



## Currently available intravenous immunoglobulin contains antibodies reacting against severe acute respiratory syndrome coronavirus 2 antigens

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**Aim:** There is a critical need for effective therapies that are immediately available to control the spread of COVID-19 disease. **Material & methods:** Gamunex<sup>®</sup>-C and Flebogamma<sup>®</sup> DIF (Grifols) intravenous immunoglobulin (IVIG) products were tested using ELISA techniques for antibodies against several antigens of human common betacoronaviruses that may crossreact with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. **Results:** Both IVIGs showed consistent reactivity to components of the tested viruses. Positive crossreactivity was seen in SARS-CoV, middle east respiratory syndrome-CoV and SARS-CoV-2. For SARS-CoV-2, positive reactivity was observed at IVIG concentrations ranging from 100 µg/ml with Gamunex-C to 1 mg/ml with Flebogamma 5% DIF. **Conclusion:** Gamunex-C and Flebogamma DIF contain antibodies reacting against SARS-CoV-2 antigens. Studies to confirm the utility of IVIG preparations for COVID-19 management may be warranted.

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The outbreak of a novel viral respiratory disease, COVID-19, is caused by infection with the severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2). Due to its extreme transmissibility, COVID-19 has spread dramatically within weeks since the first recognition in China in late December 2019 [1]. Increased human mobility as a global phenomenon has created favorable conditions for COVID-19 to become a pandemic.

Although symptoms are typically mild, in some patient groups, COVID-19 can progress to severe respiratory failure which is associated with significant morbidity and mortality. These patients with severe disease are straining the available critical care resources of the most affected countries [2]. In the short term, the lack of a vaccine and therapeutic agents of proven efficacy against SARS-CoV-2 further aggravates this trend. This critical situation demands a reliable therapy that is immediately available to control the spread of the disease. Historically, convalescent plasma or plasma-derived immunoglobulin (IG), either polyvalent IG (prepared from pooled plasma from thousands of healthy donors) or hyperimmune IG (prepared from the plasma of donors with high titers of antibody against a specific antigen), has been used as the fastest therapeutic option in outbreaks of emergent or re-emergent infections [3].

Coronaviruses are globally distributed [4] and four main common human coronavirus (HCoV) subtypes have been identified to date: HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1. SARS-CoV-2 is a novel emerging and deadly coronavirus. It joins SARS-CoV, responsible for the SARS outbreak in 2003 and middle east respiratory syndrome (MERS)-CoV, responsible for the MERS outbreak in 2012. Since coronavirus infections induce virus-neutralizing antibodies, convalescent plasma therapy was successfully used in the treatment of both SARS [5,6] and MERS [7] patients.

Common human coronaviruses are accountable for a large proportion of respiratory infections which, in most cases, are mild. Because of this ubiquity, antibodies against human common coronaviruses are present in the normal population. Since intravenous immunoglobulin (IVIG) is a polyvalent IG prepared from plasma from thousands

of donors, this product covers a large spectrum of immunity in the general population and, as expected, includes anti-coronavirus antibodies.

It is important to note that coronaviruses of the same subgroup, particularly betacoronaviruses such as HCoV-OC43, HCoV-HKU1, SARS-CoV, SARS-CoV-2 and MERS-CoV, show some crossreactivity in antigenic responses. Crossreactivity between SARS-CoV and MERS-CoV with other common human betacoronaviruses has been reported in some neutralization assays [8–10]. The fact that the new betacoronavirus SARS-CoV-2 is directly related to SARS-CoV (they share more than 90% sequence homology) [11] suggests that antigenic crossreactivity between them is possible, at least for some proteins.

To explore this potential therapeutic pathway, this study was designed to detect antibodies against common human coronaviruses in IVIG products that may crossreact with the new SARS-CoV-2 virus.

## Material & methods

### Experimental design

Gamunex<sup>®</sup>-C (Grifols Therapeutics, Inc., NC, USA) and Flebogamma<sup>®</sup> DIF (Instituto Grifols S.A., Barcelona, Spain) IVIGs were tested for crossreactivity against several betacoronaviruses, including SARS-CoV, MERS-CoV and SARS-CoV-2 antigens, using ELISA techniques.

### IVIG products

Gamunex-C and Flebogamma DIF are unmodified human IVIG products ( $\geq 98$  to 99% IgG) manufactured from plasma collected from donors in the USA and/or several European countries. Gamunex-C is manufactured at a concentration of 100 mg/ml (10%) while Flebogamma DIF is available as 50 and 100 mg/ml (5 and 10%) IgG concentrations.

### Coronaviruses IgG ELISA kits

The following kits were used for the qualitative determination of IgG class antibodies against human coronaviruses: abx052609 Human Coronavirus IgG ELISA kit (Abxexa, Cambridge, UK), against an undetermined antigen; MBS9301037, HCoV-HKU-IgG ELISA kit (MyBioSource, Inc., CA, USA), against N protein; DEIA1035; SARS Coronavirus IgG ELISA kit (Creative Diagnostics, NY, USA), against virus lysate; RV-402100-1; human anti-MERS-NP IgG ELISA Kit (Alpha Diagnostic Intl., Inc., TX, USA), against N protein; RV-402400-1, human anti-MERS-receptor-binding domain (RBD) IgG ELISA Kit (Alpha Diagnostic Intl. Inc.), against RBD of S1 subunit spike protein (S1/RBD); RV-402300-1, human anti-MERS-S2 IgG ELISA Kit (Alpha Diagnostic Intl., Inc.), against S2 subunit spike protein; RV-405200 (formerly RV-404100-1); human anti-SARS-CoV-2 virus spike 1 [S1] IgG ELISA Kit (Alpha Diagnostic Intl., Inc.), against S1 subunit spike protein; EI-2606-9601-G, Anti-SARS-CoV-2 IgG ELISA Kit (Euroimmun AG, Luebeck, Germany), against structural protein (S1 domain); DEIASL019, SARS-CoV-2 IgG ELISA Kit (Creative Diagnostics), against virus lysate. In all cases, the determinations were carried out following the manufacturer's instructions.

### Sample preparation & testing

IVIG samples were serially diluted using the buffer solutions provided in each IgG ELISA kit. With the IVIG 5% product, the dilution series was: neat (undiluted), 1:5, 1:50, 1:500, 1:1000 and 1:5000. With the IVIG 10% product, the dilution series was: neat, 1:10, 1:100, 1:1000, 1:2000 and 1:10000. Therefore, final IgG concentrations of the samples were: 50 mg/ml, 100 mg/ml, 10 mg/ml, 1 mg/ml, 100  $\mu$ g/ml, 50  $\mu$ g/ml and 10  $\mu$ g/ml. In SARS-CoV-2 tests, additional dilutions of 1:300 (333  $\mu$ g/ml) and 1:600 (167  $\mu$ g/ml) were included.

Reactivity against the coronavirus antigens in the different ELISA kits was rated as negative (–) if no reactivity was observed even with neat IVIG, or positive (+) if the lowest IVIG dilution demonstrated reactivity. Independent assays on the same lots were performed on different days: 2–3 for Gamunex-C, 2–4 for Flebogamma 10% DIF and 1–2 for Flebogamma 5% DIF.

## Results

Both Gamunex-C and Flebogamma DIF showed consistent reactivity to components of the tested viruses including a variety of virus proteins, except for the N-protein from HCoV-HKU1. There was no reactivity to this protein even with undiluted IVIG samples.

As shown in [Table 1](#), positive reactivity was particularly apparent in SARS-CoV, MERS-CoV and SARS-CoV-2.

**Table 1. Results of IgG reactivity against different coronaviruses.**

IVIg product	Country of origin of the plasma	Virus and antigen/target							
		HCoV (beta-coronavirus) Undetermined	SARS-CoV Culture lysate	MERS-CoV			SARS-CoV-2		Virus lysate
				N protein	S1 subunit/RBD	S2 subunit	S1 subunit (RV-405200 kit)	S1 subunit (EI-2606-9601-G kit)	
Gamunex-C 10%	USA	Negative	1 mg/ml	100 µg/ml	50 µg/ml	50 µg/ml	100 µg/ml	1 mg/ml	50 mg/ml
Flebogamma 5% DIF	USA	50 mg/ml	10 mg/ml	50 µg/ml	50 µg/ml	50 µg/ml	1 mg/ml	NT	NT
Flebogamma 10% DIF	Spain	100 mg/ml	10 mg/ml	100 µg/ml	50 µg/ml	100 µg/ml	167 µg/ml	10 mg/ml	100 mg/ml
Flebogamma 5% DIF	Czech Republic	50 mg/ml	10 mg/ml	1 mg/ml	1 mg/ml	100 µg/ml	NT	NT	NT
Flebogamma 5% DIF	Germany	100 µg/ml	10 mg/ml	1 mg/ml	1 mg/ml	50 µg/ml	NT	NT	NT

Concentration denotes the last intravenous immunoglobulin dilution with positive result, or no reactivity even undiluted. N = 1–4 tests.  
CoV: Coronavirus; HCoV: Human coronavirus; IVIG: Intravenous immunoglobulin; NT: Not tested; RBD: Receptor-binding domain; SARS: Severe acute respiratory syndrome.

In the case of MERS-CoV, positive reactivity was observed in IVIG samples down to 1:2000 dilution (50 µg/ml) for N protein, S1-RBD protein and S2 protein. For SARS-CoV-2 S1 protein, positive reactivity ranged from an IVIG concentration of 100 µg/ml with Gamunex-C using the RV-405200 ELISA kit to 10 mg/ml with Flebogamma 5% DIF using the EI-2606-9601-G ELISA kit (Table 1). The SARS-CoV-2 lysate showed reactivity at half-diluted Gamunex-C and undiluted Flebogamma 10% DIF.

Reactivity to HCoV (betacoronavirus undetermined antigen) was also observed, although less consistently: negative for Gamunex, but positive for Flebogamma DIF at low dilutions (Table 1).

## Discussion

The need for readily available effective therapies to combat SARS-CoV-2 infection is compelling. In this study, we considered whether IVIG treatment could contribute to COVID-19 disease management. To test this hypothesis, known currently available IVIG products, Gamunex-C and Flebogamma DIF, were tested for crossreactivity with SARS-CoV-2 and other coronaviruses, including SARS-CoV and MERS-CoV. In this first-time report, we found significant crossreactivity to components of all tested viruses including the S1 protein of SARS-CoV-2, the protein responsible for virion attachment to the host cell and neutralization [12].

The consistency of our crossreactivity results among the SARS-CoV-2, SARS-CoV and MERS-CoV viruses is noteworthy. This replicates with the new SARS-CoV-2, the crossreactivity already reported for SARS-CoV/MERS-CoV with other human betacoronaviruses [8–10]. Gamunex-C and Flebogamma DIF were confirmed to contain antibodies reacting against SARS-CoV-2 antigens, although further research is needed to prove functionality and safety for COVID-19.

ELISA results for the undetermined antigen of HCoV were also mostly positive. This was in contrast to HCoV-HKU1, which had negative reactivity. HCoV-HKU1 was discovered in 2005 in Hong Kong and, although it did not result in an outbreak and had only restricted spread, this virus is probably still circulating in the population [13,14]. However, the negativity of an IVIG reaction using a single ELISA coronavirus kit does not mean that such IVIG does not contain antibodies against this pathogen. ELISA sensitivity relies on factors such as the antigen used, the sequence, the organism used to produce it, and the amount of material coated. ELISA results should only be compared qualitatively, since the comparison of the results between different kits is difficult based on differences in sensitivity, and there is no gold standard for quantification. This was confirmed in our results for the SARS-CoV-2 S1 protein subunit antigen, in which different ELISA kits showed different reactivities. In addition, there is scarcity of tests for common coronaviruses.

It has been observed that patients who develop a more severe clinical course of SARS-CoV-2 infection have higher plasma levels of pro-inflammatory cytokines, suggesting a possible ‘cytokine storm’ associated with the disease severity [15]. IVIG products have been demonstrated to be effective in the treatment of inflammatory disorders [16]. Hence, IVIG is considered a therapeutic option for hyperinflammation in patients with severe COVID-19 [17]. To date, a number of possible mechanisms for the immunomodulatory and anti-inflammatory effects of IVIG therapy have been described [18,19], including anticomplement effects [20], anti-idiotypic neutralization of pathogenic

autoantibodies [21], immune regulation via an inhibitory Fc receptor [16,22], enhancement of regulatory T cells [23] and inhibition of Th17 differentiation [24]. Thus, IVIG may mediate a wide variety of biological and immunomodulatory effects via various types of blood cells [24]. When considered together, these immunomodulatory effects of IVIG products might be beneficial in COVID-19 disease management, but this needs to be confirmed clinically. Although IVIG may not confer protection against SARS-CoV-2, the role of antibodies crossreacting with SARS-CoV-2 has been suggested by exerting a priming effect on host immune response or through an immunomodulatory action on monocytes and tissue-resident macrophages [25].

The antibody-dependent enhancement (ADE) phenomenon associated with non-neutralizing antiviral proteins in IVIG also needs to be considered. ADE is often due to low-affinity antibodies and is well described for some viruses such as dengue, and it has also been mentioned for SARS-CoV-1 [26]. ADE has been only suggested for SARS-CoV-2 [27] and, at this stage, it cannot entirely be ruled out.

## Conclusion

The positive ELISA binding results reported should only be considered exploratory, and not generalized or extrapolated to indicate clinical utility. Rather, the findings can be viewed as a base for further evaluation of IVIG to investigate its potential *in vivo* activity in functional tests such as neutralization assays. Even with this uncertainty, in the context of the current health emergency (pandemic), the potential of IVIG as a therapy for COVID-19 is already being evaluated in a number of studies involving patients with severe SARS-CoV-2 viral infections including pneumonia [28–30].

We consistently observed crossreactivity of IVIG products with SARS-CoV-2, SARS-CoV and MERS-CoV using ELISAs from different manufacturers. This evidence supports the presence of anti-SARS-CoV-2 crossreacting antibodies in these IVIG preparations. These results, together with the known immunomodulatory and anti-inflammatory effects of IVIG, suggest a potential positive contribution of currently available IVIG products to COVID-19 disease management, although it should be confirmed by antibody functionality studies.

## Executive summary

- There is an urgent need for readily available effective therapies to combat COVID-19 disease.
- This is the first time that currently available intravenous immunoglobulins have been reported to contain antibodies that crossreact against antigens of SARS-CoV-2 and other coronaviruses.
- Further studies to confirm the functionality of intravenous immunoglobulin antibodies may be warranted.

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## Financial & competing interests disclosure

The authors are full-time employees of Grifols, the manufacturer of Gamunex-C and Flebogamma DIF. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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