European Journal of Immunology Clinical·Basic·Translational

Immunodeficiencies and autoimmunity

Clinical

Research Article

Single-cell multi-omics analysis of COVID-19 patients with pre-existing autoimmune diseases shows aberrant immune responses to infection

Anis Barmada[1,2], Louis-François Handfield[#1], Gerard Godoy-Tena[#3], Carlos de la Calle-Fabregat[#3], Laura Ciudad[3], Anna Arutyunyan[1], Eduardo Andrés-León[4], Regina Hoo[1], Tarryn Porter[1], Agnes Oszlanczi[1], Laura Richardson[1], Fernando J. Calero-Nieto[5], Nicola K. Wilson[5], Domenica Marchese[6], Carmen Sancho-Serra[1], Jorge Carrillo[7], Silvia Presas-Rodríguez[8], Cristina Ramo-Tello[8], Adolfo Ruiz-Sanmartin[9], Ricard Ferrer[9], Juan Carlos Ruiz-Rodriguez[9], Mónica Martínez-Gallo[10], Mónica Munera-Campos[11], Jose Manuel Carrascosa[11], Berthold Göttgens[5], Holger Heyn[6,12], Elena Prigmore[1], Ivette Casafont-Solé[13,14], Xavier Solanich[15], Ildefonso Sánchez-Cerrillo[16], Isidoro González-Álvaro[17], Maria Gabriella Raimondo[18,19], Andreas Ramming[18,19], Javier Martin[4], Eva Martínez-Cáceres[20,21], Esteban Ballestar[3], Roser Vento-Tormo1[1] and Javier Rodríguez-Ubreva[3]

[1] Wellcome Sanger Institute, Wellcome Genome Campus, Cambridge, United Kingdom
[2] Department of Medical Genetics, University of Cambridge, Cambridge, United Kingdom
[3] Epigenetics and Immune Disease Group, Josep Carreras Research Institute (IJC), Barcelona, Spain
[4] Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas (IPBLN-CSIC), Granada, Spain
[5] Department of Haematology and Wellcome & MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom
[6] CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain
[7] IrsiCaixa AIDS Research Institute, Hospital Germans Trias i Pujol, Badalona, Spain
[8] MS Unit, Department of Neurology, Germans Trias i Pujol University Hospital, Barcelona, Spain
[9] Department of Intensive Care, Hospital Universitari Vall d'Hebron, Shock, Organ Dysfunction and Resuscitation Research Group, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain
[10] Division of Immunology, Hospital Universitari Vall d'Hebron (HUVH), Vall d'Hebron Research Institute (VHIR), Barcelona, Spain
[11] Dermatology Service, Germans Trias i Pujol University Hospital, LCMN, Germans Trias i Pujol Research Institute (IGTP), Barcelona, Spain
[12] Universitat Pompeu Fabra (UPF), Barcelona, Spain
[13] Department of Rheumatology, Germans Trias i Pujol University Hospital, LCMN, Germans Trias i Pujol Research Institute (IGTP), Barcelona, Spain
[14] Department of Infectious Diseases, Germans Trias i Pujol University Hospital, Barcelona, Spain
[15] Department of Internal Medicine, Hospital Universitari de Bellvitge, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain
[16] Department of Immunology, Hospital Universitario La Princesa, IIS-IP, Madrid, Spain
[17] Rheumatology Service, Hospital Universitario La Princesa, IIS-IP, Madrid, Spain

Correspondence: Esteban Ballestar, Roser Vento-Tormo and Dr. Javier Rodríguez-Ubreva
e-mail: eballestar@carrerasresearch.org; jrodriguez@carrerasresearch.org; rv4@sanger.ac.uk

[#]Louis-François Handfield, Gerard Godoy-Tena, and Carlos de la Calle-Fabregat contributed equally to this study.
Esteban Ballestar, Roser Vento-Tormo, and Javier Rodríguez-Ubreva share senior authorship.

[18] Department of Internal Medicine 3, Friedrich-Alexander-University Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany
[19] Deutsches Zentrum Immuntherapie (DZI), Friedrich-Alexander-University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany
[20] Division of Immunology, Germans Trias i Pujol University Hospital, LCMN, Germans Trias i Pujol Research Institute (IGTP), Barcelona, Spain
[21] Department of Cell Biology, Physiology, and Immunology, Universitat Autònoma, Barcelona, Spain

In COVID-19, hyperinflammatory and dysregulated immune responses contribute to severity. Patients with pre-existing autoimmune conditions can therefore be at increased risk of severe COVID-19 and/or associated sequelae, yet SARS-CoV-2 infection in this group has been little studied. Here, we performed single-cell analysis of peripheral blood mononuclear cells from patients with three major autoimmune diseases (rheumatoid arthritis, psoriasis, or multiple sclerosis) during SARS-CoV-2 infection. We observed compositional differences between the autoimmune disease groups coupled with altered patterns of gene expression, transcription factor activity, and cell–cell communication that substantially shape the immune response under SARS-CoV-2 infection. While enrichment of HLA-DRlow CD14+ monocytes was observed in all three autoimmune disease groups, type-I interferon signaling as well as inflammatory T cell and monocyte responses varied widely between the three groups of patients. Our results reveal disturbed immune responses to SARS-CoV-2 in patients with pre-existing autoimmunity, highlighting important considerations for disease treatment and follow-up.

Keywords: Autoimmunity · COVID-19 · Multiple sclerosis · Psoriasis · Rheumatoid arthritis

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

Clinical features of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes COVID-19, range from mild or moderate respiratory tract disease to severe disease and respiratory failure. This range of clinical presentations can be dependent on the fitness of the host immune system and the specific immune response mounted against the virus. An effective immune response against SARS-CoV-2 requires coordination between the innate and adaptive immune systems, including the activity of granulocytes, macrophages, and T and B cells [1, 2]. Importantly, some patients develop dysregulated immune responses against SARS-CoV-2 leading to hyperinflammation. This hyperinflammation has been associated with mortality in COVID-19 patients – in whom it is frequently observed in the context of advanced age and comorbidities – suggesting an effect of underlying systemic chronic inflammation [3, 4]. Immune hyperactivation leads to excessive production of inflammatory mediators, either systemic or at the site of infection, that further exacerbate disease symptoms, causing lung tissue destruction and eventual respiratory failure [5]. Specifically, overexpression of IL-6 and IL-1β has been implicated as a marker of disease severity [6, 7]. Impairment of the type I IFN response has also been identified as a marker of COVID-19 severity, which is further associated with an exacerbated inflammatory response partially driven by NF-κB and TNF-α activation [7]. This emphasizes the importance of a balanced immune response to the infection.

Single-cell studies have shed light on the underlying immune cell-specific dysregulation in COVID-19-associated hyperinflammation [8–12]. Generally, T cells and inflammatory monocytes are particularly involved in the immune dysregulation and hyperactivation exhibited by COVID-19 patients [10]. In addition, several studies have indicated an increase in neutrophils and a decrease in nonclassical ($CD14^{low}CD16^{hi}$) monocytes in severe COVID-19 patients [7, 13]. Further research has demonstrated that transcriptomic profiles of monocytes in COVID-19 reflect defective antigen presentation and IFN responsiveness, which contrasts with the higher responsiveness to IFN signaling noted in lymphocytes [14]. Adaptive immune cells have also been shown to contribute to disease severity, for example through neutrophil recruitment and proinflammatory monocyte/macrophage polarization by Th1 and Th17 responses [2, 15, 16]. Regarding CD4+ T cells, previous studies have observed cytopenia in severe COVID-19 patients coupled with substantial heterogeneity of the molecular profile [17, 18].

There is currently only sparse and contradictory evidence regarding the risk of viral infection or evolution to severe COVID-19 forms in individuals with chronic underlying immune pathologies, including autoimmune diseases [19–23]. There further remains a need for knowledge about the specific antiviral immune responses mounted by such patients. To address this matter, we performed single-cell analysis of the transcriptome, surface proteome, and T-cell receptors of peripheral blood mononuclear cells (PBMCs) from COVID-19 patients with three distinct autoimmune

diseases – rheumatoid arthritis (RA), psoriasis (Ps), or multiple sclerosis (MS). Our results indicate the existence of highly disturbed immune responses against SARS-CoV-2 that are governed by the specific autoimmune disease context, which may ultimately influence clinical outcomes such as disease severity and the development of sequelae in these patient populations.

Materials and methods

Study participants and sample collection

Human blood samples were collected from patients under SARS-CoV-2 infection with or without pre-existing autoimmune conditions (RA, Ps, MS, and control) as previously diagnosed according to established criteria. COVID-19 individuals categorized as 'mild' were those who were located in a ward and did not need oxygen. Those placed outside of the ICU but requiring oxygen were categorized as "moderate". "Severe" disease was applicable to all patients in the ICU or those requiring noninvasive ventilation. All samples were collected when patients displayed COVID-19 symptoms (with the exception of one patient who was asymptomatic; Supporting information Table S1), thus during the progression of the disease and not at the convalescence stage after recovery. They were collected at Hospital La Princesa, Hospital Vall d'Hebron, Hospital Can Ruti, and Hospital Bellvitge (Spain). All donors received oral and written information about the possibility that their blood would be used for research purposes, and any questions that arose were answered. Patients included in this study were individuals infected with SARS-CoV-2 between March and November of 2020 in Spain. Information about which SARS-CoV-2 lineage was most frequent [24] at the time of infection for each patient (the B.1.177 variant for most patients) can be found in Supporting information Table S1. Prior to sample collection, donors signed a consent form approved by the Ethics Committee of their corresponding hospital, which adhered to the principles set out in the WMA Declaration of Helsinki. PBMCs were obtained from peripheral blood by Ficoll gradient using Lymphocyte Isolation Solution (Rafer). Once PBMCs had been isolated, all samples were stored at –150°C in fetal bovine serum (FBS) + 10% DMSO until analysis.

Single-cell capture

PBMCs were thawed rapidly in a 37°C water bath, then slowly diluted in the prewarmed growth medium, centrifuged, and resuspended in fresh FACS buffer (PBS + 3% FBS) before staining with CITE-seq antibodies (TotalSeq-C 192 antibody panel; Supporting information Table S9) and loading into 10X Chromium. In cases where PBMCs from different donors were pooled, a fraction was taken to isolate genomic DNA for genotyping and the other fraction was used to generate single-cell gel beads-in-emulsion (GEMs). Genomic DNA was isolated from PBMCs for genotyping using a Maxwell® 16 Blood DNA Purification Kit from Promega following the manufacturer's instructions. For the CITE-seq protocol, cells were resuspended in FACS buffer (PBS + 4% FBS), incubated with Fc Block for 10 min, and then with the specific mix of antibodies for 30 min at 4°C. Cells were then washed three times, filtered using a Flowmi strainer, and counted before loading into 10X Chromium to generate single-cell GEMs, following the manufacturer's instructions.

Library generation and sequencing

Libraries were constructed following the manufacturer's protocol for the Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 with Feature Barcode technology for Cell Surface Protein (10X Genomics Rev E), but with two amendments: the amount of SI primer was doubled, and the number of PCR cycles was set at 7. Samples were sequenced using the Illumina NovaSeq 6000, where cellular gene expression, T-cell clonality, and selected surface proteins in the CITE-seq protocol were simultaneously profiled.

Single-cell data alignment, quantification, and quality control

The single-cell transcriptome data were aligned and quantified by Cell Ranger v3.1 using GRCh38 (Ensembl 93) concatenated to the SARS-Cov-2 genome as a reference. Surface protein data were quantified using a dictionary of tagged antibodies. Pooled donor samples were deconvolved using Souporcell [25], which yielded a genotype variant that allowed donor identity to be matched across samples. Cells that could not be explained by a single genotype were considered doublets and removed before analysis. Additionally, Scrublet [26] was employed to detect and remove other doublets by computing a doublet score for each cell. Briefly, a Student's t-test ($p < 0.01$) was used after Bonferroni correction within fine-grained subclustering of each cluster produced by the Leiden algorithm. Thereafter, SoupX [27] was used to denoise the surface proteome data from ambient RNA. The single-cell data were then integrated and batch effects corrected using total variational inference [28] combining the transcriptome and surface proteome data with a generative model of 64 latent variables and 500 iterations. The resulting latent representation was used to compute a neighborhood graph (*scanpy.pp.neighbors*), which was further used for Louvain clustering (*scanpy.tl.louvain*) and Uniform Manifold Approximation and Projection (UMAP) visualization (*scanpy.tl.umap*), using the Scanpy toolkit [29]. Before downstream analysis, genes expressed in fewer than three cells, and cells with fewer than 200 genes or more than 20% mitochondrial gene content were removed.

Cell type identification and cluster annotation

Scanpy [29] was also used for downstream analysis following the recommended standard practices. Data were first

normalized (*scanpy.pp.normalize_per_cell*, scaling factor 10^4) and then log-transformed (*scanpy.pp.log1p*). Preliminary annotations were transferred using a logistic regression model from a published large PBMC dataset of COVID-19 patients [30], selecting only highly variable genes (*scanpy.pp.highly_variable_genes*) and then subsetting to shared genes between the datasets. Clustering was thereafter performed using the Louvain algorithm and annotations were manually refined based on the expression of known cell-specific marker genes.

T-cell receptor clonality

Single-cell TCR data were processed with the Cell Ranger v3.1 vdj pipeline using GRCh38 as a reference. Downstream analysis was then performed using Scirpy [31]. In particular, only cells with at least one α-chain and one β-chain but fewer than two full pairs of α/β chains were kept for analysis, and expanded clones were defined when a clonotype was present in more than one cell.

Differential gene expression

The limma package [32] was used to perform differential gene expression analysis between the disease groups. Each patient group was compared with all patients from the other three groups combined in order to pinpoint the specific immune responses in each disease condition, and to reduce any bias between individual groups. Simultaneously, differential expression analysis was performed on the published COVID-19 dataset [30] that had been used previously for annotation transfer, comparing male with female COVID-19 patients as well as COVID-19 patients with healthy individuals. This approach enabled us to filter out differentially expressed genes that were influenced by sex (including all Y chromosome genes using a list from Ensembl BioMart [Supporting information Table S10]) and to subset our results to genes differentially expressed due to COVID-19 (false discovery rate [FDR] < 0.05). Further, given the heterogeneity in COVID-19 disease severity in the autoimmune disease groups owing to the difficulties associated with obtaining such samples, we used the comparison between mild and severe COVID-19 patients with healthy individuals to ensure the immune signatures observed in the autoimmune disease groups are not caused by the differences in COVID-19 severity [33]. For this analysis, the two sets of cells whose expression is to be compared were each partitioned into 4 groups based on the quartile for their total UMI count, such that Wilcoxon tests would be performed on each matching quartile. For each quartile, the group of cells with the higher UMI count per cell had its counts randomly downsampled to better match the other, then the resulting 4 z-scores were combined. This analysis was performed on all identified cell types that were sufficiently represented in the four patient groups. Subsequent downstream analyses then focused on CD14+ monocytes and CD4+ T cells given the highest dysregulation observed in these subsets. Additionally, PBMC datasets of uninfected MS [34], RA [35], and Ps patients were similarly analyzed, in comparison to healthy individuals where annotations were transferred from our dataset using logistic regression as described above, to identify which of the responses observed in the COVID-19 autoimmune disease groups were specific to the infection. Together, these parallel computational analyses allowed us to control for the variability across the autoimmune disease groups and determine the specific immune signatures caused by SARS-CoV-2 infection.

Gene set enrichment analysis

Gene set enrichment analysis was performed with functions from the *clusterProfiler* R package [36]. Genes were ranked by expression $\log_2$FC of every comparison, in every cell cluster, and a normalized enrichment score (NES) was calculated for each gene set. Whole gene set collections C1 (hallmark) and C5 (ontology) were included in the analysis. *p*-values were adjusted for FDR across all gene sets.

Transcription factor activity

Discriminant Regulon Expression Analysis [37], a curated resource of TFs and their targets compiled from various sources including the literature, ChIP-Seq peaks, in silico predictions, as well as gene expression data, was used to estimate TF activities from combined expression values of gene targets.

Cell–cell communication

CellPhoneDB [38] (www.CellPhoneDB.org) was used to infer interactions between the identified cell subsets. The previously described statistical method was adjusted to identify ligand/receptor interactions that were significantly enriched between the disease groups. Specifically, differentially expressed genes (FDR < 0.05) obtained within each cell type were used to select relevant interactions instead of random shuffling. Only ligand/receptor pairs expressed by at least 10% of interacting cells were retained, and an interaction was considered enriched if at least one partner was differentially expressed. For the IFN antiviral interactions, given that ligands are produced by cells other than just those of the immune subsets, an interaction was considered to exist if at least one of the receptor subunits was differentially expressed.

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committees of Hospital Universitari Germans Trias i Pujol (PI-20-129), Hospital de Bellvitge (PI-20-139), Hospital La Princesa (4070), and Vall d'Hebron University Hospital (PR(AG)282/2020), which adhered to the principles set out in the WMA Declaration of

Helsinki. All samples were collected in compliance with the written informed consent required to participate in the study.

Results

Cellular compositional and clonal differences in COVID-19 patients with pre-existing autoimmunity

In order to investigate the specific immune responses in patients with pre-existing autoimmune conditions under SARS-CoV-2 infection, we collected peripheral blood samples from a cohort of 5 RA, 4 Ps, and 3 MS COVID-19 patients (positive for SARS-CoV-2 at the time of sample collection), as well as 10 COVID-19 patients without pre-existing autoimmunity who served as controls (Fig. 1A and Supporting information Table S1). These control COVID-19 patients, many of whom suffering from pre-existing conditions associated with a higher risk of severe COVID-19 such as obesity or hypertension, were selected based on their disease severity to test the hypothesis that COVID-19 patients with pre-existing autoimmunity are prone to exhibiting inflammatory responses characteristic of severe COVID-19. As a reference, we also inspected control COVID-19 patients with mild symptoms [33], in order to identify the specific immune responses associated with the pre-existing autoimmunity while controlling for the variable COVID-19 severity status of the autoimmune disease patients at the time of sample collection. Given that most patients involved in this study, including the control group, received immunosuppressant and/or anti-inflammatory treatment during infection, differences in the immune responses observed between the groups will likely be dependent on the pre-existing autoimmune context (including possible intertwined effects of prior immunomodulatory treatments that are typical for such patients). Thus, controlling for the treatment received during COVID-19 between the groups will help separate the likely immune signatures most characteristic of such autoimmune disease patients upon SARS-CoV-2 infection. Additionally, we focused our analysis on the common responses across all patients within each autoimmune disease group that were conserved regardless of the treatment administered. Lastly, we compared the results with uninfected autoimmune disease patients to help identify the signatures unique to these patients under SARS-CoV-2 infection. Although the uninfected MS patients were treatment-naive, both the uninfected RA and Ps control groups received similar immunomodulatory treatment (Supporting information Table S5) to the SARS-CoV-2-infected RA and Ps patients, further helping dissect the effect of the autoimmune disease from that of the immunomodulatory treatment on the immune responses.

We isolated PBMCs from the aforementioned patient groups and generated a combined single-cell transcriptomic and surface-proteomic (CITE-seq) [39] profile of immune cells. We profiled a total of 97,499 cells, comprising 29,813 cells from COVID-19 patients without pre-existing autoimmunity, 29,477 cells from RA, 19,907 cells from Ps, and 18,302 cells from MS COVID-19 patients. All datasets were integrated into a joint representation considering the transcriptome and surface proteome generated using total variational inference [28]. Our resource is available for visualization, download, and interrogation through the COVID-19 Cell Atlas web portal (https://www.covid19cellatlas.org).

Our single-cell transcriptomic analysis revealed 28 cell subsets that we annotated based on specific markers (Fig. 1B, C). In particular, we identified distinct populations of T cells (*CD3G*): CD4 naïve (*CD4* and *LEF1*); CD4 memory (*CD4* and *CCL5*); CD8 naïve (*CD8A* and *LEF1*); CD8 memory (*CD8A* and *CCL5*); regulatory (Treg: *CD4* and *FOXP3*); mucosal-associated invariant (MAIT: *TRAV1-2*); gamma-delta (γδT: *TRDV2*); proliferating (Prolif. T: *MKI67*); and natural killer T cells (NKT: *KLRF1* among others). In addition, we identified two subsets of natural killer cells (NK CD56^{dim}: *KLRF1*, *NCAM1*, and *FCGR3A*^{high}; and NK CD56^{bright}: *KLRF1*, *NCAM1*, and *FCGR3A*^{low}) along with proliferating natural killer cells (Prolif. NK: *KLRF1* and *MKI67*). Captured B cell subsets (*CD79A*) included naïve (*TCL1A*); immature (*TCL1A* and *MME*); memory (*CD27* and *TNFRSF13B*); exhausted (*TNFRSF13B* and *CR2*^{neg}); plasmablast (*JCHAIN*, *SDC1*, and *MKI67*); and plasma B cells (*JCHAIN* and *SDC1*). The myeloid fraction comprised two subsets of monocytes (CD14 mono: *CD14*; and CD16 mono: *CD14* and *FCGR3A*); conventional dendritic cells (*FLT3*) including cDC1: *CLEC9A*; cDC2: *CD1C*; and cDC3: *CD1C* and *CD14*; plasmacytoid dendritic cells (pDC: *JCHAIN* and *LILRA4*); and neutrophils (*FCGR3B*). Lastly, we identified precursor hematopoietic stem cells (HSC: *CD34*); platelets (*GP9*); and red blood cells (RBC: *HBB*). We further exploited CITE-seq to validate these cell annotations using markers from the surface proteome data (Fig. 1D).

We observed significant differences in cell proportions between the different patient groups (Fig. 2A, B, Supporting information Fig. S1 and Table S2). Within the T-cell compartment, we observed relatively more CD4+ memory T cells in RA COVID-19 patients compared with controls (Fig. 2B and Supporting information Fig. S1). In MS COVID-19 patients, we found a higher frequency of circulating MAIT cells than in the other three groups and fewer CD8+ memory T cells compared with controls. Higher proportions of MAIT cells in asymptomatic and mild SARS-CoV-2 infections have previously been reported [30]. Within the B-cell compartment, we detected significantly fewer plasmablasts and plasma cells in Ps and RA COVID-19 patients in comparison with controls, although the use of anti-CD20 therapy in some of the RA patients included in the cohort likely influences these observed differences. Control COVID-19 patients also had relatively higher proportions of platelets compared with all three autoimmune COVID-19 groups, and higher proportions of HSCs compared with the MS COVID-19 group. Relative expansion of HSCs, platelets, and plasmablasts has previously been associated with increased COVID-19 severity [30]. In addition, since monocyte-platelet aggregates have been reported in COVID-19 patients [40], the observed differences in platelet frequencies between controls and autoimmune COVID-19 patients might be caused by a specific subset of monocytes sequestering platelets as previously described [30]. Lastly, differences in some other cell subsets (neutrophils and RBCs) can further be influenced by the sample processing procedure itself.

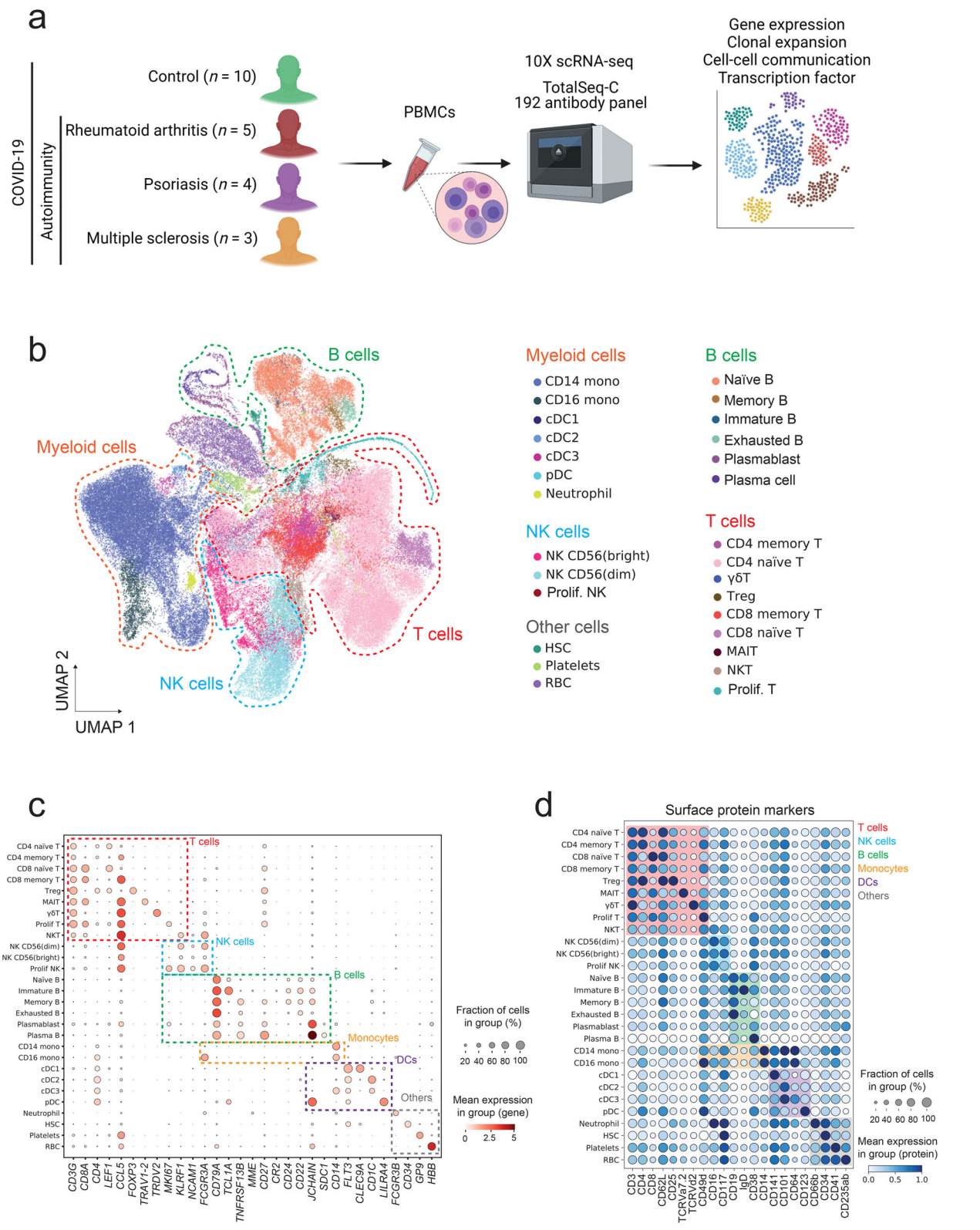

Figure 1. Immune cell subset annotation from single-cell data of COVID-19 patients with pre-existing autoimmunity and controls. (a) Schematic diagram depicting the cohort of control and autoimmune COVID-19 patients included in the study, as well as the single-cell approaches and analyses performed. (b) UMAP visualization showing the different immune cell populations identified from Louvain clustering and cell-specific marker gene expression. The T-cell compartment includes CD4+ naïve, CD4+ memory, CD8+ naïve, and CD8+ memory T cells, as well as regulatory (Treg), mucosal-associated invariant (MAIT), gamma-delta (γδT), proliferating (Prolif. T), and natural killer T cells (NKT). The NK cell compartment includes NK $CD56^{dim}$ and NK $CD56^{bright}$ along with proliferating NK cells. The B cell compartment includes naïve, immature, memory, exhausted,

Within the myeloid compartment, we focused on CD14+ monocytes given that dysregulation in this compartment upon SARS-CoV-2 infection is linked to COVID-19 severity [11]. In this regard, we identified a CD14+ HLA-DR^{low} subset in our dataset (Fig. 2C, D), whose proportion was significantly higher in all three autoimmune COVID-19 groups compared with controls (Fig. 2E).

Given the altered cellular composition identified in the T-cell compartment, we also analyzed the clonal expansion of the distinct T-cell subsets identified (Fig. 2F and Supporting information Table S3). The MS COVID-19 group had a significantly higher proportion of clonally expanded MAIT cells compared with the other three groups, consistent with the compositional analysis. The RA COVID-19 group instead had a significantly lower proportion of clonally expanded CD8+ memory T cells than the COVID-19 Ps and control groups. We also noted a trend toward a higher proportion of clonally expanded CD4+ memory T cells compared with the three other groups, consistent with the compositional analysis of this cell type.

Distinct immune gene expression profiles among COVID-19 patients with pre-existing autoimmunity

We next focused on CD14+ monocyte and CD4+ T cell responses due to their compositional and clonal alterations observed in COVID-19 patients with pre-existing autoimmunity, as well as their dysregulated responses under SARS-CoV-2 infection described by others [8, 13, 41, 42]. As such, we performed differential gene expression analysis in the COVID-19 patient groups included in the study, with special emphasis on CD14+ monocytes, as well as CD4+ naïve and memory T cells (Supporting information Table S4). To pinpoint the specific immune responses in each disease condition and reduce any bias between individual groups, cells from each patient group (control, RA, Ps, and MS) within either the CD14+ monocyte, CD4+ naïve T cell, or CD4+ memory T-cell clusters were compared with the other cells within that cluster (including cells from the three other groups combined). We further made use of a publicly available large PBMC dataset [30] to select those genes showing the most significant transcriptional changes in COVID-19 patients compared with healthy individuals (Supporting information Tables S5 and S6).

Gene set enrichment analysis revealed that multiple immune-related categories are uniquely dysregulated in COVID-19 patients with pre-existing autoimmunity (Fig. 3A). In particular, CD14+ monocytes from MS COVID-19 patients were characterized by increased responses to inflammatory cytokines such as IL-1β, IL-6, type I IFNs, and IFNγ, as well as increased innate and adaptive immune responses (Fig. 3A). This subset of CD14+ monocytes displayed significant upregulation of genes of the type I IFN pathway, including members of the IFN-induced protein with tetratricopeptide repeats (IFIT) family (e.g., *IFIT2*), members of the IFN-induced transmembrane protein (IFITM) family, such as *IFITM1*, as well as IFN-stimulated genes like *ISG15* (Fig. 3B and Supporting information Table S4). Additionally, CD14+ monocytes showed significant upregulation of genes of the inflammasome pathway such as *NAIP*, *NLRC4*, *CASP1*, and *CASP4*, as well as IL-6 production-related genes such as *CD36*, *LGALS9*, *STAT3*, and *IL17RA*, among others (Fig. 3B and Supporting information Table S4). In Ps COVID-19 patients, CD14+ monocytes displayed greater enrichment of the hypoxia and TNF-α/NF-κB pathways (Fig. 3A). Consistently, CD14+ monocytes from Ps COVID-19 patients displayed upregulation of hypoxia-related genes including *HIF1A*, *HK2*, and *MAFF* in comparison with MS and, to a lower extent, RA COVID-19 patients (Fig. 3B).

In the case of CD4+ T cells, we also found alterations in the expression of multiple relevant genes for all three groups of patients with pre-existing autoimmunity upon SARS-CoV-2 infection. MS COVID-19 individuals showed significant enrichment of the type I IFN response category in both CD4+ naïve and memory T cells (Fig. 3A). In this regard, we observed upregulation of type I IFN-related genes such as *IFI35*, *IFNAR2*, and *IFITM2* among others in CD4+ naïve T cells (Fig. 3C). It is of note that lymphocyte antigen 6 complex, locus E (*LY6E*), a previously described ISG [43] that has recently been found to impair coronavirus fusion and restrict its entry into cells [44, 45], was also upregulated in MS COVID-19 patients in CD14+ monocytes and CD4+ naïve T cells (Supporting information Table S4). Remarkably, the specific IFN signature observed in CD14+ monocytes and CD4+ naïve T cells of MS COVID-19 patients is likely specific to the autoimmune condition under infection, as no differences in sampling time relative to COVID-19 onset are seen compared with controls. Similarly, no significant differences were observed in sampling time relative to COVID-19 onset in the Ps and RA groups compared with controls (Supporting information Table S1). In Ps COVID-19 patients, CD4+ naïve T cells displayed enrichment of the TNF-α/NF-κB signaling pathway, as well as a pronounced hypoxia signature. Consistent with these results, we observed upregulation of TNF-α/NF-κB signaling pathway genes such as *NFKB1*, *CEBPD*, and *CD83* (Fig. 3C). In RA COVID-19 patients, CD4+ memory T cells displayed enrichment of the TNF-α/NF-κB signaling pathway, as well as higher levels of hypoxia, IL-12, and IFN-γ responses (Fig. 3A). Additionally, this cell compartment displayed upregulation of several TNF-α/NF-κB signaling-related genes, including *RELA*, *RELB*, and *ICAM1*, among others, as well as upregulation of several genes related to the IFN-γ response, such as *IRF1*, *TRIM21*, and *PIAS1* (Fig. 3C).

plasmablast, and plasma B cells. The myeloid fraction includes CD14+ and CD16+ monocytes, as well as conventional dendritic cells including cDC1, cDC2, and cDC3, plasmacytoid dendritic cells (pDC), and neutrophils. Additionally, we identified precursor hematopoietic stem cells (HSC), platelets, and red blood cells (RBC). (c) Dot plot depicting the expression of selected marker genes in the cell populations identified. The scale represents the mean gene expression in the cell subset; the circle size represents the percentage of cells in the subset expressing the gene. (d) Dot plot depicting the expression of selected protein markers in the cell populations identified. The scale represents the standardized mean protein expression in the cell subset; the circle size represents the percentage of cells in the subset expressing the protein.

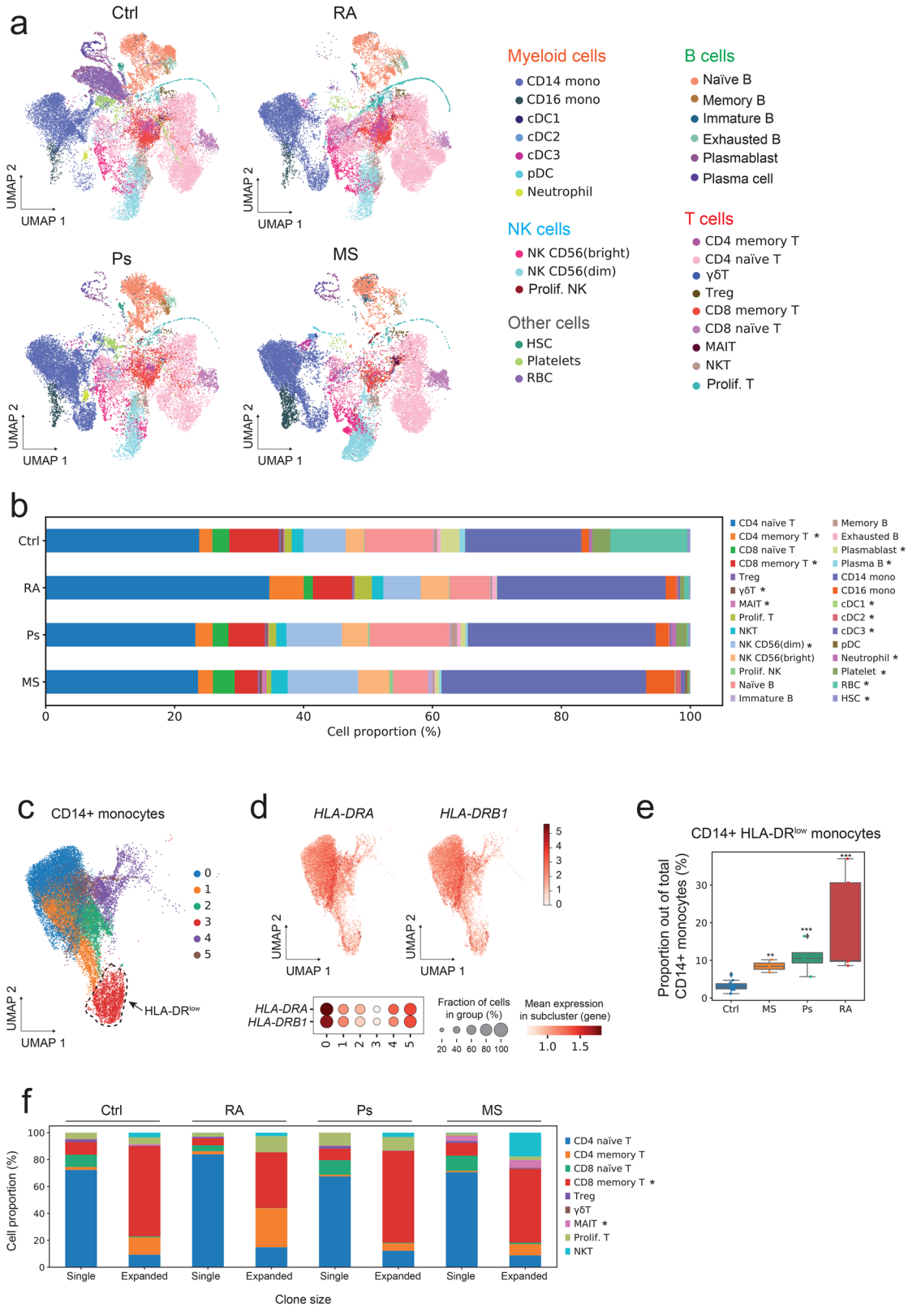

Figure 2. Alterations in cell composition and T cell receptor clonality in patients with pre-existing autoimmunity under SARS-CoV-2 infection. (A) UMAP visualization shows the distribution of the immune cell populations in the patient groups and controls included. (B) Bar plot depicting the average cell proportions of the immune cell types in each patient group. Significant differences in cell proportions between any two groups are marked with an asterisk next to the cell type (unpaired, two-sided Wilcoxon rank-sum test; see Supporting information Table S2 for statistically significant *p*-values between each pair of groups). (C) UMAP visualization depicting the sub-clustering of the CD14+ monocyte compartment,

Importantly, the upregulation of the aforementioned genes was not observed in CD14+ monocytes or CD4+ T cells of uninfected RA, Ps, or MS patients compared with healthy donors (Supporting information Tables S5 and S6), suggesting that the observed gene signatures specifically originate in response to SARS-CoV-2 infection. Moreover, many of these upregulated genes in COVID-19 with pre-existing autoimmunity (many of whom displayed mild COVID-19 at the time of sample collection compared with COVID-19 controls) were shown to be less expressed in mild compared with severe COVID-19 controls (Supporting information Fig. S2A, B), suggesting that such responses are unique to the autoimmune disease patients under infection regardless of COVID-19 severity.

Some of the autoimmune disease patients included in this study received rituximab treatment (anti-CD20 monoclonal antibody) before SARS-CoV-2 infection. To gain further insights into the possible effects of this immunotherapy on the immune response to SARS-CoV2 infection, we compared those rituximab-treated patients in our cohort (3 RA and 1 MS) versus patients from the same disease groups who did not receive rituximab as controls (2 RA and 2 MS). Our results indicate that several genes involved in IFN and antiviral immune responses that are upregulated in monocytes during SARS-CoV-2 infection such as *IFITM1*, *IFI16*, *IRF1*, *ISG15*, *OAS1*, and *MX2*, together with genes involved in inflammasome activation and IL-1β production such as *NAIP*, *CASP1*, and *PYCARD*, had significantly reduced expression in monocytes from rituximab-treated patients compared with the controls (Supporting information Table S6).

Overall, CD14+ monocytes and CD4+ T cells show altered immune signatures associated with inflammation, hypoxia, as well as IFNs and TNF responses for all three autoimmune disease patents under SARS-CoV-2 infection that might condition the function and polarization of these cells to specific phenotypes.

Altered transcription factor activity in autoimmune patients supports diverging responses to SARS-CoV-2

We next analyzed transcription factor (TF) activities in the different COVID-19 patient groups using Discriminant Regulon Expression Analysis, a comprehensive resource of curated TFs and their gene targets [37].

In MS COVID-19 patients, both CD14+ monocytes and CD4+ T cells displayed stronger activation of type I IFN-associated TFs – namely STAT1, STAT2, IRF9, and IRF1 – compared with Ps, RA, and control COVID-19 patients, in line with our previous differential gene expression analysis (Fig. 4A, B). This signature was only observed in MS patients under SARS-CoV-2 infection and not in uninfected MS patients (analysis performed on published dataset [34]; Supporting information Fig. S2C and Tables S5 and S6), supporting that the observed IFN signature specifically originates in response to infection. Additionally, CD14+ monocytes of Ps COVID-19 individuals displayed a significantly lower level of activity of several regulatory factor X (RFX) TFs, particularly in comparison to the MS COVID-19 group (Fig. 4A). RFX TFs are involved in the transcriptional regulation of HLA genes [46], including HLA-DR, which is known to be downregulated in dysfunctional HLA-DR^{low}CD163^{high} and HLA-DR^{low}S100A^{high} CD14+ monocytes in severe COVID-19 [11]. We also inspected hypoxia-associated TFs such as hypoxia-inducible factor 1-alpha (HIF-1α) and EPAS1 (HIF-2α), as they have been related to tolerogenic phenotype in monocytes [47] and with an increase in glycolysis involved in the acquisition of trained immunity in myeloid cells during inflammation [48]. We detected predominant and significant hyperactivation of these two TFs in CD14+ monocytes of Ps COVID-19 patients compared with the other patient groups (Fig. 4A). These signatures for HIF-1α and EPAS1, but not for RFX TFs, only occurred in Ps patients under SARS-CoV-2 infection and not in uninfected ones (Supporting information Fig. S2C and Tables S5 and S6), supporting that the observed hypoxia signature in Ps patients specifically originates in response to the infection.

In Ps COVID-19 patients, both CD4+ naïve and memory T cells presented a specific activation of the GATA3 pathway (Fig. 4B), which might be associated with higher polarization toward a Th2 response. In the MS COVID-19 group, GATA3 pathway TFs were only modestly upregulated in CD4+ memory T cells. Notably, CD4+ naïve T cells of Ps COVID-19 patients showed significant protein upregulation of CD124 (Supporting information Table S7), the IL-4 receptor, which is involved in Th2 polarization of CD4+ T cells [49]. We also noted significant protein upregulation of the IL-21 receptor (CD360) (Supporting information Table S7), whose ligand IL-21 is known to impair Th1 polarization and amplify the Th2 response [50].

We also detected higher levels of activity of the AP-1 subunits JUND and FOS, and NF-κB pathway TFs in CD4+ naïve T cells of Ps COVID-19 patients (Fig. 4B). Both of these TF pathways are downstream of TNF signaling [51], consistent with the enrichment of the TNF-α signaling category observed earlier in the same cell compartment of Ps COVID-19 patients. Instead, the activation of NF-κB in the RA group was seen in CD4+ memory

where subcluster 3 corresponding to CD14+ HLA-DR^{low} monocytes is shown in red. (D) UMAP visualization showing the gene expression of HLA-DR subunits (*HLA-DRA* and *HLA-DRB1*) in CD14+ monocytes. The dot plot depicts the gene expression of the two HLA-DR subunits in the subclusters identified within CD14+ monocytes. (E) Box plot depicting the average proportion of HLA-DR^{low} cells within CD14+ monocytes between the different groups. Boxes represent the interquartile range (IQR), horizontal bars depict the median, whiskers extend to 1.5 × IQR, and dots denote the values for each donor where outliers are additionally marked with grey labels. Statistical significance was determined using the unpaired, two-sided Wilcoxon rank-sum test of comparisons with controls ($p = 2.20 \times 10^{-3}$ vs. RA, $p = 1.12 \times 10^{-2}$ vs. MS, $p = 7.21 \times 10^{-3}$ vs. Ps). (F) Bar plot of the average proportions of the T cell subsets corresponding to single or expanded clones in the different groups. Significant differences in cell proportions of expanded clones between the groups by the unpaired, two-sided Wilcoxon rank-sum test are marked with an asterisk (increased MAIT in MS: $p = 2.53 \times 10^{-2}$ vs. RA, $p = 3.39 \times 10^{-2}$ vs. Ps, $p = 4.25 \times 10^{-2}$ vs. control; reduced CD8+ memory T cells in RA: $p = 1.43 \times 10^{-2}$ vs. Ps, $p = 7.05 \times 10^{-3}$ vs. control).

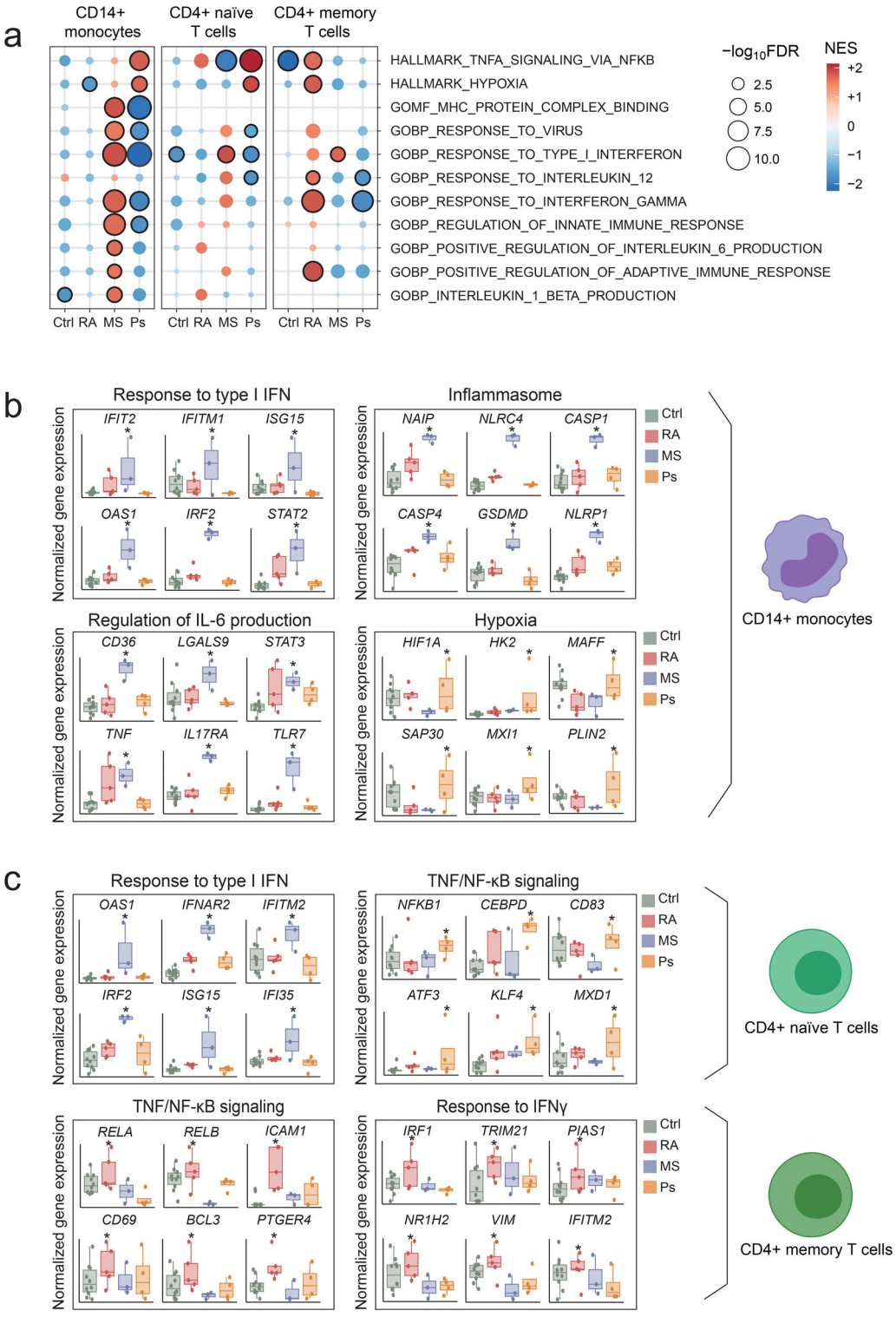

Figure 3. Genes differentially expressed in patients with pre-existing autoimmunity under SARS-CoV-2 infection. (A) Dot plots depicting the gene set enrichment analysis (GSEA) in CD14+ monocytes, CD4+ naïve T cells, and CD4+ memory T cells of differentially expressed genes between the COVID-19 patient groups (control, RA, MS, and Ps). The scale represents the normalized enrichment score (NES); the circle size indicates the -$\log_{10}$FDR value, where the circle edge represents the statistical significance of the enrichment (black: significant; no edge: not significant). (B) Box plots depicting the normalized mean expression of differentially expressed genes (false discovery rate [FDR] < 0.05) between the four COVID-19 patient groups that are involved in pathways implicated in (A) in CD14+ monocytes (see Supporting information Table S4 for FDR values). The asterisk indicates the significantly different patient group. (C) Box plots depicting the normalized mean expression of genes differentially expressed (FDR < 0.05) between the four COVID-19 patient groups involved in the pathways implicated in (A) in CD4+ naïve and memory T cells (see Supporting information Table S4 for FDR values). Similarly, the asterisk indicates a significantly different patient group.

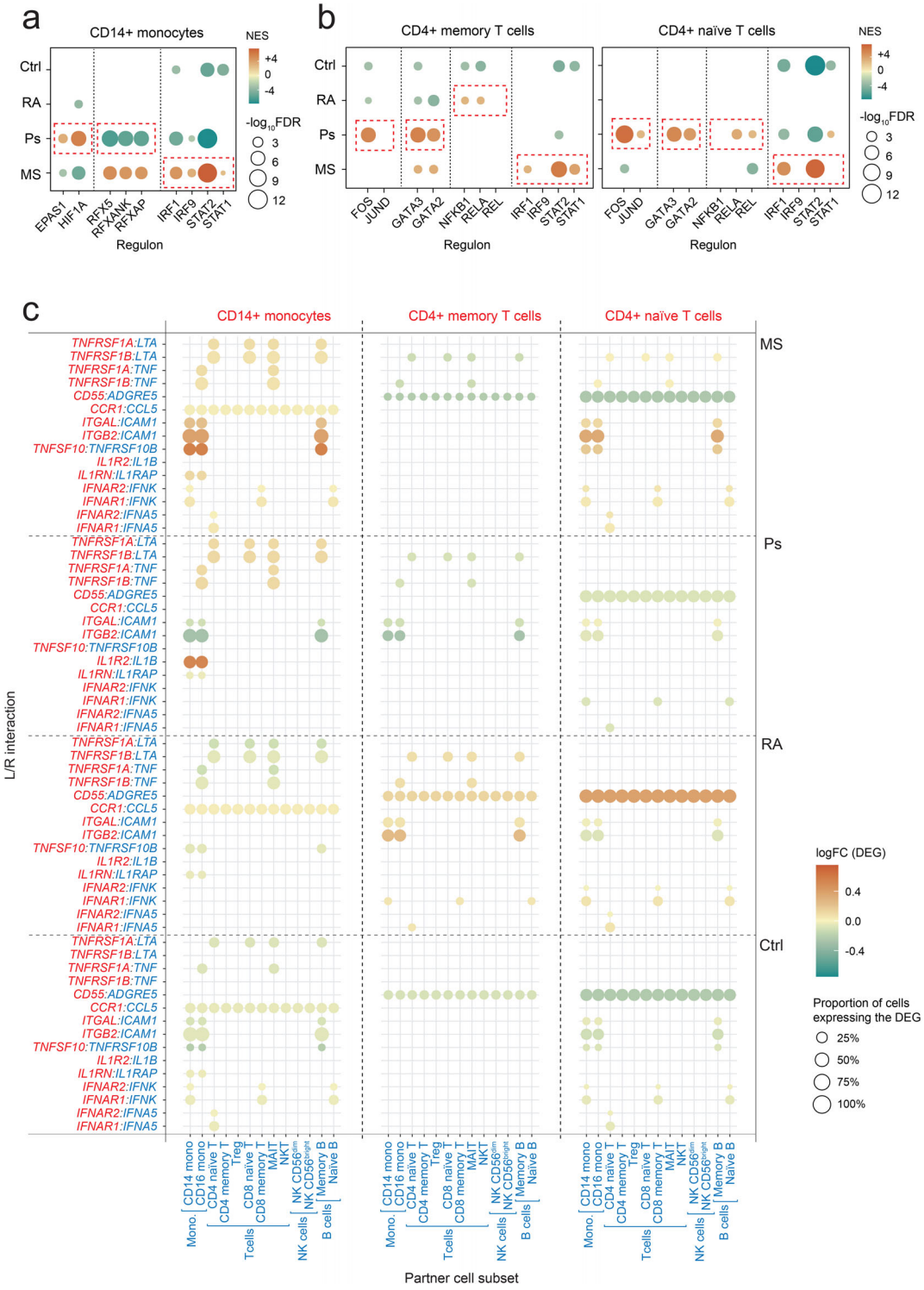

Figure 4. Transcription factor activity and cell–cell communication alterations in patients with pre-existing autoimmunity under SARS-CoV-2 infection. (A) Dot plot showing selected transcription factor activities of different regulons in CD14+ monocytes from the different COVID-19 patient groups (control, RA, Ps, and MS). The scale represents the normalized enrichment score (NES); the circle size indicates the $-\log_{10}$FDR value. (B) Dot plots showing selected transcription factor activities of different regulons in CD4+ naïve and memory T cells from each patient group. The scale represents the normalized enrichment score (NES); the circle size indicates the $-\log_{10}$FDR value. (c) Dot plot of selected dysregulated ligand/receptor (L/R) interactions between CD14+ monocytes or CD4+ T cells (naïve and memory) and other immune cell compartments in the COVID-19 patient groups. The scale indicates the $\log_2$(FC) in gene expression of differentially expressed genes (FDR < 0.05); the circle size represents the percentage of cells expressing the differentially expressed gene. Molecules of the L/R pairs expressed in the inspected immune subset (CD14+ monocyte, CD4+ naïve T cell, or CD4+ memory T cell) are shown in red, and molecules of the L/R pairs expressed in the immune cell partner are shown in blue. Assays were performed at the mRNA level, but results were extrapolated to protein interactions.

T cells (Fig. 4B), which was also the cell subset displaying TNF-α signaling enrichment as observed earlier. In contrast to the GATA3 signature that was already detectable in uninfected Ps individuals (which may likely be the consequence of immunomodulatory treatment in such patients [52]), the signatures for AP-1 in Ps patients and NF-κB in Ps and RA patients were only observed under SARS-CoV-2 infection and not in uninfected individuals (RA analysis performed on published dataset [35]; Supporting information Fig. S1D and Tables S5 and S6), suggesting that they originate specifically in response to the infection.

Overall, these results reveal altered TF activities associated with IL-4, type I IFN, TNF, and hypoxia pathway unique to the autoimmune conditions under SARS-CoV-2 infection.

Defects in cell–cell communication in COVID-19 patients with pre-existing autoimmunity

To systematically analyze the effect of cell–cell communication on immune responses upon SARS-CoV-2 infection, we used CellPhoneDB [38, 53], which infers specific cell–cell interactions based on the expression of ligands and receptors. We identified alterations in several ligand-receptor pairs involved in the immune and inflammatory responses (Fig. 4C and Supporting information Table S8). For instance, in MS COVID-19 patients, we detected a dysregulated higher interaction between TRAIL (*TNFSF10*), expressed in CD14+ monocytes and CD4+ naïve T cells, and its receptor *TNFRSF10B*, expressed in monocytes and memory B cells (Fig. 4C), which is probably a consequence of the upstream IFN activation [54]. A similar dysregulated interaction pattern in the same cell subsets was observed with respect to LFA-1 integrin (consisting of *ITGB2* and *ITGAL* subunits) and its ligand CD54 (*ICAM1*) (Fig. 4C). Enhanced interaction between the CCL5 ligand, expressed in all the immune cells inspected, and the CD191 (*CCR1*) receptor, whose inhibition potentially suppresses immune hyperactivation in critical COVID-19 [55], was noted in the MS and RA COVID-19 groups for CD14+ monocytes. Our analysis also revealed, in RA COVID-19 patients, a stronger interaction between CD55, expressed in CD4+ T cells, and its ligand CD97 (*ADGRE5*) (Fig. 4C), which has been described to enhance T cell activation [56]. While a stronger TNF/TNFR interaction was observed in the CD14+ monocyte subset of MS and Ps COVID-19 patients, such interaction predominated in the CD4+ memory T cells of the RA group (Fig. 4C), which is consistent with the previously observed downstream activation of NF-κB in the same cell type. Additionally, in Ps COVID-19 patients, we observed a stronger autocrine/paracrine interaction between IL-1β, expressed in CD14+ and CD16+ monocytes, and its inhibitory receptor (*IL1R2*), expressed in CD14+ monocytes (Fig. 4C).

Consistent with the overarching immune pattern revealed in this study, we also found stronger interactions with type I IFN receptors (*IFNAR1* and *IFNAR2*) in the CD4+ T cell subsets for MS and RA COVID-19 patients, as well as in CD14+ monocytes predominantly for the MS COVID-19 group (Fig. 4C), which most likely explains the general type I IFN signature seen.

In summary, we found a dysregulated network of cell–cell communication events in autoimmune disease patients in response to infection involving inflammatory cytokines, cell adhesion molecules, and cell activation regulators, which likely modulate the downstream TFs pathways and may impact the antiviral immune responses triggered in these patients.

Discussion

Here, we analyzed the immune response changes in the peripheral blood of three groups of autoimmune disease patients during the course of COVID-19 by combining single-cell transcriptome and surface proteome profiles. Our study revealed that these patients display aberrant immune responses to SARS-CoV-2 infection that are most likely dependent on the context of the autoimmune disease.

Very few studies have addressed the implications of chronic underlying inflammatory conditions in relation to the severity of COVID-19, focusing on clinical or epidemiological analyses rather than immunological insights. A meta-analysis of 62 observational studies, covering more than 300,000 patients with autoimmune diseases, suggested that these patients had an increased risk of COVID-19 [21]. More recently, another study has shown that patients with immune-mediated inflammatory diseases were at higher risk of being hospitalized with COVID-19 [23]. Conversely, another study suggested that patients with autoimmunity who required hospital admission owing to SARS-CoV-2 infection had a lower risk of developing severe disease and were less likely to require a stay in the intensive care unit or mechanical ventilation [22]. It has been shown that MS patients with advanced age and disease, as well as those with greater disability, are at an increased likelihood of developing severe and even fatal COVID-19 [57]. In addition, as viral respiratory infections are recognized to increase the risk of relapse in MS patients [58], there is a concern that COVID-19 might exacerbate symptoms in MS individuals, though this remains an open question [59]. For RA patients, it has also been described that such patients face an increased risk of severe COVID-19, particularly those with related interstitial lung disease [60]. Furthermore, several studies have consistently revealed that unvaccinated individuals with RA show an elevated risk of COVID-19 hospitalization and mortality [61–63]. For Ps patients, it has been suggested that although individuals can have an increased risk of contracting SARS-CoV-2 infection, they may not exhibit increased susceptibility to the complications of COVID-19 [64]. Given such divergent scenarios, our study aimed at exploring the particular immune signatures to SARS-CoV-2 infection in individuals with pre-existing autoimmune diseases.

Our analysis of cell proportions revealed that COVID-19 patients with pre-existing autoimmunity do not display features characterizing severity, such as expanded HSCs, platelets, or plasmablasts [30]. However, the three autoimmune groups studied showed a marked expansion of HLA-DR^{low} CD14+ monocytes. Expansion of these dysfunctional HLA-DR^{low} monocytes has recently been described in patients with severe COVID-19 [8,

11]. Regardless of whether this subset of monocytes is already present at baseline in autoimmune disease patients or if instead it is expanded under SARS-CoV-2 infection, the high proportion of HLA-DR^{low} CD14+ monocytes in COVID-19 with pre-existing autoimmunity might interfere with a proper antiviral response, given that these monocytes are characterized by displaying an immunosuppressive phenotype [65–68]. Remarkably, MS COVID-19 patients had higher proportions, as well as clonal expansion, of circulating MAIT cells. Higher proportions of MAIT cells have been reported in asymptomatic and mild infections [30], and their expansion may enhance the antiviral immune response in these patients [69].

We also identified a predominant upregulation of the type I IFN pathway along with its downstream transcription factors (i.e. STAT1, STAT2, and IRF9) in CD14+ monocytes and CD4+ T cells of MS COVID-19 patients. This signature most likely stems from a stronger ligand/receptor (IFNAR1 and IFNAR2) interaction, as identified in our cell–cell communication analysis. Whether this feature may be beneficial or detrimental to these patients in the context of SARS-CoV-2 infection remains unclear. Several studies have identified impaired type I IFN activity in patients with severe COVID-19 [7, 70–74], and COVID-19 patients treated with inhaled nebulized IFN beta-1a (SNG001) showed greater odds of improvement and recovered more rapidly from SARS-CoV-2 infection [75]. On the other hand, it has been suggested that such a robust type I IFN response potentially exacerbates hyperinflammation, facilitating the development of severe COVID-19 [76, 77]. This predominant IFN signature in MS COVID-19 patients may therefore be a double-edged sword, simultaneously promoting antiviral immune responses and exacerbating immune hyperactivation in these patients. Angiotensin-converting enzyme 2 (*ACE2*), whose protein product binds to the SARS-CoV-2 spike (S) protein and promotes cellular entry of the virus, was initially described as a human ISG [78], but these results were challenged shortly after [79, 80]. Indeed, increased SARS-CoV-2 cell entry leading to higher viral loads is ultimately associated with increased COVID-19 severity and mortality [81, 82]. In this regard, the ISG *LY6E*, which has been reported to impair coronavirus fusion and restrict its entry into cells [44, 45], was upregulated in MS COVID-19 patients relative to the other patient groups in both CD14+ monocytes and CD4+ naïve T cells. Notably, our analysis of uninfected MS patients did not show this predominant IFN signature, suggesting that it originates specifically in response to the infection.

In Ps COVID-19 patients, we found a higher level of GATA3 pathway activity in the CD4+ T-cell compartment. GATA3 is a crucial TF involved in Th2 polarization [83], which is induced by IL-4. In this regard, we have also detected a higher level of the IL-4 receptor protein (CD124) in the CD4+ T cells of these patients, which likely explains the observed greater activity of GATA3. The Th2 immune response has been linked to a fatal outcome of COVID-19 [16]. Higher proportions of Th2 cells have been further detected in poor-prognosis COVID-19 patients [84], with cytological signals of the Th2 immune response in peripheral blood from COVID-19 patients who required intensive care [85]. As such, a high Th2 polarization in Ps patients during SARS-CoV-2 infection, which might be caused or enhanced by the specific treatments received and/or the underlying immune condition (86), might represent an adverse immunological feature in these patients.

Beyond CD4+ T cells, we identified altered immune responses in the myeloid compartment of Ps COVID-19 patients. For instance, the CD14+ monocytes of Ps COVID-19 individuals showed a higher level of activation of the HIF pathway. HIF factors are upregulated at low oxygen concentrations and are involved in the regulation of immune responses [87]. Although control COVID-19 patients also showed activation of the HIF pathway, Ps COVID-19 patients displayed significantly higher levels of activity in this HIF pathway, predominantly in CD14+ monocytes. This pathway has been associated with the acquisition of an immunosuppressive phenotype in contexts characterized by altered immune responses such as sepsis [47]. Taken together, these results suggest that Ps patients may confront a SARS-CoV-2 infection with inefficient responses in several immune compartments that affect their innate and adaptive immune systems.

For RA COVID-19 patients, in addition to increased proportions and a trend toward clonal expansion of CD4+ memory T cells, this immune subset displayed the highest level of dysregulation in this patient group. In particular, we observed significant activation of TNF-α/NF-κB signaling as well as upregulation of the IL-12 and IFN-γ responses. Synergistic activation of these cytokines occurs in CD4+ T cells and is associated with the exacerbated inflammatory response in severe and critical COVID-19 [7, 88]. Additionally, inhibition of NF-κB and TNF-α is thought to have a therapeutic potential for alleviating the cytokine storm and COVID-19 severity [89].

The appearance of sequelae in some COVID-19 patients after the acute phase of infection is a paramount clinical aspect that has become apparent during the pandemic. Such sequelae include manifestations like pulmonary damage, thromboembolism, and neuroinflammation among others [90]. Pulmonary sequelae, ranging from dyspnea to fibrotic damage and persistent oxygen requirement, might be a consequence of elevated inflammatory cytokine production and lung invasion by neutrophils and monocytes [90]. In this regard, our results indicate that the IL-6 pathway is significantly upregulated in CD14+ monocytes of MS COVID-19 patients, which may ultimately facilitate a fibrotic state [91]. Furthermore, LFA-1 was upregulated in the CD14+ monocytes of MS COVID-19 patients, potentially favoring monocyte extravasation into the alveolar space [92]. Conversely, CD14+ monocytes of Ps COVID-19 patients and CD4+ memory T cells of RA COVID-19 patients displayed hypoxia features, which are related to thrombotic complications in COVID-19 patients [93].

The limited number of patients included in this study reflects the difficulty in collecting samples from such patient populations at the time of infection, especially given that patients classified as at-risk, including autoimmune disease patients, significantly reduced their frequency of medical visits during the COVID-19 pandemic [94, 95]. Additionally, although we perform several analyses to control for the inherent heterogeneity between groups

(accounting for differences in COVID-19 disease course and severity, sex, treatment, timing, and infection status; see Methods), such differences should be considered for broad conclusions. Nevertheless, our cohort is unique and valuable by including COVID-19 patients with pre-existing autoimmunity in a prevaccination state, and it allowed us to explore unresolved questions in the field of immunology and virology. One of the unique strengths of our work is the validation of the immune responses observed across separate computational analyses and tools, particularly from immune ligand/receptor interactions and downstream pathway activity, providing consistent and complementary evidence of immune dysregulation.

While our analysis focused on identifying shared responses among all patients within each autoimmune disease group to minimize the influence of specific prior immunomodulatory treatments on the observed transcriptional changes upon SARS-CoV-2 infection, this is a limitation that could potentially impact the findings. Nevertheless, it is worth noting that individuals with autoimmune diseases often have a history of immunomodulatory treatments whose effects become intertwined with the chronic autoimmune state in these patients. These combined factors together can then shape how these patients respond to SARS-CoV-2 infection. Therefore, our study provides valuable insights into the immune responses to infection in patients with pre-existing autoimmunity on immunomodulatory treatment, which is most representative of typical autoimmune disease patients.

In our cohort of patients with pre-existing autoimmune conditions, some patients received rituximab prior to SARS-CoV-2 infection. Published evidence suggested that such rituximab treatment may be linked to unfavorable COVID-19 outcomes [96, 97]. Our analysis may be consistent with this hypothesis revealing that monocytes from such rituximab-treated patients have reduced expression of several genes involved in the antiviral response and inflammasome activation during SARS-CoV-2 infection. Nonetheless, patients on anti-CD20 treatment are still able to generate robust T-cell responses following COVID-19 vaccination, including CD8+ T-cell responses linked to milder COVID-19 outcomes, despite the impaired humoral responses, which may be important in reducing the risk of complications associated with severe COVID-19 [98, 99].

Our results highlight the presence of altered and diverging immune responses in autoimmune disease patients that are specific to SARS-CoV-2 infection, and that may affect the course of the disease. Future molecular and clinical studies building on our work, including expanded cohorts, will shed light on the ultimate consequences of the range of altered immune responses observed, including whether they are ultimately beneficial or harmful for these patients. The influence of these specific immune responses on the risk of evolution to severe forms of COVID-19 and/or clinical sequelae after the acute phase of SARS-CoV-2 infection, which may help find tailored treatments for different autoimmune patient populations, is particularly important to consider. This study presents a substantial and critical step toward that end. Notably, given the overlapping features of immunopathology between COVID-19 and other infections, such as influenza [100, 101], the implications of the altered immune responses observed here may further extend the study on how autoimmune disease patients cope with other viral infections.

Acknowledgements: This publication is part of the Human Cell Atlas (https://www.humancellatlas.org/publications/). The authors gratefully acknowledge the Sanger Cellular Genetics Informatics team, particularly A. Predeus and S. Murray for their assistance with aligning the published raw data from uninfected MS patients, as well as S. van Dongen, M. Prete, and Q. Lin for their help with online data hosting. We acknowledge the members of the Vento-Tormo and Ballestar groups for useful discussions. A.B. received additional support from a Gates Cambridge Scholarship. This research was funded/supported by the R+D+i project of the Spanish Ministry of Science and Innovation (grant number PID2020-117212RB-I00/ MICIN/AEI/10.13039/501100011033), the Chan Zuckerberg Initiative (grant 2020–216799) and Wellcome Sanger core funding (WT206194). This publication has been supported by the Unstoppable campaign of the Josep Carreras Leukaemia Foundation. B.G., F.J.C.-N., and N.K.W. were funded by Wellcome (206328/Z/17/Z), the MRC (MR/S036113/1), and the Aging Biology Foundation.

Conflict of interest: I.G.-A. has received research support from Sanofi, Roche, and Lilly. H.H. is a co-founder and shareholder of Omniscope. The remaining authors declare no conflicts of interest.

Author contributions: Esteban Ballestar, Roser Vento-Tormo, and Javier Rodríguez-Ubreva conceived and designed the study; Gerard Godoy-Tena, Laura Ciudad, Tarryn Porter, Laura Richardson, Carmen Sancho-Serra, and Javier Rodríguez-Ubreva performed sample preparation and 10x experiments; Regina Hoo, Tarryn Porter, Agnes Oszlanczi, Laura Richardson, Carmen Sancho-Serra, and Elena Prigmore generated libraries and performed the sequencing; Eduardo Andrés-León and Javier Martin performed patient genotyping; Anis Barmada, Louis-François Handfield, Carlos de la Calle-Fabregat, Anna Arutyunyan, Eduardo Andrés-León, and Javier Rodríguez-Ubreva performed the computational analyses; Fernando J. Calero-Nieto, Nicola K. Wilson and Berthold Göttgens optimized the CITE-seq protocol; Domenica Marchese and Holger Heyn provided support with the 10x experiments; Jorge Carrillo, Silvia Presas-Rodríguez, Cristina Ramo-Tello, Adolfo Ruiz-Sanmartin, Ricard Ferrer, Juan Carlos Ruiz-Rodriguez, Mónica Martínez-Gallo, Mónica Munera-Campos, Jose Manuel Carrascosa, Ivette Casafont-Solé, Xavier Solanich, Ildefonso Sánchez-Cerrillo, Isidoro González-Álvaro, Maria Gabriella Raimondo, Andreas Ramming, and Eva Martínez-Cáceres provided patient samples and analyzed clinical data; Anis

Barmada, Louis-François Handfield, Gerard Godoy-Tena, Carlos de la Calle-Fabregat, Regina Hoo, Javier Martin, Esteban Ballestar, Roser Vento-Tormo, and Javier Rodríguez-Ubreva analyzed and interpreted the data; Anis Barmada and Javier Rodríguez-Ubreva wrote the manuscript with contributions from Gerard Godoy-Tena, Carlos de la Calle-Fabregat, Esteban Ballestar, and Roser Vento-Tormo; Esteban Ballestar, Roser Vento-Tormo and Javier Rodríguez-Ubreva supervised the study; all authors read and approved the final manuscript.

Data availability statement: All raw single-cell data used in this study have been deposited under controlled access at the European Genome-Phenome Archive (EGA) under accession number EGAD00001007982, where access requests can be submitted through the Sanger Data Access Committee. To enable direct re-analysis of the single-cell dataset, processed data, as an h5ad file containing raw and normalized counts along with interactive visualization, are further available through the COVID-19 Cell Atlas web portal (https://www.covid19cellatlas.org/index.patient.html; download under "COVID-19 Autoimmunity PBMCs").

References

1 **Schultze, J. L. and Aschenbrenner, A. C.**, COVID-19 and the human innate immune system. *Cell*. 2021. **184**: 1671–1692.

2 **Sette, A. and Crotty, S.**, Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell*. 2021. **184**: 861–880.

3 **Wang, C., Xie, J., Zhao, L., Fei, X., Zhang, H., Tan, Y., Nie, X. et al.**, Alveolar macrophage dysfunction and cytokine storm in the pathogenesis of two severe COVID-19 patients. *EBioMedicine*. 2020. **57**: 102833.

4 **Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L. et al.**, Chronic inflammation in the etiology of disease across the life span. *Nat. Med*. 2019. **25**: 1822–1832.

5 **Tay, M. Z., Poh, C. M., Rénia, L., Macary, P. A. and Ng, L. F. P.**, The trinity of COVID-19: immunity, inflammation and intervention. *Nat. Rev. Immunol.* 2020. **20**: 363–374.

6 **Henry, B. M., De Oliveira, M. H. S., Benoit, S., Plebani, M. and Lippi, G.**, Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin. Chem. Lab. Med.* 2020. **58**: 1021–1028.

7 **Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., Péré, H. et al.**, Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science*. 2020. **369**: 718–724.

8 **Silvin, A., Chapuis, N., Dunsmore, G., Goubet, A.-G., Dubuisson, A., Derosa, L., Almire, C. et al.**, Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19. *Cell*. 2020. **182**: 1401–1418.e18.e18.

9 **Guo, C., Li, B., Ma, H., Wang, X., Cai, P., Yu, Q., Zhu, L. et al.**, Single-cell analysis of two severe COVID-19 patients reveals a monocyte-associated and tocilizumab-responding cytokine storm. *Nat Commun*. 2020. **11**().

10 **Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Qi, Y., Sun, R. et al.**, Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. *Natl. Sci. Rev.* 2020. **7**: 998–1002.

11 **Schulte-Schrepping, J., Reusch, N., Paclik, D., Baßler, K., Schlickeiser, S., Zhang, B., Krämer, B. et al.**, Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell*. 2020. **182**: 1419–1440.e23.e23.

12 **Tian, Y., Carpp, L. N., Miller, H. E. R., Zager, M., Newell, E. W. and Gottardo, R.**, Single-cell immunology of SARS-CoV-2 infection. *Nat. Biotechnol.* 2022. **40**: 30–41.

13 **Sánchez-Cerrillo, I., Landete, P., Aldave, B., Sánchez-Alonso, S., Sánchez-Azofra, A., Marcos-Jiménez, A., Ávalos, E. et al.**, COVID-19 severity associates with pulmonary redistribution of CD1c+ DCs and inflammatory transitional and nonclassical monocytes. *J Clin Invest*. 2020. **130**: 6290–6300.

14 **Yao, C., Bora, S. A., Parimon, T., Zaman, T., Friedman, O. A., Palatinus, J. A., Surapaneni, N. S. et al.**, Cell-type-specific immune dysregulation in severely Ill COVID-19 patients. *Cell Rep*. 2021. **34**: 108590.

15 **Wong, J. J. M., Leong, J. Y., Lee, J. H., Albani, S. and Yeo, J. G.**, Insights into the immuno-pathogenesis of acute respiratory distress syndrome. *Ann. Transl. Med*. 2019. **7**: 504–504.

16 **Gil-Etayo F. J., Suàrez-Fernández P., Cabrera-Marante O., Arroyo D., Garcinuño S., Naranjo L., Pleguezuelo D. E. et al.**, T-helper cell subset response is a determining factor in COVID-19 progression. *Front. Cell Infect. Microbiol*. 2021. **11**: 624483.

17 **Giamarellos-Bourboulis, E. J., Netea, M. G., Rovina, N., Akinosoglou, K., Antoniadou, A., Antonakos, N., Damoraki, G. et al.**, Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe*. 2020. **27**: 992–1000.e3.e3.

18 **Meckiff, B. J., Ramírez-Suástegui, C., Fajardo, V., Chee, S. J., Kusnadi, A., Simon, H., Eschweiler, S. et al.**, Imbalance of regulatory and cytotoxic SARS-CoV-2-reactive CD4+ T cells in COVID-19. *Cell*. 2020;**183**: 1340–1353.e16.e16.

19 **Freites Nuñez, D. D., Leon, L., Mucientes, A., Rodriguez-Rodriguez, L., Font Urgelles, J., Madrid García, A., Colomer, J. I. et al.**, Risk factors for hospital admissions related to COVID-19 in patients with autoimmune inflammatory rheumatic diseases. *Ann. Rheum. Dis*. 2020;**79**: 1393–1399.

20 **Willis, M. D. and Robertson, N. P.**, Multiple sclerosis and the risk of infection: considerations in the threat of the novel coronavirus, COVID-19/SARS-CoV-2. *J. Neurol*. 2020. **267**: 1567–1569.

21 **Akiyama, S., Hamdeh, S., Micic, D. and Sakuraba, A.**, Prevalence and clinical outcomes of COVID-19 in patients with autoimmune diseases: a systematic review and meta-analysis. *Ann. Rheum. Dis*. 2021. **80**: 384–391.

22 **Sarmiento-Monroy, J. C., Espinosa, G., Londoño, M. C., Meira, F., Caballol, B., Llufriu, S., Carrasco, J. L. et al.**, A multidisciplinary registry of patients with autoimmune and immune-mediated diseases with symptomatic COVID-19 from a single center. *J. Autoimmun*. 2021. **117**: 102580.

23 **Eder, L., Croxford, R., Drucker, A. M., Mendel, A., Kuriya, B., Touma, Z., Johnson, S. R. et al.**, COVID-19 hospitalizations, intensive care unit stays, ventilation, and death among patients with immune-mediated inflammatory diseases compared to controls. *J. Rheumatol*. 2022. **49**: 523–530. Available from: http://www.jrheum.org/content/49/5/523.abstract

24 **Troyano-Hernáez, P., Reinosa, R. and Holguín, Á.**, Evolution of SARS-CoV-2 in Spain during the first two years of the pandemic: circulating variants, amino acid conservation, and genetic variability in structural, non-structural, and accessory proteins. *Int. J. Mol. Sci*. 2022. **23**: 6394.

25 **Heaton, H., Talman, A. M., Knights, A., Imaz, M., Gaffney, D. J., Durbin, R., Hemberg, M. et al.**, Souporcell: robust clustering of single-cell RNA-seq data by genotype without reference genotypes. *Nat. Methods*. 2020. **17**: 615–620.

26 **Wolock, S. L., Lopez, R. and Klein, A. M.**, Scrublet: Computational identification of cell doublets in single-cell transcriptomic data. *Cell Syst*. 2019. **8**: 281–291.e9.

27 **Young, M. D. and Behjati, S.**, SoupX removes ambient RNA contamination from droplet-based single-cell RNA sequencing data. *Gigascience*. 2020. **9**: giaa151.

28 **Gayoso, A., Steier, Z., Lopez, R., Regier, J., Nazor, K. L., Streets, A. and Yosef, N.**, Joint probabilistic modeling of single-cell multi-omic data with totalVI. *Nat. Methods*. 2021. **18**: 272–282.

29 **Wolf, F. A., Angerer, P. and Theis, F. J.**, SCANPY: Large-scale single-cell gene expression data analysis. *Genome Biol*. 2018. **19**.

30 **Stephenson, E., Reynolds, G., Botting, R. A., Calero-Nieto, F. J., Morgan, M. D., Tuong, Z. K., Bach, K. et al.**, Single-cell multi-omics analysis of the immune response in COVID-19. *Nat. Med*. 2021: 904–916.

31 **Sturm, G., Szabo, T., Fotakis, G., Haider, M., Rieder, D., Trajanoski, Z. and Finotello, F.**, Scirpy: a Scanpy extension for analyzing single-cell T-cell receptor-sequencing data. *Bioinformatics*. 2020. **36**: 4817–4818.

32 **Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W. and Smyth, G. K.**, Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015. **43**: e47.

33 **Ren, X., Wen, W., Fan, X., Hou, W., Su, B., Cai, P., Li, J. et al.**, COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. *Cell*. 2021. **184**: 1895–1913.e19.e19.

34 **Schafflick, D., Xu, C. A., Hartlehnert, M., Cole, M., Schulte-Mecklenbeck, A., Lautwein, T., Wolbert, J. et al.**, Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis. *Nat. Commun*. 2020. **11**: 247.

35 **Ranjan, B., Sun, W., Park, J., Mishra, K., Schmidt, F., Xie, R., Alipour, F. et al.**, DUBStepR is a scalable correlation-based feature selection method for accurately clustering single-cell data. *Nat. Commun*. 2021. **12**: 5849.

36 **Yu, G., Wang, L. G., Han, Y. and He, Q. Y.**, ClusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS: J. Integr. Biol*. 2012. **16**: 284–287.

37 **Garcia-Alonso, L., Holland, C. H., Ibrahim, M. M., Turei, D. and Saez-Rodriguez, J.**, Benchmark and integration of resources for the estimation of human transcription factor activities. *Genome Res*. 2019. **29**: 1363–1375.

38 **Efremova, M., Vento-Tormo, M., Teichmann, S. A. and Vento-Tormo, R.**, CellPhoneDB: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes. *Nat. Protoc*. 2020. **15**: 1484–1506.

39 **Stoeckius, M., Hafemeister, C., Stephenson, W., Houck-Loomis, B., Chattopadhyay, P. K., Swerdlow, H., Satija, R. et al.**, Simultaneous epitope and transcriptome measurement in single cells. *Nat. Methods*. 2017. **14**: 865–868.

40 **Hottz, E. D., Azevedo-Quintanilha, I. G., Palhinha, L., Teixeira, L., Barreto, E. A., Pão, C. R. R., Righy, C. et al.**, Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. *Blood*. 2020. **136**: 1330–1341.

41 **Grifoni, A., Weiskopf, D., Ramirez, S. I., Mateus, J., Dan, J. M., Moderbacher, C. R., Rawlings, S. A. et al.**, Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell*. 2020. **181**: 1489–1501.e15.e15.

42 **Sekine, T., Perez-Potti, A., Rivera-Ballesteros, O., Strålin, K., Gorin, J. B., Olsson, A., Llewellyn-Lacey, S. et al.**, Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell*. 2020. **183**: 158–168.e14.e14.

43 **Xu, X., Qiu, C., Zhu, L., Huang, J., Li, L., Fu, W., Zhang, L. et al.**, IFN-stimulated gene LY6E in monocytes regulates the CD14/TLR4 pathway but inadequately restrains the hyperactivation of monocytes during chronic HIV-1 infection. *J. Immunol*. 2014. **193**: 4125–4136.

44 **Pfaender, S., Mar, K. B., Michailidis, E., Kratzel, A., Boys, I. N., V'kovski, P., Fan, W. et al.**, LY6E impairs coronavirus fusion and confers immune control of viral disease. *Nat. Microbiol*. 2020. **5**: 1330–1339.

45 **Zhao, X., Zheng, S., Chen, D., Zheng, M., Li, X., Li, G., Lin, H.** et al, LY6E restricts entry of human coronaviruses, including currently pandemic SARS-CoV-2. *J. Virol*. 2020. **94**: e00562–20.

46 **Johnson, J. K., Wright, P. W., Li, H. and Anderson, S K.**, Identification of trophoblast-specific elements in the HLA-C core promoter. *HLA*. 2018. **92**: 288–297.

47 **Shalova, I. N., Lim, J. Y., Chittezhath, M., Zinkernagel, A. S., Beasley, F., Hernández-Jiménez, E., Toledano, V. et al.**, Human monocytes undergo functional re-programming during sepsis mediated by hypoxia-inducible factor-1α. *Immunity*. 2015. **42**: 484–498.

48 **Cheng, S. C., Quintin, J., Cramer, R. A., Shepardson, K. M., Saeed, S., Kumar, V. et al.**, MTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science (80-)*. 2014. **345**.

49 **Swain, S. L., Weinberg, A. D., English, M. and Huston, G.**, IL-4 directs the development of Th2-like helper effectors. *J. Immunol*. 1990. **145**: 3796–3806. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2147202

50 **Wurster, A. L., Rodgers, V. L., Satoskar, A. R., Whitters, M. J., Young, D. A., Collins, M. and Grusby, M. J.**, Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon γ-producing Th1 cells. *J. Exp. Med*. 2002. **196**: 969–977.

51 **Hayden, M. S. and Ghosh, S.**, Regulation of NF-κB by TNF family cytokines. *Semin. Immunol*. 2014. **26**: 253–266.

52 **Quaglino, P., Bergallo, M., Ponti, R., Barberio, E., Cicchelli, S., Buffa, E., Comessatti, A. et al.**, Th1, Th2, Th17 and regulatory T cell pattern in psoriatic patients: Modulation of cytokines and gene targets induced by etanercept treatment and correlation with clinical response. *Dermatology*. 2011. **223**: 57–67.

53 **Vento-Tormo, R., Efremova, M., Botting, R. A., Turco, M. Y., Vento-Tormo, M., Meyer, K. B., Park, J. E. et al.**, Single-cell reconstruction of the early maternal–fetal interface in humans. *Nature*. 2018. **563**: 347–353.

54 **Peteranderl, C. and Herold, S.**, The impact of the interferon/TNF-related apoptosis-inducing ligand signaling axis on disease progression in respiratory viral infection and beyond. *Front. Immunol.* 2017. **8**: 313.

55 **Chua, R. L., Lukassen, S., Trump, S., Hennig, B. P., Wendisch, D., Pott, F., Debnath, O. et al.**, COVID-19 severity correlates with airway epithelium–immune cell interactions identified by single-cell analysis. *Nat. Biotechnol*. 2020. **38**: 970–979.

56 **Capasso, M., Durrant, L. G., Stacey, M., Gordon, S., Ramage, J. and Spendlove, I.**, Costimulation via CD55 on human CD4 + T cells mediated by CD97. *J. Immunol*. 2006. **177**: 1070–1077.

57 **Arrambide, G., Llaneza-González, M. Á., Costa-Frossard França, L., Meca-Lallana, V., Díaz, E. F., Moreno-Torres, I., García-Domínguez, J. M. et al.**, SARS-CoV-2 infection in multiple sclerosis: results of the Spanish neurology society registry. *Neurol. Neuroimmunol*. 2021. **8**: e1024.

58 **Barzegar, M., Vaheb, S., Mirmosayyeb, O., Afshari-Safavi, A., Nehzat, N. and Shaygannejad, V.**, Can coronavirus disease 2019 (COVID-19) trigger exacerbation of multiple sclerosis? A retrospective study. *Mult. Scler. Relat. Disord*.2021. **52**: 102947.

59 **Muñoz-Jurado, A., Escribano, B M., Agüera, E., Caballero-Villarraso, J., Galván, A. and Túnez, I.**, SARS-CoV-2 infection in multiple sclerosis patients: interaction with treatments, adjuvant therapies, and vaccines against COVID-19. *J. Neurol.* 2022. **269**: 4581–4603.

60 **Figueroa-Parra, G., Gilbert, E. L., Valenzuela-Almada, M. O., Vallejo, S., Neville, M. R., Patel, N. J., Cook, C. et al.**, Risk of severe COVID-19 out-

comes associated with rheumatoid arthritis and phenotypic subgroups: a retrospective, comparative, multicentre cohort study. *Lancet Rheumatol*. 2022. **4**: e765–e774.

61 **Bournia, V. K., Fragoulis, G. E., Mitrou, P., Mathioudakis, K., Tsolakidis, A., Konstantonis, G., Tseti, I. et al.**, Different COVID-19 outcomes among systemic rheumatic diseases: a nation-wide cohort study. *Rheumatology (Oxford)*. 2023. **62**: 1047–1056.

62 **Bower, H., Frisell, T., Di Giuseppe, D., Delcoigne, B., Ahlenius, G. M., Baecklund, E., Chatzidionysiou, K. et al.**, Impact of the COVID-19 pandemic on morbidity and mortality in patients with inflammatory joint diseases and in the general population: a nationwide Swedish cohort study. *Ann. Rheum. Dis*. 2021. **80**: 1086–1093.

63 **Shin, Y Ho, Shin, J Il, Moon, S. Y., Jin, H. Y., Kim, So Y, Yang, J. M., Cho, S Ho et al.**, Autoimmune inflammatory rheumatic diseases and COVID-19 outcomes in South Korea: a nationwide cohort study. *Lancet Rheumatol*. 2021. **3**: e698–e706.

64 **Kridin, K., Schonmann, Y., Onn, E., Bitan, D. T., Weinstein, O., Shavit, E. and Cohen, A. D.**, Nineteen months into the pandemic, what have we learned about COVID-19-related outcomes in patients with psoriasis? *J. Cosmet. Dermatol*. 2022. **21**: 6549–6553.

65 **Lin, Yi, Gustafson, M. P., Bulur, P. A., Gastineau, D. A., Witzig, T. E. and Dietz, A. B.**, Immunosuppressive CD14+HLA-DRlow/- monocytes in B-cell non-Hodgkin lymphoma. *Blood*. 2011. **117**: 872–881.

66 **Mengos, A. E., Gastineau, D. A. and Gustafson, M P.**, The CD14+HLA-DrlO/NEG monocyte: an immunosuppressive phenotype that restrains responses to cancer immunotherapy. *Front. Immunol.* 2019. **10**: 1147.

67 **Reyes, M., Filbin, M. R., Bhattacharyya, R. P., Sonny, A., Mehta, A., Billman, K., Kays, K. R. et al.**, Plasma from patients with bacterial sepsis or severe COVID-19 induces suppressive myeloid cell production from hematopoietic progenitors in vitro. *Sci Transl Med*. 2021. **13**: eabe9599.

68 **Wilk, A. J., Lee, M. J., Wei, B., Parks, B., Pi, R., Martínez-Colón, G. J., Ranganath, T. et al.**, Multi-omic profiling reveals widespread dysregulation of innate immunity and hematopoiesis in COVID-19. *J. Exp. Med*. 2021. **218**: e20210582.

69 **Parrot, T., Gorin, J-B., Ponzetta, A., Maleki, K. T., Kammann, T., Emgård, J., Perez-Potti, A. et al.**, MAIT cell activation and dynamics associated with COVID-19 disease severity. *Sci. Immunol*. 2020. **5**: eabe1670.

70 **Trouillet-Assant, S., Viel, S., Gaymard, A., Pons, S., Richard, J. C., Perret, M., Villard, M. et al.**, Type I IFN immunoprofiling in COVID-19 patients. *J. Allergy. Clin. Immunol*. 2020. **146**: 206–208.e2.e2.

71 **Bastard, P., Rosen, L. B., Zhang, Q., Michailidis, E., Hoffmann, H. H., Zhang, Y., Dorgham, K. et al.**, Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science*. 2020. **370**: eabd4585.

72 **Zhang, Q., Bastard, P., Liu, Z., Le Pen, J., Moncada-Velez, M., Chen, J., Ogishi, M. et al.**, Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science*. 2020. **370**: eabd4570.

73 **Arunachalam, P. S., Wimmers, F., Mok, C. K. P., Perera, R A. P. M., Scott, M., Hagan, T., Sigal, N. et al.**, Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. *Science*. 2020. **369**: 1210–1220.

74 **Combes, A. J., Courau, T., Kuhn, N. F., Hu, K. H., Ray, A., Chen, W. S., Chew, N. W. et al.**, Global absence and targeting of protective immune states in severe COVID-19. *Nature*. 2021. **591**: 124–130.

75 **Monk, P. D., Marsden, R. J., Tear, V. J., Brookes, J., Batten, T. N., Mankowski, M., Gabbay, F. J. et al.**, Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Respir. Med*. 2021. **9**: 196–206.

76 **Lee, J. S. and Shin, E. C.**, The type I interferon response in COVID-19: implications for treatment. *Nat. Rev. Immunol*. 2020. **20**: 585–586.

77 **King, C. and Sprent, J.**, Dual nature of type I interferons in SARS-CoV-2-induced inflammation. *Trends Immunol*. 2021. **42**: 312–322.

78 **Ziegler, C. G.K., Allon, S. J., Nyquist, S. K., Mbano, I M., Miao, V. N., Tzouanas, C. N., Cao, Y. et al.**, SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. *Cell.* 2020. **181**: 1016–1035.e19.e19.

79 **Onabajo, O. O., Banday, A. R, Stanifer, M. L., Yan, W., Obajemu, A., Santer, D. M., Florez-Vargas, O. et al.**, Interferons and viruses induce a novel truncated ACE2 isoform and not the full-length SARS-CoV-2 receptor. *Nat Genet*. 2020. **52**: 1283–1293.

80 **Saffern, M.**, ACE2 is not induced by interferon. *Nat Rev Immunol*. 2020. **20**: 521.

81 **Fajnzylber, J., Regan, J., Coxen, K., Corry, H., Wong, C., Rosenthal, A., Worrall, D. et al.**, SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun*. 2020. **11**: 5493.

82 **Liu, Y., Yan, L. M., Wan, L., Xiang, T. X., Le, A., Liu, J. M. et al.**, Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect. Dis*. 2020. **20**. 656–657.

83 **Zhu, J., Yamane, H., Cote-Sierra, J., Guo, L. and Paul, W. E.**, GATA-3 promotes Th2 responses through three different mechanisms: Induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. *Cell Res.* 2006. **16**: 3–10.

84 **Lin, W. L., Jing, W. W., Xi, C. D. and Xu, B.**, Dysregulation of the immune response affects the outcome of critical COVID-19 patients. *J. Med. Virol*. 2020. **92**: 2768–2776.

85 **Roncati, L., Nasillo, V., Lusenti, B. and Riva, G.**, Signals of Th2 immune response from COVID-19 patients requiring intensive care. *Ann. Hematol*. 2020. **99**: 1419–1420.

86 **Ghoreschi, K., Thomas, P., Breit, S., Dugas, M., Mailhammer, R., Van Eden, W., Van Der Zee, R. et al.**, Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. *Nat Med*. 2003. **9**: 40–46.

87 **Palazon, A., Goldrath, A. W., Nizet, V. and Johnson, R. S.**, HIF transcription factors, inflammation, and immunity. *Immunity*. 2014. **41**: 518–528.

88 **Fara, A., Mitrev, Z., Rosalia, R. A. and Assas, B. M.**, Cytokine storm and COVID-19: a chronicle of pro-inflammatory cytokines: cytokine storm: the elements of rage!. *Open Biol.* 2020. **10**: 200160.

89 **Hariharan, A., Hakeem, A. R., Radhakrishnan, S., Reddy, M. S. and Rela, M.**, The role and therapeutic potential of NF-kappa-B pathway in severe COVID-19 patients. *Inflammopharmacology*. 2021. **29**: 91–100.

90 **Nalbandian, A., Sehgal, K., Gupta, A., Madhavan, M. V., Mcgroder, C., Stevens, J. S., Cook, J. R. et al.**, Post-acute COVID-19 syndrome. *Nat. Med.* 2021. **27**: 601–615.

91 **Le, T-T. T., Karmouty-Quintana, H., Melicoff, E., Le, T-T. T., Weng, T., Chen, N. Y., Pedroza, M. et al.**, Blockade of IL-6 trans signaling attenuates pulmonary fibrosis. *J. Immunol*. 2014. **193**: 3755–3768

92 **Teh, Y. C, Ding, J. L., Ng, L. G. and Chong, S. Z.**, Capturing the fantastic voyage of monocytes through time and space. *Front. Immunol.* 2019. **10**: 3389.

93 **Thachil, J.**, Hypoxia—An overlooked trigger for thrombosis in COVID-19 and other critically ill patients. *J. Thromb. Haemost.* 2020. **18**: 3109–3110.

94 **Chuah, S. L., Teh, C. L., Wan Mohd Akbar, S. A., Cheong, Y. K. and Sachdev Manjit Singh, B.**, Impact of COVID-19 pandemic on hospitalisation of patients with systemic lupus erythematosus (SLE): report from a

tertiary hospital during the peak of the pandemic. *Ann. Rheum. Dis*. 2020. 81: e144.

95 **George, M. D., Venkatachalam, S., Banerjee, S., Baker, J. F., Merkel, P. A., Gavigan, K., Curtis, D. et al.**, Concerns, healthcare use, and treatment interruptions in patients with common autoimmune rheumatic diseases during the COVID-19 pandemic. *J. Rheumatol*. 2021. **48**: 603–607.

96 **Avouac, J., Drumez, E., Hachulla, E., Seror, R., Georgin-Lavialle, S. and El Mahou, S.**, COVID-19 outcomes in patients with inflammatory rheumatic and musculoskeletal diseases treated with rituximab: a cohort study. *Lancet Rheumatol*. 2021. **3**: e419–e426.

97 **Burgener, S., Rochat, P., Dollenmaier, G., Benz, G., Kistler, A. D., Fulchini, R. et al.**, Progression of COVID-19 in a patient on anti-CD20 antibody treatment: case report and literature review. *Case Rep. Infect. Dis*. 2022. **2022**: 1–5.

98 **Verstegen, N. J., Hagen, R. R., van den Dijssel, J., Kuijper, L. H., Kreher, C., Ashhurst, T. et al.**, Immune dynamics in SARS-CoV-2 experienced immunosuppressed rheumatoid arthritis or multiple sclerosis patients vaccinated with mRNA-1273. *eLife*. 2022. **11**: e77969.

99 **Madelon, N., Lauper, K., Breville, G., Sabater Royo, I., Goldstein, R., Andrey, D. O., Grifoni, A. et al.**, Robust T-cell responses in anti-CD20-treated patients following COVID-19 vaccination: a prospective cohort study. *Clin. Infect. Dis*. 2022. **75**: e1037–e1045.

100 **Lee, J. S., Park, S., Jeong, H. W., Ahn, J. Y., Choi, S. J., Lee, H., Choi, B. et al.**, Immunophenotyping of covid-19 and influenza highlights the role of type i interferons in development of severe covid-19. *Sci. Immunol*. 2020. **5**: eabd1554.

101 **Alon, R., Sportiello, M., Kozlovski, S., Kumar, A., Reilly, E C., Zarbock, A. et al.**, Leukocyte trafficking to the lungs and beyond: lessons from influenza for COVID-19. *Nat. Rev. Immunol*. 2021. **21**: 49–64.

Abbreviations: **HIF-1α**: hypoxia-inducible factor 1-alpha · **MS**: multiple sclerosis · **PBMCs**: peripheral blood mononuclear cells · **Ps**: psoriasis · **RA**: rheumatoid arthritis · **RFX**: regulatory factor X · **SARS-CoV-2**: severe acute respiratory syndrome coronavirus 2 · **scRNA-seq**: single-cell RNA sequencing · **TF**: transcription factor

Full correspondence: Esteban Ballestar and Dr. Javier Rodríguez-Ubreva, Epigenetics and Immune Disease Group, Josep Carreras Research Institute (IJC), 08916 Badalona, Barcelona, Spain
e-mail: eballestar@carrerasresearch.org; jrodriguez@carrerasresearch.org
Roser Vento-Tormo, Wellcome Sanger Institute, Wellcome Genome Campus, Cambridge, United Kingdom
e-mail: rv4@sanger.ac.uk

Received: 28/6/2023
Revised: 29/9/2023
Accepted: 3/10/2023
Accepted article online: 6/10/2023