Convalescent plasma-mediated resolution of COVID-19 in a patient with humoral immunodeficiency

Graphical Abstract

Highlights

- Convalescent plasma clinically benefited a severely ill immunodeficient patient
- The transfer of high-titer neutralizing antibodies led to rapid clinical recovery
- Neutralizing activity was low in most convalescent plasmas but high in recipients
- Neutralizing activity should be tested in both plasma donors and recipients

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In Brief

An immunodeficient patient with protracted COVID-19 unable to mount a humoral immune response rapidly recovered following transfusion with convalescent plasma. Honjo et al. found that immunotherapy with high-titer neutralizing antibodies was clinically beneficial. However, since neutralizing activity of convalescent plasmas varies widely, units should be tested prior to therapy.

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Report

Convalescent plasma-mediated resolution of COVID-19 in a patient with humoral immunodeficiency

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SUMMARY

Convalescent plasma (CP) is widely used to treat COVID-19, but without formal evidence of efficacy. Here, we report the beneficial effects of CP in a severely ill COVID-19 patient with prolonged pneumonia and advanced chronic lymphocytic leukemia (CLL), who was unable to generate an antiviral antibody response of her own. On day 33 after becoming symptomatic, the patient received CP containing high-titer (ID50 > 5,000) neutralizing antibodies (NAbs), defervesced, and improved clinically within 48 h and was discharged on day 37. Hence, when present in sufficient quantities, NAbs to SARS-CoV-2 have clinical benefit even if administered relatively late in the disease course. However, analysis of additional CP units revealed widely varying NAb titers, with many recipients exhibiting endogenous NAb responses far exceeding those of the administered units. To obtain the full therapeutic benefits of CP immunotherapy, it will thus be important to determine the neutralizing activity in both CP units and transfusion candidates.

INTRODUCTION

One strategy to treat coronavirus disease 2019 (COVID-19) is to use convalescent plasma (CP) from individuals who have successfully cleared severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and established humoral immunity.1 Historical evidence indicates that CP mitigates human infectious diseases,2 including those caused by the related SARS-CoV-1 (SARS) and Middle East respiratory syndrome (MERS) coronaviruses.3,4 CP therapy is widely used and was initiated as an investigational new drug (IND) by the US Food and Drug Administration (FDA) through a nationwide expanded access treatment protocol.5 Considered safe,6,7 it was approved for emergency use authorization (EUA) on August 23, 2020.8 However, as of yet there is no evidence for efficacy,9–12 thus rendering this decision controversial.13 This may be because correlates of immune protection are lacking, which render the identification of appropriate convalescing donors and recipients difficult. A key component of CP is neutralizing antibodies (NAbs) that impede SARS-CoV-2 entry into human cells, usually by inhibiting nanomolar affinity interactions between the receptor-binding domain (RBD) of the viral spike (S-protein) and the angiotensin-converting enzyme 2 (ACE2) receptor.14,15 Recent reports indicate, but do not prove, that CPs containing high NAb titers can be beneficial when administered within a few days of hospitalization.9–11 Given later or in lower amounts, CP NAbs may be unable to meaningfully supplement endogenous NAb responses produced during seroconversion.

Here, we report the successful administration of CP to a COVID-19 patient who was unable to generate her own antiviral
antibodies (Abs) due to underlying B cell chronic lymphocytic leukemia (CLL). The CP contained high-titer NAbs (ID50 >5,000) and was given on day 33 after symptom onset. Her prolonged clinical illness and fever resolved rapidly and she was discharged 4 days later, providing compelling evidence for a curative anti-viral effect of the administered NAbs, even though they were given late in the disease course.

RESULTS

Presentation of the case and clinical course

A 72-year-old female with a 20-year history of CLL developed a dry cough following exposure to her daughter (relative 1) who had contracted COVID-19 (Figure 1). The patient had a history of humoral immunodeficiency with chronic sinopulmonary infections requiring monthly intravenous immunoglobulin (IVIg) infusions for over 4 years. Given the progression of her CLL with Rai stage III disease in December 2019, obinutuzumab anti-CD20 B cell depletion immunotherapy was initiated. Her last treatment was 23 days before symptom onset and she had received IVIg 9 days earlier. Relative 1 developed a dry cough 17 days after a holiday in Key West, FL (Figure S1A), presented to a local hospital with headache, ageusia, and diarrhea, tested positive for SARS-CoV-2 by quantitative real-time polymerase chain reaction (PCR) analysis of a respiratory swab sample (A). Notable symptoms at days post onset (DPO), hospitalization course, relevant interventions, and body weight are indicated. IVIg denotes intravenous immunoglobulin; CT, computed tomography; PNA, pneumonia; CXR, chest radiograph; CP, convalescent plasma.

(B–D) Longitudinal analysis of serum albumin and aspartate aminotransferase (AST) (B), hemoglobin and platelet counts (C), and maximum body temperatures (D) are shown after symptom onset. The date of CP infusion (B–D) is indicated by a red arrow.

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(B–D) Longitudinal analysis of serum albumin and aspartate aminotransferase (AST) (B), hemoglobin and platelet counts (C), and maximum body temperatures (D) are shown after symptom onset. The date of CP infusion (B–D) is indicated by a red arrow.
received 218 mL (3.6 mL/kg) on DPO 33. She rapidly defervesced over the next 48 h, became SARS-CoV-2 virus negative on DPO 36, was discharged at DPO 37, recovered her weight, and her hepatic function and anemia resolved by DPO 119 (Figures 1A–1D). Repeat chest radiography showed resolution of her pneumonia at DPO 59 (Figure 2D).

Transfer of high-titer neutralizing antibodies has curative potential

Plasma samples from the CLL patient, convalescing relatives 1 and 2, a third unexposed relative (relative 3), and the remnant CP from relative 2 were tested for SARS-CoV-2 anti-S-protein IgG binding Abs by ELISA. High-titer Abs were present in plasma from relatives 1 and 2 as well as in the remnant CP but were absent in relative 3 (Table 1). As expected, anti-S-protein Abs were not initially present in the immunodeficient patient but became detectable 1 day after CP transfusion.

The same plasma samples were then tested for SARS-CoV-2 NAbs using an HIV-1-based pseudovirus system.16 An additional assay for Ab-mediated inhibition of the SARS-CoV-2 RBD/ACE2 interaction generated highly concordant data (Figure S2). Consistent with the ELISA findings, high-titer NAbs and ACE2/RBD inhibitory Abs were present in the plasma from relatives 1 and 2 and the remnant CP unit, but not in the pretreatment plasma from the CLL patient (Table 1; Figure S3). However, NAbs were clearly detectable on four consecutive days after CP transfusion with titers consistent with an ~1:10 dilution. Analysis of the CP for receptor binding domain (RBD) reactivity revealed primarily IgG and IgM isotype responses, with IgG1 and IgG3 dominant among the IgG subclasses (Figure S4). The CP contained ~112 µg/mL of RBD-specific IgG1, corresponding to ~24 mg in the transfused unit. Thus, the transfer of a CP unit with a NAb ID50 titer of 5,700 from a convalescent donor to an immunodeficient recipient resulted in the loss of detectable virus in nasal swabs, a dramatic clinical improvement, discharge from the hospital within 4 days, and resolution of an advanced pneumonia (Figures 1 and 2).

Most CP units do not reach pre-transfusion neutralizing antibody levels of hospitalized recipients

To assess how this case compared to other CP transfusions, we measured NAb titers in 38 remnant CP units (RCPUs) from the American Red Cross and other regional blood services that were transfused at UAB under the FDA IND indication for COVID-19 patients (Figure 3).17 All CP donors were SARS-CoV-2 PCR or Ab positive and donated plasma at least 14 days after symptom resolution.17 Only 37% of the RCPUs had NAb titers >250, and only 29% inhibited RBD/ACE2 binding. NAbs were undetectable in four RCPUs (ID50 <20). Based on the FDA criterion for ID50 titer >250,18 a high proportion of these plasmas were inadequate for transfusion. We also analyzed a different set of 26 CP units from LifeSouth Community Blood Centers (LSCP) that were pre-screened with a SARS-CoV-2 spike immunoassay (Ortho-VITROS). Those CPs were generally of higher titer (median NAb ID50 456), with 47% exceeding the >250 FDA cutoff and 58% inhibiting ACE2/RBD binding. However, only 8 of the 26 LSCP units had ID50 values >1,000 and none exceeded the 5,700 titer of the CP plasma given to the CLL patient.

To examine how CP transfusion affected plasma NAb activity, we analyzed 17 CP recipients (CPR) for whom pre- and/or post-transfusion blood samples were available. These patients were...
hospitalized, mostly in the intensive care unit (Table S1). At baseline, 53% had ID₅₀ values >250, with 7 exceeding titers of 3,000. Overall, the mean ID₅₀ for this cohort before transfusion was 10.2-fold higher than for the RCPU products (p < 0.001), 3-fold higher than the LSCP units (p = 0.003), and 5.2-fold higher than all units combined (p < 0.001) (Figure 3A). Similar results were obtained analyzing ACE2/RBD inhibition (Figure 3B). For 16 CPs we could analyze, CP transfusion had no significant impact on the pre-existing NAb titers or ACE2/RBD inhibition (p = 0.50 and p = 0.25, respectively) (Figures 3C and 3D). In contrast, CP transfusion of our CLL patient resulted in an obvious rise in NAb titers (Figures 3A–3D, green samples) to levels that are protective in animal studies.19–22

**Table 1. Anti-S-protein and neutralizing antibodies in plasma from the index case and her relatives**

| Plasma sample                      | Collection date (DPO)ᵃ | EC₅₀   | Endpoint | Neutralization (ID₅₀)ᵇ | ACE2/RBD binding (1:25)ᵈ |
|-----------------------------------|------------------------|--------|----------|-------------------------|---------------------------|
| Relatives                         |                        |        |          |                         |                           |
| Relative 1 (infected daughter)    | 52                     | 44,770 | 762,070  | 6,710                   | 7%                        |
| Relative 2 (infected son-in-law/donor) | 44                   | 28,350 | 499,970  | 13,190                  | 11%                       |
| Relative 3 (infection-naive daughter) | –                    | <100   | <100     | <20                     | 109%                       |
| Remnant convalescent plasma (CP)  | 38                     | 24,360 | 266,470  | 5,720                   | 16%                       |
| Index case recipient              |                        |        |          |                         |                           |
| Before CP transfusion (day 0)     | 33                     | <100   | <100     | <20                     | 106%                       |
| Day 1 after transfusion           | 34                     | 1,340  | 11,680   | 570                     | 83%                       |
| Day 2 after transfusion           | 35                     | 1,150  | 12,230   | 500                     | 89%                       |
| Day 3 after transfusion           | 36                     | 670    | 12,870   | 370                     | 98%                       |
| Day 4 after transfusion           | 37                     | 820    | 12,880   | 360                     | 102%                      |

ᵃDPO, days post-onset of symptoms.
ᵇSARS-CoV-2 IgG S-protein ELISA EC₅₀ and endpoint titers are rounded to the nearest 10, and values of <100 are considered negative.
ᶜNeutralization ID₅₀ titters are rounded to the nearest 10, and values <20 are considered negative.
ᵈA reduction in the binding of the angiotensin converting enzyme 2 (ACE2) to the receptor binding domain (RBD) to <90% of control indicates inhibition and values are rounded to the nearest whole number.

Our findings confirm the wide disparity in neutralizing activity found for donor plasma in other studies,29 including international randomized controlled clinical trials that failed to find efficacy for CP immunotherapy.¹¹,¹²,¹⁸ Thus, beyond reliably identifying suitable high-titer plasma donors, clinical trials that investigate correlates of immune protection will be critical for defining titers more likely to achieve clinical efficacy. In this context, CP studies in non-human primates may also be informative.¹² The evolving FDA guidelines and implementation of improved screening procedures that more accurately define neutralizing activity should improve the CP unit supply quality. However, since NAb levels gradually decline over time,³⁰ retesting will be needed for repeat donations.

The strongest plasma NAb titers are found in the sickest patients, and they increase over time during the first
~20–30 days after developing symptoms. Thus, the timing of CP transfusions is another critical consideration given that this treatment is likely to be most effective if given before endogenous NAbs develop. Indeed, other recent studies have shown that CP therapy may only be beneficial when it is used very early after hospitalization and is enriched with high-titer IgG. Ideally, CP use should consider the NAb titers in both donors and recipients, taking into account the substantial dilution factors involved. The failure to consider these issues may account for why early small-scale CP trials have generated non-definitive outcomes and why the recombinant NAb bamlanivimab lacked efficacy in hospitalized COVID-19 patients but may show promise by lowering viral loads in outpatients.

In summary, our case report demonstrates that transfusing a high NAb titer CP into a B cell-deficient patient rapidly resolved her severe COVID-19 illness. CP is a readily available therapeutic option that can potentially provide an immediate benefit. However, the generally low NAb titers in most donors, as well as high-titer baseline NAbs in many recipients, highlight the importance of first testing the CPs, and also the recipients. Doing so should optimize the clinical benefit and reduce the effort spent when CP therapy is not appropriate.

**Limitations of study**

One of the limitations of this report is the sample size of a single index patient. An analysis of neutralizing activity in a larger series of immunodeficient recipients transfused with CP units harboring well-defined NAb titers would be helpful for validating these findings. However, while NAbs may serve as a useful correlate of immune protection, the NAb titer threshold required to achieve clinical efficacy still remains unknown. Although the index case was unable to mount a humoral immune response, we did not examine whether the patient developed SARS-CoV-2-specific cell-mediated immunity and whether this contributed to her clinical improvement. Another limitation is that qPCR Ct values of the index case were not available, thus precluding quantitative analyses of SARS-CoV-2 viral loads prior to and after the CP infusion. Finally, we were unable to examine the clinical outcomes of CP recipients who received units with low NAb titers. Thus, it remains unclear whether CP transfusions have beneficial effects in addition to providing Abs capable of neutralizing SARS-CoV-2. These data will ultimately be required to determine the full clinical benefits of CP immunotherapy for COVID-19.
STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Human samples
  - Cell lines
- **METHOD DETAILS**
  - SARS-CoV-2 S-protein ELISA
  - SARS-CoV-2 RBD ELISA
  - SARS-CoV-2 Pseudovirus Neutralization Assay
  - ACE2/RBD Binding Inhibition Assay
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2020.100164.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.L.H., B.H.H., and R.S.D.; Methodology, K.H., T.J.K., T.H., P.D.B., J.P.M., and R.S.D.; Validation, K.H., R.M.R., D.T.R., and R.S.D.; Investigation, K.H., R.M.R., R.L., W.L., R.S., E.M.T., and Y.H.; Resources, L.P., A.N.K., S.S., M.B.M., J.L.L., C.M.L., T.P.M., T.J.K., T.H., P.D.B., J.P.M., P.A.G., S.L.H., and R.S.D.; Writing – Original Draft, R.S.D.; Writing – Review & Editing, M.B.M., J.P.M., P.A.G., S.L.H., B.H.H., and R.S.D.; Supervision, M.B.M., P.A.G., S.L.H., B.H.H., and R.S.D.; Funding Acquisition, J.P.M., P.A.G., S.L.H., B.H.H., and R.S.D.

DECLARATION OF INTERESTS

R.S.D. and K.H. have submitted an intellectual property disclosure related to the ACE2/RBD assay.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Goat anti-human IgG Fc-HRP | SouthernBiotech | Cat# 2048-05; RRID: AB_2795688 |
| Goat anti-human IgG-HRP | SouthernBiotech | Cat# 2045-05; RRID:AB_2795676 |
| Goat anti-human IgM-HRP | SouthernBiotech | Cat# 2023-05; RRID:AB_2795622 |
| Goat anti-human IgA-HRP | SouthernBiotech | Cat# 2053-05; RRID:AB_2795715 |
| Mouse anti-human IgG1-HRP (clone HP6069) | Invitrogen | Cat# A-10648; RRID:AB_2534051 |
| Mouse anti-human IgG1-HRP (clone 4E3) | SouthernBiotech | Cat# 9052-05; RRID:AB_2796619 |
| Mouse anti-human IgG2-HRP (clone 31-7-4) | SouthernBiotech | Cat# 9060-05; RRID:AB_2796633 |
| Mouse anti-human IgG3-HRP (clone HP6050) | SouthernBiotech | Cat# 9210-05; RRID:AB_2796699 |
| Mouse anti-human IgG4-HRP (clone HP6025) | SouthernBiotech | Cat# 9200-05; RRID:AB_2796691 |
| Mouse anti-human IgA1-HRP (clone B3506B4) | Southern Biotech | Cat# 9130-05; RRID:AB_2796654 |
| Goat anti-human ACE2-biotin | R&D systems | Cat# BAF933; RRID:AB_2305177 |
| Human IgG1 anti-Spike-RBD (clone CR3022) | Obtained from the lab of Dr. James Kobie | |
| Human IgM anti-Spike-RBD-hlgM (clone CR3022) | Invivogen | Cat# srbd-mab5 |
| Human IgA1 anti-Spike-RBD-hIgA1 (clone CR3022) | Invivogen | Cat# srbd-mab6 |
| **Bacterial and virus strains** |        |            |
| MAX Efficiency Stbl2 Competent Cells | ThermoFisher | Cat# 10268019 |
| **Biological samples** |        |            |
| Human serum | Millipore Sigma | Cat# H5667 |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate | Sigma-Aldrich | Cat# T0440-1L |
| 3,3',5,5'-Tetramethylbenzidine (TMB) | BioLegend | Cat# 421501 |
| Sulfuric Acid (1N) | ICCA | Cat# 8300-32 |
| SARS CoV2 His-tag | Brouwer et al., Produced In House | PMID: 32540902 |
| SARS CoV2 Strep-tag | Brouwer et al., Produced In House | PMID: 32540902 |
| SARS CoV2 Avi-tag | Brouwer et al., Produced In House | PMID: 32540902 |
| Recombinant human ACE2 | Raybiotech | Cat# 230-30165 |
| Recombinant SARS-CoV-2 S1 subunit (RBD) | Raybiotech | Cat# 230-30162 |
| DMEM | ThermoFisher | Cat# 10566024 |
| Characterized Fetal Bovine Serum, US Origin | HyClone | Cat# SH30071.02 |
| Penicillin-Streptomycin-Glutamine (100X) | ThermoFisher | Cat# 10378016 |
| FuGENE 6 Transfection Reagent | Promega | Cat# E2692 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Randall S. Davis (rsdavis@uab.edu).

Materials availability
This study did not generate new unique reagents.

Data and code availability
This study did not generate any unique datasets or code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human samples
Blood samples were collected following institutional review board (IRB) approval by the University of Alabama at Birmingham (IRB #130821005 and 160125005) and written informed consent was obtained from all participants including the index patient (72 year old female) and relatives (Relative 1, 50 year old female; Relative 2, 58 year old male; and Relative 3, 53 year old female) (Table 1). Diagnosis of SARS-CoV-2 infection was determined by real-time polymerase-chain-reaction (PCR) analysis of respiratory swab specimens37 and/or detection of nucleocapsid IgG antibodies in the blood (Abbott)38 performed in a clinical diagnostic laboratory.

Seronegative healthy controls (n = 11) were obtained from cryopreserved samples prior to Dec 31, 2020. Sera from CP recipients (n = 16) and units (n = 38) was obtained from remnant blood specimens or discarded plasma bags and tubing. Details of the gender and age for CP recipients is provided in Table S1. Deidentified samples from banked SARS-CoV-2 recovered donor CP units (n = 26) were obtained from LifeSouth Blood Bank (Gainesville, FL).

Cell lines
HEK293T (ATCC) and 293T-ACE2-Clone13 cells were cultured in DMEM supplemented with 10% fetal bovine serum, L-glutamine, and penicillin/streptomycin at 37°C and 5% CO2.

METHOD DETAILS

SARS-CoV-2 S-protein ELISA
The design of the soluble, His-tagged, stabilized SARS-CoV-2 spike (S)-protein (residues 1-1138 of the Wuhan strain) has been described previously.39 Test wells of Corning Clear Polystyrene 96-Well Microplates (FisherScientific #07-200-642) were coated

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Critical commercial assays |  |  |
| Nano-Glo Luciferase Assay System | Promega | Cat# N1150 |
| SARS-CoV-2 IgG | Abbott Laboratories | Cat# 6R86 |
| Experimental models: cell lines |  |  |
| Human embryo kidney (HEK) 293T cells | ATCC | ATCC CRL-3216 |
| 293T-ACE2-Clone13 | Schmidt et al. | PMID: 32692348 |
| Recombinant DNA |  |  |
| pHIV-1NL4ΔEnv-NanoLuc | Schmidt et al. | PMID: 32692348 |
| pSARS-CoV-2-SΔ19 | Schmidt et al. | PMID: 32692348 |
| Software and algorithms |  |  |
| Graphpad Prism version 8 | Graphpad | https://www.graphpad.com:443/ |
| Other |  |  |
| Corning Clear Polystyrene 96-Well Microplates | FisherScientific | Cat# 07-200-642 |
| Corning 96-Well, High Binding, Flat-Bottom, Half-Area Microplate | FisherScientific | Cat# 07-200-37 |
| Streptavidin-HRP | SouthernBiotech | Cat# 7105-05 |
with 300 ng of S-protein in PBS (100 µl) overnight at 4°C. All subsequent incubation steps were carried out at 37°C in volumes of 100 µl. The plates were blocked with blocking buffer (5% non-fat milk powder in PBS + 0.05% Tween 20) for 1 h incubation. Plasma samples were 5-fold serially diluted in blocking buffer and added to the plates for 1 h incubation. Plates were then washed five times with washing buffer (PBS + 0.1% Tween 20) and incubated for 1 h with a goat anti-human IgG horseradish peroxidase (HRP) conjugated antibody (SouthernBiotech #2048-05) diluted 1:5,000 in blocking buffer. Plates were again washed five times with washing buffer. For color development, the TMB substrate (Sigma-Aldrich #T0440-1L) was added for 10 min before the reaction was stopped with an equal volume of 1N H2SO4. Absorbance was read at 450 nm using a Synergy 4 (BioTek) spectrophotometer. For each serum dilution, the average OD450 value from three background control wells (no serum) was subtracted from the S-protein coated sample wells. In addition, the average OD450 value plus 2 standard deviation derived from 28 pre-pandemic sera from UAB was subtracted for each serum dilution. Midpoint (EC50) values were calculated by a nonlinear-regression fit of a 4-parameter sigmoid function to the corrected OD450 values and the logarithmic dilution factors (the lower plateau was set to 0; GraphPad Prism software). The end-point titers were read from the fitted curve at a corrected OD450 cut-off of 0.1.

SARS-CoV-2 RBD ELISA

High-binding 96-well plates (Corning #3690) were coated at 4°C overnight with 50 µl of recombinant RBD (RayBiotech) diluted at 1 µg/ml in PBS. The following day, plates were washed 3 times with 0.1% Tween 20 in PBS (PBST). A blocking solution of 100 µl per well of 3% non-fat dry milk in PBST was added and incubated at room temperature for 1 h, washing plates 3 times with PBST. Plasma samples were inactivated at 56°C for 30 min and initially diluted at 1:25, then serially diluted 4-fold in 1% non-fat dry milk in PBST before adding 50 µl per well, and incubating at room temperature for 2 h. Plates were then washed 3 times with PBST, and a 50 µl per well mixture of horseradish peroxidase (HRP) conjugated goat anti-human IgG (1:8000), goat anti-human IgM (1:10000), goat anti-human IgA (1:5000), mouse anti-human IgG1 (clone 4E3, 1:1000), mouse anti-human IgG2 (31-7-4, 1:500), mouse anti-human IgG3 (HP6050, 1:4000), mouse anti-human IgG4 (HP6025, 1:3000) (all from Southern Biotech) in PBST was added to the wells and incubated at room temperature for 1 h to measure S-RBD Ab responses. For quantitative measurements of RBD-specific antibodies, 50 µl of serially diluted human IgG1 anti-Spike-RBD (clone CR3022, a gift from Dr. James Kobie), human IgM anti-Spike-RBD (CR3022, Invitrogen), and human IgA1 anti-Spike-RBD (CR3022, Invitrogen) in 1% non-fat dry milk in PBST were used as standards, and plate bound Abs were detected by HRP conjugated goat anti-human IgM (1:20000, Southern Biotech), mouse anti-human IgG1 (HP6069, 1:750, Invitrogen), and mouse anti-human IgA1 (B3506B4, 1:1200, Southern Biotech) in PBST. Plates were washed 4 times with PBST, developed with 50 µl per well of HRP substrate 3, 3', 5', 5' tetramethyl benzidine (TMB, Biolegend) at room temperature for 10 min. The reaction was stopped by addition of 50 µl of 1N H2SO4. The optical density (OD) was measured at 450 nm with a SPECTROstar omega (BMG Labtech) microplate reader. EC50 values and endpoints were determined as detailed for the SARS-CoV-2 S-protein ELISA.

SARS-CoV-2 Pseudovirus Neutralization Assay

Plasma samples were tested for SARS-CoV-2 neutralizing antibodies as previously described. Briefly, pseudovirus was generated by co-transfection in HEK293T cells, using an HIV-1 nanoluciferase reporter construct and an expression plasmid for the SARS-CoV-2 Spike (Wuhan 1), containing a 19-amino acid cytoplasmic tail truncation. To test neutralization, 1.5x10⁴ 293T-ACE2 cells (clone 13) were seeded in 96-well plates. The following day, five-fold serial dilutions of plasma were incubated with pseudovirus for one hour at 37°C prior to the addition to target cells. Two days post infection, cells were washed with PBS, lysed, and nanoluciferase activity was determined according to manufacturer’s instructions (NANO-Glo Luciferase Assay System). Luciferase activity in wells with virus and no patient plasma were set to 100%, and the dilution of plasma at which luminescence was reduced to 50% (ID50) was calculated.

ACE2/RBD Binding Inhibition Assay

Plasma samples were analyzed with an ACE2/receptor binding domain (RBD) binding inhibition assay. High-binding 96-well plates (Corning #3690) were coated with 50 µl per well of recombinant RBD (RayBiotech) diluted at 1 µg/ml in PBS at 4°C overnight. The following day, plates were washed 3 times with PBS + 0.1% Tween 20 (PBST), and wells were blocked with 100 µl per well of 3% non-fat dry milk in PBST by incubation at room temperature for 1 h. After washing the blocked wells 3 times with PBST, either 50 µl of serum or plasma samples serially diluted in 1% non-fat dry milk in PBST, or 1% non-fat dry milk in PBST alone as a no inhibition control, was added to wells, and incubated at room temperature for 2 h. Heat-inactivated plasma samples (56°C for 30 min) were initially diluted at 1:25, then serially diluted 2-fold for the assay to 1:400. After incubation, plates were washed 3 times with PBST, then 50 µl per well of recombinant human ACE2 (RayBiotech) diluted at 0.1 µg/ml in PBST was added to the wells. Plates were incubated at room temperature for 1 h, washed 4 times with PBST, and then 50 µl per well of HRP-conjugated streptavidin (Southern Biotech) (1:2000 in PBST) was added to the wells, and incubated at room temperature for 30 min. Plates were washed 5 times with PBST, developed with 50 µl per well of 3,3',5,5'-tetramethylbenzidine TMB substrate (Biolegend) at room temperature for 8 min, and the reaction was stopped by addition of 50 µl of 1N H2SO4. The OD was measured at 450 nm with a SPECTROstar omega (BMG Labtech) microplate reader. ACE2 binding was expressed as a percentage of OD values relative to OD value of a no inhibition control. Binding values of < 90% at a 1:25 dilution of plasma were used to calculate inhibitory activity.
QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed and visualized using GraphPad Prism v8 software. Correlations between ACE2/RBD binding (1:25 dilution) and neutralization activity (ID\textsubscript{50}) were analyzed using the Pearson correlation coefficient. Comparisons among plasma sample cohorts were analyzed by ANOVA with Tukey’s multiple comparisons and Brown-Forsythe test correction. Comparisons between CP recipients pre and post transfusion were analyzed with Student’s paired t test. Statistical parameters are provided in Figure S2 and Figure 3. P values below 0.05 were considered significant.