Omicron breakthrough infection after heterologous prime-boost vaccination induces a vigorous antibody response

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Running title: Immunogenicity of breakthrough infection

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ABSTRACT

Infection by the SARS-CoV-2 variant Omicron is usually asymptomatic or mild and appears to be poorly immunogenic at least in unvaccinated individuals. Here, we found that healthcare workers vaccinated with two doses of Sputnik V and a booster dose of ChAdOx1 mount a vigorous neutralizing-antibody response after Omicron breakthrough infection.

Keywords: COVID-19; SARS-CoV-2; Omicron; neutralizing antibodies

Notes

Financial support. This work was supported by grants from the Fondo Nacional para la Investigación Científica y Tecnológica (PICT 2017-1616 and PICT 2018–02844 to J. G.) and the Universidad de Buenos Aires (20020170100573BA to J. G.).

Potential conflicts of interest. All authors reported no conflicts of interest.

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BACKGROUND
The emergence of the variant of concern (VOC) Omicron with 37 amino acid substitutions in the spike protein has challenged the immunity against SARS-CoV-2 conferred by both vaccination and previous infection [1]. It also severely compromised the therapeutic activity of most monoclonal antibodies directed to the receptor-binding motif of the spike protein [2]. Pioneering studies have reported that plasma from convalescent patients or vaccinated individuals with two doses of the different anti-SARS-CoV-2 vaccine platforms (mRNA, viral vectors, and virus inactivated vaccines) show very low levels of neutralizing antibodies or no neutralizing activity when assessed against the Omicron variant [1,2]. A booster vaccine dose has shown to increase the neutralizing response against Omicron and hence it has been incorporated to the vaccination scheme applied in adults worldwide [3,4]. In this context, with the emergence of new viral variants and a decline in the memory immune response, rational criteria to guide the administration of additional booster doses is needed. Omicron infection itself could act in a similar way to a booster dose, however, its immunogenicity has not been clearly defined in previously vaccinated individuals. Here we analyzed the immunogenicity of Omicron breakthrough infection in a cohort of healthcare workers vaccinated with two doses of Sputnik V and a booster dose of ChAdOx1.

METHODS
Our study was approved by the Ethics Committee at Hospital “Alejandro Posadas”, Hospital Central de San Isidro “Melchor A. Posse”, Hospital de Clínicas “José de San Martín”, and Hospital de Villa Mercedes "Juan Domingo Perón", Argentina, in accordance with the Declaration of Helsinki. Written informed consent was obtained from all donors. Healthcare workers vaccinated with two doses of Sputnik V (dose interval mean 24 days (range 18-56) in December 2020 or January 2021 were initially recruited in August and September 2021 (n=113). A serum sample was collected 129 to 225 days after full vaccination. Participants were
followed up after receiving a heterologous ChAdOx-1 booster dose on November 2021 (mean interval between second and booster dose was 301 days, range 215-347). Individuals with previous documented infection and/or detectable SARS-CoV-2 nucleocapsid-specific antibodies were excluded from the analysis (n=28). A second serum sample was collected on February 2022, and samples were grouped in participants with no record of infection (n=48) and those with PCR-confirmed SARS-CoV-2 infection (n= 37) in the period between December 24, 2021 and January 31, 2022, period in which more than 99% of new infections in Argentina were attributed to the VOC Omicron (BA.1 lineage), according to the reports of the National Ministry of Health [5]. Mean age of the cohort was 46.2 years (range 28-69) and 45.5 years (range 28-64), for uninfected and infected participants, respectively. Gender distribution (female: male ratio) was 36:12 and 27:10, respectively. No statistical differences were observed regarding the age, gender distribution or dose intervals between the second and booster doses, between groups. The interval between booster dose and breakthrough infection was 41 days (range 7-54) and the interval between infection (PCR-positive test) and sampling was 38 days (range 11-76). All infections were mild and no participant required hospitalization. Informed consent was obtained from all study participants. Blood samples were collected in dry tubes and serum was separated and stored at -20°C until use. Spike-specific IgG titers were determined by 2-fold serial dilutions using a two-step COVIDAR ELISA kit following manufacturer’s instructions [6]. Nucleocapsid-specific IgG were detected using two-step ELISA. Serum neutralizing capacity was evaluated using the ancestral SARS-CoV-2 reference strain 2019 B.1 (GISAID Accession ID: EPI_ISL_499083) and the VOC Omicron (BA.1 lineage) (GISAID accession ID: EPI_ISL_10633761). Vero cells (ATCC) were cultured at 37°C in 5% CO2 in Dulbecco’s Modified Eagle’s high glucose medium (DMEM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (GIBCO). Serum samples were heat-inactivated (30 min, 56°C) and serial dilutions (1/4 to 1/16384) were incubated for 1 h/37°C with SARS-CoV-2 in DMEM 2% FBS. Fifty µl of the mixtures were then incubated with Vero cell monolayers for 1 h/37°C
(MOI = 0.01). Then, the media was removed and replaced by DMEM 2% FBS. After 72 h of culture, cells were fixed with PFA 4% (4°C/20 min) and stained with crystal violet solution in methanol. The viral cytopathic effect on the monolayer of Vero cells was analyzed and the neutralization titer was defined as the highest serum dilution that prevent any cytopathic effect. Multiple comparisons were analyzed by non-parametric Kruskal-Wallis test and Dunn’s posttest and for two group comparisons Mann Whitney test or Wilcoxon pair-matched test were used. Data were analyzed using GraphPad Prism 8.4.3 software.

RESULTS
Serum anti-spike IgG titers in healthcare workers who received two doses of Sputnik V (GMT=71.6) increased 13.7 times after booster vaccination (GMT=979.2). Breakthrough infection by Omicron further increased IgG titers, reaching values 3.9-fold higher (GMT=3859.3), compared with uninfected boosted individuals (Figure 1A). We then analyzed neutralizing activity against the original Wuhan (B.1) variant and Omicron (BA.1). Serum neutralizing titers against the Wuhan variant in individuals vaccinated with two doses of Sputnik V (GMT=10.3) increased 32.2 times upon booster vaccination (GMT=331.9), reaching values 7–fold higher (GMT=2335.0) after Omicron breakthrough infection (Figure 1B, left panel). Analysis of neutralizing activity against Omicron showed that most of the samples from individuals vaccinated with two doses of Sputnik V were seronegative (GMT=2.6). After receiving the booster dose, neutralizing titers increased more than 13.4 times while Omicron breakthrough infection in boosted individuals further increased neutralizing titers reaching values 19.8-fold higher compared with uninfected boosted ones (Figure 1B, right panel). Analysis of paired samples further confirmed that Omicron breakthrough infection in boosted individuals reduced Omicron escape to neutralizing antibodies (Figure 1C).
DISCUSSION

The SARS-CoV-2 variant Omicron shows a high ability to infect vaccinated and convalescent individuals, however booster vaccination has shown to efficiently protect against severe infection [7]. This response appears to be mediated, at least in part, by an enhanced production of neutralizing antibodies with increased potency and breadth compared to the response induced after vaccination with two doses [8].

While Omicron infection in unvaccinated individuals appears to induce a poor antibody response that shows little cross-reactivity with the earlier variants [9,10], recent studies have analyzed the immunogenicity of breakthrough infections in individuals vaccinated with either mRNA vaccines (RNA-1273 and BNT162b2) or Ad26.COV2.S. By studying individuals infected by Omicron sub-lineage BA.1 at 2-3 weeks after infection, with or without previous vaccination with Pfizer-BNT162b2 or Ad26.CoV2.S, Khan and coworkers reported that previously vaccinated individuals mount a strong neutralizing response not only against BA.1, but also against other variants including Omicron BA.2, Delta, Beta, and the ancestral virus [10]. By contrast, only a limited cross-protection was observed in infected unvaccinated individuals [10]. Not only breakthrough infections by Omicron but also by Delta, has shown to result in a marked increase in the antibody response against different VOCs, as reported by Kitchin and coworkers in individuals previously vaccinated with Ad26.Cov2.S [11]. In this regard, however, it should be mentioned the observations reported by Servellita and coworkers showing that Omicron breakthrough infections appear to be less immunogenic than those produced by Delta[12]. Interestingly, breakthrough infections by Omicron in boosted vaccinated individuals have also shown to result in a marked increase in the serum neutralizing activity against Omicron as well as the VOCs Alpha, Beta and Delta, as reported by Woldemeskel and coworkers[13].
In agreement with these observations, by studying healthcare workers vaccinated with a heterologous scheme that included two doses of Sputnik V and a third dose of ChAdOx1, we found that breakthrough infection markedly increased serum neutralizing titers against both, the original Wuhan variant and Omicron. Since breakthrough infections occurred in a period in which more than 99% of new infections in Argentina were attributed to Omicron (BA.1 lineage), we assume that this lineage was responsible for the infection of all the individuals recruited in our study. However, we recognize that the inability to confirm Omicron infection by sequence analysis represent a limitation of the study. Further studies are needed to establish the durability of the antibody response induced by Omicron breakthrough infection and whether this response is actually associated to a better protection against both, infection by different Omicron lineages and progression to severe COVID-19.

ACKNOWLEDGMENTS

We thank Facundo Di Diego, Ignacio Mazzitelli, Ana Paletta and Dr. Mauricio Carobene for isolation and characterization of SARS-CoV-2 Omicron VOC. We thank all team members of the “Hospital de Clínicas José de San Martín”, “Hospital Alejandro Posadas”, “Hospital Central de San Isidro Melchor A. Posse” and “Hospital de Villa Mercedes Juan Domingo Perón”, Argentina. Most of all, we are indebted to all health care workers that were participants in our study.
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Figure 1.- Analysis of antibody response induced by Omicron breakthrough infection. (A) Titers of serum IgG anti-spike antibodies were analyzed by ELISA. The geometric means with 95% confidence intervals are shown. B) Serum neutralizing titers against the ancestral variant B.1 and Omicron (BA.1) were analyzed using isolated variants. The geometric means with 95% confidence intervals are shown. (C) Paired B.1 and BA.1 neutralization titers for each sample. Kruskal-Wallis test and Dunn test for multiple comparisons were used in panels A and B. Wilcoxon pair-matched test was used for panel C. Statistical significance is depicted as: *p=0.05, **p=0.005, ***p=0.0005 and ****p=0.0001.
Figure 1

126x229 mm (.31 x DPI)