Rapidly Increasing Severe Acute Respiratory Syndrome Coronavirus 2 Neutralization by Intravenous Immunoglobulins Produced From Plasma Collected During the 2020 Pandemic

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Immunoglobulin lots (N = 176) released since March 2020 were tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing antibodies, with first positive results for September 2020 lots (mean, 1.7 IU/mL; 46% of lots positive). From there, values steadily increased, in correlation with the cumulative coronavirus disease 2019 (COVID-19) incidence, to reach a mean of 31.2 IU/mL and 93% of lots positive by January 2021. Extrapolating the correlation, immunoglobulins could reach an anti–SARS-CoV-2 potency of approximately 345 IU/mL by July 2021. At that stage, prophylactic immunoglobulin treatment for primary/secondary immunodeficiency could contain similar doses of anti–SARS-CoV-2 as convalescent plasma that is used for treatment of COVID-19.

Keywords. primary immunodeficiency; secondary immunodeficiency; SARS-CoV-2; SARS-CoV-2 antibody potency; neutralizing antibodies; COVID-19; intravenous immune globulin; immunoglobulin; plasma; prophylaxis.

People with primary immunodeficiency (PID) and secondary immunodeficiency (SID) need substitution therapy with antibodies prepared from the plasma of healthy donors in the form of immunoglobulin (IG) preparations. For emerging agents, however, the plasma donor community is initially seronaive, and thus IG preparations cannot afford protection against these new infectious agents.

After the emergence of another zoonotic coronavirus in humans, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it was initially unclear whether past infection with other, seasonally circulating human coronaviruses (HCoVs) would have induced cross-protective antibodies to the new virus. Using the gold standard virus neutralization test, this was shown not to be the case; that is, intravenous immunoglobulins (IVIGs) produced from plasma collected before the coronavirus disease 2019 (COVID-19) pandemic did not neutralize SARS-CoV-2 [1].

By the end of 2020, close to 100 million COVID-19 cases had been reported globally, with almost a quarter of them in the United States (US) alone [2]. The US is quantitatively the most important origin of plasma for fractionation [3], and thus an increasing proportion of the plasma supply will be collected from donors post–COVID-19, plasma that is now expected to also carry antibodies to SARS-CoV-2.

Here, we report the results from an investigation into the seroconversion of the US supply of plasma for fractionation, through testing of IVIGs derived exclusively from US plasma for SARS-CoV-2 neutralization, which revealed rapidly increasing antibody titers that correlated with cumulative COVID-19 incidence. Together with the characterization of SARS-CoV-2 neutralizing antibody (nAb) titers in a large collection of COVID-19 convalescent plasma (CP) donations, this permits a near-term projection of potentially protective SARS-CoV-2 antibody levels in future IG lots, particularly when used in a prophylactic setting for substitution therapy.

MATERIALS AND METHODS

Immunoglobulin Preparations

A total of 176 IVIG lots (Gammagard Liquid, Baxter Healthcare, Westlake Village, California) released between March 2020 and January 2021 from plasma collected by plasmapheresis (source plasma) in the US were analyzed. For a subset of 12 of these IVIG lots, information about the dates of plasma collection was obtained.

COVID-19 Convalescent Plasma Samples

Four hundred thirty-eight COVID-19 CP samples collected between March and July 2020 were obtained from Austrian (n = 300) and US (n = 138) plasma donation centers (BioLife). The samples originated from donors who had polymerase chain reaction (PCR)–confirmed SARS-CoV-2 infections or who described a disease progression consistent with COVID-19 and for which SARS-CoV-2 neutralization was confirmed.
Information on disease severity was requested from each donor. Donors provided written informed consent.

Measurement of SARS-CoV-2 and HCoV-229E Neutralizing Antibodies
SARS-CoV-2 and HCoV-229E nAb titer were determined using materials and methods previously reported [1]. In brief, 2-fold serially diluted samples were incubated with equal volumes of SARS-CoV-2 (strain BavPat1/2020, Charité Berlin, Germany) or HCoV-229E (for IVIG samples, catalog number VR-740, American Type Culture Collection [ATCC], Rockville, Maryland) at 10^4.0 tissue culture infectious doses 50% per milliliter (TCID₅₀/mL) and incubated for 150 minutes before titration on Vero cells (for SARS-CoV-2; catalog number 84113001, European Collection of Authenticated Cell Culture, Porton Down, Salisbury, United Kingdom) or MRC-5 cells (for HCoV-229E; catalog number CCL-171, ATCC) in 8-fold replicates per dilution. The virus-induced cytopathic effect was determined after 5–7 days of incubation. The reciprocal sample dilution resulting in 50% virus neutralization (NT₅₀; SARS-CoV-2 detection limit in IVIG: ≤1:2) was determined using the Spearman–Kärber formula, and the calculated neutralization titer for 50% of the wells reported as 1:X. For further analyses, samples with a neutralization titer below the detection limit were assigned a value of 0.5 times the detection limit. The National Institute of Biological Standards and Control (Potters Bar, United Kingdom) World Health Organization (WHO) International Standard 20/136, for which a potency in international units (IU) has recently been assigned [4], was included in the study, and the concentration of SARS-CoV-2 nAbs is therefore reported in IU/mL.

Testing was done using a fully validated analytical method (for SARS-CoV-2 nAbs) or a controlled assay that included several validity criteria, that is, confirmatory titration of input virus infectivity and cell viability (for HCoV-229E nAbs).

Statistical Analysis
Overall COVID-19 incidence in the US was taken from the Centers for Disease Control and Prevention COVID-19 data tracker [2]. Data analysis and visualization were done using GraphPad Prism version 8.1.1 (San Diego, California), R Studio version 1.1.383 (Boston, Massachusetts), Mininab version 17.3.1 (State College, Pennsylvania), and Microsoft Excel (Redmond, Washington).

Mean SARS-CoV-2 antibody values for IVIG lots released in a month were correlated against the cumulative incidence of COVID-19 in the US that was recorded 6 months prior to IVIG release; for example, the mean antibody potency measured for IVIG lots released in September 2020 was correlated against cumulative COVID-19 incidence in the US in March 2020. A log-transformed linear model and a polynomial regression model indicated comparable quality of fit, and both were used to calculate an extrapolation beyond the period for which antibody measurements were made. The slope at the highest observed cumulative incidence (ie, July 2020; 1.39%) was used for extending the models in a linear manner.

RESULTS
SARS-CoV-2 and HCoV-229E Neutralizing Antibodies in Commercial IVIG Preparations
SARS-CoV-2 nAbs were undetectable for IVIG lots released to the market between March and August 2020 (n = 63). For IVIG lots released in September 2020, 12 of 26 lots (46%) were sero-positive with a mean SARS-CoV-2 nAb concentration of 1.7 IU/mL (Figure 1A). From there onward, the proportion of SARS-CoV-2 nAb–positive IVIG lots steadily increased, with mean nAb concentrations of 2.7 IU/mL (n = 7/13 [54%]) in October 2020, 4.2 IU/mL (n = 20/30 [67%]) in November 2020, 10.2 IU/mL (n = 16/17 [94%]) in December 2020, and 31.2 IU/mL (n = 25/27 [93%]) in January 2021 (Figure 1A).

HCoV-229E nAb titers of the same IVIG lots remained at similar levels throughout the period surveyed (Figure 1A), and the observed variations in HCoV-229E nAb titers were similar to previously observed ranges [1].

COVID-19 Incidence in the US and Development of SARS-CoV-2 Antibody Content
For the first 12 IVIG lots that contained measurable SARS-CoV-2 nAbs, the time of collection for the several thousand plasma units used for production was analyzed. On average, plasma was collected 6 months before IVIG lot release. Thus, the cumulative incidence as the underlying cause for the seroprevalence in any given month would be expected to determine the level of antibodies present in IVIG lots released 6 months later. The low SARS-CoV-2 antibody content of IVIG lots released in September 2020 would thus on average be derived from plasma collected in March 2020, when the cumulative COVID-19 incidence was 0.06% of the US population (Figure 1A). By July 2020, the cumulative incidence had increased to 1.39% (ie, about 20-fold), and similarly, the mean SARS-CoV-2 antibody concentration increased, from 1.7 IU/mL for September 2020 IVIG lots to 31.2 IU/mL for IVIG lots released in January 2021 (Figure 1A). Based on the increasing cumulative COVID-19 incidence since August 2020, an estimation of SARS-CoV-2 nAb concentrations in future IVIG lots is possible (Figure 1B). Given a cumulative incidence of 7.94% in January 2021, IG lots to be released in July 2021 can be expected to contain a mean SARS-CoV-2 nAb concentration of around 345 IU/mL (Figure 1B).

SARS-CoV-2 Neutralizing Antibodies in COVID-19 Convalescent Plasma Samples
A large collective of COVID-19 CP units (n = 438) was tested with the gold standard neutralization assay for anti–SARS-CoV-2 potency and the results were reported in relation to...
the newly assigned WHO standard [4]. The mean SARS-CoV-2 nAb concentration was 255 IU/mL (Figure 2: IU/mL histogram, median 132, 20th percentile 63, 80th percentile 300, range <2–5879). A mean of 437 IU/mL was determined for all units above the median (n = 218) and a mean of 801 IU/mL for the top 20% of CP samples characterized in this study (n = 88). No SARS-CoV-2 nAbs were detected in the sample of 1 donor who had PCR-confirmed SARS-CoV-2 infection. Most donors had experienced asymptomatic or mild COVID-19 (75% of samples), and around 9% of samples originated from donors who had recovered from severe COVID-19.

DISCUSSION

The cumulative incidence of past COVID-19 cases, that is, the proportion of individuals expected to be SARS-CoV-2 antibody positive, in the US in any given month forms the basis for seropositivity in IG lots released to the market approximately 6 months later. This prediction assumes a constant proportion of asymptomatic infections to COVID-19 cases as they, too, are expected to result in antibody-positive plasma donors.

For the extrapolation of future SARS-CoV-2 nAb concentrations in IG, consideration of only past COVID-19 cases is very conservative. With large vaccination campaigns under way at this moment, approximately 9.6% of the US population...
has already been vaccinated by the end of January 2021 [2]. Vaccine-induced antibodies will thus further increase the anti–SARS-CoV-2 potency of future lots of IGs, and based on the current cumulative incidence of 7.94% in addition to a 9.6% vaccination rate, and higher messenger RNA (mRNA) vaccine–induced antibody titers than post–COVID-19 [5, 6], the anti–SARS-CoV-2 potency of future IG products may potentially be higher than the SARS-CoV-2 potency extrapolated here (Figure 1B).

To date, the level of SARS-CoV-2 nAb titers required in IG to provide protection against COVID-19 has not been determined. A comparison of the antibody potency contained in CP to those expected in IG soon may provide some perspective.

In a large cohort of COVID-19 convalescents, a median anti–SARS-CoV-2 potency of 132 IU/mL was determined (Figure 2). The original US Food and Drug Administration Emergency Use Authorization for transfusion of CP required use of plasma at a potency of above the median, as determined by a high-throughput binding assay [7, 8]. The mean neutralization potency of above-median units of the cohort of samples tested here is 437 IU/mL. Jöyner et al [7] did, however, report significantly better clinical success when using CP above the 80th percentile, the mean of which in neutralization potency is 801 IU/mL. With about 200 mL used for CP transfusion, this would equate to SARS-CoV-2 nAb doses of 87 400 IU and 160 200 IU, respectively.

By July 2021, IGs can be extrapolated to contain a mean potency of approximately 345 IU/mL, of which the standard prophylaxis regimen for PID and SID would apply approximately 500 mg IG/kg, resulting in the administration of 350 mL IG for a 70-kg person, or a dose of around 121 000 IU. While the total doses would be quite similar, it has become evident that CP treatment was more successful when administered at early stages of COVID-19 [9, 10], that is, before extensive virus spread within respiratory and other organs. Regular IG substitution therapy for the treatment of PID and SID represents prophylaxis (ie, antibody administration even before virus exposure), and thus should have a significantly better likelihood of success. This prospect is of particular importance for PID patients, for whom a 10-fold higher COVID-19 mortality rate has been reported [11]. The above calculations are quite conservative, as in the US already now the proportion of vaccinated individuals has surpassed those with post–COVID-19, a trend that can be expected to accelerate in the months to come. In addition, currently available data indicate superior levels of SARS-CoV-2 antibodies after mRNA-based vaccines as currently in use in the US [5, 6]. Cumulatively, these circumstances would appear to support rapidly increasing levels of SARS-CoV-2 antibodies in IG, so that IG-mediated protection against COVID-19 for regularly substituted PID/SID seems quite possible. More research to confirm the extrapolations from this study is currently under way. The ongoing emergence of SARS-CoV-2 variants will add further complexity to the anti–SARS-CoV-2 potency of IG, as initially these virus variants may be less well neutralized by antibodies generated against earlier virus strains. At a later point, though, antibodies against newer virus variants will also appear in these IG preparations.

For COVID-19 treatment, rather than prophylaxis, a hyper-IgIVIG manufactured exclusively from plasma of COVID-19 convalescent donors is currently under evaluation in a phase 3 clinical trial [12].
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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