

Medicinal Immunoglobulin G Products (2020) Show High Infectivity Neutralizing Activity Against Seasonal Influenza Virus Strains Selected for Future Vaccines (2020–2022)

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Immunoglobulin (Ig)G medicinal products manufactured in 2020 were tested for infectivity neutralization and hemagglutination inhibition against World Health Organization-selected influenza strains included in worldwide vaccines 2020–2022. The IgG batches (from US plasma) showed potent activity. Intravenous immunoglobulin could potentially add to therapies for serious influenza cases in immunocompromised patients. Further study is warranted.

Keywords. antibody neutralization; hemagglutination; IgG medicinal products; influenza; vaccination.

Seasonal influenza viruses are usually a major annual global public health problem. Recent estimates of global influenza mortality are 290 000–650 000 deaths annually [1, 2]. However, the 2020–2021 influenza season was very mild overall [3], likely due, in part, to public health measures for controlling coronavirus disease 2019 (COVID-19) (masking, physical distancing, restrictions on large gatherings) [4, 5]. With the decline in COVID-19 cases in some regions, public health measures in place for the last 2 years are being lifted. This could lead to a rebound for 2021–2022 and subsequent seasons with influenza case levels well above a normal influenza season [3].

Vaccination is one of the best countermeasures against influenza. Antiviral drugs complement vaccination as treatment and prophylaxis. Influenza still results in significant morbidity and mortality in immunocompromised patients [6]. Other

treatment options are needed that can be used in combination with current therapies.

Approved immunoglobulin (Ig)G medicinal products reflect the immunological history of thousands of individual healthy plasma donors of different ages from different geographical areas with unique histories regarding influenza exposure. Intravenous immunoglobulin (IVIg) manufactured from donated plasma would contain antibodies arising from natural influenza infections and immunizations in past years.

Previous studies have demonstrated the activity of IVIg against influenza viruses *in vitro* and in animal models [7–10]. In this study, IVIg solutions manufactured in June 2020 (from plasma collected in the prior 6 months) were tested for activity against 4 influenza strains recommended by the World Health Organization (WHO) in February 2020 for the Northern Hemisphere 2020–2021 influenza season [11], the strains recommended in September 2020 for the Southern Hemisphere 2021 influenza season [12], and the strains recommended in February 2021 for the Northern Hemisphere 2021–2022 influenza season [13]. Activity was measured using neutralization and hemagglutination inhibition assays.

METHODS

Influenza Virus Strains

Influenza virus strains used in these studies were obtained from National Institute for Biological Standards and Controls ([NIBSC] Ridge, Herts, UK). Strains selected by the WHO for the quadrivalent vaccine usually contain 2 A strains (H1N1 and H3N2) and 2 B strains (Victoria lineage and Yamagata lineage). The 4 strains selected for the Northern Hemisphere 2020–2021 influenza season were as follows: A - Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like; A - Hong Kong/2671/2019 (H3N2)-like; B - Washington/02/2019 (Victoria lineage)-like; B - Phuket/3073/2013 (Yamagata lineage)-like [11]. The 4 strains recommended for the Southern Hemisphere 2021 influenza season were the same except for the A/H1N1 strain, which was Victoria/2570/2019 (H1N1)pdm09-like virus [12]. The 4 recommended strains for the Northern Hemisphere's 2021–2022 influenza season were as follows: A - Victoria/2570/2019 (H1N1)pdm09-like virus; A - Cambodia/e0826360/2020 (H3N2)-like virus; B - Washington/02/2019 (B/Victoria lineage)-like virus; and B - Phuket/3073/2013 (B/Yamagata lineage)-like virus. Three of these strains were recommended in previous vaccines, the exception being the Cambodia/e0826360/2020 (H3N2)-like virus [13].

Immunoglobulin G Products

Intravenous immunoglobulin solutions (10%), Flebogamma DIF (batch A4GLE00311) and Gamunex (batches

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Table 1. Hemagglutination Inhibition and Infectivity Neutralization Titers for Seasonal IVIG (2020) Against Influenza Strains Recommended by the WHO for 2020–2022 Influenza Seasons in the Northern and Southern Hemispheres

Recommended Strains	Product	Infectivity Neutralization Titer, TCID ₅₀ ID ₅₀ (IC ₅₀ µg/mL)	Infectivity Neutralization Titer, Luminometry ID ₅₀ (IC ₅₀ µg/mL)	Inhibition of Hemagglutination
Northern Hemisphere 2020–2021 Influenza Season				
Influenza A-(H1N1) Guangdong-Maonan/SWL1536/2019	Flebogamma DIF	1:468 (214)	1:2399 (41.7)	1:800–1:400
	Gamunex C	1:468 (214)	1:5754 (17.4)	1:400–1:800
Influenza A/(H3N2) Hong Kong/2671/2019	Flebogamma DIF	1:4266 (23.4)	1:6768 (14.7)	1:3200
	Gamunex C	1:4266 (23.4)	1:4230 (23.6)	1:3200
Influenza B/Victoria lineage Washington/02/2019	Flebogamma DIF	1:2138 (46.8)	1:4075 (24.5)	1:320
	Gamunex C	1:862 (53.7)	1:2908 (34.4)	1:400
Influenza B/Yamagata lineage Phuket/3073/2013	Flebogamma DIF	1:3631 (27.5)	1:21 428 (4.67)	1:800-1:1600
	Gamunex C	1:4169 (24)	NT	1:1600
Southern Hemisphere 2021 Season (Changes From Above Recommendation)				
Influenza A-(H1N1) Victoria/2570/2019 (H1N1)pdm09-like virus	Flebogamma DIF	1:1413 (70.8)	NT	1:400
	Gamunex C	1:1413 (70.8)	NT	1:400
Northern Hemisphere 2021–2022 Influenza Season (Changes From Above Recommendation)				
Influenza A (H3N2) Cambodia/e826360/2020	Flebogamma DIF	1:1413 (70.8)	1:7102 (14.1)	NA
	Gamunex C	1:1413 (70.8)	1:4645 (21.5)	NA

Abbreviations: IC₅₀, product concentration producing 50 percent infectivity neutralization; ID₅₀, product dilution producing 50 percent infectivity neutralization; IVIG, intravenous immunoglobulin; NA, not available; NT, not tested; TCID₅₀, 50% tissue culture infective dose; WHO, World Health Organization.

B2GGE00063 and B1GJE00153), manufactured in June 2020 by Grifols (Barcelona, Spain, and Clayton, NC, respectively) were used in this study.

Microneutralization

Viral neutralization studies were performed as described in the [Supplementary Material](#). The ID₅₀ (the dilution producing 50 percent neutralization) and IC₅₀ (the concentration producing 50 percent neutralization) values were calculated using Prism software (GraphPad, San Diego, CA).

Inhibition of Hemagglutination

Assessment of hemagglutination inhibition was performed using a solution of 0.75% chicken (*Gallus gallus domesticus*) erythrocytes (Innovative Research, Novi, MI; Seguridad y Salud Animal SL., Barcelona, Spain) diluted in Alsever's solution (Sigma-Aldrich, St. Louis, MO) as previously published [14, 15] and described in the [Supplemental Material](#). Results were reported as the highest dilution of IVIG that inhibited hemagglutination.

Patient Consent Statement

This study does not include any factors that necessitate obtaining patient consent.

RESULTS

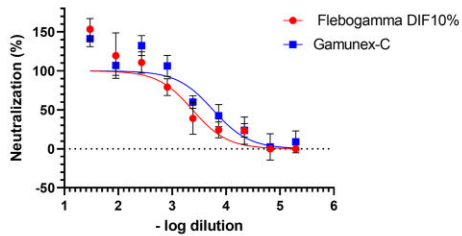
[Table 1](#) shows neutralization and hemagglutination inhibition titers for seasonal IVIG (June 2020) tested against the influenza strains selected by the WHO for the influenza seasons in the Northern and Southern Hemispheres in 2020–2022. These

results show that the IVIG solutions produced in June 2020 contained antibodies against the 4 influenza strains recommended by the WHO for the Northern Hemisphere 2020–2021 influenza season. The same IVIG solutions showed similar activity against the influenza strain recommended by the WHO for the Southern Hemisphere 2021 season and the strain recommended for the Northern Hemisphere 2021–2022 season.

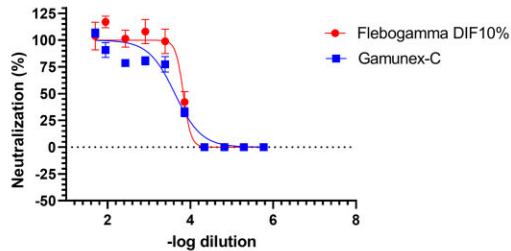
The concentration-response curves for IVIG (June 2020) neutralization against the WHO-selected influenza strains selected for the Northern Hemisphere 2020–2021 influenza season are shown in [Figure 1](#). These curves were used to calculate the IC₅₀ values given in [Table 1](#). Neutralization activity (IC₅₀) was similar for the selected influenza strains. Neutralization titers were in the range of 1:2399–1:21 428 and IC₅₀ values ranged from 4.67 to 41.7 µg/mL. The higher end of IC₅₀ potency was against the influenza B/Yamagata lineage Phuket/3073/2013 strain: 4.67–24 µg/mL. Potency was similar against 3 of the other strains (14.7–41.7 µg/mL): influenza A/(H3N2) Hong Kong/2671/2019, influenza B/Victoria lineage Washington/02/2019, and influenza A-(H1N1) Guangdong-Maonan/SWL1536/2019 strain.

Hemagglutination inhibition titers for seasonal IVIG against the selected strains of influenza (Northern Hemisphere 2020–2021) were high: 1:320–1:3200. The same IVIG product batches (June 2020) showed similar titers against the H1N1 influenza A virus recommended for the Southern Hemisphere 2021 influenza season (Victoria/2570/2019 pdm09-like) and the H3N2 influenza A virus recommended for the Northern Hemisphere 2021–2022 influenza season (Cambodia/e826360/2020).

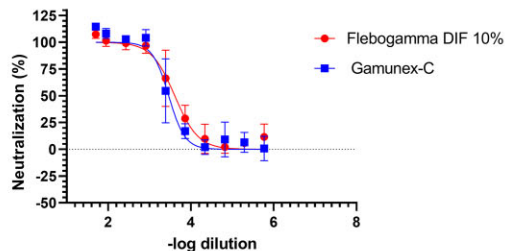
A Influenza A Guangdong-Maonan/SWL1536/2019 (H1N1)



B Influenza A Hong Kong/2671/2019 (H3N2)



C Influenza B Washington/02/2019 (Victoria-lineage)



D Influenza B Phuket/3073/2013 (Yamagata-lineage)

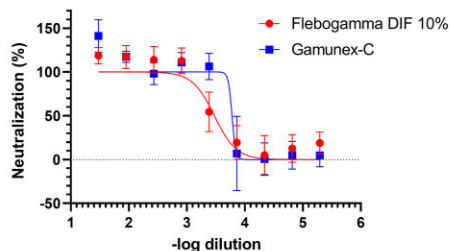


Figure 1. Concentration-neutralization curves for the influenza strains recommended by the World Health Organization for the 2020–2021 influenza season [11] for neutralization by seasonal intravenous immunoglobulin (IVIg) (Flebogamma DIF and Gamunex) manufactured in June 2020. (A) Effects of IVIG in the influenza A Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like strain. (B) Effects of IVIG in the influenza A Hong Kong/2671/2019 (H3N2)-like strain. (C) Effects of IVIG in the influenza B Washington/02/2019 (Victoria lineage)-like strain. (D) Effects of IVIG in the influenza B Phuket/3073/2013 (Yamagata lineage)-like strain.

DISCUSSION

Immunoglobulin (IG) products are produced from pooled human plasma collected from thousands of healthy donors. These plasma pools and the resulting IG products mirror the

immunological history of the donor population and their exposure to influenza viruses. They reflect donors of different ages and geographic origins each having their own influenza exposure history. The anti-influenza antibodies in IG products arise through a combination of infections and vaccinations over the years in each donor.

Influenza viruses have evolved 2 different mechanisms (antigenic drift and antigenic shift) to escape natural immunity. These mechanisms lessen the efficacy of influenza vaccines [16]. As a result, surveillance programs were established by the WHO to constantly monitor antigenic changes in influenza viruses around the world. Based on these programs, the WHO recommends the strains targeted by vaccines for the Northern and Southern Hemisphere influenza seasons.

Vaccination strategies are typically aimed at the hemagglutinin surface protein. As a result, standard measures of anti-influenza antibody titers are directed towards hemagglutination and hemagglutination inhibition. Hemagglutination inhibition detects binding antibodies that may be neutralizing. In general, hemagglutination inhibition titers $\geq 1:40$ are considered protective [17, 18]. All hemagglutination titers in this study were well above this value. In theory, in usual IgG replacement therapy dosing (200 mg IgG/kg bodyweight), the titers in these products would be sufficient to provide a high enough recipient plasma titer ($\geq 1:40$) to consider IVIG solutions to be protective against these influenza viruses.

Moreover, there are other influenza surface proteins that may be targeted by neutralizing antibodies (eg, neuraminidase). Directly measuring infectivity neutralization detects not only neutralizing antibodies towards hemagglutinin, but also against different influenza surface proteins. This method would detect neutralizing antibodies to neuraminidase, the target of some anti-influenza drug therapies.

Intravenous immunoglobulin solutions manufactured in June 2020 (from plasma collected in the prior 6-months) were tested in the current study for activity against the 4 influenza strains recommended for the Northern Hemisphere 2020–2021 influenza season [11], the strains recommended for the Southern Hemisphere 2021 influenza season [12], and the strains recommended for the Northern Hemisphere 2021–2022 season [13]. These IgG products were found to have a very high infectivity neutralization activity against all the strains.

Seasonal influenza epidemics vary from year to year but frequently extract a heavy global toll in terms of morbidity and mortality. Vaccination is the mainstay for controlling these influenza epidemics. In addition, antiviral drugs play an important role in postexposure prophylaxis and treatment of influenza infections. However, a limited efficacy and the development of viral resistance can influence their effectiveness. Despite these treatments, significant morbidity and mortality can still occur especially in immunocompromised patients [6].

Although there are limited data showing a benefit of hyperimmune IVIG in treating influenza [19, 20], whether patients with immunodeficiencies (primary immunodeficiency or secondary immunodeficiency, hematological malignancy patients, transplant recipients) could benefit of IVIG products for prophylaxis as well as therapy for active disease needs further investigation.

A recent study found that the breadth of the antibody response from a natural infection was quite different from that produced by vaccination [21]. This could theoretically increase the diversity of antibodies in IVIG by inclusion of both infection- and vaccination-induced antibodies. The diversity of antibodies and the cross-reactivity between antibodies to different influenza strains could lead to broad activity for IVIG against influenza infections. This broad-based activity may be reflected in the activity of a single seasonal IVIG against all strains selected for 3 consecutive influenza seasons.

CONCLUSIONS

Due to viral resistance and/or a lack of effectiveness of current influenza treatments, additional prophylactic or treatment options are needed. These data show that this seasonal IVIG had high hemagglutination inhibition and neutralization titers against the strains selected for inclusion in seasonal influenza vaccines. These results suggest that IVIG could be used as an adjunct to existing treatments for influenza. Immunocompromised patients, those with comorbidities, and older populations could potentially benefit from IgG treatment. Randomized, controlled trials are needed to determine whether IVIG could be beneficial in influenza as postexposure prophylaxis or treatment.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. All authors are employees of Grifols.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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