these cells to the pathogenesis of RA-ILD remains elusive.

Transcriptome analysis represents an added dimension to understanding the dynamics of gene expression and the pathological roles of immune cells. In general, transcriptome analysis in autoimmune diseases including systemic lupus erythematous (SLE) and RA has identified prominent IFN gene signatures [19]. In IPF, it has been demonstrated that S100 calcium binding protein A12 is highly expressed in monocytes and as a result has been proposed as a prognostic biomarker [20]. In RA-ILD, a recent study investigated gene expression of whole blood of RA-ILD *vs* RA without ILD in 12 female participants. Demonstrating nine significantly upregulated genes, most of which have been implicated in the pathogenesis of fibrosis, the results of this study provided preliminary support for the use of gene expression profiling as a tool in the early detection of RA-ILD [21]. Whether transcriptomic analysis of circulating monocytes might also identify informative biomarkers in RA-ILD has not been subject to intensive investigation.

The objective of this study, therefore, was to characterize the gene expression profile of peripheral blood $CD14^+$ monocytes in RA-ILD patients in comparison with patients with RA without lung disease, patients with IPF without autoimmunity, and a comparator group of controls without lung disease or evidence of systemic autoimmunity. In addition to providing new disease biomarkers, the identification of unique or overlapping gene expression profiles could inform disease pathogenesis and potentially identify future therapeutic targets for RA-ILD.

Methods

Peripheral blood samples

Blood samples from participants were obtained after receiving written informed consent and following approval of the Institutional Review Board at the Veterans Affairs (VA) Nebraska-Western Iowa Health Care System and University of Nebraska Medical Center (UNMC). Blood was collected between January 2022 and January 2023. Details of the enrolment criteria have been previously published [9]. Briefly, all patients with RA fulfilled the 1987 American College of Rheumatology classification criteria [22]. The presence of RA-ILD was confirmed by a board-certified subspecialist (pulmonologist or rheumatologist) and the presence of supportive chest CT findings. Patients were defined as having RA without ILD in the absence of a clinical diagnosis of ILD or past chest imaging findings suggestive of ILD. All patients with IPF without clinically apparent autoimmune disease were diagnosed by a board-certified pulmonologist and had confirmatory findings of UIP by chest CT imaging. However, these IPF patients were not systematically tested for circulating autoantibodies. A convenience sample of 'control' comparator patients was recruited from the VA allergy clinic and included patients with allergic rhinitis on specific allergy desensitization and in the absence of clinically apparent lung disease and/or autoimmunity. Venous blood samples were collected in EDTA tubes and processed within 2 h of collection.

Dyspnoea symptom assessment and clinical data

Participants completed the University of California, San Diego (UCSD) Shortness of Breath Questionnaire (SOBQ), a 24-item dyspnoea questionnaire with scores ranging from 0 to 120, with higher scores indicating greater dyspnoea [23]. For patients with RA, clinical data, disease severity measures and medication use were recorded by the treating rheumatologists. Lung function indices for RA-ILD and IPF were abstracted from patients' medical records.

$CD14^+$ monocyte and RNA isolation

Peripheral blood mononuclear cells (PBMCs) were first isolated from peripheral blood by Histopaque-1077 (Millipore-Sigma, Burlington, MA, USA) density gradient centrifugation. PBMCs were washed and $CD14^+$ monocytes were selected using the MACS magnetic microbead positive selection kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Post-selection monocyte purity was determined using Diffquick staining and enumeration of cytospin preparations. Monocyte purity ranged from 85.6% to 90.6%. Next, $CD14^+$ selected monocytes were washed and lysed with RLT buffer containing β-mercaptoethanol for RNA isolation as per the manufacturer's instructions with Qiagen RNAeasy Micro Kit (Qiagen, Germantown, MD, USA).

NanoString nCounter system

Quality and quantity of total RNA was evaluated using a Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA) and Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA), respectively. Total RNA (>4 ng/μl) was hybridized and processed per the manufacturer's suggested protocol with capture and reporter probes to prepare target-probe complexes using reagents from the Human Innate Immune Myeloid (770 genes) and Human Fibrosis (770 genes) profiling panel (NanoString, Seattle, WA, USA). There were 175 overlapping genes between panels and gene data from the first panel (human innate immune myeloid panel) were utilized for combined analyses, noting congruency between panels. Sequencing libraries were generated by the UNMC Next Generation Sequencing Core beginning with 500 ng of total

RNA from each sample using the NuGEN Universal Plus mRNA-Seq library kit from TECAN (Redwood City, CA, USA) following the manufacturer's recommended procedure. Resulting libraries were assessed for size of insert by analysis of an aliquot of each library on a bioanalyser instrument (Agilent Technologies). Each library had a unique indexing identifier barcode allowing the individual libraries to be multiplexed together for efficient sequencing. Multiplexed libraries were sequenced on a single 150-cycle mid-output flow cell of the NextSeq550 Sequencer (Illumina, San Diego, CA, USA) using a 2×75 bp paired end protocol to generate a total of ~50 million pairs of reads for each sample.

Statistics

For NanoString analysis, the nSolver Analysis Software was utilized to manage and analyse data. After importing raw data, experimental properties included the following: background subtraction parameters: 30; normalized mean type: geometric mean; positive control normalization, threshold minimum: 0.3 and threshold maximum: 3.0; code set content normalization threshold minimum: 0.1 and maximum: 10; ratio data parameters: all pairwise ratios. Analysis properties included normalization of data to housekeeping genes, utilization of gene-specific *z*-scores, exclusion of controls, and preset exclusion of genes with <10% of samples outside of the interval specified by the upper and lower bound values (myeloid innate panel lower bound 1.0 to upper bound 323 700 and fibrosis panel lower bound 1.0 to upper bound 64 364). In total, this included 736 for myeloid innate panel and 301 for fibrosis panel. Using unsupervised, machine-based, hierarchical agglomerative clustering with settings of linkage type of average and metric type of Pearson correlation, clustering by participant was performed with corresponding data visualization by heat map. Next, using the same settings for individual participant clustering, gene transcript average counts were normalized to housekeeping genes by participant group. Subsequent hierarchical agglomerative clustering was conducted for the entirety of the gene set as well as for the 30 genes with the highest average gene transcripts, which is referred to as 'top differentially expressed genes' (DEGs). Next, ratio data to include $\log_2$ ratio with *P*-value determined from NSolver software analysis was exported for subsequent supervised analyses. Volcano plots and heatmaps of statistically significant (*P*-value <0.05 or $-\log_{10}$ *P*-value >1.3) and $\log_2$-fold changes were generated using GraphPad Prism software, version 10.2.2 (GraphPad Software, Boston, MA, USA). Gene enrichment analyses were performed using Ingenuity Pathway Analysis (IPA; Qiagen Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis). The R package *GOPlot* was utilized to visualize the relationship between genes and enriched pathways.

Results

Participant characteristics

Characteristics of the 19 study participants (five with RA-ILD, five with RA in absence of ILD, five with IPF and four controls) are summarized in Table 1. Participants with RA-ILD, RA, and IPF were older than controls, though age was overlapping among the disease groups. Participants across groups were predominately male and self-reported White race, reflecting characteristics of the larger VA population. A history of ever smoking was higher among participants with RA and RA-ILD followed by those with IPF and lowest in controls. Shortness of breath, as assessed by the UCSD-SOBQ, although generally low, demonstrated increased symptoms (total score) in patients with RA-ILD, RA and IPF *vs* controls with the highest symptomatic dyspnoea observed in those with IPF. Based on CT findings, RA-ILD patients were categorized as having (i) typical UIP pattern with honeycombing, reticular pattern with peripheral traction, and/or fibrosis ($n = 4$, 80%) or (ii) probable UIP pattern (indeterminate phenotype) with bibasilar interstitial thickening and subpleural reticulation ($n = 1$, 20%) [24, 25]. Further characteristics of patients with RA and RA-ILD are listed in Supplementary Table 1, available at *Rheumatology* online.

Unsupervised hierarchical agglomerative clustering of DEGs of CD14$^+$ monocytes across RA-ILD, RA, IPF and control participants

Gene expression profiles of circulating CD14$^+$ monocytes from patients with RA-ILD were compared with those with RA without lung disease, IPF without autoimmunity and controls for 770 myeloid innate genes and 770 fibrosis genes. Heatmap analyses with corresponding hierarchical dendrograms demonstrate the unsupervised clustering of the

Table 1. Participant characteristics

	Control ($n = 4$)	RA ($n = 5$)	RA-ILD ($n = 5$)	IPF ($n = 5$)
Age, median (range), years	53 (40–57)	68 (39–82)	75 (66–81)	79 (73–85)
Race, *n* (%)				
White American	4 (100)	5 (100)	3 (60)	15 (100)
Black/African American	0 (0)	0 (0)	2 (40)	0 (0)
Sex, *n* (%)				
Male	2 (50)	4 (80)	5 (100)	5 (100)
Female	2 (50)	1 (20)	0 (0)	0 (0)
Cigarette Smoking, *n* (%)				
Never	3 (75)	1 (20)	3 (60)	4 (80)
Ever	1 (25)	3 (60)	1 (20)	1 (20)
Current	0 (0)	1 (20)	1 (20)	0 (0)
UCSD SOBQ total score, median (IQR)	3.5 (1–7)	23 (11–63)	28 (9–36)	65 (27–103)
Lung function, median (IQR)				
FVC, % predicted	—	—	73 (65–81)	55 (49–71)
DLCO, % predicted	—	—	50 (48–71)	33 (22–53)

DLCO: Diffusion capacity of the lungs for carbon monoxide; FVC: Forced vital capacity; ILD: interstitial lung disease; IPF: idiopathic pulmonary fibrosis; IQR: interquartile range; UCSD SOBQ: University of California, San Diego Shortness of Breath Questionnaire.

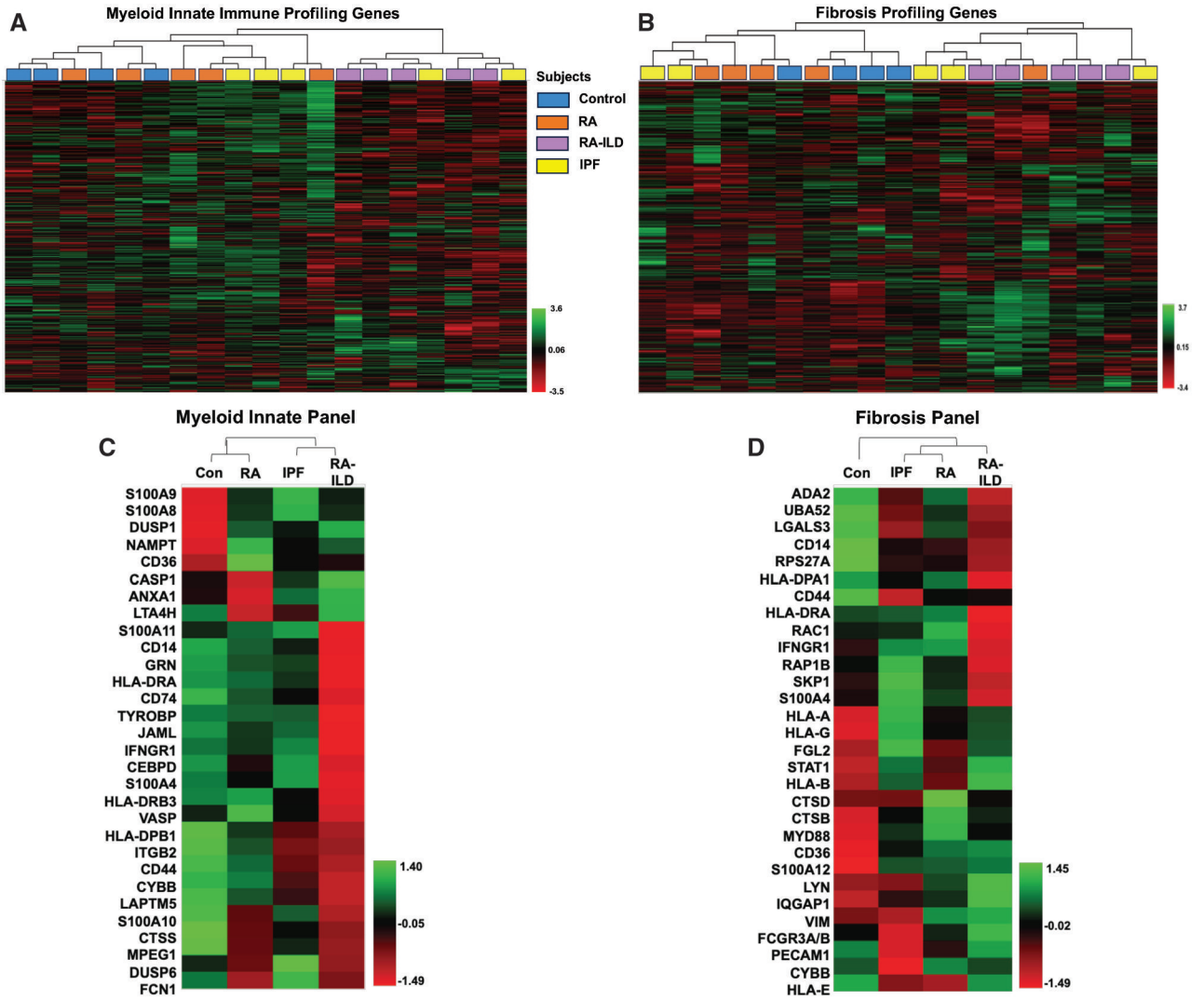

Figure 1. Differential expression analysis of monocyte genes across RA-ILD, RA, IPF and controls. (**A** and **B**) Heatmaps of unsupervised hierarchical agglomerative clustering of expression pattern of genes of the NanoString human myeloid innate (**A**) and fibrosis (**B**) profiling panel of each monocyte sample ($n = 19$) according to their expression and similarity. Subject group is colour coded as blue (control), orange (RA), purple (RA-ILD) and yellow (IPF). (**C** and **D**) Unsupervised clustering of the expression pattern of the top 30 differentially expressed genes (DEGs) by innate (**C**) and fibrosis (**D**) panels normalized to housekeeping genes by average gene transcript count. Analysis by NSolver with linkage type of average and metric type of Pearson correlation. The scheme represents the *Z*-score distribution from low to high expression. ILD: interstitial lung disease; IPF: idiopathic pulmonary fibrosis

NanoString human myeloid innate (Fig. 1A) and fibrosis (Fig. 1B) profiling panels of the gene set of each subject as well as the top 30 DEGs by myeloid innate (Fig. 1C) and fibrosis (Fig. 1D) panels. Note, this hierarchical dendrogram clustering by participant group was the same for the full gene set (data not shown) and top 30 DEGs shown here. Unsupervised myeloid innate gene analysis of monocytes demonstrated a clear stratification of patients with RA-ILD clustering together with IPF as opposed to RA in the absence of ILD (RA alone) or controls whereas RA alone aligned more closely with controls. In comparison, the fibrosis genes of monocytes from each patient with RA-ILD clustered together without clear associations with any of the other comparator patient groups. Controls and patients with RA alone demonstrated visual stratification together whereas patients with IPF did not demonstrate observable clustering of monocytes by the fibrosis genes interrogated.

By these machine-based analyses, the relative expression of inflammatory innate immune genes (*DUSP1*, *NAMPT*, *CASP1*, *ANXA1*, *LTA3*) and fibrosis genes (*LYN*, *IQGAP1*, *VIM*, *FCGR3A/B*, *PECAM1*, *STAT1*, *HLA-A/G/BE*) were increased in the monocytes of RA-ILD patients. Relative to the comparator groups, there was reduced DEG expression in RA-ILD monocytes for a large number of genes.

Fold-change in DEGs of circulating monocytes in RA-ILD, RA and IPF vs controls

DEGs of circulating $CD14^+$ monocytes of RA-ILD, RA and IPF participants were compared with controls with manual curation of the significant ($P < 0.05$) top upregulated and top downregulated genes identified by myeloid innate and fibrosis profile panels (Fig. 2A). By fold-change ($\log_2$-fold) of participant group to controls, 27, 35 and 37 myeloid innate (panel) and 77, 15 and 32 fibrosis (panel) up-regulated genes

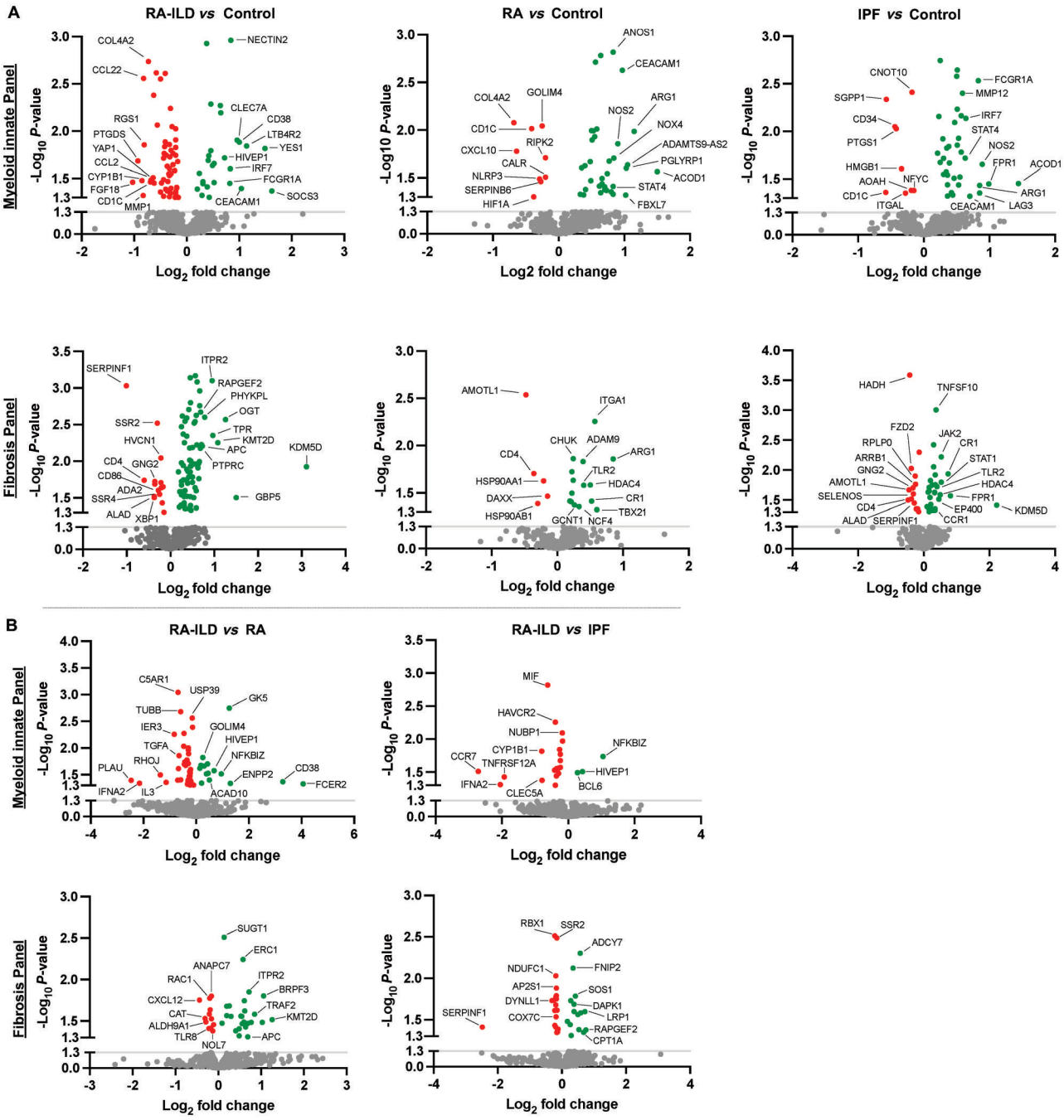

Figure 2. Differential gene expression of circulating monocytes in RA-ILD, RA, IPF and controls. Volcano plots showing the distribution of $\log_2$-fold change and *P*-value for myeloid innate (top panel) and fibrosis (bottom panel) profile differentially expressed genes of (**A**) patient group *vs* control participants and (**B**) RA-ILD *vs* RA and IPF. Dots represent significantly ($P < 0.05$ or $-\log_{10}$ *P*-value >1.3) upregulated (positive) and downregulated (negative) genes (*vs* control) with up to the top 10 genes by fold-change named. ILD: interstitial lung disease; IPF: idiopathic pulmonary fibrosis

with 50, 9 and 11 myeloid innate and 17, 5 and 14 fibrosis downregulated genes were identified in monocytes of RA-ILD, RA and IPF (respectively) (Supplementary Tables 2–7, available at *Rheumatology* online). Correspondingly, comparisons were also conducted for RA-ILD *vs* RA and RA *vs* IPF by myeloid innate and fibrosis profile panels (Fig. 2B, Supplementary Tables 8–11, available at *Rheumatology* online). For RA-ILD *vs* RA, there were 15 and 25 upregulated and 28 and 12 downregulated genes (myeloid innate and fibrosis panels, respectively). For RA-ILD *vs* IPF, there were 3 and 25 upregulated and 19 and 20 downregulated (myeloid innate and fibrosis panels, respectively).

The top 10 DEGs from each panel identified in RA-ILD patients (*vs* controls) are summarized in Table 2 (and shown in Fig. 2) with name and/or main biological processes. Genes identified include those involved in regulating inflammation and fibrosis (*SOCS3*, *CECAM1*, *LTB4R2*, *CLEC7A*, *IRF7*, *PHYKPL*, *GBP5*, *RAPGEF2*, *HIVEP1*), epigenetic

Table 2. Top differentially expressed genes identified in RA-ILD patients ($n = 5$)

Gene	Name and main biological process
Myeloid innate panel[a]	
SOCS3	Suppressor of cytokine signalling 3; negative regulator of cytokine signalling
YES1	Src family tyrosine kinase, regulation of cell growth and survival, apoptosis
LTB4R2	Leukotriene B4 receptor; regulate inflammation, reactive oxygen species production
FCGR1A	CD64, high-affinity IgG Fc receptor 1; link of humoral and cell mediated responses
CD38	Cyclic ADP ribose hydrolase; cell adhesion, signal transduction, calcium signalling
CLEC7A	Dectin-1, C-type lectin domain family; pattern recognition receptor
NECTIN2	Type 1 membrane glycoprotein, cell polarization, proliferation, differentiation, survival
IRF7	IFN regulatory factor, regulates type 1 interferon genes
CEACAM1	CD66a, adhesion molecule; potentiates inflammation and fibrosis
HIVEP1	Zinc finger protein 40; negative regulator of NF-κB and cytokine signalling
Fibrosis panel[a]	
KDM5D	Histone lysine demethylase, zinc finger protein, sex chromosome encoded
GBP5	Guanylate binding protein, IFN-inducible GTPAse, inflammasome assembly activator
OGT	Glycotransferase
KMT2D	Histone lysine methyltransferase
TPR	Translocated promoter region gene, nuclear export of mRNAs
ITPR2	Inositol trisphosphate receptor; regulator of calcium transportation, proliferation
PHYKPL	Phosphohydroxy-lysine phospholyase, related to ECM and collagen organization
PTPRC	CD45, protein tyrosine phosphatase; regulator of T and B cell signalling
APC	Tumour suppressor gene, regulator of β-catenin and cadherin, cell cycle
RAPGEF2	Rap GTPase, Ras activator, cell maturation, inflammation

Genes were ranked according to the fold-change of significantly ($P < 0.05$) up- or down-regulated genes (RA-ILD *vs* control).
[a] NanoString targeted gene profile panels. ECM: extracellular matrix; NF-κB: nuclear factor κB.

modification (*KDM5D*, *KMT2D*, *OGT*), and M1-like monocyte/macrophage activation (*GBP5*, *RAPGEF2* [Ras-activators], *FCGR1A*, *SOCS3*).

Total DEGs expressed of patients with RA-ILD overlap more with IPF than RA

Next, the DEGs for patients (RA-ILD, RA and IPF) *vs* controls for the myeloid innate and fibrosis profile panel were combined for a total of 1365 unique genes. In total, there were 103 upregulated and 66 downregulated genes for RA-ILD, 66 upregulated and 14 downregulated for RA, and 64 upregulated and 25 downregulated for IPF, with shared genes across patient groups represented by Venn diagram (Fig. 3). There were 12 upregulated genes shared by RA-ILD, RA and IPF (*FCGR1A*, *CEACAM1*, *ANOS1*, *NOS2*, *HDAC4*, *CFLAR*, *GCNT1*, *STATE3*, *TLR2*, *CHUK*, *PTPN1*, *NUMB*), 3 upregulated genes shared by RA-ILD and RA (*SERPINE 3*, *NECTIN2*, *KDM3B*) and 12 upregulated genes shared by RA-ILD and IPF (*IRF7*, *PTPRC*, *JAK2*, *JAK3*, *IL4R*, *ACAD10*, *RICTOR*, *MAPK14*, *EP400*, *CASP4*, *CDKLN2C*, *CSNK1D*). There were fewer shared downregulated genes, with two downregulated genes shared by RA-ILD, RA and IPF (*CD1C*, *CD4*), one gene (*COL4A2*) shared by RA-ILD and RA, and eight genes (*CD34*, *LGALS3*, *NFYC*, *SERPINF1*, *ALAD*, *GNG2*, *RPLP0*, *HADH*) shared by RA-ILD and IPF. Thus, there were numerically more DEGs shared between patients with RA-ILD and IPF as compared with RA.

Top canonical pathways across RA-ILD, RA and IPF patients

To achieve further insights into the functional consequences of differential gene expression in CD14$^+$ monocytes of RA-ILD participants as well as that observed in RA and IPF, a pathway enrichment analysis using all the combined DEGs from the myeloid innate immunity and fibrosis profile panels was performed. For RA-ILD participants, this analysis confirmed the differential regulation of genes involved in macrophage differentiation and activation in RA with the following signalling pathways implicated: IL-12, glucocorticoid receptor, and IL-27 signalling pathways; neuroinflammatory pathways were also implicated (Fig. 4). Pathways involved in regulating epithelial mesenchymal transition by growth factors were also implicated, potentially suggesting a role for macrophages in orchestrating wound repair, regulation and fibrosis important in ILD development. For RA patients without lung disease, perturbation of the following signalling pathways was demonstrated: pathogen induced cytokine, immunogenic cell death, Th1 and Th2, macrophage classical activation, IL-27, and interferon regulatory pathways (Supplementary Fig. 1, top panel, available at *Rheumatology* online). For IPF patients, neuroinflammation, production of nitric oxide and reactive oxygen species and inducible nitric oxide synthase (iNOS), IL-12, IL-27 and macrophage alternative activation signalling pathways were significantly affected (Supplementary Fig. 1, bottom panel, available at *Rheumatology* online). Thus, alteration of the IL-12, IL-27 and neuroinflammation signalling pathways was shared between RA-ILD and IPF.

Discussion

The most over-represented cause of death in RA is ILD [26, 27]; yet, the key cellular players, biomarkers of disease and efficacious therapeutic modalities remain limited. Numbers of peripheral blood monocytes have been associated in at least one study with poor outcomes in RA-ILD [11], and prior work demonstrated increased numbers of total monocytes in RA-ILD with expansion of several monocyte subpopulations including intermediate monocytes, non-classical monocytes and mMDSCs [9]. However, to our knowledge, there have been no prior transcriptomic analyses of circulating monocytes derived from individuals with RA-ILD. Herein we show unique transcriptomic profiles of circulating CD14$^+$ monocytes in RA-ILD that include the upregulation of M1-like macrophage genes with inflammatory and fibrotic potential, epigenetic modulatory genes, and inflammatory and suppressive regulatory genes that are likely important in driving disease. Notably, the monocyte DEG patterns of RA-ILD were more aligned to patients with IPF than RA in the absence of lung disease, suggesting that a transitioning,

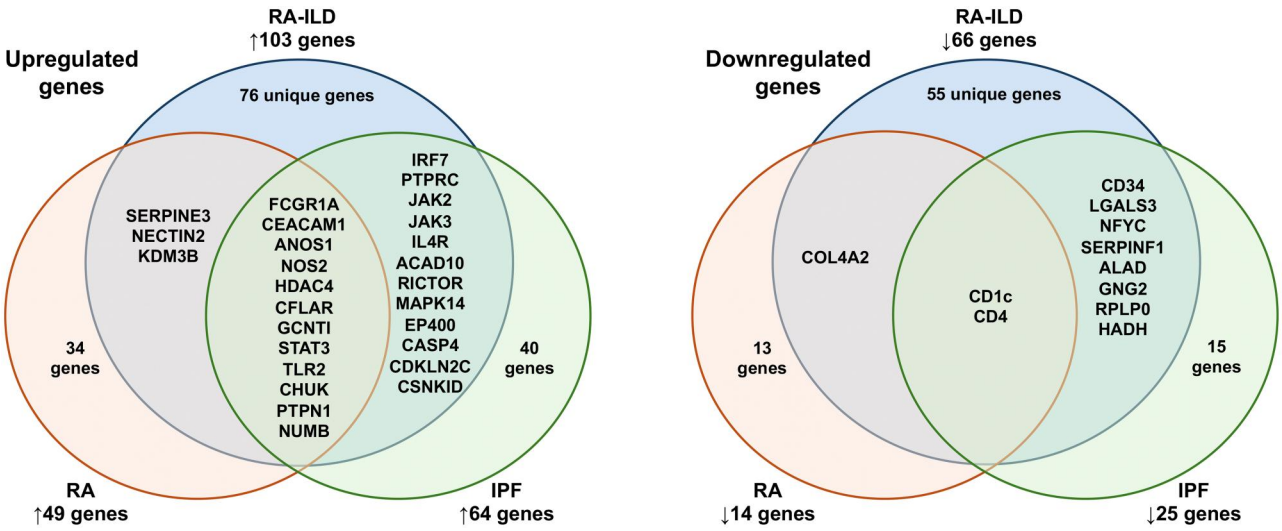

Figure 3. Combined total differentially expressed genes (DEGs) of circulating monocytes shared across RA-ILD, RA and IPF. Venn diagram demonstrating shared upregulated and downregulated genes (*vs* control with statistically significant *P*-value <0.05) that patients with RA-ILD share with RA and/or IPF. All significantly upregulated and downregulated DEGs listed in Supplementary Tables 2–7, available at *Rheumatology* online. ILD: interstitial lung disease; IPF: idiopathic pulmonary fibrosis

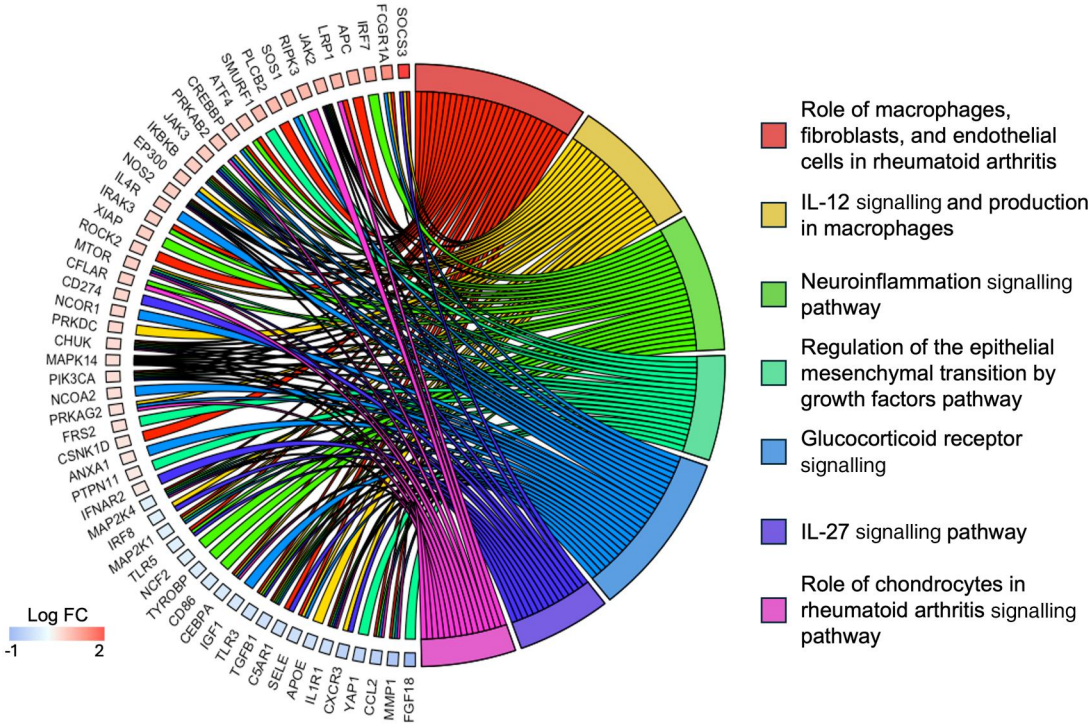

Figure 4. Top canonical pathways and implicated differentially expressed genes (DEGs) of RA-ILD monocytes. Chord plot showing the combined DEGs ($P < 0.05$) from myeloid innate immunity and fibrosis panels and the top canonical pathways (adjusted $P < 0.05$) implicated by ingenuity pathway analysis (IPA). FC: fold change; ILD: interstitial lung disease

activated monocyte to macrophage cell phenotype may orchestrate and skew RA towards lung disease.

To date, there has been one study of whole blood DEGs analysed from female patients with RA-ILD *vs* RA without ILD that found nine upregulated genes most implicated in fibrosis including *ARG1*, *TYMS*, *SORT1*, *MKI67*, *BIRC5*, *OLFM4*, *MS4A4A*, *CLEC12A* and *LINC029667* [21]. In our approach, focused on circulating monocytes, a C-type lectin domain family member was also identified, Dectin-1 (*CLEC7A*), which represented a unique gene upregulated in patients with RA-ILD. Dectin-1 is a β-glucan receptor involved in anti-fungal defence expressed primarily on phagocytes [28]. Activation of Dectin-1 rapidly converts monocytes towards a pro-inflammatory, M1-like macrophage state, important in phagocytosis, the production of reactive oxygen species and inflammatory cytokines, Th17-type adaptive responses, and autoimmunity [29]. Dectin-1 signalling is regulated by CD45 (*PTPRC*) [29], another upregulated gene

identified in our RA-ILD (and IPF) participants. The Dectin-1 signalling pathway has been proposed to directly or indirectly contribute to the pathogenesis of fibrosing ILD [30]. Our results, coupled with observations from other existing reports, suggest that further studies are warranted to more precisely understand the role of C-type lectin domain family member signalling pathways in RA-ILD.

In focusing on the uniquely upregulated innate immune genes in monocytes of RA-ILD patients, elevated *SOCS3* expression may be particularly noteworthy. SOCS3 is typically regarded as a negative regulator of cytokine signalling, but SOCS3^{+} monocytes/macrophages have also been recognized to restore M1 polarization following an inflammatory insult [31]. In addition, SOCS3 is critical in antigen processing and presentation, IFN-γ signalling, regulation of leucocyte activation, and was previously identified as a significantly upregulated gene in PBMCs from RA patients [32]. Leukotriene B4 (*LTB4R2* gene), upregulated in our RA-ILD participants, has been implicated in the induction of pain and bone damage in RA [33], and the LTB4 receptor pathway has been implicated in experimental autoimmune encephalomyelitis and uveitis [34].

The upregulation of *IRF7* gene expression in our RA-ILD as well as IPF study participants has also been described in non-classical monocytes of patients with scleroderma demonstrating aberrant IFN responses [35]. Correspondingly, dysregulated type I IFNs have long been linked to various autoimmune diseases [36]. In addition, Janus kinases (JAK) were upregulated in participants with both RA-ILD and IPF. Based on results of observational studies, JAK inhibitors including tofacitinib have been suggested to have a potential therapeutic role in RA-ILD [37, 38].

Epigenetic and post-translational protein modifications of proteins have been well characterized in both RA and RA-ILD. Several DEGs potentially implicated in histone modification and glycotransferase were upregulated in the monocytes of RA-ILD patients including *KDM5D*, *KMT2D*, *GBP5* and *OGT*. Interestingly, *KDM5D* is a histone lysine demethylase that is sex chromosome encoded (Y-chromosome) with associations with autoantigens in SLE [39]. KDM5 molecules suppress interferon production in experimental autoimmune encephalomyelitis [40]. *KMT2D* encodes histone lysine methyltransferase, and mutations in *KMT2D* characterize Kabuki syndrome, an inherited malformation syndrome with endocrine disorders, immune deficiency and autoimmunity [41], but less has been described with autoimmunity alone. *GBP5* (guanylate binding protein) has been recognized as a marker of IFN-γ-induced activated M1-like monocytes/macrophages [42] and an activator of inflammasome assembly [43]. *O*-GlcNAcylation is a post-translational modification of proteins regulated by *O*-GlcNAc transferase (*OGT*), and OGT activity has been described as inducing maturation of monocytes to immature dendritic cells [44]. The role of these histone and protein modifiers in RA-ILD may represent a novel direction of research to better understand the development and progression of ILD in RA.

The enriched biological pathways observed in our study, suggest possible roles for other inflammatory and/or regulatory mediators in RA-ILD. Whereas the top pathway highlighted the role of macrophages, fibroblasts and endothelial cells, which is interpreted to potentially impact fibrotic processes, a neuroinflammation pathway was also highlighted. A growing area of inquiry surrounds the bidirectional cross talk of lung inflammation and sensory neurons with their associated neuropeptides, and activation/regulator pathways to modify disease development and severity. As the machine-based algorithms identified neuroinflammatory pathways in both RA-ILD and IPF, future studies exploring this avenue may be warranted to understand the key drivers in this pathway and potentially unlock novel therapeutic approaches. The IL-27 signalling pathway (implicated in RA-ILD, RA and IPF in the current study) has nuanced roles as it is recognized for its suppressive function related to cancer biology but is also recognized to prime and/or amplify airway inflammatory responses of fibroblasts and epithelial cells [45, 46]. IL-27 inhibitors (i.e. SRF388; CHS-388, casdozokitug) are currently being investigated in early-stage clinical trials as therapeutics in cancer [47]. The IL-12 signalling pathway, highlighted in both RA-ILD and IPF in our study, is a well-recognized pathway involved in inflammatory and autoimmune diseases. Although ustekinumab is an IL-12/23 inhibitor currently approved for psoriasis, psoriatic arthritis and Crohn's disease, this agent lacks efficacy in RA [48] and ILD has been reported as a rare adverse event with its use [49, 50], which may limit enthusiasm for targeting this pathway.

There are limitations to this study. We did not systematically rule out the possibility of subclinical ILD via advanced imaging or pulmonary function testing in RA participants. We also did not systematically test IPF for circulating autoantibodies, which would be interesting to evaluate in future studies. Additionally, control participants were not age-matched to the diseased groups, and all had allergic rhinitis on specific therapy, which could impact circulating myeloid cell subpopulations. RA and RA-ILD participants were older and male predominant, which may limit the generalizability. Our sample size number was also limited, preventing subgroup analyses based on characteristics such as age, sex, articular disease activity, autoantibody status, active treatments, environmental factors such as cigarette smoking, and RA-ILD pattern. As our study included patients with typical or probable UIP, these results (including the striking overlap observed with IPF) may not be generalizable to RA patients with other ILD patterns, and future studies would be warranted to make comparisons across lung disease findings in RA.

In conclusion, circulating monocytes in participants with RA-ILD demonstrate unique gene expression profiles with innate immune features more aligned with IPF as opposed to RA in the absence of lung disease and fibrosis gene features that were distinct from RA and IPF. Collectively, the monocytic upregulated genes are involved in regulating inflammation and fibrosis, epigenetic modification (histone modification, glycotransferase), and M1-like activated macrophages. The top canonical pathways for RA-ILD included macrophage differentiation and activation and IL-12, neuroinflammatory, glucocorticoid receptor, and IL-27 pathways. These studies provide information for potential biomarkers and/or therapeutic targets that are much needed for patients with RA-ILD.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

All data used in this research study are already included in the article and supplementary files.

Contribution statement

Conceptualization (J.A.P., T.R.M., G.M.T., B.R.E.); Methodology and Design (J.A.P., T.R.M., G.M.T., B.R.E., A. J.N., M.J.D.), Investigation/Acquisition of data (J.A.P., T.R. M., G.M.T., B.R.E., A.J.N., A.S., A.G., M.J.D., D.J.R., K.J. B., D.H., J.V.D.G., S.M.M., R.W., B.K.), Analysis/ Interpretation of data (J.A.P., T.R.M., G.M.T., B.R.E., A.J. N., A.S., A.G., M.J.D., D.J.R., K.J.B., D.H., J.V.D.G., S.M. M.), Funding acquisition (J.A.P., T.R.M., G.M.T., B.R.E.), Project administration (J.A.P., T.R.M.), Writing original draft (J.A.P., T.R.M., G.M.T., A.J.N.), Writing-review and editing and final approval (J.A.P., T.R.M., G.M.T., B.R.E., A.J.N., AS, AG, M.J.D., D.J.R., K.J.B., D.H., J.V.D.G., S.M. M., R.W., B.K.).

Funding

The main funding support for this work is from a grant by the Department of Defence, PR200793 (J.A.P., T.R.M.). Other funding support includes National Institute of Occupational Safety and Health (R01OH012045; J.A.P., D. J.R.), V.A. (BLR&D Merits I01 BX004660 and I01 BX003635, T.R.M. and CSR&D CX002203 B.R.E.), National Institute of Health (2U54GM115458, T.R.M.), and Rheumatology Research Foundation (T.R.M., B.R.E.).

Disclosure statement: J.A.P. has received research reagents (anti-IL-33/ST2 blocking antibody reagent, no monies) from AstraZeneca. J.A.P., J.V.D.G., S.M.M. and R.W. are site recruiters for clinical industry studies for asthma, sinus disease and urticaria (GlaxoSmithKline, AstraZeneca, Regeneron Pharmaceuticals, CellDex Therapeutics). B.R.E. has consulted with and received research support from Boehringer-Ingelheim. T.R.M. has consulted for Horizon Therapeutics, Otaltech Therapeutics, Pfizer and UCB and receives research support from Horizon.

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs, the Department of Defence, or the United States government.

Acknowledgements

We would like to thank all the study participants. The authors also acknowledge the UNMC Genomics Core Facility including assistance by James Eudy (Core Director), Jennifer Bushing, Alok Dhar and Greg Kubik at the University of Nebraska Medical Center for aiding with transcriptomic studies.

References

1. Duarte AC, Porter JC, Leandro MJ. The lung in a cohort of rheumatoid arthritis patients-an overview of different types of involvement and treatment. Rheumatology (Oxford) 2019;58:2031–8.
2. Van Kalsbeek D, Brooks R, Shaver D *et al.* Peripheral blood biomarkers for rheumatoid arthritis-associated interstitial lung disease: a systematic review. ACR Open Rheumatol 2023;5:201–26.
3. Kadura S, Raghu G. Rheumatoid arthritis-interstitial lung disease: manifestations and current concepts in pathogenesis and management. Eur Respir Rev 2021;30:210011.
4. England BR, Hershberger D. Management issues in rheumatoid arthritis-associated interstitial lung disease. Curr Opin Rheumatol 2020;32:255–63.
5. Manfredi A, Luppi F, Cassone G *et al.* Pathogenesis and treatment of idiopathic and rheumatoid arthritis-related interstitial pneumonia. The possible lesson from COVID-19 pneumonia. Expert Rev Clin Immunol 2020;16:751–70.
6. Brooks R, Baker JF, Yang Y *et al.* The impact of disease severity measures on survival in U.S. Veterans with rheumatoid arthritis-associated interstitial lung disease. Rheumatology (Oxford) 2022; 61:4667–77.
7. Raimundo K, Solomon JJ, Olson AL *et al.* Rheumatoid arthritis–interstitial lung disease in the United States: prevalence, incidence, and healthcare costs and mortality. The Journal of rheumatology 2019;46:360–9.
8. Shaw M, Collins BF, Ho LA, Raghu G. Rheumatoid arthritis-associated lung disease. Eur Respir Rev 2015;24:1–16.
9. Poole JA, Cole KE, Thiele GM *et al.* Expansion of distinct peripheral blood myeloid cell subpopulations in patients with rheumatoid arthritis-associated interstitial lung disease. Int Immunopharmacol 2024;127:111330.
10. Kreuter M, Lee JS, Tzouvelekis A *et al.* Monocyte count as a prognostic biomarker in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2021;204:74–81.
11. Saku A, Fujisawa T, Nishimoto K *et al.* Prognostic significance of peripheral blood monocyte and neutrophil counts in rheumatoid arthritis-associated interstitial lung disease. Respir Med 2021; 182:106420.
12. Prajzlerová K, Kryštůfková O, Komarc M *et al.* The dysregulation of monocyte subpopulations in individuals at risk of developing rheumatoid arthritis. Rheumatology (Oxford) 2021;60:1823–31.
13. Rossol M, Kraus S, Pierer M, Baerwald C, Wagner U. The CD14 (bright) CD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. Arthritis Rheum 2012;64:671–7.
14. Kawanaka N, Yamamura M, Aita T *et al.* CD14+,CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum 2002;46:2578–86.
15. Cole K, Al-Kadhimi Z, Talmadge JE. Role of myeloid-derived suppressor cells in tumor recurrence. Cancer Metastasis Rev 2023; 42:113–42.
16. Navashenaq JG, Shabgah AG, Hedayati-Moghadam M *et al.* The role of myeloid-derived suppressor cells in rheumatoid arthritis: an update. Life Sci 2021;269:119083.
17. Liu T, Gonzalez De Los Santos F, Rinke AE *et al.* B7H3-dependent myeloid-derived suppressor cell recruitment and activation in pulmonary fibrosis. Front Immunol 2022;13:901349.
18. Fernandez IE, Greiffo FR, Frankenberger M *et al.* Peripheral blood myeloid-derived suppressor cells reflect disease status in idiopathic pulmonary fibrosis. Eur Respir J 2016;48:1171–83.
19. Nagafuchi Y, Yanaoka H, Fujio K. Lessons From Transcriptome Analysis of Autoimmune Diseases. Front Immunol 2022;13:857269.
20. Li Y, He Y, Chen S *et al.* S100A12 as biomarker of disease severity and prognosis in patients with idiopathic pulmonary fibrosis. Front Immunol 2022;13:810338.
21. Wierczeiko A, Linke M, Friedrich JP *et al.* A call for gene expression analysis in whole blood of patients with rheumatoid arthritis (RA) as a biomarker for RA-associated interstitial lung disease. J Rheumatol 2024;51:130–3.
22. Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
23. Eakin EG, Resnikoff PM, Prewitt LM, Ries AL, Kaplan RM. Validation of a new dyspnea measure: the UCSD Shortness of Breath Questionnaire. University of California, San Diego. Chest 1998;113:619–24.

24. Lynch DA, Sverzellati N, Travis WD *et al.* Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. Lancet Respir Med 2018;6:138–53.
25. Giles JT, Danoff SK, Sokolove J *et al.* Association of fine specificity and repertoire expansion of anticitrullinated peptide antibodies with rheumatoid arthritis associated interstitial lung disease. Ann Rheum Dis 2014;73:1487–94.
26. England BR, Sayles H, Michaud K *et al.* Chronic lung disease in U.S. Veterans with rheumatoid arthritis and the impact on survival. Clin Rheumatol 2018;37:2907–15.
27. Kelly CA, Saravanan V, Nisar M *et al.*; British Rheumatoid Interstitial Lung (BRILL) Network. Rheumatoid arthritis-related interstitial lung disease: associations, prognostic factors and physiological and radiological characteristics—a large multicentre UK study. Rheumatology 2014;53:1676–82.
28. Reid DM, Gow NA, Brown GD. Pattern recognition: recent insights from Dectin-1. Curr Opin Immunol 2009;21:30–7.
29. Erkelens MN, Goverse G, Konijn T *et al.* Intestinal macrophages balance inflammatory expression profiles via vitamin A and dectin-1-mediated signaling. Front Immunol 2020;11:551.
30. Effendi WI, Nagano T, Hasan H, Yudhawati R. Immunoregulatory property of C-type lectin-like receptors in fibrosing interstitial lung diseases. Int J Mol Sci 2020;21.
31. Yoshimura A, Suzuki M, Sakaguchi R, Hanada T, Yasukawa H. SOCS, inflammation, and autoimmunity. Front Immunol 2012; 3:20.
32. Song X, Zhang Y, Zhao L *et al.* Analyzation of the peripheral blood mononuclear cells atlas and cell communication of rheumatoid arthritis patients based on single-cell RNA-seq. J Immunol Res 2023;2023:6300633.
33. Zheng LX, Li KX, Hong FF, Yang SL. Pain and bone damage in rheumatoid arthritis: role of leukotriene B4. Clin Exp Rheumatol 2019;37:872–8.
34. Kihara Y, Yokomizo T, Kunita A *et al.* The leukotriene B4 receptor, BLT1, is required for the induction of experimental autoimmune encephalomyelitis. Biochem Biophys Res Commun 2010; 394:673–8.
35. Villanueva-Martin G, Acosta-Herrera M, Carmona EG *et al.* Non-classical circulating monocytes expressing high levels of microsomal prostaglandin E2 synthase-1 tag an aberrant IFN-response in systemic sclerosis. J Autoimmun 2023;140:103097.
36. Wahren-Herlenius M, Dorner T. Immunopathogenic mechanisms of systemic autoimmune disease. Lancet 2013;382:819–31.
37. Baker MC, Liu Y, Lu R *et al.* Incidence of interstitial lung disease in patients with rheumatoid arthritis treated with biologic and targeted synthetic disease-modifying antirheumatic drugs. JAMA Netw Open 2023;6:e233640.
38. Tardella M, Di Carlo M, Carotti M *et al.* A retrospective study of the efficacy of JAK inhibitors or abatacept on rheumatoid arthritis-interstitial lung disease. Inflammopharmacology 2022; 30:705–12.
39. Ramezankhani R, Minaei N, Haddadi M, Solhi R, Taleahmad S. The impact of sex on susceptibility to systemic lupus erythematosus and rheumatoid arthritis; a bioinformatics point of view. Cell Signal 2021;88:110171.
40. Fazazi MR, Ruda GF, Brennan PE, Rangachari M. The X-linked histone demethylases KDM5C and KDM6A as regulators of T cell-driven autoimmunity in the central nervous system. Brain Res Bull 2023;202:110748.
41. Wang YR, Xu NX, Wang J, Wang XM. Kabuki syndrome: review of the clinical features, diagnosis and epigenetic mechanisms. World J Pediatr 2019;15:528–35.
42. Fujiwara Y, Hizukuri Y, Yamashiro K *et al.* Guanylate-binding protein 5 is a marker of interferon-gamma-induced classically activated macrophages. Clin Transl Immunology 2016;5:e111.
43. Shenoy AR, Wellington DA, Kumar P *et al.* GBP5 promotes NLRP3 inflammasome assembly and immunity in mammals. Science 2012;336:481–5.
44. Weiss M, Anderluh M, Gobec M. Inhibition of O-GlcNAc transferase alters the differentiation and maturation process of human monocyte derived dendritic cells. Cells 2021;10:3312.
45. Dong S, Zhang X, He Y *et al.* Synergy of IL-27 and TNF-alpha in regulating CXCL10 expression in lung fibroblasts. Am J Respir Cell Mol Biol 2013;48:518–30.
46. Su Y, Yao H, Wang H *et al.* IL-27 enhances innate immunity of human pulmonary fibroblasts and epithelial cells through upregulation of TLR4 expression. Am J Physiol Lung Cell Mol Physiol 2016;310:L133–41.
47. Składanowska K, Bloch Y, Strand J *et al.* Structural basis of activation and antagonism of receptor signaling mediated by interleukin-27. Cell Rep 2022;41:111490.
48. Smolen JS, Agarwal SK, Ilivanova E *et al.* A randomised phase II study evaluating the efficacy and safety of subcutaneously administered ustekinumab and guselkumab in patients with active rheumatoid arthritis despite treatment with methotrexate. Ann Rheum Dis 2017;76:831–9.
49. Cioffi V, Di Napoli G, Tozzi P *et al.* Non-infectious pneumonitis and acute respiratory distress syndrome in a patient on ustekinumab treatment: case report and literature review. Life (Basel) 2024;14:394.
50. Mistry S, Kumarapeli AR, Mudrakola HV. A rare pulmonary manifestation of Crohn's disease: acute fibrinous and organizing pneumonia presenting as multifocal nodules. Respir Med Case Rep 2024;48:A5323.

Rheumatology, 2025, 64, 37–46
https://doi.org/10.1093/rheumatology/keae572
Original Article