

Plasma from post-COVID-19 and COVID-19-Vaccinated Donors Results in Highly Potent SARS-CoV-2 Neutralization by Intravenous Immunoglobulins

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SUMMARY: A rapidly increasing proportion of the US population, including plasma donors, now carries SARS-CoV-2 neutralizing antibodies, a consequence of earlier COVID-19 or – for many more – from vaccination against it. The widely used mRNA vaccines induce higher antibody titers than COVID-19, and thus a proportion of immunoglobulin lots fractionated from US-origin plasma now contain SARS-CoV-2 neutralizing antibody titers even more potent than earlier produced COVID-19 hyperimmune globulin products.

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Footnotes

Potential conflicts of interest

Authors are employees of Baxter AG, Vienna, Austria, now part of the Takeda group of companies. MK, MRF, NP, JL, JMS, HT and TRK have Takeda stock interest.

Financial support

This study was funded by Baxter AG, now a Takeda Company.

Presented in part

The results have not been presented anywhere else.

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Abstract

From September 2020, some immunoglobulin (IG) lots from US plasma contained neutralizing antibodies against the newly emerged SARS-CoV-2. Paralleled by the increasing numbers of post-COVID-19 donors, IG lot antibody positivity increased to 93% by January 2021, at a mean titer of ~30 IU/mL. The correlation predicted anti-SARS-CoV-2 potency to reach 345 IU/mL by July 2021. In addition to post-COVID-19 donors, the rapidly increasing number of COVID-19 vaccinated plasma donors now result in a mean antibody titer of >600 IU/mL already in July 2021 IG lots, with SARS-CoV-2 antibody titers for several lots even higher than earlier produced hyperimmune globulin products.

Keywords

Primary immunodeficiency; Secondary immunodeficiency; SARS-CoV-2; SARS coronavirus 2 antibody potency; neutralizing antibodies; COVID-19; intravenous immune globulin; immunoglobulin; plasma; prophylaxis; hyperimmune globulin; CoVIg-19

Background

Based on the increasing percentage of post-COVID-19 plasma donors in the US, 46% of US plasma-derived immunoglobulin (IG) lots released in September 2020 contained detectable SARS-CoV-2 antibodies, at a mean potency of 1.7 IU/mL [1]. Subsequently, in parallel with the rising number of COVID-19 cases in the US, SARS-CoV-2 neutralizing antibodies also became more prevalent in IG, with 93% antibody positive for January 2021 lots at a mean potency of 31.2 IU/mL [1]. Based on COVID-19 incidence and observed SARS-CoV-2 potency in IG, extrapolation of correlation predicted mean SARS-CoV-2 antibody levels of 345 IU/mL for July 2021 IG lots, which would have resulted in IG doses routinely used for prophylactic treatment of people with immune deficiencies or under immune suppression containing an anti-SARS-CoV-2 potency similar to the highest 20% of convalescent plasma units as used therapeutically for the treatment of COVID-19 [1].

Since the calculation of this correlation, effective vaccination campaigns were rolled-out in the US and elsewhere, with the predominantly used mRNA-vaccines reported to induce SARS-CoV-2 antibody titers even higher than COVID-19 itself [2,3]. In addition, more US citizens have by now been vaccinated against COVID-19 rather than having gone through the disease [4], and thus it seemed plausible that the earlier predictions might not reflect the current state. As people with primary and secondary immunodeficiencies (PID / SID) have a higher risk of severe COVID-19 consequences [5], and thus critically depend on the levels of neutralizing SARS-CoV-2 antibodies in their IG treatment, the question of developing antibody levels in IG was revisited. In addition, the relative contributions of plasma sourced from post-COVID-19 versus COVID-19 vaccinated donors to the development of SARS-CoV-2 neutralizing antibodies in plasma pools for fractionation were evaluated, based on the development of antibodies to the spike (S) as well as the nucleoprotein (N) after the former, versus only S antibodies by the currently licensed vaccines.

Methods

Samples from COVID-19 vaccinees

Sera of staff members (N=24) were collected >19 days after second vaccination with SARS-CoV-2 mRNA vaccine Comirnaty (BioNTech/Pfizer) and analyzed for SARS-CoV-2 neutralizing antibodies. All subjects received two vaccinations according to EMA approval and informed consent was obtained from all study subjects.

Immunoglobulin preparations and anti-SARS-CoV-2 hyperimmune globulin

311 IG lots (Gammagard Liquid; Baxalta US Inc., Lexington, MA) released between September 2020 and July 2021, manufactured from US plasma collected by plasmapheresis (source plasma, N=252) or obtained from whole blood donations (recovered plasma, N=59) were analyzed.

As part of the CoVIg-19 Plasma Alliance, Takeda manufactured anti-SARS-CoV-2 hyperimmune globulin (HIG) lots (N=16) for clinical evaluation as inpatient treatment for COVID-19 [6]. Plasma was collected exclusively from convalescent donors after confirmed COVID-19, the IG purified with the same licensed and fully validated IG manufacturing process used for Gammagard Liquid [7], conceptually similar to others [8] and SARS-CoV-2 neutralizing potency determined.

Measurement of SARS-CoV-2 antibodies

SARS-CoV-2 neutralizing antibody titers were determined using materials and methods previously reported [1], using a fully validated analytical method. The National Institute of Biological Standards and Control (NIBSC, Potters Bar, UK) WHO International Standard 20/136, for which a potency in international units has recently been assigned [9], was

included in the study and the concentration of SARS-CoV-2 neutralizing antibodies therefore reported in IU/ml.

The Abbott SARS-CoV-2 IgG and the SARS-CoV-2 IgG II Quant chemiluminescent microparticle immunoassays (CMIA; Abbott Laboratories, Abbott Park, IL, USA), were used for detection of SARS-CoV-2 IgG anti-N and anti-S antibodies in plasma pools for IG fractionation (N=202). Testing was done on ARCHITECT i2000SR equipment (Abbott, IL, USA) with a sample volume of 75 μ L and following the manufacturers' instructions, as previously reported [10].

Categorization of plasma donors

To estimate the contribution of COVID-19 vaccinated versus convalescent (i.e., post-COVID-19) US plasma donors, information from donor questionnaires was obtained. During the donor qualification process, individuals disclose whether they have received any vaccination during the past two months. As the FDA emergency use authorizations for mRNA vaccines were granted mid-December 2020, the group of 'vaccinated' plasma donors can be expected to comprise a substantial number of individuals having received these vaccines in the subsequent months. Further, donor questionnaires comprise a question relating to any known COVID-19 infection in the past.

Graphs and statistical analysis

The incidence of COVID-19 and the proportion of SARS-CoV-2 vaccinated individuals in the US are based on the Centers for Disease Control and Prevention (CDC) COVID data tracker [4] and the US Census Bureau Population Clock [11]. Data analysis and visualization

was done using GraphPad Prism v8.1.1 (San Diego, CA), R Studio v1.1.383 (Boston, MA), and Microsoft Excel.

Results

SARS-CoV-2 neutralizing antibodies in COVID-19 convalescent and -vaccinated plasma donors

Plasma collected from post-COVID-19 donors had geometric mean SARS-CoV-2 neutralizing potency of 140 IU/mL (N=438; [1]). When plasma of mRNA vaccinees was tested in the same assay system, vaccination was found to induce a geometric mean antibody potency of 482 IU/mL (N=24), i.e. 3.4-fold higher.

Cumulative COVID-19 incidence and COVID-19 vaccination rates in the US, and the development of high SARS-CoV-2 antibody levels in lots of commercial IG

By September 2020, approximately half of the IG lots manufactured from plasma collected in the US contained detectable but low levels of SARS-CoV-2 neutralizing antibodies [1]. At the time when the plasma manufactured into these IG lots was collected, the US CDC had reported a cumulative incidence of post-COVID-19 of not even 0.1% [4]. Quite likely, the real case numbers were significantly higher, with one serosurvey reporting positivity estimates of 4.6% for the same time, with subgroup estimates ranging from 1.1% to 14.2% [12].

The positivity rate as well as the mean SARS-CoV-2 neutralization titers of IG lots have gradually increased over time, with a more dramatic upward change for IG lot potency starting with several IG lots released in May 2021 (Figure 1). This trend reflects the effective

COVID-19 vaccination campaigns in the US, which resulted in a higher percentage of the US population being SARS-CoV-2 antibody positive, at the comparably higher antibody levels induced by vaccination as opposed to COVID-19.

Currently licensed SARS-CoV-2 vaccines only induce antibodies to the spike protein of the virus (S), as opposed to COVID-19 which – amongst others – also induces nucleocapsid (N) antibodies. The relative contributions of vaccinees and post-COVID-19 plasma donors to the rising SARS-CoV-2 antibody titers seen in IGs can thus be differentiated by analyzing the ratio of anti-N versus anti-S antibodies. The ratio of these two antibodies in plasma pools composed of several thousand donations started to change rather dramatically from May 2021 (Figure 2), driven by the rapidly increasing anti-S antibody levels induced by vaccination. Of note, no samples were prepared during the period between March 6th and May 3rd, 2021, and thus samples tested from May reflect plasma collections from approximately two months earlier. From the beginning of April 2021, a sharp increase in plasma donors who received a vaccine in the two months prior to donation was registered, a large proportion of which COVID-19 vaccinations, as reflected in the concurrent rapid increase in anti-S antibody levels in plasma pools for fractionation (Figure 2).

Anti-COVID-19 hyperimmune globulin

As others have reported [8], the enrichment factors of SARS-CoV-2 neutralizing antibodies from convalescent plasma into the final HIG were disappointing. While the concentration of total IgG was increased approximately 13-fold by the IG manufacturing process, the increase of SARS-CoV-2 neutralizing antibody titers was only 3-fold. Takeda HIG lots nevertheless contained a highly potent geometric mean of 1,287 IU/mL SARS-CoV-2 antibodies, with a range of 488 to 2,202 IU/mL. By comparison, the SARS-CoV-2 neutralizing antibody titers of regular IG lots released to the market in July 2021 were surprisingly high. The three

highest -SARS-CoV-2 neutralizing IG lots released in this month were manufactured from recovered plasma (Figure 1), with a geometric mean potency of 4,740 IU/mL (range 4,605 – 5,022 IU/mL) significantly ($p < 0.0001$; Welch's t-test) higher than the lots manufactured from source plasma with a geometric mean potency of 1,045 IU/mL (range 157 – 3,256 IU/mL).

Discussion

mRNA vaccines are now understood to induce SARS-CoV-2 neutralizing antibody levels that are several-fold more potent than following COVID-19 infection [2,3]. The results of the current investigation, i.e. a 3.4-fold anti-SARS-CoV-2 titer of mRNA vaccine recipients compared to unvaccinated post-COVID-19 individuals, are consistent with an earlier study, where we have also shown that vaccination of individuals after convalescence from COVID-19 induces SARS-CoV-2 neutralizing antibody titers that are more than 10-fold higher than vaccination of persons without prior exposure to SARS-CoV-2 [13].

For the US, mRNA vaccines account for approx. 96% of all the COVID-19 vaccine doses administered, i.e. 338 million doses around the end of July 2021 [4]. It is thus not surprising to now see SARS-CoV-2 neutralizing antibody titers in commercial IG preparations that are significantly higher than predicted based on the cumulative incidence of COVID-19, and that these higher antibody titers were also achieved earlier than predicted [1].

The SARS-CoV-2 antibody titers in several lots of commercial IG released in July 2021 were even more potent than the earlier produced lots of an investigational HIG. These findings are quite plausible in context though, through higher antibody titers induced by vaccination now, versus only COVID-19 infections earlier. In addition, for the investigational HIG, plasma was collected from post COVID-19 donors relatively soon after their disease, i.e. at a stage when it would have contained significant proportions of neutralizing IgM and IgA antibodies

against the just recently encountered new virus, both Ig subclasses which are removed during the production process from the virtually pure IgG in the final IG product.

The higher SARS-CoV-2 antibody titers contained in IG lots manufactured from recovered plasma is quite possibly the result of the comparatively higher age of blood versus plasma donors [14], with advanced age a risk factor for more severe COVID-19 which then results in stronger immune responses and consequentially higher antibody titers after full recovery [10].

Vaccination rates in the US have increased greatly over the past few months (Figure 1).

Plasma collected now will thus contain even higher SARS-CoV-2 neutralizing antibody titers, a development that within the typical time interval between plasma collection and final IG lot release of a few months is expected to result in a further increase of SARS-CoV-2 neutralizing potency of IG. This fact itself is surely encouraging for people with immune deficiencies or under immune suppression, as the IG they are prophylactically treated with would contain higher doses of SARS-CoV-2 neutralizing antibodies than earlier used for therapeutic treatment of COVID-19 by convalescent plasma. However, it is important to stress that clinically protective doses remain to be determined, and thus it would still be prudent to observe additional precautionary measures for these more vulnerable individuals.

Funding

No additional financial support was received.

Acknowledgments

The contributions of the entire Global Pathogen Safety team, most notably Simone Knotzer, Jasmin de Silva, Stefan Pantic, Brigitte Kainz, Melanie Graf, Elisabeth List and Petra Gruber, (neutralization assays), Veronika Sulzer, Sabrina Brandtner (cell culture), Eva Ha and Alexandra Schlapschy-Danzinger (virus culture), Kathrin Nagl (IG logistics) are gratefully acknowledged. Alla Kazy and Kaitlin Wagner (BioLife US) supported Abbott CMIA testing and Amanda McClendon (Plasma Operations, Takeda) provided plasma pool information.

SARS-CoV-2 was sourced via EVAg (supported by the European Community, agreement no. 871029) and kindly provided by Christian Drosten and Victor Corman (Charité Universitätsmedizin, Institute of Virology, Berlin, Germany).

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FIGURE LEGENDS

Figure 1. SARS-CoV-2 neutralizing antibody concentration of commercial intravenous immunoglobulin (IVIG) lots produced from source (○) or recovered (●) plasma and released between September 2020 and July 2021. Cumulative SARS-CoV-2 incidence (◇; % of population) as well as cumulative SARS-CoV-2 incidence plus fully vaccinated individuals (◇; % of population) in the US population are shown. Abbreviations: SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; IU/ml, international units per milliliter; COVID-19, Coronavirus disease 2019.

Figure 2. Ratio of SARS-CoV-2 antibodies against the spike (S) and nucleocapsid (N) protein in plasma pools used for intravenous immunoglobulin (IVIG) manufacturing (●), as well as cumulative numbers of US plasma donors that were categorized (based on donor qualification questionnaires) into either 'post-COVID-19' (▼), or 'vaccinated' (i.e., having received any vaccination in the past 2 months). Data from September 2020 until July 2021 is shown with a differentiation of 'vaccinated' donors before (○) or after (●) the first mRNA vaccine received FDA emergency use authorization. Temporal development of the anti-S: anti-N ratio was modeled by exponential growth (least squares fit; $R^2 = 0.877$).

Figure 1

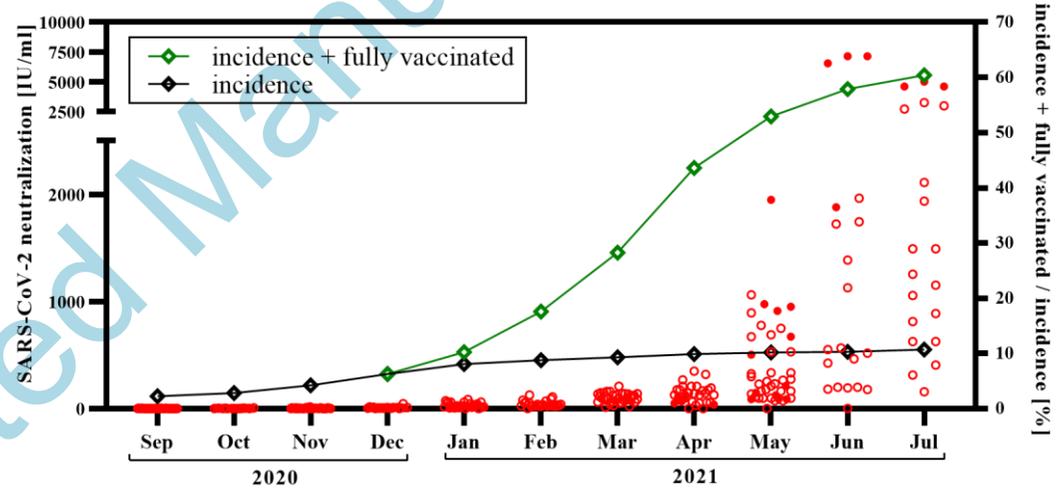


Figure 2

