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CASE REPORT
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Longitudinal Analysis of Biologic Correlates of COVID-19 Resolution: Case Report

Natalie Bruiners[1,2], Valentina Guerrini[1,2], Rahul Ukey[1], Ryan J. Dikdan[1], Jason H. Yang[3,4], Pankaj Kumar Mishra[1], Alberta Onyuka[5], Deborah Handler[5], Joshua Vieth[6], Mary Carayannopoulos[7], Shuang Guo[8], Maressa Pollen[7], Abraham Pinter[1,2], Sanjay Tyagi[1,2], Daniel Feingold[9], Claire Philipp[8], Steven K. Libutti[6] and Maria Laura Gennaro[1,2]**

[1] Rutgers New Jersey Medical School, Public Health Research Institute, Newark, NJ, United States, [2] Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ, United States, [3] Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers New Jersey Medical School, Newark, NJ, United States, [4] Center for Emerging and Re-emerging Pathogens, Rutgers New Jersey Medical School, Newark, NJ, United States, [5] Global Tuberculosis Institute, Rutgers New Jersey Medical School, Newark, NJ, United States, [6] Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ, United States, [7] Department of Pathology and Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, United States, [8] Division of Hematology, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, United States, [9] Department of Surgery, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, United States

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***Correspondence:**
Steven K. Libutti
steven.libutti@cinj.rutgers.edu
Maria Laura Gennaro
marila.gennaro@rutgers.edu

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While the biomarkers of COVID-19 severity have been thoroughly investigated, the key biological dynamics associated with COVID-19 resolution are still insufficiently understood. We report a case of full resolution of severe COVID-19 due to convalescent plasma transfusion. Following transfusion, the patient showed fever remission, improved respiratory status, and rapidly decreased viral burden in respiratory fluids and SARS-CoV-2 RNAemia. Longitudinal unbiased proteomic analysis of plasma and single-cell transcriptomics of peripheral blood cells conducted prior to and at multiple times after convalescent plasma transfusion identified the key biological processes associated with the transition from severe disease to disease-free state. These included (i) temporally ordered upward and downward changes in plasma proteins reestablishing homeostasis and (ii) post-transfusion disappearance of a subset of monocytes characterized by hyperactivated Interferon responses and decreased TNF-α signaling. Monitoring specific dysfunctional myeloid cell subsets in peripheral blood may provide prognostic keys in COVID-19.

Keywords: severe COVID-19, RNAemia, convalescent plasma therapy, plasma proteomics, single-cell transcriptomics

INTRODUCTION

Two years into the COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) worldwide research effort has defined the immunopathology of COVID-19 (1, 2). The immune responses associated with severe and fatal COVID-19 manifestations include defective antiviral mechanisms such as type I interferon (IFN) responses (3, 4), imbalanced T cell responses (5, 6), abnormal expansion of potentially dysfunctional B cells (7), exuberant inflammation driven by altered myeloid responses (8, 9), and production of potentially pathogenic antibodies (autoantibodies and/or proinflammatory antibodies) (10–12).

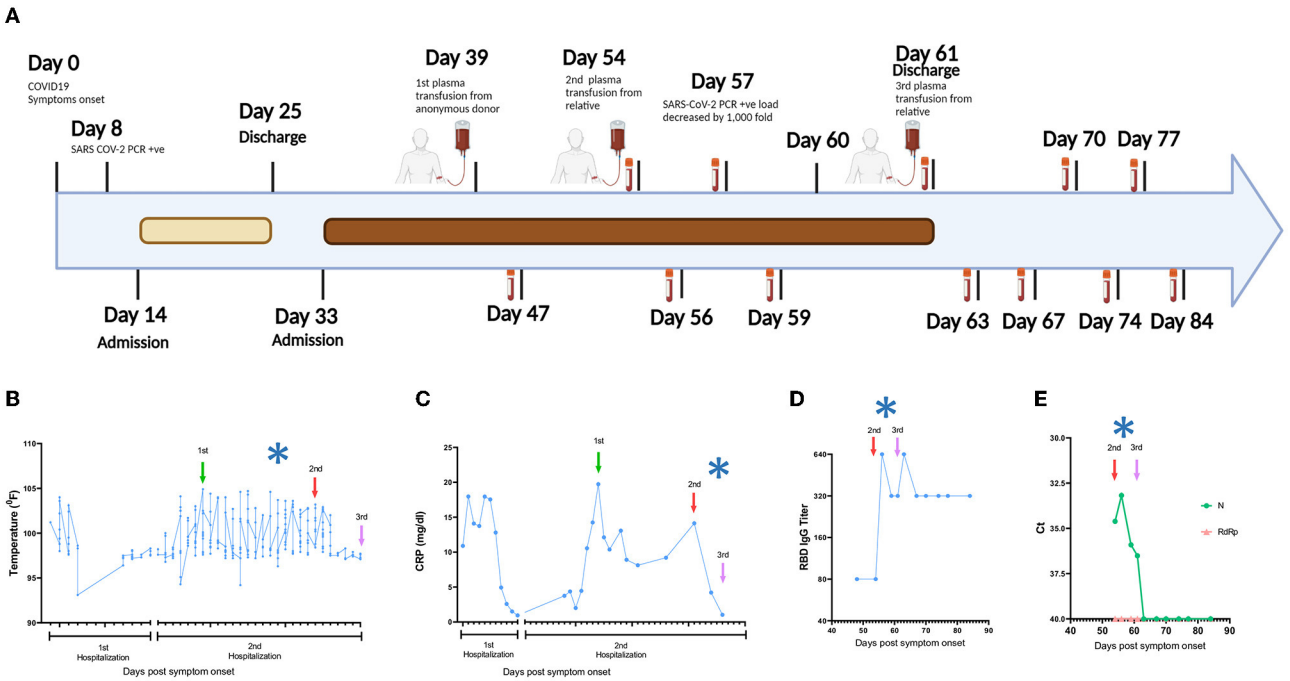

FIGURE 1 | Timeline of the clinical course. **(A)** Day 0 indicates the date of the COVID-19 symptom onset. The days post-onset at which COVID-19 related symptoms, hospitalization course, RT-PCR test results, convalescent plasma transfusions, and interventions are indicated. **(B–E)** Longitudinal analysis of **(B)** maximum body temperature, **(C)** C- Reactive protein (CRP) in plasma, **(D)** Titers of IgG against SARS-CoV-2 Spike Receptor binding domain (RBD), and **(E)** SARS-CoV-2 RNA in plasma. The y axis in each panel indicates the corresponding measurement unit. In all panels, the x axis indicates the day numbering as depicted in **(A)**. In **(B,C)**, the timeline in the x axis highlights the first hospitalization (days 14–25) and the second hospitalization (days 33–61), since the corresponding measurements were performed only in the hospital. In all panels, the vertical arrows indicate convalescent plasma transfusions from anonymous donor (1st) and from a patient's relative (2nd and 3rd). The asterisk indicates the drop in viral load recorded at day 57 **(A)**. The low-level anti-RBD IgG seen pre-transfusion **(D)** are consistent with the lack of B cell detection in the patient's peripheral blood by clinical flow cytometry tests (data not shown), which are presumably due to the use of rituximab and/or methotrexate for MAS, since treatment with these drugs can reduce B cell frequencies and humoral immune responses (13, 14). In **(E)**, Ct, cycle threshold; N, SARS-CoV-2 nucleocapsid gene; RdRP, SARS-CoV-2 RNA-dependent RNA polymerase gene.

Some studies leveraged biosamples collected serially from the same patients, thus providing a dynamic representation of the biologic parameters associated with disease worsening [for example (6)]. Much less attention has been placed on characterizing the biological events associated with the clinical resolution of severe COVID-19, even though understanding disease resolution at the molecular and cellular level might contribute new targets of therapeutic interventions against severe COVID-19.

Here we report the first, in-depth, longitudinal analysis of viral and host biologic correlates of the clinical resolution of COVID-19 in a patient who received convalescent plasma therapy during the first pandemic wave in the United States. We find that disease resolution was associated with temporally ordered upward and downward changes in plasma proteins reestablishing homeostasis. Moreover, a subset of monocytes characterized by hyperactivated IFN responses and decreased TNF-α signaling uniquely disappeared following transfusion, implying a key role of this monocyte population in COVID-19 severity and prognosis. Our report of COVID-19 resolution following convalescent plasma therapy in a patient unable to generate humoral responses to SARS-CoV-2 infection, together with a previous report of convalescent plasma-associated COVID-19 resolution in a patient with humoral immunodeficiency (15), strongly supports the use of well-characterized convalescent plasma for therapeutic use in COVID-19 patients who exhibit humoral immunocompromise due to underlying immunological defects or immunosuppressive therapies.

CLINICAL COURSE AND TRANSFUSION EFFECTS ON VIRAL LOAD AND CLINICAL PARAMETERS

In March 2020, a 52-year-old white woman with multiple autoimmune syndrome (MAS), including rheumatoid arthritis and systemic lupus erythematosus developed flu-like symptoms (**Figure 1A**). Her treatment for MAS included daily hydroxychloroquine, weekly methotrexate, and every 5–6 months Rituximab infusion. Three days after symptom onset, she presented to the emergency department (ED), where the nasopharyngeal fluid PCR test was positive for SARS-CoV-2 and chest X ray (CXR) examination demonstrated early right lower lung infiltrates. She was discharged from the ED with

Azithromycin dose pack. On day 14, she returned to the ED with fever (101.2°F) and hypoxia (pulse oximetry of 87% on ambient air). CXR showed bilateral lung opacities with interval progression from the previous exam. She was placed on 4-liter supplemental oxygen and admitted to a hospital with a COVID-19 diagnosis. On day 16, she was started on a 10-day remdesivir course. The hospital course was complicated by hypotension (68/44 mm Hg) and hypothermia on day 17 (93.1°F) (**Figure 1B**), requiring transfer to the medical intensive care unit and pharmacologic blood pressure support. On day 22, a computed tomography (CT) angiogram of the chest showed bilateral COVID-19 pneumonia and a segmental artery pulmonary embolism; anticoagulation was increased from prophylactic to therapeutic dosing. Fevers resolved, and the patient was weaned off of supplemental oxygen at rest and discharged after completing 10-day course of remdesivir. On day 33, she was re-admitted to the hospital with a fever of 102°F and pulse oximetry of 93% on ambient air. She was diagnosed with severe COVID-19, according to US National Institute of Health classification (https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/). She was started empirically on supplemental oxygen via nasal cannula, piperacillin/tazobactam, vancomycin, and prednisone. She continued to have high fevers twice daily, accompanied by increasing C-reactive protein (CRP) levels (**Figures 1B,C**). Given the recent remdesivir course and the recurrence of severe COVID-19 symptoms, the patient received alternative management with 400 mL of COVID-19 convalescent plasma from an anonymous donor on day 40; however, fevers persisted daily reaching maximum temperature of 104.7°F and CT of the chest with contrast on day 45 showed worsened bilateral ground-glass lung opacities compared to 1 week prior. She continued to require supplemental oxygen via nasal cannula (2 liters). On day 48, plasma samples from the patient and family members were analyzed in our research laboratory for antibody testing. Plasma from a patient's relative, who had recovered from COVID-19, showed high anti-SARS-CoV-2 RBD IgG titers (**Supplementary Figure S1**) and was selected for donation (600 mL; on day 54). Oxygen saturation improved

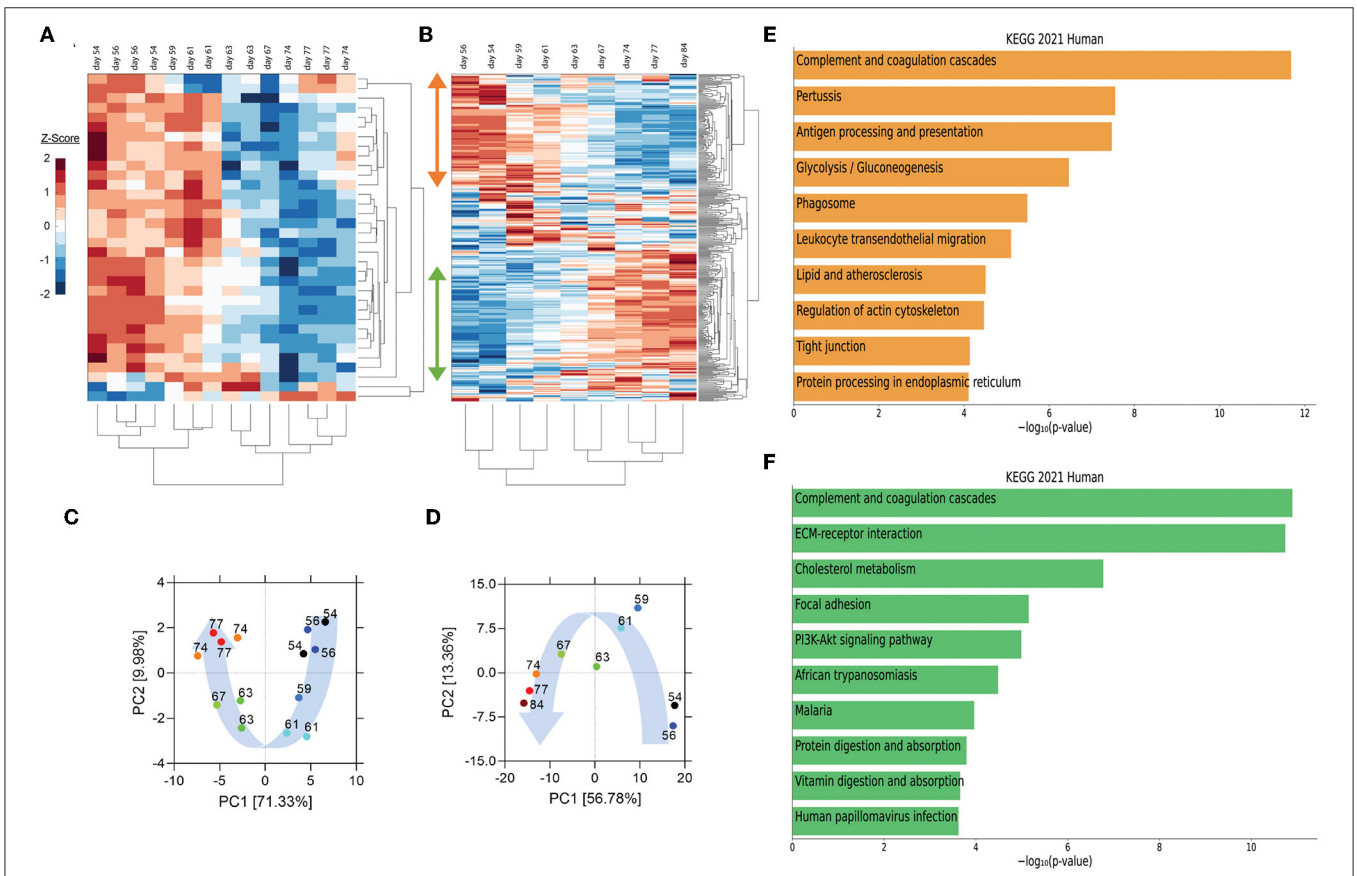

FIGURE 2 | Unsupervised hierarchical clustering and principal component analysis (PCA) of cytokines and proteins in the recipient's plasma before and after convalescent plasma transfusion. **(A,B)** Unsupervised hierarchical clustering was performed on plasma cytokine profile and proteomic data using MATLAB. Vertical arrows in **(B)** mark the set of proteins that decrease (orange arrow) or increase (green arrow) following transfusion. **(C,D)** PCA was conducted on plasma cytokine profile **(C)** and proteome **(D)**. The variance accounted for by PC1 is shown on the x-axis and the variance accounted for by PC2 on the y-axis. Numbers indicate days post-symptom onset as shown in **(A,B)**. Blue arrows illustrate the distribution of PC1 scores, which were self-organized in a temporal trajectory. **(E,F)** The top 10 enriched KEGG pathways for proteins that decrease (orange bars) (referred to as module A in **Supplementary Figure S2** and in the text) or increase (green bars) (module B in **Supplementary Figure S2** and in the text) using EnrichR Pathway Analysis.

TABLE 1 | Proteins in the top three KEGG pathways that decrease (module A) or increase (module B) post-transfusion.

		Kegg term	Symbol (name)
Module A	Post-transfusion decrease	Complement and coagulation cascades	C4A (complement component 4A); C5 (complement component 5); SERPINA1 (alpha-1 antitrypsin); CFHR1 (complement factor H related 1); C9; CFHR3 (complement factor H related 3); CFHR5 (complement factor H related 5); SERPING1 (C1 inhibitor); C4BPA (complement component 4 binding protein alpha); C4BPB (complement component 4 binding protein beta)
		Pertussis	C4A (complement component 4A); C5 (complement component 5); CFL1 (COFILIN 1); SERPING1 (C1 inhibitor); C4BPA (complement component 4 binding protein alpha); C4BPB (complement component 4 binding protein beta); CD14 (cluster of differentiation 14)
		Antigen processing and presentation	HSPA8 (heat shock protein family a member 8); HSP90AB1 (heat shock protein 90 alpha family class B member 1); HSPA1L (heat shock protein family A member 1 Like); HLA-B (human leukocyte antigen B); HLA-A (human leukocyte antigen A); CALR (calreticulin); B2M (beta-2-microglobulin)
Module B	Post-transfusion increase	Complement and coagulation cascades	C7 (complement 7); SERPINC1 (serpin Family C Member 1); F12 (coagulation factor XII); PLG (plasminogen); F13B (coagulation factor XIII B Chaign); A2M (alpha 2-macroglobulin); CLU (clusterin)
		ECM-receptor interaction	COMP (cartilage oligomeric matrix protein); TNXB (tenascin-X); COL1A2 (collagen type I alpha 2 chain); LAMA2 (laminin subunit alpha-2); COL6A1 (collagen, type VI, alpha 1); COL4A5 (collagen Type IV alpha 5 chain); COL6A3 (collagen type VI alpha 3 chain); HSPG2 (heparan sulfate proteoglycan 2); CD44 (cluster of differentiation 44); THBS4 (thrombospondin 4)
		Cholesterol metabolism	LRP1 (lipoprotein receptor-related protein 1); APOH (Apolipoprotein H); APOC1 (Apolipoprotein C-I); APOA1 (apolipoprotein A-I); APOC3 (Apolipoprotein C-III); APOA4 (Apolipoprotein A4)

and patient no longer required supplemental oxygen at rest on day 54. Three days later, on day 57, SARS-CoV-2 PCR analysis of a nasopharyngeal sample showed a ∼1,000-fold decrease [10 cycle threshold (Ct) values] in viral burden relative to the preceding test result. Concurrently, fevers abruptly resolved on day 57 (**Figure 1B**) and plasma markers of inflammation, such as CRP, decreased rapidly (**Figure 1C**). SARS-CoV-2 PCR testing of a nasopharyngeal sample became negative on day 59 and lowly positive on day 60. The patient remained afebrile. Oxygen saturation remained normal at rest. She received a second transfusion of 400 mL of her relative's convalescent plasma on day 61 and was discharged from the hospital without further recurrence of COVID-19 symptoms or re-hospitalization. The SARS-CoV-2 PCR test remained stably negative after this transfusion for >20 days (**Figure 1A**), after which testing was discontinued. Antibody binding assays performed for >20 days after her discharge showed stable levels of anti-RBD IgG (**Figure 1D**). The SARS-CoV-2 RNAemia was observed before the beneficial transfusion and became undetectable post-transfusion (**Figure 1E**). In summary, administration of the relative's convalescent plasma resulted in fever remission, improved respiratory status, return to normal of plasma indicators of inflammation, and drastic decrease of viral RNA levels in plasma and upper respiratory tract fluids.

PLASMA CYTOKINE AND PROTEOME SELF-ORGANIZE TEMPORALLY AND IDENTIFY KEY BIOLOGICAL PROCESSES DURING DISEASE RESOLUTION

Utilizing peripheral blood samples collected from the patient at multiple times pre- and post-transfusion with her relative's plasma (as indicated in **Figure 1A**), we performed cytokine and proteomic analysis of plasma collected from the patient collected from the patient on the day of (prior to) the first transfusion (i.e., day 54 post-symptom onset), and multiple times thereafter (see **Figure 1A** for blood collection points). Unbiased hierarchical clustering analysis and principal component analysis revealed dynamic changes in plasma cytokine and protein concentrations over four temporal phases following transfusion (**Figures 2A–D**). Changes in the abundance of plasma proteins became detectable between days 56 and 61 (**Figures 2A,B**)—a few days after the transfusion, and slightly preceded those in plasma cytokines. Between days 61 and 67, the concentration of most plasma cytokines decreased sharply, showing that disease resolution was linked with countering the hyperproduction of inflammatory cytokines typically associated with severe COVID-19 (6). Plasma proteins exhibited a more complex behavior, with some proteins decreasing (**Figure 2B**, orange vertical arrow) and others increasing (**Figure 2B**, green vertical arrow) following transfusion. We call these two protein subsets Module A and Module B, respectively (see **Supplementary Figure S2** for contribution to Principal Component 1 of the two Modules). By day 74 post-transfusion, plasma cytokines and proteins reached their steady-state concentrations, indicating therapeutic resolution. Bioinformatic analyses of plasma proteins in Modules A and B are presented below.

Pathway enrichment analysis using the Enrichr tool (16) identified "complement and coagulation cascades" as top term in both Module A and Module B (**Figures 2E,F**), reflecting the known complement and coagulation disorders associated with severe disease by SARS-CoV-2 and other pathogenic coronaviruses (17, 18). Proteins included in this term in Module A (**Figure 2E**) were predominantly components of the complement cascade (**Table 1**), consistent with complement hyperactivation potentially causing host tissue damage and disease (19) and being associated with severe COVID-19 (20). The second top term in Module A is "pertussis" (**Figure 2E**). In addition to proteins also present in "complement and coagulation cascades", this pathway included cluster of differentiation 14 (CD14) (**Table 1**). Soluble CD14 (sCD14) and sCD163, which

we also find decreased in the patient's plasma following transfusion, contribute to monocyte-macrophage activation and increase with COVID-19 severity (21). Indeed, CD14 is currently being explored as a COVID-19 therapeutic target (clinical trial NCT04391309). The third pathway in Module A was "antigen processing and presentation" (**Figure 2E**). This pathway includes heat shock proteins (HSP) (**Table 1**) that have been described as "facilitators" of infections with several viruses, including coronaviruses and Zika (22, 23). Some, such as HSP90, have been proposed as targets of therapeutic strategies against viral replication (24, 25). The "antigen processing and presentation" pathway also included human leukocyte antigen (HLA) class I molecules and β2-microglobulin (β2-m), which is essential for the conformation of the major histocompatibility complex (MHC) class I protein complex (**Table 1**). Blood levels of soluble HLA antigens are elevated during chronic infection with hepatitis virus B and C and decrease following treatment of chronic viral infections (26), suggesting an association with viral persistence. Moreover, high β2-m levels in blood have been associated with several viral infections, including COVID-19 (27, 28). An additional member of the "antigen processing and presentation" pathway is Calreticulin (**Table 1**), a molecular chaperone ensuring proper folding and functioning of proteins in the endoplasmic reticulum (ER) (29). Since all coronaviruses use the ER to replicate, severe infection with these viruses and the consequent massive production of viral proteins may induce ER stress and activate a compensatory mechanism known as the unfolded protein response, which can further enhance viral replication (30). Taken together, the analysis of the top three terms in Module A shows that clinical resolution of COVID-19 due to convalescent plasma transfusion was accompanied by the longitudinal decrease of plasma markers associated with pathogenic or harmful functions. These included (i) dampening of the excessive activation of the complement cascade and hyperinflammatory responses, with consequent reduction of their tissue- and organ-damaging effects, and (ii) decreased expression of antigen presentation and various protein modifying systems that may favor viral replication.

As mentioned above, the top term in the pathway analysis in Module B, which increased with convalescent plasma transfusion, was also "complement and coagulation cascades" (**Figure 2F**). Proteins assigned to this term in Module B included mostly coagulation factors that were low prior to the convalescent plasma transfusion, and serine protease inhibitors (**Table 1**). In addition, this pathway included components of the kallikrein-kinin system (**Table 1**), which participates in coagulation and control of blood pressure and may contribute to lung angioedema in severe COVID-19 (31). The second pathway in Module B is "Extracellular matrix (ECM) receptor interaction" (**Figure 2F**; **Table 1**). Interactions between ECM constituents and cells, which are typically mediated by transmembrane molecules, contribute to the regulation of key cellular functions, including adhesion, migration, and differentiation (32). Proteins associated with ECM receptors and focal adhesion (the fourth topmost pathway in Module B, **Figure 2F**) have been found decreased in the lung tissue of patients deceased with COVID-19 pneumonia, suggesting dysregulation of the lung extracellular microenvironment (33). The observed increased plasma levels of these proteins following convalescent plasma transfusion—if they reflect changes occurring also in organ microenvironments—could then be considered a meaningful indication of COVID-19 resolution. Furthermore, Module B contains proteins in the "cholesterol metabolism" pathway (**Figure 2F**; **Table 1**), which participate in high-density lipoprotein (HDL) cholesterol metabolism (34, 35). These data are consistent with a report of low HDL cholesterol levels in patients with severe COVID-19 (36). Taken together, the longitudinal increase in protein sets in Module B during disease resolution point to key organ and cellular functions required to reestablish tissue homeostasis and a disease-free state.

CONVALESCENT PLASMA TRANSFUSION IS ASSOCIATED WITH THE DISAPPEARANCE OF DYSFUNCTIONAL MONOCYTES

We next utilized single-cell RNA sequencing to analyze transcriptional changes occurring in peripheral blood cells collected from the patient on the day of (prior to) the first transfusion with her relative's plasma (i.e., day 54 post-symptom onset), and multiple times thereafter (see **Figure 1A** for blood collection points). Unsupervised clustering identified one data cluster that was observed pre-transfusion but was absent at subsequent days (**Figure 3A**). Cell type identification analysis using the Seurat package (37) identified this data cluster as being constituted by monocytes (**Figure 3B**). We next performed Hallmark gene set enrichment analysis to identify genes that best distinguished the monocyte cluster unique to the pre-transfusion state from monocytes in other data clusters. The top hits among upregulated genes [positive normalized enrichment score (NES)] included genes involved in responses to interferon (IFN) alpha (type I), IFN gamma (type II), inflammatory responses, and complement and coagulation (**Figure 3C**). While type I IFNs are key mediators of antiviral responses (38), their excessive activation can exacerbate hyperinflammation and the associated severe manifestations of COVID-19 (39). Similar considerations can be made for type II IFN, which may be important for antiviral defense but may worsen systemic inflammation and increase organ damage when produced at persistently high levels (38). Thus, the hyperactivation of IFN-γ observed in this patient may have contributed to hyperinflammation and COVID-19 severity. The top hits among downregulated genes (negative NES) comprised MYC targets and signaling by cytokines such as TGF-β and TNF-α (**Figure 3C**). The downregulation of MYC targets is difficult to interpret given the highly diverse cellular functions of the ∼ 400 genes in this gene set. The finding that TNF-α signaling is decreased in the monocyte cluster unique to the pre-transfusion state leads to two sets of considerations. First, the pre-transfusion high levels of TNF-α in plasma (data included in **Figure 2A**) were likely produced by cell types other than this unique monocyte cluster. Second, an imbalance between signaling by type I

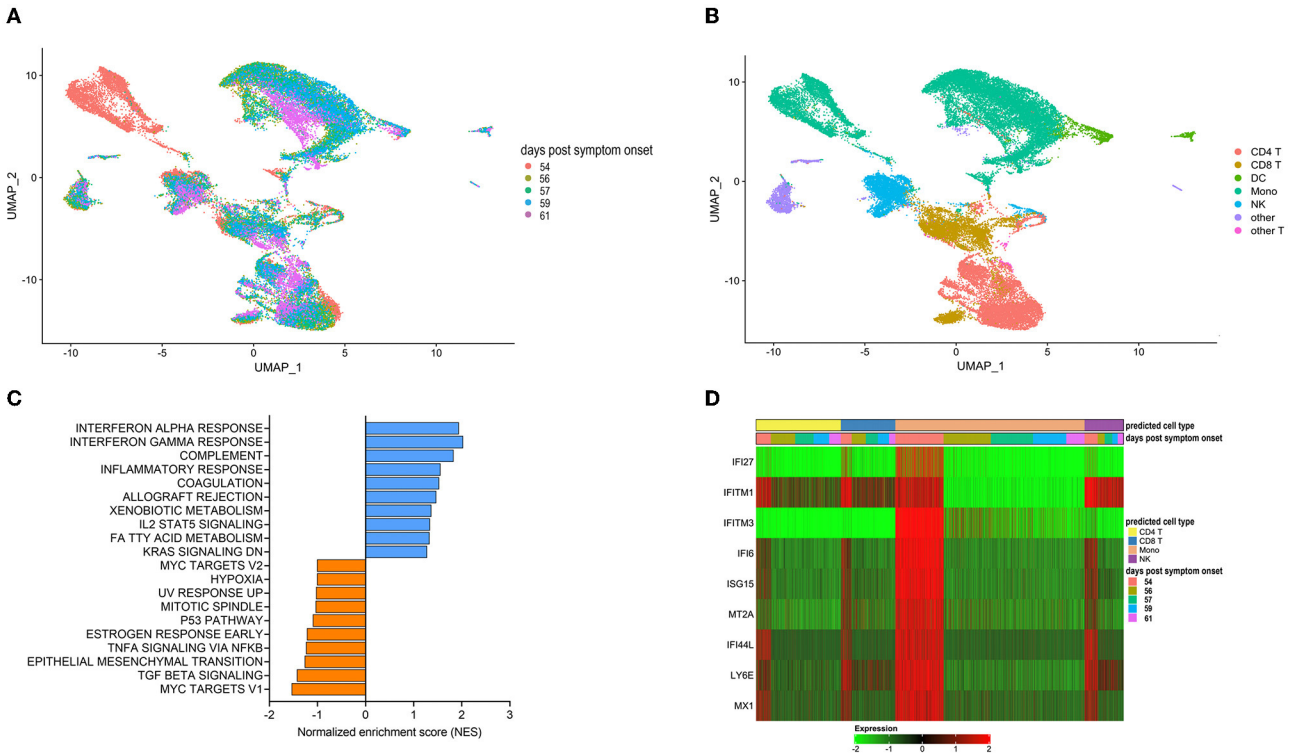

FIGURE 3 | Single cell transcriptomics of recipient's peripheral blood mononuclear cells before and after convalescent plasma transfusion. **(A)** Uniform Manifold Approximation and Projection (UMAP) distribution of single cells grouped by time-post-transfusion sample. Day 0 is the sample obtained just before the first transfusion with the relative's convalescent plasma. **(B)** Cell types were determined using Seurat's MapQuery function along with their PBMC reference dataset. It is worth noting that none of the cell types identified in **(B)** could be assigned to B cells, both prior to the transfusion and for the duration of the post-transfusion analysis, in agreement with flow cytometry data recorded in the patient's hospital chart, as mentioned in the legend to **Figure 1**. **(C)** Histogram of Hallmark gene set enrichment analysis (GSEA) using differentially expressed genes of the unique monocyte cluster identified at day 0 compared to monocytes across other data clusters. **(D)** Heatmap showing the expression levels of type I Interferon-regulated genes in predicted T cells, monocytes, and NK cells. The color code of the "days post-transfusion" bar corresponds to the color code in **(A)**.

IFN (increased) and TNF-α (decreased) in monocytic cells, as observed in this patient, has been proposed to contribute to loss of protective innate immune functions in hyperinflammatory or autoimmune conditions (40) and has been described in severe COVID-19 (41). Of note, upregulation of both type I IFN and TNF-α responses (42) and imbalances in the opposite direction (dampened type I IFN and elevated TNF-α signaling) (43, 44) have also been reported in severe COVID-19, suggesting that immune dysregulation phenotypes may vary in different patients, presumably depending on context. Furthermore, when we analyzed individual Interferon stimulated gene (ISG) expression, we observed that typical ISGs, such as IFI6, ISG15, IFI44L, and LY6E were most prominently elevated in monocytes prior to transfusion but were also increased in NK cells and T cell subsets, and that this strong IFN response was dampened post-transfusion also in these cell types (**Figure 3D**). Together, these data show that the beneficial effects of the relative's plasma transfusion were accompanied by the elimination of a unique population of disease-associated, hyperinflammatory monocytes.

DISCUSSION

The present report constitutes, to our knowledge, the first longitudinal molecular and cellular in-depth analysis of COVID-19 resolution. In the study patient, severe COVID-19 was associated with SARS-CoV-2 RNAemia, high levels of pro-inflammatory mediators and other potentially harmful plasma factors, dysregulation of coagulation cascades, and abnormally low levels of proteins related to systemic and tissue homeostasis. Following convalescent plasma transfusion from a family member, the plasma protein landscape was characterized by temporally organized clusters of cytokines and other plasma proteins, indicative of dynamic trajectories toward disease resolution. Disease resolution was also associated with post-transfusion disappearance of monocytes characterized by hyperactivation of type I and type II IFN cascades and dampening of TNF-α signaling. Even though we cannot rigorously ascribe disease resolution to the relative's convalescent plasma transfusion, the temporal relationship between transfusion and the dramatic improvement of all virologic and clinical

parameters, which had not occurred with any of the previous empirical treatments, is compelling. Indeed, it is plausible that, by neutralizing virus, the beneficial convalescent plasma caused a drop of viral load in respiratory fluids, effectively dampening IFN responses and the downstream noxious effects of IFN hyperactivation (39). These include dysregulation of complement and coagulation functions, potentially leading to lung damage and respiratory failure (45). Lung damage might be further compounded by dysfunctional ECM receptors and focal adhesion pathways, as revealed by plasma proteomics, which may underlie the lung injury seen in severe and fatal cases of COVID-19 (33). Plasma factors and immune cell types partaking in the changes observed post-transfusion likely identify cellular mechanisms critically associated with disease resolution. The characterization of the temporal dynamics of biological processes associated with COVID-19 resolution may identify targets of therapeutic interventions against severe COVID-19, even beyond convalescent plasma therapy. Furthermore, since disease resolution in the study patient was associated with convalescent plasma transfusion, we also characterized key properties of donor plasma, such as virus neutralizing capacity, which are too often ignored when assessing the value of convalescent plasma therapy for COVID-19, as previously discussed (15).

Our study has limitations. One is the study of a single patient. The particular characteristics of the patient, including the underlying immune dysfunction, undoubtedly played a role in both the clinical outcome of the convalescent plasma donation and the ways in which disease resolution occurred. However, most of our findings are corroborated by the rapidly evolving knowledge of the pathways associated with COVID-19 disease and its severity, as presented in *Results*. Thus, we are reasonably confident that our findings are applicable beyond the particular patient under study. A second limitation of our work lies in our inability – despite multiple attempts – to access the plasma obtained from the first, anonymous donor, which had no beneficial effect on the recipient's disease. A comparison between the two sets of convalescent plasma samples might have shed light on key therapeutic properties of convalescent plasma for COVID-19. Thirdly, we do not know which organ/cell(s) the viral transcripts we detected in plasma are derived from. Care of the patient required no organ biopsies, and we found no viral RNA in peripheral blood cells (not shown). Nevertheless, our data strongly point to RNAemia as a proxy for severe infection, as also proposed by others (46), and perhaps as a tool to monitor viral clearance systemically to identify cases of post-acute viral persistence, which may potentially lead to protracted symptoms (47).

ETHICS STATEMENT

We confirm that all relevant ethical guidelines have been followed and all study activities were approved by the Rutgers Institutional Review Board (Pro2020000655). We also confirm that written consent to publish the present work was obtained from the patient. The signed consent is available for review by the editorial team. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

VG, RU, SL, MG, and NB: conceptualization. RD and ST: methodology. JY, RD, and NB: formal analysis. RU, VG, RD, JV, NB, and PM: investigation. AO, DH, MC, SG, MP, CP, AP, and SL: resources. JV, NB, VG, RU, RD, ST, JY, and MG: data curation. NB and MG: writing—original draft. MG, NB, VG, RU, RD, JY, SG, MP, SL, and CP: writing—review and editing. NB, RU, VG, RD, and JY: visualization. MG and SL: supervision. JY and MG: funding. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.915367/full#supplementary-material

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