

A shift towards an immature myeloid profile in peripheral blood of critically ill COVID-19 patients

Keiko Taniguchi-Ponciano

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Eduardo Vadillo

Unidad de Investigación Médica en Enfermedades Oncológicas, UMAE Hospital de Oncología, Instituto Mexicano del Seguro Social.

Constantino Lopez-Macias

Unidad de Investigación Médica en Inmunoquímica, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Roberto Carvente-Garcia

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Hector Mayani

Unidad de Investigación Médica en Enfermedades Oncológicas, UMAE Hospital de Oncología, Instituto Mexicano del Seguro Social.

Eduardo Ferat-Osorio

Division de Investigacion en Salud, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Guillermo Flores-Padilla

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Javier Torres

Unidad de Investigación Médica en Enfermedades Infecciosas, UMAE Hospital de Pediatría, Instituto Mexicano del Seguro Social.

Cesar Raul Gonzales-Bonilla

Titular, Coordinación de Investigación en Salud, Instituto Mexicano del Seguro Social.

Abraham Majluf

Unidad de Investigación Médica en trombosis, hemostasia y aterogenesis, Instituto Mexicano del Seguro Social.

Alejandra Albarran-Sanchez

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Juan Carlos Galan

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Eduardo Peña-Martínez

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Gloria Silva-Román

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Sandra Vela-Patiño

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Aldo Ferreira-Hermosillo

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Claudia Ramirez-Renteria

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Nancy Adriana Espinoza-Sanchez

Unidad de Investigación Médica en Enfermedades Oncológicas, UMAE Hospital de Oncología, Instituto Mexicano del Seguro Social.

Rosana Pelayo-Camacho

Centro de Investigación Biomedica de Oriente, Instituto Mexicano del Seguro Social.

Laura Bonifaz

Unidad de Investigación Médica en Inmunoquímica, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Lourdes Arriaga-Pizano

Unidad de Investigación Médica en Inmunoquímica, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Carlos Mata-Lozano

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Sergio Andonegui-Elguera

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Niels Wachter-Rodarte

Unidad de Investigación Médica en Epidemiología Clínica, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Roberto De-Lira-Barraza

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Humberto Villanueva-Compean

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Alejandra Esquivel-Pineda

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Rubén Ramírez-Montes-de-Oca

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Carlos Anda-Garay

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Maura Noyola-García

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Luis Guizar-García

Servicio de Medicina Interna, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Arturo Cerbulo-Vazquez

Unidad de Investigación Médica en Inmunoquímica, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Horacio Zamudio-Meza

Unidad de Investigación Médica en Inmunoquímica, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Daniel Marrero-Rodríguez (✉ dan.mar57@gmail.com)

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Moises Mercado (✉ mmercadoa@yahoo.com)

Unidad de Investigación Médica en Enfermedades Endocrinas, UMAE Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Research Article

Keywords: COVID-19, SARS-CoV-2, scRNAseq, immune cell profile, critically ill

Posted Date: June 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-38042/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

SARS-CoV-2, the etiological agent causing COVID-19, has infected more than 8.7 million people with over 461000 deaths worldwide since its emergence in December 2019. Factors for severe disease, such as diabetes, hypertension and obesity have been identified however, the precise pathogenesis is poorly understood. In order to understand its pathophysiology and to develop effective therapeutic strategies, it is essential to define the prevailing immune cellular subsets. We performed circulating immune cells scRNAseq from five critically ill COVID-19 patients. Immature myeloid populations, such as promyelocytes-myelocytes, metamyelocytes, band neutrophils, monocytoid precursors and activated monocytes predominated. Trajectory with pseudotime analysis supported the finding of immature cell states. Gene ontology showed myeloid cell activation in immune response, DNA and RNA processing, defense response to virus and response to type 1 interferon. Lymphoid lineage was scarce. Our results uncover a transcriptomic profiles related to immature myeloid lineages and suggest the potential induction of trained immunity.

Introduction

Since its emergence in Wuhan, China in December 2019 the coronavirus disease (COVID-19) epidemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly progressed into a devastating pandemic with serious social and economic consequences¹. According to the World Health Organization, more than 8.7 million people have been diagnosed with COVID-19 and over 461 000 persons have died worldwide^{2,3}. The clinical presentation of COVID-19 varies widely. However, a subset of patients may evolve into an acute respiratory distress syndrome (ARDS), requiring intubation and mechanical ventilation, that is frequently complicated by a peculiar form of coagulopathy and eventually multiorgan failure and death. This complicated course occurs in the context of a “cytokine storm” characterized by overproduction of TNF, IL6, IL1 β and G-CSF and generalized vascular hyperpermeability⁴. Although risk factors such as diabetes, obesity and hypertension have been associated with the development of life-threatening COVID-19, a non-negligible proportion of healthy young individuals without comorbidities develops the severe form of the disease. The immunopathogenesis of this severe form of COVID-19 is poorly understood⁵. Viral particles spread through the respiratory mucosa infecting other cells, unleashing a series of immune responses, characterized by a reduction of T and B lymphocytes and an increment of monocytes and neutrophils^{6, 7-9}. A detailed understanding of this altered immunological state is crucial for the design and development of effective therapeutic strategies. In the present work we performed single cell RNAseq from circulating immune response cells from critically ill COVID-19 patients requiring invasive mechanical ventilation.

Results

Clinical characteristics of critically ill COVID-19 patients

A pool of peripheral blood samples from five patients with severe SARS-Cov-2 infection was created and used for the scRNAseq experiment, their main clinical and biochemical characteristics are depicted in table 1. The five patients were males with a mean age of 47.8 ± 6.6 years (range 41–57). Two patients were overweight, one had grade 1 obesity and two had previously been diagnosed with type 2 diabetes mellitus; no history of hypertension was found in any of them and two patients had no known risk factors for severe SARS-Cov-2 infection. All patients had radiological evidence of alveolar occupation and ground glass appearance on imaging studies and required intubation and invasive mechanical ventilation 3 to 7 days after admission as well as hemodynamic collapse that required vasopressor support. They all had significantly elevated D-dimer, fibrinogen, procalcitonin and C-reactive protein levels. Besides respiratory and hemodynamic support, all five patients were treated with enoxaparin, hydroxychloroquine, azithromycin or clarithromycin as well as lopinavir and ritonavir; three received high dose glucocorticoids (hydrocortisone or dexamethasone). One of the five patients developed secondary bacterial and fungal infections for which he received meropenem, vancomycin and voriconazole; no major arterial or venous thrombotic events were recorded in any of them. Three of the five patients died of ARDS and multiorgan failure. Of note, the majority of patients showed increased myeloid absolute numbers after seven days of hospitalization.

Immature myeloid cell populations predominance in critically ill COVID-19 patients

A total of 10884 immune cells from critically ill COVID-19 patients and 4056 from healthy donors were studied. After normalization and clustering of the gene expression matrix, 12 cell populations clusters were identified in critically ill COVID-19 patients whereas 5 cell populations clusters were present in healthy controls. Cells of myeloid origin predominated in critically ill COVID-19 patients whereas cells of lymphoid origin were more prevalent among healthy controls. Interestingly, in COVID-19 patients with severe SARS-Cov-2 infection, immature myeloid cell populations predominate, such as band neutrophils (10.74%), metamyelocytes (19.10%), promyelocytes-myelocytes (11.14%), monocytoïd precursor (3.30%), immature monocytes (19.0%). Mature lineages such as segmented neutrophils (5.01%), mature monocytes (7.65%) and finally monocyte-macrophages (13.82%) were also observed. Lymphoid cell populations, on the other hand, including B (1.77%) and T (4.78%) lymphocytes as well as NK cells (2.37%) are present in low frequencies in these patients. In contrast, healthy individuals displayed higher proportions of lymphoid cell lineages such as T (45%), Th1 (5%) and B (12.72%) lymphocytes as well as NK cells (14.91%) compared to monocytes (21.52%) in the myeloid cell lineage (Figure 1). Transcriptome analysis from cell populations identified in peripheral blood (PB) of critically ill COVID-19 patients resembled immature lineages in the bone marrow (BM). Hence, the comparison and coincidence of most of the transcripts in the different populations characterized the different clusters. Thus, the promyelocytes-myelocyte cluster was defined by the expression of genes such as ACPP, GLB1, CTSS, SCL11A1, ITM2B, P2RX1, TREM1, TRIM22, SP110, IRF1, STAT1 and NCOA1. Metamyelocytes by the expression of the following genes: ITGAM, GGH, CR1, MGAM, MMP9, TIMP2, TNFAIP6, VNN2, ANPEP,

DYSF and PLXNC1. Genes such as GNS, VNN1, ITGAM, C3AR1, CLEC5A, ADAM8, CD58, CR1, FCER1G, MGAM, MMP8, MMP9, SIGLEC5, VNN2, DYSF, LILRB3, TLR8 and CCR are characteristically expressed by band neutrophils, whereas segmented neutrophil cells were defined by the expression of MGAM, MMP25, SIGLEC9, VENTX, NLRP1 and VENTX. Monocytoid precursor were identified by the expression of GATA2, HOXA10, LYZ, FCN1 and S100A9 and the cell cycle genes PCLAF, STMN1, MKI67, TOP2A and CDK1. The expression of genes such as TLR2, FUT4, CCR1, CCR3, PIAS, TRIM22, TRIM38, IRF1 and JUNB identified immature monocytes, this cluster also showed tendency towards M1 phenotype, while monocyte-macrophage (M1) were characterized by the expression of STAT2, EGR2, CREB5, TREM1, TL2, FCGR3B, ITGAX and NLRP1. Mature monocytes were defined by the presence of CD14, LYZ and S100A9. The expression of genes such as CD79A, CD79B and CD19 defined the presence of B lymphocytes. T lymphocytes express CD3E and CD3D whereas NK cells express NKG7 and GZMA. (Figure 2).

Differentiation profiles support the existence of immature cell populations in critically ill, COVID–19 patients

Trajectory analysis along with its respective estimation of pseudotime were carried out to infer potential differentiation states in the cell populations found in critically ill COVID–19 patients. Node 2 which gathers several of the immature cell populations, among them metamyelocytes, promyelocytes-myelocytes and immature monocytes showed time 0, follow throughout the trajectory path to node 1 where it bifurcates into two nodes, one end-node representing and “other” cells, and the second bifurcation end which includes the mature monocytes at the end of the node. As expected, B and T lymphocytes, along with NK cells were among the most mature cells found in this analysis (Figure 3). These trajectory results support our findings of diverse immature cell state populations and their potential transitional states in critically ill patients with COVID–19.

Gene expression alterations in cell populations from COVID–19 patients were related to key cellular processes

Cellular alterations of the different myeloid cell population subsets were divided in four groups: those related to 1) myeloid cell activation in immune response as well as granulocyte and leukocyte activation, 2) iron metabolism and anion homeostasis, 3) cell cycle control, DNA and RNA processing and 4) defense response to virus, response to type 1 interferon and innate immune response. The lymphoid cellular subset alterations were mostly related to ribosome biogenesis as well as RNA processing and modification (Figure 4).

Evidence of a TLR-related activation in circulating immature myeloid cells of severe COVID–19 patients

Traditionally, it was thought that immunological memory was limited to adaptative immune responses. Recent evidence has shown that primitive hematopoietic subpopulations, together with its progeny, can be trained to effectively produce myeloid cells and to respond more efficiently to a second challenge, respectively ¹⁰. This novel phenomenon is dependent on the transcription factor C/EBP β which is needed to maintain accessibility to open chromatin regions and to promote TLR-related responses. We sought to evaluate the expression of C/EBP β along with other transcripts involved in TLR-dependent signaling (Figure 5). When comparing normal monocytes to the immature lineages present in COVID-19 samples revealed that the expression of CEBP β was higher, also, the expression of TLR-related transcripts such as IRF1 and FOSL2 was upregulated, while ATF3 expression on immature granulocytes and monocytes was diminished. Therefore, our results suggest that immature circulating myeloid cells of severe COVID-19 patients are enriched in transcripts related to TLR signaling that resemble the CRBP β -dependent open chromatin regions observed during lipopolysaccharide-dependent trained immunity of primitive populations.

Discussion

The SARS-CoV-2 is the etiological agent causing COVID-19 which has infected more than 4.7 million people with more than 316 000 deaths since its emergence in December 2019². Defining which are the immune cell subsets that prevail in the circulation of these patients and what is their molecular activation status is crucial for understanding the immune clearance mechanisms that operate in these patients and thus, for the development of new and effective therapeutic strategies. Among the molecular alterations found in the immune response cells from critically ill patients with COVID-19 was the abnormal type 1 interferon response ¹¹ and could potentially represent a target for molecular therapy ¹². Typical PBMC in healthy individuals include ~80% of T lymphocytes (CD3D), ~6% of NK cells (NKG7), ~6% of B lymphocytes (CD79A) and ~7% of myeloid cells (S100A9) ¹³. During viral acute infection the single-stranded RNA from SARS-CoV-2 can be recognized by TLR-7 and TLR-8 during replication, while TLR3, RIG-like receptors, and MDA-5 can sense double-stranded RNA which is a replication intermediary ¹⁴; the viral Spike protein can be recognized by TLR2 ¹⁵, TLR4 and TLR6 ¹⁶. While the recognition of viral patterns induces strong immune responses, it is also known that human stem and progenitor cells (HSPC) express TLR7, TLR8, TLR2, TLR3, and TLR4 and upon stimulation, granulopoiesis and monopoiesis are induced and perpetuated ¹⁷⁻²¹.

Using a scRNAseq approach we have found a distinctive predominance of cell lineages of myeloid origin and a diminished number of lymphoid lineages in patients with severe atypical pneumonia and life-threatening respiratory distress syndrome due to SARS-CoV-2, which is in accordance with recently reported data ⁷⁻⁹. Not only increased myeloid lineage was favored in COVID-19 critical patients, but also immature cell states were observed. Atypical morphological alterations in monocytes from patients in intensive care unit has been reported²², and the presence of immature granulocytes such as metamyelocytes and band neutrophils has been reported ⁹. It is noteworthy that a similar blood profile has been observed in patients with acute parvovirus B19, cytomegalovirus and Epstein-Barr viral

infection²³⁻²⁵. Thus, the presence of immature myeloid cells in peripheral blood of patients in response to acute and severe viral infection is a common feature.

Our results suggest that severe SARS-Cov-2 infection induce immature granulocyte and monocyte release from the BM since most of the transcripts corresponded to an immature state of differentiation of granulocytic and monocytic programs²⁶⁻²⁹. In line with our findings, peripheral blood (PB) smears from critical COVID-19 patients have shown to have morphological abnormalities that correlate with maturation asynchrony³⁰. Overproduction of myeloid cells could be the result of increased proliferation/differentiation of hematopoietic stem and progenitor cells in response to SARS-CoV-2 (potential emergency hematopoiesis) which currently single cell transcriptome results could support. We have previously reported a proliferative state of immune cells from COVID-19 patients (submitted). Also, the massive release of immature myeloid cells -already present in bone marrow as a result of increased levels of cytokines such as G-CSF, which is upregulated⁴.

Although more than 5 different populations of PB monocytes have been described³¹, in our study only one of these cell populations was present in blood from healthy individuals. In contrast, in blood samples from critically ill patients with COVID-19 we found four different monocyte clusters. One of these clusters which we named monocytoid precursor cluster, displays monocytic transcripts as well as transcripts implicated in cell cycle regulation. We believe this cluster displays a more immature transcriptome which prompts us to speculate that it as a precursor population of the mature monocyte cluster and the M1 monocyte-macrophage.

Lineage-specific acquisition of function is achieved during cell maturation. Our analysis showed that lineage-specific molecules, such as S100A8/A9, are expressed in all stages of differentiation of myeloid cells in patients with COVID-19. These transcripts encode the myeloid-related proteins 8/14 which are known to be released and recognized in an autocrine fashion by TLR4 for effective diapedesis³². Integrins (LFA-1 and Mac-1) are fundamental for myeloid migration and function³³ and they are acquired during the latest stages of differentiation. In line with our results, ITGAL (LFA-1) and ITGAM (MAC-1) were also poorly expressed in all immature lineages of COVID-19 samples while they are fully expressed in mature myelocytes.

In accordance with the left shift seen in patients with COVID-19^{9,34} our results show severe lymphopenia reflected by at least a 7-fold reduction in the percentage of B and T lymphocytes and NK cells. During acute inflammatory conditions, the production of B lymphocytes and NK cells is drastically inhibited, favoring myelopoiesis^{21,35-37}. In this regard, it is interesting to note that our two surviving patients had normal lymphocyte counts, while those who died had severe lymphopenia.

Recently, the trained-immunity of HSPC has been shown to be dependent on epigenetic events which induce the maintenance of open chromatin regions that persist in a CEBP β -dependent fashion. Importantly, these epigenetic changes are conserved during differentiation and are used by myeloid cells during the immune response¹⁰. Therefore, we searched in our analysis for the expression of CEBP β and

found it in all stages of granulocytic and monocytic differentiation in samples from patients with severe COVID-19. Furthermore, we looked for additional factors that are known to be involved in TLR signaling and that are recruited to the open chromatin regions in conjunction with CEBP β . It is noteworthy that in comparison to healthy donor-derived monocytes, transcripts related to TLR responses such as IRF1 and FOSL2 were upregulated in patients with severe COVID-19, reflecting perhaps a state of innate immune cell activation, which is in line with our gene ontology results. Further supporting this notion is our finding of very few cells expressing RUNX1 which is in agreement with the absence of the most primitive HSPC in peripheral blood. Altogether, CEBP β and IRF1 and FOSL2 upregulation, denote an activation state related to TLR-dependent responses during severe COVID-19.

In COVID-19 patients a left shift is observed, our results suggests potential emergency hematopoiesis has occurred, supported by the presence of proinflammatory cytokines observed in this disease⁴ along with the exposure to viral PAMPs and our own data suggesting a TLR-dependent activation. Other ligands such as Poly I:C (a TLR3 ligand) can also sensitize HSPC to a secondary challenge¹⁰. Since during SARS-CoV-2 replication, TLR3 can recognize dsRNA, it is reasonable to think that possibly an epigenetic reprogramming of primitive cells (trained immunity) could occur which could contribute to a protection to a later SARS-CoV-2 challenge though the mounting of a more efficient ability to mount innate immune responses.

In summary our results show the presence of immature myeloid lineages in peripheral blood in critically ill COVID-19 patients potentially suggesting a state of emergency myelopoiesis. The molecular signatures of this phenomenon are consistent with activation of TLR-induced responses and perhaps the acquisition of trained immunity.

Materials And Methods

Patients and tissue samples

Blood samples from five critically ill patients with COVID-19 infection were collected in EDTA-coated tubes. tissues were collected from patients diagnosed, treated and followed at the Medicina Interna department of the Hospital de Especialidades, Centro Médico Nacional Siglo XXI of the Instituto Mexicano del Seguro Social in April 2020. A family member of all participating patients signed an informed consent and the study protocol was approved by our Institutional Ethics and Scientific committees. SARS-CoV-2 infection was corroborated by RT-qPCR at an official federal government reference laboratory.

Sample preparation, scRNAseq library generation and sequencing

Peripheral blood from the five critical COVID-19 patients was collected in EDTA-coated tubes, and immune cells were isolated according to standard centrifugation methods, 3500 rpm during 15 min, followed by red blood cell lysis.

Chromium Next GEM Single Cell 3' Reagent Kits v3.1 and protocol from 10X Genomics was followed as recommended by manufacturer's instructions, briefly described. The five patients immune cells were pooled in a single tube and cells were diluted in 1x phosphate buffered saline (PBS) to 700–1200 cells per μl . Cell suspension was loaded in Chromium Next GEM Chip G and sorted in the Chromium Controller from 10X Genomics. The Cell-Gel Beads in Emulsion (GEMs) were then incubated to generate the barcoded cDNA. cDNA was cleaned using Dynabeads and washed, followed by cDNA amplification and SPRIselection. The retrieved cDNA was enzymatically fragmented, end-repaired, poly-A tailed and ligated. Size selection, adaptor ligation and amplification were done. Sequencing was done using NextSeq 550 System High-Output Kit (300 cycles) in NextSeq 500 system (Illumina) according to 10X Genomics specifications: Read 1 = 28 cycles, Read 2 = 91 cycles, Index 1 = 8 cycles. All quality control steps were carried out using 4200 TapeStation System (Agilent) with High Sensitivity D1000 Screen Tape, whereas the concentration was calculated using Qubit 2.0 Fluorometer with Kit High Sensitivity assays.

scRNAseq bioinformatic analysis

Partek Flow software was used with scRNAseq toolbox. First the tags were trimmed and then the reads were aligned using STAR 2.7.3a algorithm to human genome hg38. UMI's were deduplicated and barcode filtered. Following criteria were then applied to each cell, i.e., gene number between 200 and 6000, UMI count above 300 and mitochondrial gene percentage below 20%. To quantify the transcriptome human hg38 Ensembl transcripts release 99 was used. Counts per million, Add 1.0 Log 2.0 were the normalization parameters. Data has been deposited in Sequence Read Archive hosted by National Center for Biotechnology Information.

Markers used to circumscribe cell populations

Clusters were categorized by analyzing differentially expressed genes according to previously published data obtained from human samples^{26–29,38,39}.

Dimensionality reduction and clustering

The filtered and normalized gene-barcode matrix was analyzed by principal components, then graph based and t-distributed stochastic neighbor embedding (t-SNE) using default parameters was carried out.

Trajectory and pseudotime analysis

Monocle2 algorithm located within the Partek Flow scRNAseq toolbox was used with default parameters to calculate the transition states and pseudotime from cell populations identified.

Gene ontology analysis

WebGestalt (<http://www.webgestalt.org>) was used for understanding the biological meaning behind the resulting list of genes, to obtain gene ontology for significantly de-regulated genes in COVID-19 patients cell populations.

Declarations

Acknowledgments

DMR is a recipient of the National Council for Science and Technology Fellowship “Catedra CONACyT” program. This work was partially supported by grants 289499 from Fondos Sectoriales Consejo Nacional de Ciencia y Tecnologia Mexico and R-2015-785-015 from Instituto Mexicano del Seguro Social (MM). We would like to thank to Xiaowen Wang from Partek Inc. for the exceptional technical support provided.

Author contributions

MM, DMR and KTP conceived, designed and coordinated the project, performed scRNAseq experiments, analyzed, discussed data and prepared the manuscript. EV, RCG, HM, JT, CRGB, AM, EPM, GSR, SVP, AFH, CRR, NAES, RP, LB, LAP, CML, SAE and NW performed scRNAseq experiments, analyzed, discussed and interpreted biological data, wrote the paper.

CLM, EFO, GFP, AAS, JCG, RDLB, HVC, AEP, RRMO, CAG, MNG, LGG and HZM provided the biological sample, retrieved the immune cells and collect clinical data.

Competing interest

RCG and CML work for Analitek S.A. de C.V. which supplied research reagents. The rest of the authors declare not competing interests.

Data availability

Data has been deposited in Sequence Read Archive hosted by National Center for Biotechnology Information under accession number PRJNA635580.

References

1 Bi, Q. *et al.* Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. *Lancet Infect Dis*, 30287-30285 (2020).

- 2 Hou, Y. J., Okuda, K., Edwards, C. & al, e. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. *Cell*, 10.1016/j.cell.2020.1005.1042 (2020).
- 3 World Health Organization. Coronavirus disease (COVID-2019) situation reports 153. (2020).
- 4 Jose, R. & Manuel, A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med*, 30216-30212 (2020).
- 5 Wang, F. *et al.* Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis*, 10.1093/infdis/jiaa1150 (2020).
- 6 Chen, N. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*, 507-513 (2020).
- 7 Guo, C., Li, B., Ma, H. & al, e. Tocilizumab treatment in severe COVID-19 patients attenuates the inflammatory storm incited by monocyte centric immune interactions revealed by single-cell analysis. *Preprinted in bioRxiv*, 10.1101/2020.1104.1108.029769 (2020).
- 8 Wen, W. *et al.* Immune Cell Profiling of COVID-19 Patients in the Recovery Stage by Single-Cell Sequencing. *Cell Discov*, 10.1038/s41421-41020-40168-41429 (2020).
- 9 Zini, G., Bellesi, S., Ramundo, F. & d'Onofrio, G. Morphological anomalies of circulating blood cells in COVID-19. *Am J Hematol*, 10.1002/ajh.25824 (2020).
- 10 de Laval, B. *et al.* C/EBPbeta-Dependent Epigenetic Memory Induces Trained Immunity in Hematopoietic Stem Cells. *Cell Stem Cell* **26**, 793, doi:10.1016/j.stem.2020.03.014 (2020).
- 11 Hadjadj, J., Yatim, N., Barnabei, L. & al, e. Impaired type I interferon activity and exacerbated inflammatory responses in severe Covid-19 patients. *Preprinted in medRxiv*, 10.1101/2020.1104.1119.20068015 (2020).
- 12 Sallard, E., Lescure, F., Yazdanpanah, Y., Mentre, F. & Peiffer-Smadja, N. Type 1 interferons as a potential treatment against COVID-19. *Antiviral Res*, 10.1016/j.antiviral.2020.104791 (2020).
- 13 Zheng, G., Terry, J., Belgrader, P. & al, e. Massively parallel digital transcriptional profiling of single cells. *Nat Commun*, 14049-14061 (2017).
- 14 Kindler, E., Thiel, V. & Weber, F. Interaction of SARS and MERS Coronaviruses with the Antiviral Interferon Response. *Adv Virus Res* **96**, 219-243, doi:10.1016/bs.aivir.2016.08.006 (2016).
- 15 Dosch, S. F., Mahajan, S. D. & Collins, A. R. SARS coronavirus spike protein-induced innate immune response occurs via activation of the NF-kappaB pathway in human monocyte macrophages in vitro. *Virus Res* **142**, 19-27, doi:10.1016/j.virusres.2009.01.005 (2009).

- 16 Choudhury, A. & Mukherjee, S. In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. *J Med Virol*, doi:10.1002/jmv.25987 (2020).
- 17 Takizawa, H., Boettcher, S. & Manz, M. G. Demand-adapted regulation of early hematopoiesis in infection and inflammation. *Blood* **119**, 2991-3002, doi:10.1182/blood-2011-12-380113 (2012).
- 18 Vadillo, E. & Pelayo, R. [Toll-like receptors in development and function of the hematopoietic system]. *Rev Invest Clin* **64**, 461-476 (2012).
- 19 Sioud, M. & Floisand, Y. TLR agonists induce the differentiation of human bone marrow CD34+ progenitors into CD11c+ CD80/86+ DC capable of inducing a Th1-type response. *Eur J Immunol* **37**, 2834-2846, doi:10.1002/eji.200737112 (2007).
- 20 Sioud, M., Floisand, Y., Forfang, L. & Lund-Johansen, F. Signaling through toll-like receptor 7/8 induces the differentiation of human bone marrow CD34+ progenitor cells along the myeloid lineage. *J Mol Biol* **364**, 945-954, doi:10.1016/j.jmb.2006.09.054 (2006).
- 21 De Luca, K. *et al.* The TLR1/2 agonist PAM(3)CSK(4) instructs commitment of human hematopoietic stem cells to a myeloid cell fate. *Leukemia* **23**, 2063-2074, doi:10.1038/leu.2009.155 (2009).
- 22 Zhang, D. *et al.* COVID-19 infection induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, the severity of which correlate with patient outcome. *Preprinted in medRxiv*, 10.1101/2020.1103.1124.20042655 (2020).
- 23 Kirby, M., Weitzman, S. & Freedman, M. Juvenile chronic myelogenous leukemia: differentiation from infantile cytomegalovirus infection. *Am J Pediatr Hematol Oncol*, 292-296 (1990).
- 24 Herrod, H., Dow, L. & Sullivan, J. Persistent Epstein-Barr virus infection mimicking juvenile myelogenous leukemia: immunologic and hematologic studies. *Blood*, 1098-1104 (1983).
- 25 Yetgin, S., Cetin, M., Yenicesu, I., Ozaltin, F. & Uckan, D. acute parvovirus B19 infection mimicking juvenile myelomonocytic leukemia. *Eur J Haematol*, 276-278 (2000).
- 26 Novershtern, N. *et al.* Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell* **144**, 296-309, doi:10.1016/j.cell.2011.01.004 (2011).
- 27 Tang-Huau, T. L. *et al.* Human in vivo-generated monocyte-derived dendritic cells and macrophages cross-present antigens through a vacuolar pathway. *Nat Commun* **9**, 2570, doi:10.1038/s41467-018-04985-0 (2018).
- 28 Lambert, C., Preijers, F., Yanikkaya Demirel, G. & Sack, U. Monocytes and macrophages in flow: an ESCCA initiative on advanced analyses of monocyte lineage using flow cytometry. *Cytometry B Clin*

Cytom **92**, 180-188, doi:10.1002/cyto.b.21280 (2017).

- 29 Grassi, L. *et al.* Dynamics of Transcription Regulation in Human Bone Marrow Myeloid Differentiation to Mature Blood Neutrophils. *Cell Rep* **24**, 2784-2794, doi:10.1016/j.celrep.2018.08.018 (2018).
- 30 Mitra, A. *et al.* Leukoerythroblastic reaction in a patient with COVID-19 infection. *Am J Hematol*, 10.1002.25793 (2020).
- 31 Hamers, A. A. J. *et al.* Human Monocyte Heterogeneity as Revealed by High-Dimensional Mass Cytometry. *Arterioscler Thromb Vasc Biol* **39**, 25-36, doi:10.1161/ATVBAHA.118.311022 (2019).
- 32 Pruenster, M. *et al.* Extracellular MRP8/14 is a regulator of beta2 integrin-dependent neutrophil slow rolling and adhesion. *Nat Commun* **6**, 6915, doi:10.1038/ncomms7915 (2015).
- 33 Schnoor, M., Alcaide, P., Voisin, M. B. & van Buul, J. D. Crossing the Vascular Wall: Common and Unique Mechanisms Exploited by Different Leukocyte Subsets during Extravasation. *Mediators Inflamm* **2015**, 946509, doi:10.1155/2015/946509 (2015).
- 34 Chen, G. *et al.* Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* **130**, 2620-2629, doi:10.1172/JCI137244 (2020).
- 35 Vadillo, E. *et al.* Adult, but not neonatal, human lymphoid progenitors respond to TLR9 ligation by producing functional NK-like cells. *Exp Hematol* **42**, 562-573 e563, doi:10.1016/j.exphem.2014.03.008 (2014).
- 36 Welner, R. S. *et al.* Lymphoid precursors are directed to produce dendritic cells as a result of TLR9 ligation during herpes infection. *Blood* **112**, 3753-3761, doi:10.1182/blood-2008-04-151506 (2008).
- 37 Nagai, Y. *et al.* Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. *Immunity* **24**, 801-812, doi:10.1016/j.immuni.2006.04.008 (2006).
- 38 Hoogendijk, A. J. *et al.* Dynamic Transcriptome-Proteome Correlation Networks Reveal Human Myeloid Differentiation and Neutrophil-Specific Programming. *Cell Rep* **29**, 2505-2519 e2504, doi:10.1016/j.celrep.2019.10.082 (2019).
- 39 Itelman, E. *et al.* Clinical Characterization of 162 COVID-19 patients in Israel: Preliminary Report from a Large Tertiary Center. *Isr Med Assoc J* **22**, 271-274 (2020).

Table

Table 1: Clinical and biochemical characteristics of critically ill patients with COVID-19 infection (OW: overweight, DM: type 2 diabetes mellitus, BMI: body mass index)

-	<u>Patient 1</u>	<u>Patient 2</u>	<u>Patient 3</u>	<u>Patient 4</u>	<u>Patient 5</u>
Age, yrs	57	46	43	41	52
BMI, Kg/cm ²	29	26.3	31	24	29
Comorbidities	OW	None	DM, OW	None	DM, OW
Admission to intub, days	4	3	5	3	5
Leukocytes per mm ³	9130	8760	7760	8900	5060
Lymphocytes, x 10 ³ /mm ³	650	520	160	1130	1600
Hb, g/dL	16.4	15.9	17	12.9	15.8
Platelets, x 10 ³ /mm ³	193	357	380	523	169
CRP, mg/L	23.6	35	11.9	27	11.6
Procalcitonin, ng/mL	2.32	1.87	0.07	0.22	0.33
D-Dimer, ng/mL	2410	3320	1100	2980	2020
Fibrinogen, mg/dL	834	788	753	789	773
Final outcome	Dead	Dead	Dead	Alive	Alive

Figures

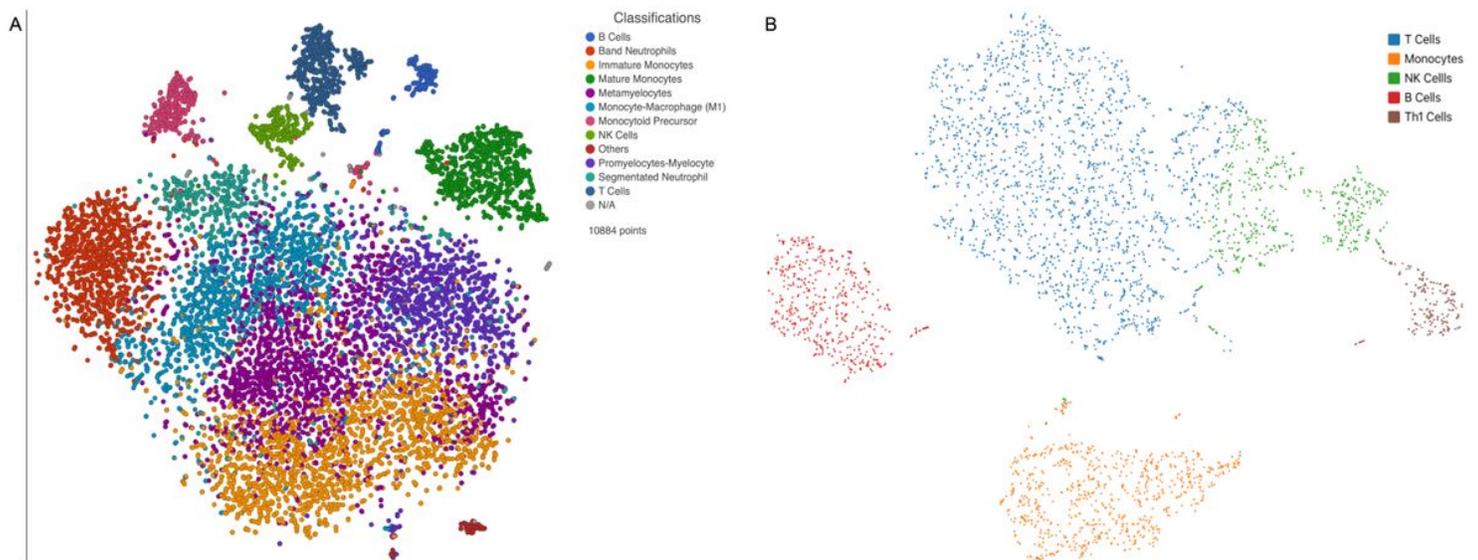


Figure 1

Cell populations identified in critically ill COVID-19 patients.

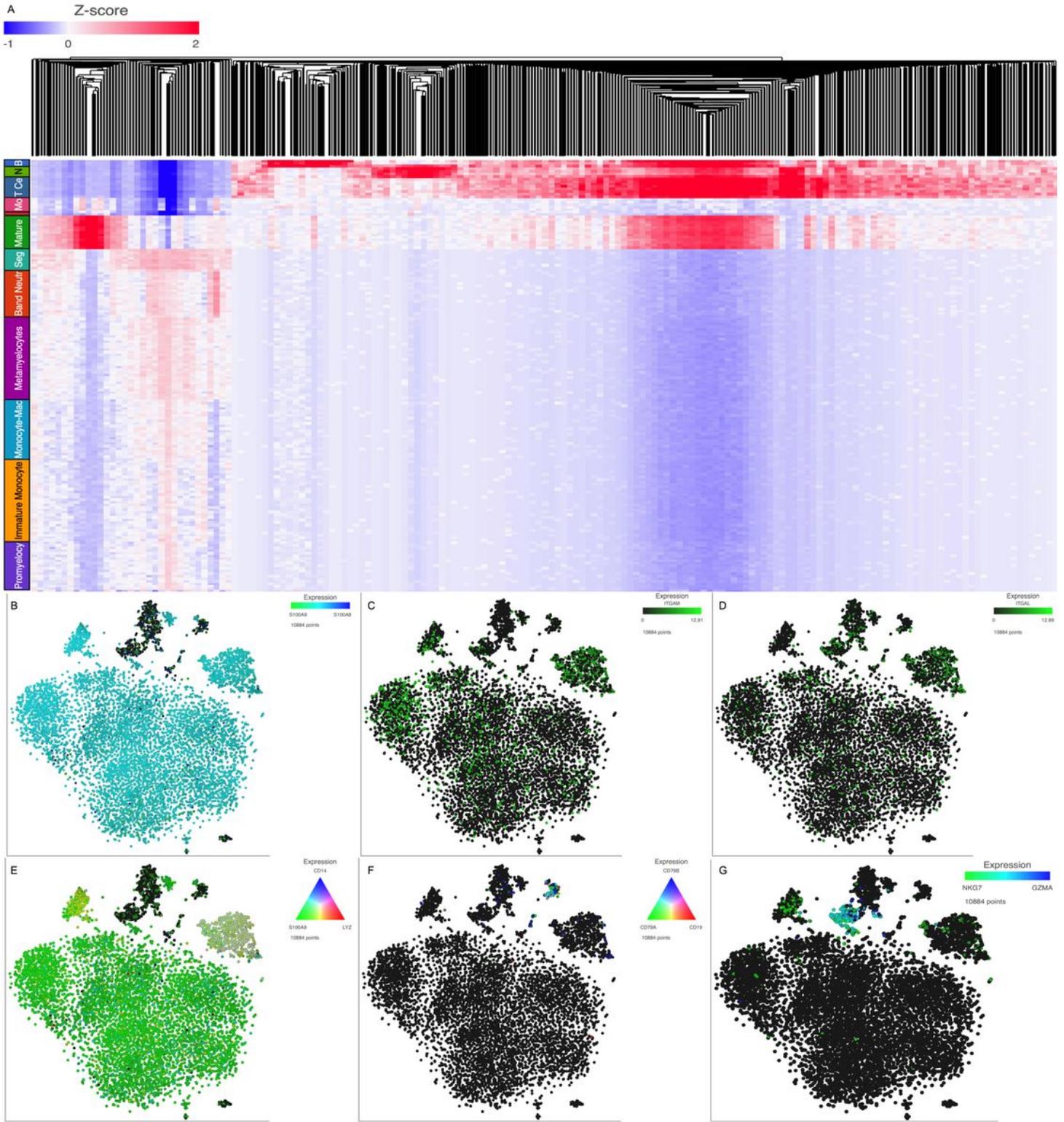


Figure 2

Molecular markers identifying cell clusters.

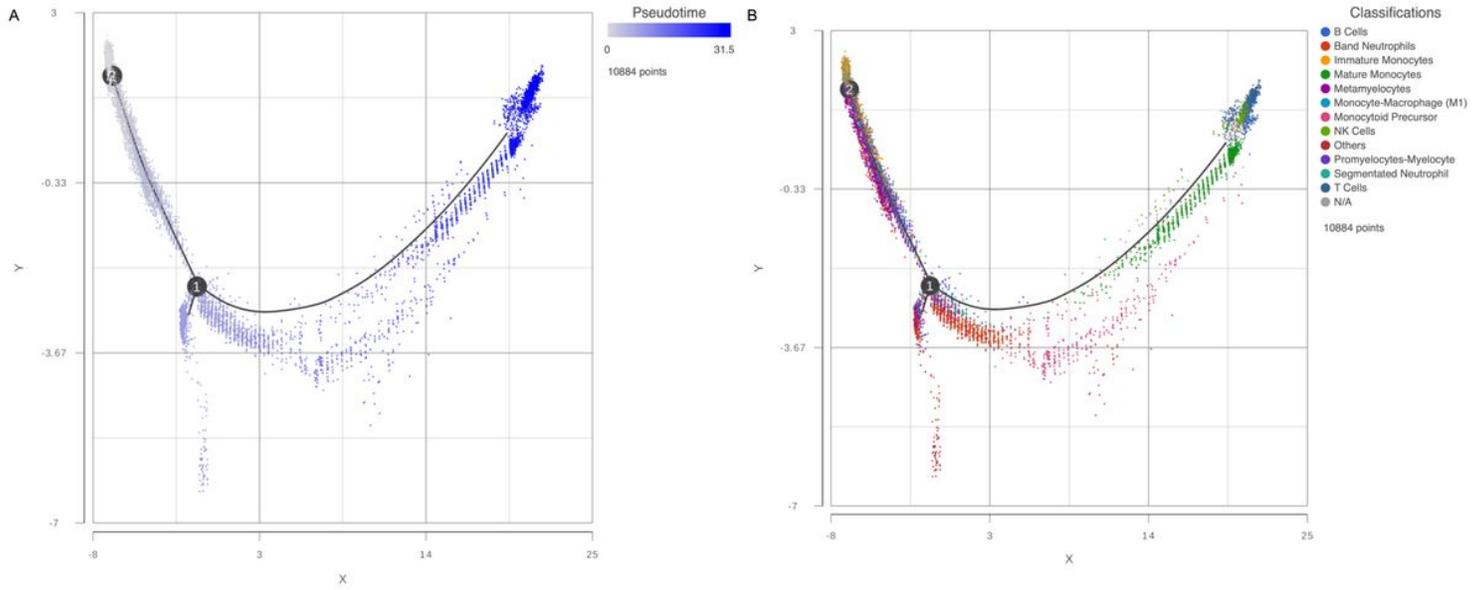


Figure 3

Trajectory analysis

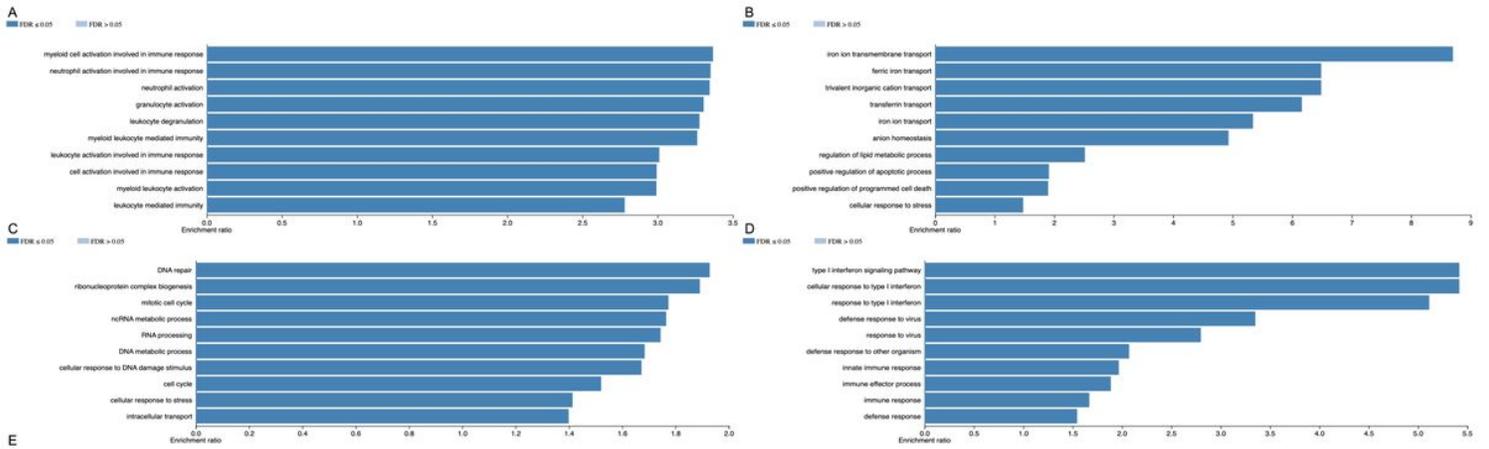


Figure 4

Gene Ontology terms.

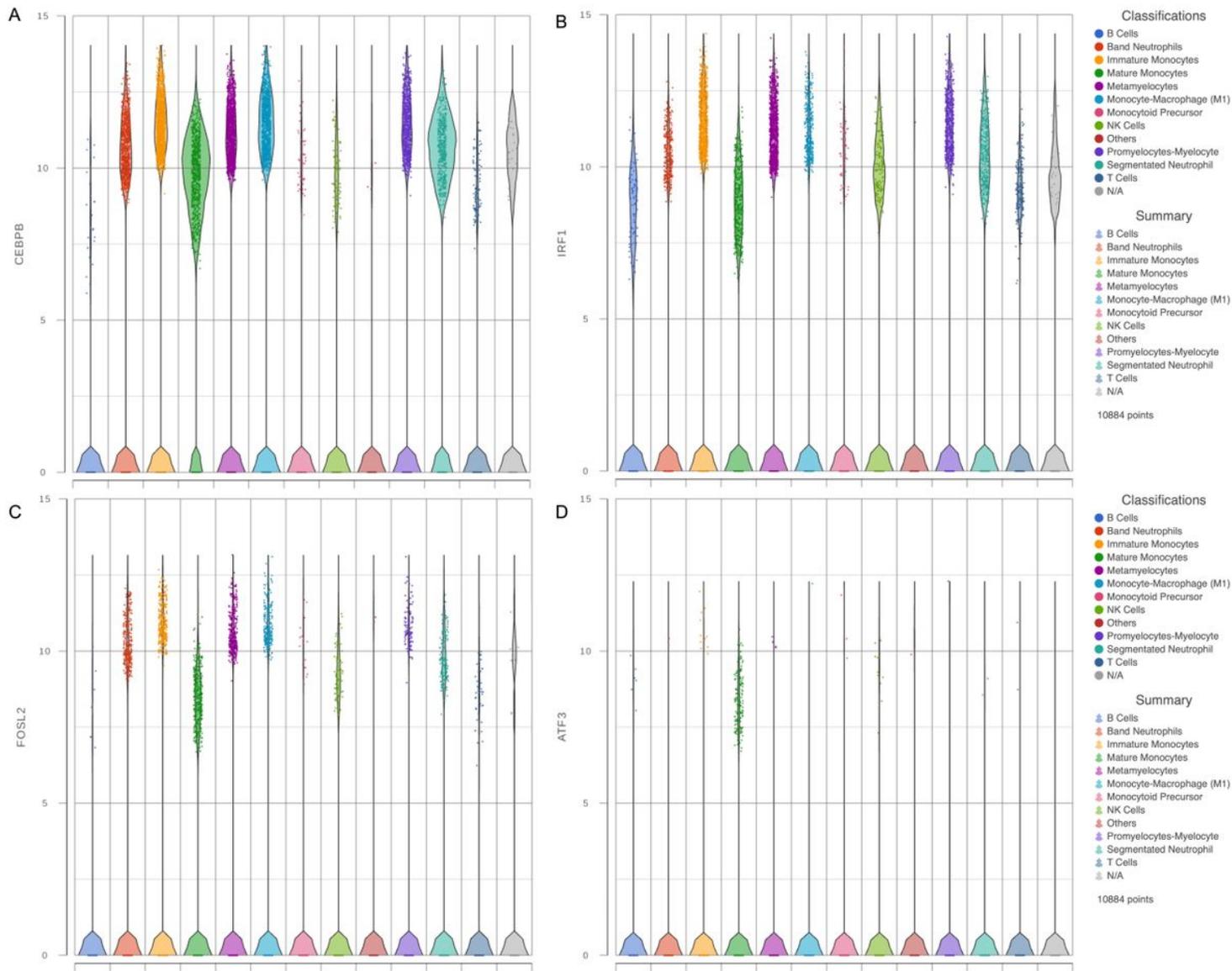


Figure 5

Immunity trained gene expression.