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BRIEF REPORT

TRANSFUSION

High-throughput detection of antibodies targeting the SARS-CoV-2 Spike in longitudinal convalescent plasma samples

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Abstract

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus is the cause of the ongoing coronavirus disease 2019 (COVID-19) pandemic, infecting millions of people and causing more than two million deaths. The SARS-CoV-2 Spike glycoproteins mediate viral entry and represent the main target for antibody responses. Humoral responses were shown to be important for preventing and controlling infection by coronaviruses. A promising approach to reduce the severity of COVID-19 is the transfusion of convalescent plasma. However, longitudinal studies revealed that the level of antibodies targeting the receptor-binding domain (RBD) of the SARS-CoV-2 Spike declines rapidly after the resolution of the infection.

Study Design and Methods: To extend this observation beyond the RBD domain, we performed a longitudinal analysis of the persistence of antibodies targeting the full-length SARS-CoV-2 Spike in the plasma from 15 convalescent donors. We generated a 293T cell line constitutively expressing the SARS-CoV-2 Spike and used it to develop a high-throughput flow cytometry-based assay to detect SARS-CoV-2 Spike-specific antibodies in the plasma of convalescent donors.

Results and Conclusion: We found that the level of antibodies targeting the full-length SARS-CoV-2 Spike declines gradually after the resolution of the infection. This decline was not related to the number of donations but strongly correlated with the decline of RBD-specific antibodies and the number of days post-symptom onset. These findings help to better understand the decline of humoral responses against the SARS-CoV-2 Spike and provide important information on when to collect plasma after recovery from active infection for convalescent plasma transfusion.

KEYWORDS

antibodies, convalescent plasma, coronavirus, COVID-19, flow cytometry, high-throughput screening, RBD, SARS-CoV-2, spike glycoproteins

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1 | INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and, as of February 2021, has caused over two million deaths worldwide (https://www.worldometers.info/coronavirus/). A limited number of studies showed that the transfusion of convalescent plasma for the treatment of respiratory infections caused by coronaviruses, such as SARS-CoV-1, has been successful in improving patient outcome.[1] Its use has now been initiated as an adjunctive therapy for patients with COVID-19, and several clinical trials are underway (e.g., NCT04412486 and NCT04342182). Preliminary findings have suggested improvements in the patients' clinical status after convalescent plasma treatment.[2–5]

Currently, the dynamics of the humoral response to SARS-CoV-2 are under investigation. Of importance is the highly immunogenic trimeric Spike (S) glycoprotein, which is the target of neutralizing antibodies (Abs) and facilitates SARS-CoV-2 entry into host cells via its receptor-binding domain (RBD) that interacts with angiotensin-converting enzyme 2 (ACE2).[6,7] The neutralization activity of plasma from convalescent donors has been suggested to be important for clinical improvement and is a factor of consideration in screening convalescent plasma.[2,3,8,9] However, several studies have shown that Ab titers and neutralization activity against Spike, including RBD-specific Abs, decrease during the first weeks after resolution of infection.[10–12] Furthermore, despite most neutralizing Abs being RBD-specific,[12–14] studies have isolated potent neutralizing Abs that are specific to other epitopes on the S trimer, mainly directed against the N-terminal domain (NTD) of the S1 subunit.[15] In addition, the bulk of the Ab responses elicited by SARS-CoV-2 infection were found to target two major immunodominant regions on the S protein, the fusion peptide region and heptad repeat 2 of the S2 subunit.[16,17] Thus, current plasma screening processes using only recombinant RBD to determine seropositivity and Ab titers for convalescent plasma therapy could overlook Abs specific to multiple epitopes on the viral Spike. Here, we have developed a high-throughput flow cytometry assay that is based on the recognition of the full-length SARS-CoV-2 S protein expressed on the surface of 293T cells. This method allows for the detection of Abs binding to various conformations and domains of the Spike and could be used as a confirmatory assay for seroprevalence studies that are currently underway.

A recent study characterized longitudinal convalescent plasma samples from 15 donors to determine the Ab response against the RBD of SARS-CoV-2.[18] As this analysis was only limited to Abs targeting one domain of the Spike, we used our new high-throughput flow cytometry assay to determine the Ab response against the full Spike over time.

2 | MATERIAL AND METHODS

2.1 | Convalescent plasma donors

Recovered COVID-19 patients were recruited mostly following self-identification and through social media. All participants have received a diagnosis of COVID-19 from the Québec Provincial Health Authority and met the donor selection criteria for plasma donation in use at Héma-Québec. They donated plasma at least 14 days after complete resolution of COVID-19 symptoms. Men and women with no history of pregnancy meeting the above criteria were invited to donate plasma after informed consent. A volume of 500–750 ml of plasma was collected by plasmapheresis (TRIMA Accel®, Terumo BCT, Lakewood, CO). Seropositive donors donated additional plasma units every 6 days for a maximum of 12 weeks. All work was conducted in accordance with the Declaration of Helsinki in terms of informed consent and approval by an appropriate institutional board. Convalescent plasmas were obtained from donors who consented to participate in this research project at Héma-Québec (REB # 2020–004).

2.2 | Transfection and transduction of 293T cells

The 293T human embryonic kidney cells (obtained from ATCC, Manassas, VA) were maintained at 37°C under 5% CO_2 in Dulbecco's modified Eagle's medium (Wisent, St. Bruno, QC, Canada) containing 5% fetal bovine serum (VWR, Radnor, PA) and 100 μg/ml of penicillin–streptomycin (Wisent). Cells were cultured for no more than 10 passages. The plasmid expressing the full-length SARS-CoV-2 Spike was kindly provided by Stefan Pöhlmann and was previously reported.[7] The 293T cells were transfected with 10 μg of Spike expressor and 2 μg of a green fluorescent protein (GFP) expressor (pIRES-GFP) for 2×10^6 293T cells using the standard calcium phosphate method. For the generation of 293T cells stably expressing the SARS-CoV-2 S protein, transgenic lentiviruses were produced in 293T using a third-generation lentiviral vector system. Briefly, 293T cells were cotransfected with two packaging plasmids (pLP1 and pLP2), an envelope plasmid (pSVCMV-IN-VSV-G), and a lentiviral transfer plasmid coding for a GFP-tagged SARS-CoV-2 Spike (pLV-SARS-CoV-2 S C-GFPSpark tag)

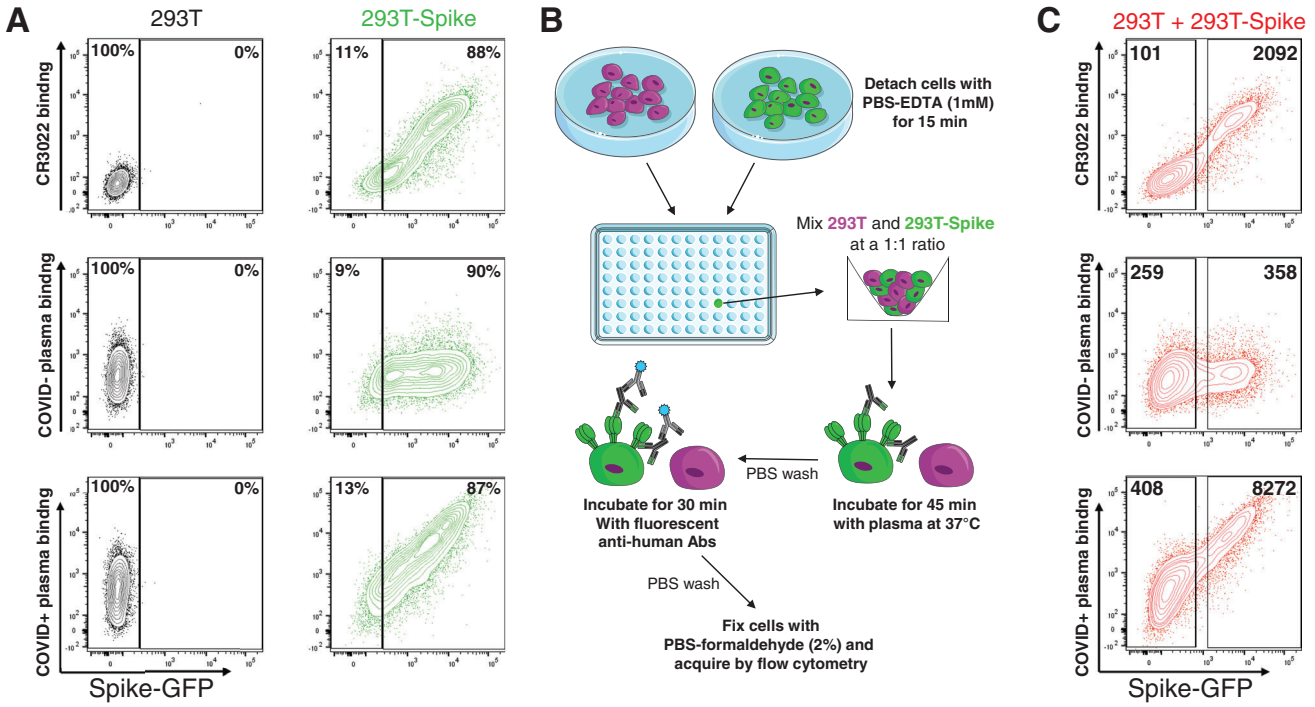

FIGURE 1 Characterization of the 293T-Spike cell line. (A) Dot plots depicting representative stainings of the parental 293T (left) or the 293T-Spike cell lines (right) using CR3022 mAb, a representative COVID-19-negative and COVID-19-positive plasma. Percentages represent the proportion of green fluorescent protein (GFP)+ and GFP- cells on the total cell population. (B) A schematic representation of the experimental procedures used to perform high-throughput screening (HTS) of plasma samples for their specific binding to SARS-CoV-2 Spike. (C) Dot plots depicting representative staining of pooled cell lines used for HTS assay (equal ratio of parental 293T (GFP-) and the 293T-Spike cells (GFP+)) using CR3022 mAb, a COVID-19-negative plasma, and a COVID-19-positive plasma. Median fluorescence intensities obtained on GFP- and GFP+ cell populations are indicated [Color figure can be viewed at wileyonlinelibrary.com]

(Sino Biological, Beijing, China). A supernatant containing lentiviral particles was used to transduce more 293T cells in the presence of 5 μg/ml polybrene. The 293T cells stably expressing SARS-CoV-2 Spike (GFP+) were sorted by flow cytometry.[10,11]

2.3 | Cell surface staining and flow cytometry analysis

The 293T cells transfected with a Spike expressor or 293T-Spike cells were stained with the anti-RBD CR3022 monoclonal Ab (5 μg/ml) or plasma (1:250 dilution). AlexaFluor-647-conjugated goat anti-human IgG (H + L) (Invitrogen, Rockford, IL) were used as secondary antibodies. The percentage of transfected/transduced cells (GFP+ cells) was determined by manually gating the living cell population based on viability dye staining (Aqua Vivid, Invitrogen). For our high-throughput flow cytometry-based assay, parental 293T and 293T-Spike cells were counted and mixed at an equal ratio; this ratio was empirically determined. Samples were acquired on an LSRII cytometer (BD Biosciences, Mississauga, ON, Canada), and data analysis was performed using FlowJo v10.5.3 (Tree Star, Ashland, OR). The seropositivity threshold was established using the following formula: (mean median fluorescence intensity (MFI) of all COVID-19-negative plasma + [3 standard deviations of the mean MFI of all COVID-19-negative plasma] + interassay coefficient of variability). The anti-RBD CR3022 monoclonal Ab was included in each run as a positive control.

2.4 | Statistical analyses

Statistics were analyzed using GraphPad Prism version 8.4.3 (GraphPad, San Diego, CA). Every dataset was tested for statistical normality, and this information was used to apply the appropriate (parametric or nonparametric) statistical test. p values <.05 were considered significant; significance values are indicated as * $p < .05$, ** $p < .01$, *** $p < .001$, and **** $p < .0001$. In Figure 2(A),(B), statistical significance was tested using one-way ANOVA with a Holm-Sidak post-test. In Figure 2(C),(D), statistical significance was tested using a Pearson correlation test or a Spearman rank correlation test based on statistical normality.

3 | RESULTS

3.1 | Generation and characterization of a 293T-Spike cell line

To develop a high-throughput flow cytometry assay able to detect anti-SARS-CoV-2 Spike Abs in plasma from convalescent donors, we generated a cell line stably expressing the full-length S glycoprotein. Third-generation transgenic lentiviruses encoding for SARS-CoV-2 Spike were used to transduce 293T cells. As the S glycoprotein is fused to a C-terminal GFP tag, 293T-Spike cells were sorted by flow cytometry based on GFP expression. The presence of cell-surface Spike was confirmed

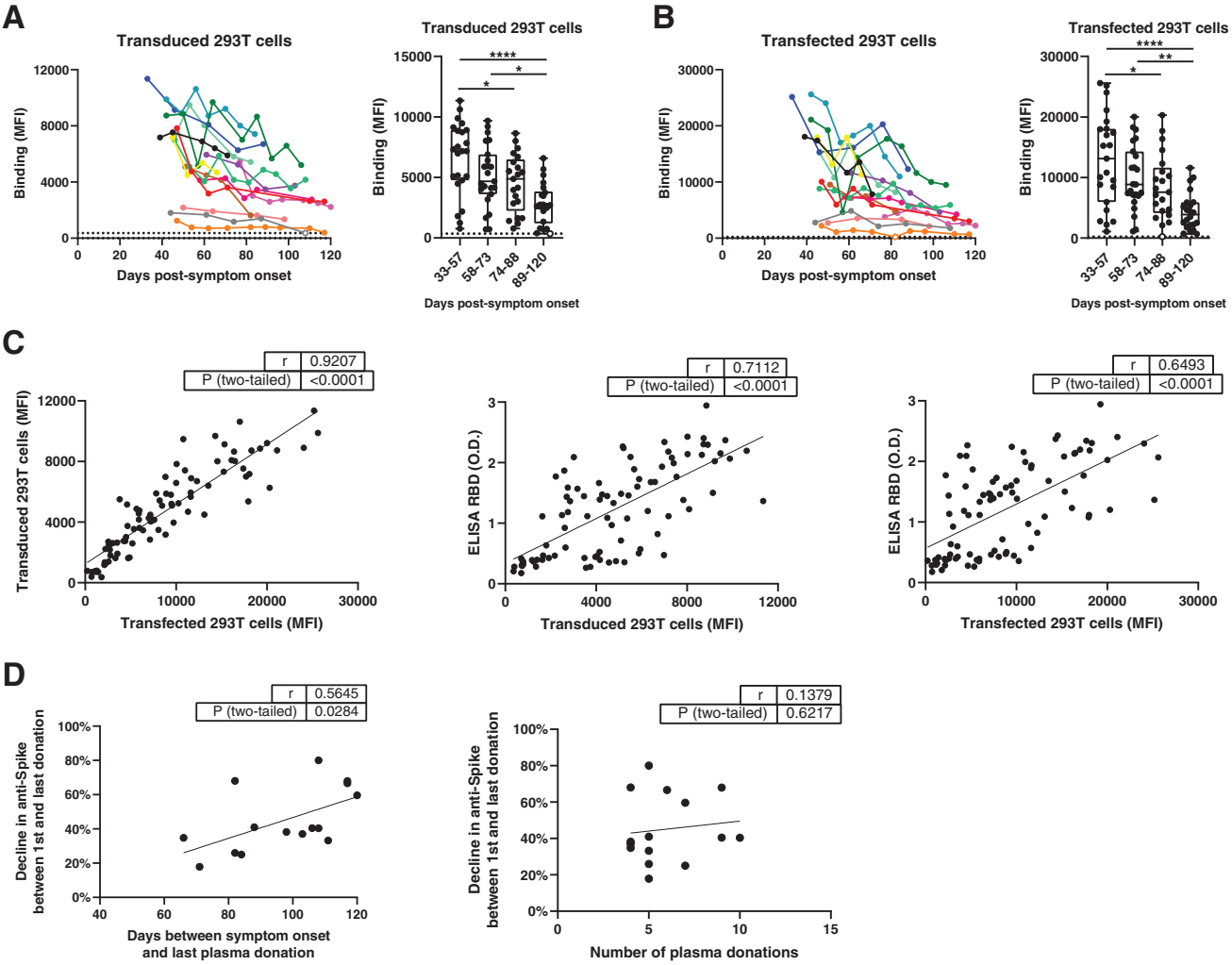

FIGURE 2 Decline of Spike-specific antibodies in longitudinal convalescent plasma. The level of anti-Spike antibodies in plasma from COVID+ donors was determined by flow cytometry using (A) 293T transduced cells or (B) 293T transfected cells expressing SARS-CoV-2 Spike. (A–B, left panels) Each curve represents the median fluorescence intensity (MFI) obtained with the plasma of one donor at every donation (4–10 donations per donor) as a function of the days after symptom onset. Undetectable measures are represented as white symbols, and limits of detection are plotted. (A-B, right panels) The time post-symptom onset (33–120 days) was divided in quartiles containing similar numbers (between 21 and 23) of plasma samples obtained from the 15 COVID-19-positive donors. Boxes and horizontal bars denote interquartile range (IQR), while horizontal lines in boxes correspond to a median of MFI values. Whisker endpoints are equal to the maximum and minimum values below or above the median ± 1.5 times the IQR. Statistical significance was tested using one-way ANOVA with a Holm-Sidak post-test (* $p < .05$; ** $p < .01$; **** $p < .0001$. (C) Correlations between the levels of recognition of SARS-CoV-2 full-length Spike evaluated by flow cytometry using transduced or transfected 293T cells and levels of RBD recognition of SARS-CoV-2 RBD evaluated by indirect ELISA. (D) Correlations between the overall decline in Spike-specific antibody levels as measured by flow cytometry with transduced 293T cells (as calculated using the following formula: 1-[MFI at the last donation/MFI obtained at first donation] × 100) and the number of days between symptom onset and the last donation or the number of donations by each donor. (C–D) Statistical significance was tested using a Pearson correlation test or a Spearman rank correlation test based on statistical normality [Color figure can be viewed at wileyonlinelibrary.com]

using the anti-RBD CR3022 monoclonal Ab and plasma from SARS-CoV-2-infected individuals. Specificity was confirmed using prepandemic healthy donor plasma (Figure 1(A)). For our high-throughput flow cytometry-based assay, parental 293T and 293T-Spike cells were mixed at an equal ratio and incubated with plasma from convalescent donors. Spike-specific Abs were detected by adding a fluorescent anti-human IgG (H + L) secondary antibody. The signal was measured by flow cytometry, and background signal measured on parental 293T cells (GFP negative) was subtracted for specificity. Signals obtained with plasma from 10 prepandemic COVID-19-negative donors were used to define a limit of detection for seropositivity (Figure 1(B),(C)).

3.2 | Longitudinal decline of Spike-specific Abs in plasma from convalescent donors

Recently, a longitudinal analysis was performed to measure the RBD-specific antibody response in convalescent plasma from 33 to 114 days post-symptom onset using a semiquantitative enzyme-linked immunosorbent assay (ELISA).[18] This cohort consisted of 11 men and 4 women (median age, 56 years old), and plasma was donated at least four times. A total of 83 plasma samples were analyzed. A decrease in RBD-specific Ab titers between the first and last donations was observed for all 15 donors tested, and this decline was shown to depend on time post-recovery but not on the number of donations. To extend this observation beyond the RBD domain, we used our high-throughput flow cytometry-based assay using the 293T-Spike cells to measure the persistence of Abs targeting the full-length SARS-CoV-2 Spike in these convalescent plasma samples. Abs against Spike also decreased over time in these plasma samples, with the decrease being significant ~74 days post-symptom onset onward (Figure 2(A)). This finding was corroborated using a previously characterized flow cytometry method to quantify SARS-CoV-2 Spike-specific Abs using 293T cells transiently transfected with a plasmid encoding the full-length Spike[10,11,19–21] (Figure 2(B)), and the MFI obtained from both these methods correlated significantly ($r = 0.9207$, $p < .0001$) (Figure 2(C)). Results obtained with both flow cytometry assays, using transduced or transfected 293T cells, were also positively correlated with the levels of RBD-specific Abs as quantified by ELISA in the recently published study using the same cohort[18] (Figure 2(C)). Of note, the decline of total anti-Spike Abs did not correlate with the number of donations ($r = 0.1379$, $p = .6217$) but rather correlated with the time elapsed between onset of symptoms and last donation ($r = 0.5645$, $p = .0284$) (Figure 2(D)).

4 | DISCUSSION

There are many serodiagnosis platforms that have recently been approved for emergency use authorization by the U.S. Food and Drug Administration (FDA). In this study, we developed a high-throughput flow cytometry-based serodiagnosis tool by developing a cell line stably expressing the SARS-CoV-2 Spike to screen for anti-Spike Abs in the plasma of COVID-19 patients. Although our study shows data with plasma from only 15 donors, this assay can be readily adapted to a large-scale plasma screening with a high-throughput screening plate reader for flow cytometry. This method could also be used as a confirmatory assay for seroprevalence studies that are currently underway. A potential advantage of our assay using 293T-Spike cells over current plasma screening assays using only recombinant RBD is the detection of Abs binding to various conformations and domains on the trimeric Spike expressed in its natural environment (cell membrane). Assays using soluble Spike mostly use trimers stabilized by the addition of two to six proline substitutions and an altered cleavage site. Emerging evidence indicates that these alterations to the trimer might have a profound impact on Spike conformation,[22] and this has also been previously observed with other class I virus fusion proteins, such as the HIV-1 Envelope.[23,24] In addition, we also expanded on recent findings showing a decrease in RBD-specific Abs in convalescent plasma over time by showing that the level of Abs targeting the full-length SARS-CoV-2 Spike also declines gradually after resolution of infection. These findings help to better understand the decline of humoral responses against the SARS-CoV-2 Spike and suggest that plasma should be collected rapidly after recovery from active infection to keep high levels of anti-Spike Abs, which are supposed to provide a clinical benefit in convalescent plasma transfer.

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CONFLICT OF INTERESTS

The authors declare no competing interests.

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