Short Communication

Humoral and adaptive immune responses to the SARS-CoV-2 vaccine

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ABSTRACT

Vaccines against SARS-CoV-2 ameliorate infection and adverse outcomes from SARS-CoV-2. Elicitation of high affinity and durable protective antibody responses is a hallmark of a successful humoral immune response to vaccination. To assess the relevance of serum levels of SARS-CoV-2 specific antibodies and to further characterize the immune response to SARS-CoV-2 vaccines, we report i) the levels of spike-binding and neutralizing antibodies to SARS-COV-2 in the sera of 30 healthy volunteers at nine months after the second vaccination dose of mRNA vaccine and one month after the booster dose; ii) the levels of IFN-γ production by blood T cells exposed to SARS-CoV-2 spike antigen (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529 variants); and iii) the specific phenotype of T cells related with exposure to SARS-CoV-2 spike antigen. We observed that the booster dose induced increased humoral and adaptive immune responses and led to early activation of the memory CD8+ T subset.

Introduction

Vaccines against SARS-CoV-2 prevent infection and adverse outcomes from SARS-CoV-2 (Olliaro et al., 2021). Elicitation of high affinity and durable protective antibody responses is a hallmark of a successful humoral immune response to vaccination (Turner et al., 2021). Antibody responses decline sharply at six months, particularly after SARS-CoV-2 mRNA vaccines (Collier et al., 2021). A recent study showed that after 20 weeks or more, the vaccination with two doses is effective against COVID-19–related hospitalization and death with a waning of the clinical protection in older adults and fragile/co-morbid patients (Andrews et al., 2022).

Methods

To assess the relevance of serum levels of SARS-CoV-2 specific antibodies and to further characterize the immune response to SARS-CoV-2 vaccines, we report i) the levels of spike-binding and neutralizing antibodies to SARS-CoV-2 in the sera of 30 healthy volunteers at nine months after the second vaccination dose of mRNA vaccine (Comirnaty; Pfizer Australia Pty Ltd) and one month after the booster dose (Table 1); ii) the levels of Interferon-γ (IFN-γ) production by blood T cells exposed to SARS-CoV-2 spike antigen (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529 variants); and iii) the specific phenotype of T cells induced by SARS-CoV-2 spike antigen presentation. The extensive methods are reported in the Supplementary Materials.
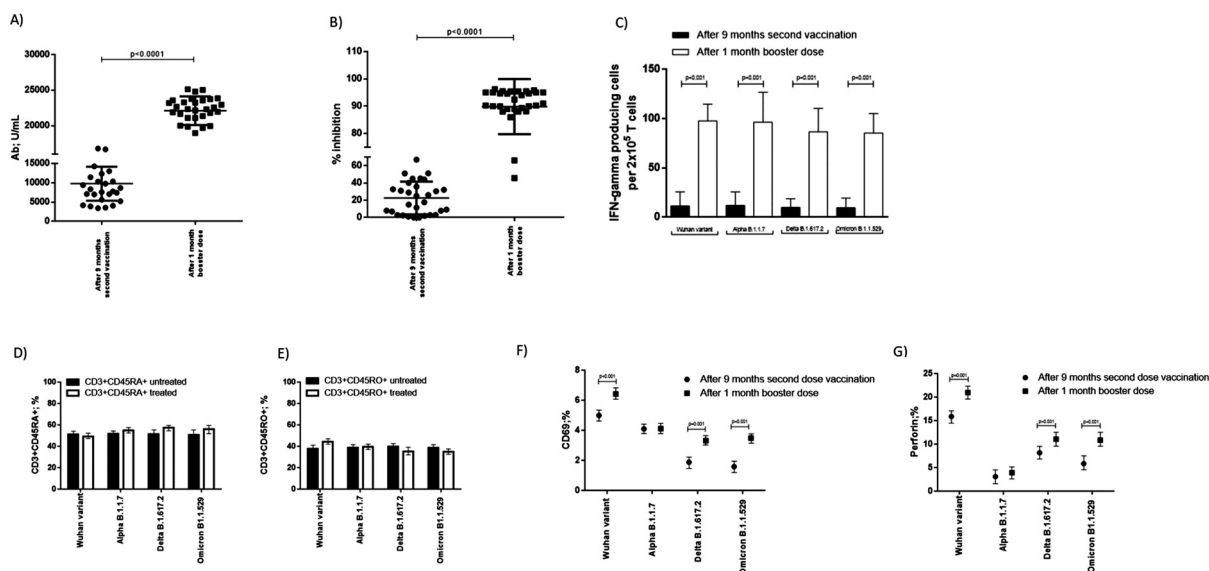
Results

High variability of spike-binding and neutralizing antibody levels was found in the sera of healthy donors nine months after the second vaccination (Figure 1A, B). Both spike-binding and neutralizing antibody levels significantly increased one month after the booster dose (Figure 1A, B). These data confirm that the booster dose is effective in enhancing the levels of spike-binding and neutralizing antibodies.

Because the specific adaptative immune response is a key element in the protective immune response to vaccines (Teijaro and Farber, 2021), we investigated the T cell responses to spike proteins from SARS-CoV-2 variants by measuring the percentage of T lymphocytes releasing IFN-γ when *ex-vivo* exposed to SARS-CoV-2 spike antigens. After the booster dose, we observed that the T lym-

Figure 1. (A) Levels of anti-spike SARS-CoV-2 RBD IgG (Ab) in the plasma samples of 30 healthy individuals nine months after the second vaccination and one month after the booster dose. The results are reported as mean ± SD. P-values were evaluated by Student's t-test. (B) Percentage of inhibition of SARS-CoV-2 infection of Calu-3 (ATCC HTB-55) human lung cell line in the presence of patients' plasma samples of 30 healthy individuals nine months after the second vaccination and one month after the booster dose. The results are reported as mean ± SD. P-values were evaluated by Fisher's exact test. (C) Number of IFN-γ secreting T lymphocytes obtained from peripheral blood of 30 healthy individuals nine months after the second vaccination and one month after the booster dose, stimulated with Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529 variants. The results are reported as mean ± SD. P-values were evaluated by Student's t-test. Percentage of (D) naïve T cells (CD3+CD45RA+) and (E) memory T cells (CD3+CD45RO+) untreated or stimulated with Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529 variants. T lymphocytes were obtained from peripheral blood of 30 healthy individuals one month after the booster dose. The results are reported as mean ± SD. P-values were evaluated by Fisher's exact test. Percentage of (F) CD69 and (G) perforin positive memory T cells lymphocytes obtained from peripheral blood of 30 healthy individuals nine months after the second vaccination and one month after the booster dose and stimulated with Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529 variants. The results are reported as mean ± SD. P-values were evaluated by Fisher's exact test.

IFN-γ = Interferon-γ; IgG (Ab) = Immunoglobulin G Antibody; RBD = Receptor-Binding Domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Table 1
Demographic and clinical characteristics of the study population.

Female, % (n)	50 (15)
Age (years), mean (SD)	38 (10)
Health care workers % (n)	100 (30)
Smoker, % (n)	0 (0)
Co-morbidities % (n)	0 (0)
mRNA vaccine[a] % (n)	100 (30)
Blood sampling after second immunization (months ± SD)	9 ± 0.2
Blood sampling after booster dose (months ± SD)	1 ± 0.1

SD = standard deviation.

[a] All the subjects received three doses of Comirnaty (Pfizer Australia Pty Ltd).

phocyte IFN-γ production was significantly enhanced toward all four SARS-CoV-2 spike variants (Figure 1C). These data further emphasize the importance of the booster dose to reactivate humoral but also cellular-mediated immune response to the vaccine.

CD8+ T cells are recognized to have an important role in viral eradication, including SARS-CoV-2 (Rha and Shin, 2021), and the induction of memory CD8+ T cells (i.e., expressing CD45RO) (Tomiyama et al., 2002) is important for the effectiveness of vaccines (Turner et al., 2021). Therefore, we further evaluated T lymphocyte responses by measuring naïve (CD45RA+) and memory (CD45RO+) CD3+CD8+ blood cells and the expression in these cells of surface CD69 and intracellular perforin as markers of early activation (Sancho et al., 2005) and cytotoxic activity (Voskoboinik et al., 2015), respectively. After stimulation of blood samples with spike variants, we found no difference in the proportion of naïve versus memory cells one month after the booster dose compared with 9 months after the second vaccination (Figure 1D, E). However, when we looked at T cell activation, we

found differences in response to the different SARS-CoV-2 variants. Activated (CD69+) memory T cells percentage was increased after the booster dose when challenged with Wuhan, Delta B.1.617.2, and Omicron B1.1.529 variants (Figure 1F; P <0.001, Fisher's exact test). The Wuhan variant challenge induced the highest percentage of activated memory T cells, in both nine months after the second vaccination and booster dose time points, compared with the Delta B.1.617.2 and Omicron B1.1.529 variants challenge (Figure 1F; P <0.001; Fisher's exact test). Activated memory T cells percentage was not increased by the booster dose when challenged with the Alpha B.1.1.7 variant but maintained the levels reached nine months after the second vaccination dose (Figure 1F). Similarly, the perforin+ memory T cells percentage was increased after the booster dose when challenged with Wuhan, Delta B.1.617.2, and Omicron B1.1.529 variants (Figure 1G; P <0.001, Fisher's exact test). The Wuhan variant challenge induced the highest percentage perforin+ memory T cells, in both 9 months after the second vaccination and booster dose time points, compared with the Delta B.1.617.2 and Omicron B1.1.529 variants challenge (Figure 1G; P <0.001; Fisher's exact test). The perforin+ memory T cells percentage was slightly increased by the booster dose when challenged with the Alpha B.1.1.7 variant (Figure 1G). These data indicate that at one month after the booster dose, there are no increased levels of memory CD8+ cells, but the repeated doses lead to early activation of these cells toward SARS-CoV-2 spike variants.

Discussion

Vaccines are important for public health, and the World Health Organization estimates that SARS-CoV-2 vaccination is preventing millions of deaths (World Health Organization, 2021). However, vaccination always raises concerns about the real efficacy of the

immune response. We evaluated the levels of spike-binding and neutralizing antibodies to SARS-CoV-2 at nine months after the second vaccination dose of mRNA vaccine and one month after the booster dose (Comirnaty; Pfizer Australia Pty Ltd). We observed that both spike-binding and neutralizing antibody levels were significantly increased one month after the booster dose, confirming the efficacy of the booster dose in enhancing the levels of spike-binding and neutralizing antibodies.

Because the specific adaptative immune response is a key element in the protective immune response to vaccines (Teijaro and Farber, 2021), we investigated the T cell responses to spike proteins from SARS-CoV-2 variants by measuring the percentage of T lymphocytes releasing IFN-γ when *ex-vivo* exposed to spike antigens from different SARS-CoV-2 variants (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529). We observed an increased production of IFN-γ by T lymphocytes obtained after the booster dose and challenged with the four SARS-CoV-2 variants.

The challenge with the different SARS-CoV-2 variants did not affect the percentage of naïve and memory T cells. Wuhan, Delta B.1.617.2, and Omicron B1.1.529 spike variants enhanced the activation (CD69+perforin+) of memory T cells obtained one month after the booster dose. The Wuhan variant challenge induced the highest increase in the percentage of activated T cells, in both 9 months after the second vaccination and booster dose time points, compared with the Delta B.1.617.2 and Omicron B1.1.529 variants challenge. The Alpha B.1.1.7 variant challenge slightly induced memory T cell activation.

In conclusion, the mRNA booster vaccine expressing Wuhan-Hu-1-like antigens induces increased neutralizing antibodies and enhanced memory T cell activation toward all the analyzed variants (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, and Omicron B1.1.529). The efficacy of the Wuhan variant in enhancing memory T cell activation suggests the presence of a vaccine-related immune imprinting, which boost the immune responses to the viral variant initially encountered by the immune system. These data are of extreme importance, suggesting a decreased efficacy of the Wuhan-Hu-1-like antigens-based vaccine toward the new viral variants, sustaining the need for updated vaccine-encoding sequences from one or more circulating variants. These data are still optimistic, as most vaccine-elicited T cell responses remain capable of recognizing all known SARS-CoV-2 variants. Nevertheless, it is of extreme importance to maintain strict surveillance of the variant evolution that could result in further reduction of T cell responses.

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Ethical approval

The research was approved by the Ethics Committee of the Area Vasta Emilia Centro della Regione Emilia-Romagna (CE-AVEC) with the number 122/2021/Oss/AOUFe.

Author contribution

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Data availability

All the data are available at request.

Declaration of Competing Interest

The authors have no competing interests to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.06.020.

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