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Comparison of immune activation of the COVID vaccines: ChAdOx1, BNT162b2, mRNA-1273, BBIBP-CorV, and Gam-COVID-Vac from serological human samples in Hungary showed higher protection after mRNA-based immunization

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Abstract. – OBJECTIVE: To gain insight into the different protective mechanisms of approved vaccines, this study focuses on the comparison of humoral and cellular immune responses of five widely used vaccines including ChAdOx1 (AZD1222, AstraZeneca), BNT162b2 (Pfizer), mRNA-1273 (Moderna), BBIBP-CorV (Sinopharm), and Gam-COVID-Vac (Sputnik V).

MATERIALS AND METHODS: Isolated plasma from 95 volunteers' blood samples was used to measure anti-SARS-CoV-2 humoral and cellular immune responses. Positive controls were recovered patients from COVID-19 (unvaccinated). Specific quantification kits for anti-nucleocapsid IgG, anti-Spike protein IgG, neutralizing antibodies as well as specific SARS-CoV-2 antigens for T-cell activation were used and Spearman correlation and matrix analyses were performed to compare overall immune responses.

RESULTS: Nucleocapsid antibodies were significantly higher for the BBIBP-CorV and convalescent group when compared to other vaccines. In contrast, subjects vaccinated with BNT162b2 and mRNA-1273 presented significantly higher anti-spike IgG. In fact, 9.1% of convalescent, 4.5% of Gam-COVID-Vac, 28.6% of ChAdOx1, and 12.5% of BBIBP-CorV volunteers did not generate anti-spike IgG. Similarly, a positive correlation was observed after the neutralization assay. T-cell activation studies showed that mRNA-based vaccines induced a T-cell driven immune response in all cases, while 55% of convalescents, 8% of BNT162b1, 12,5% of mRNA-1273, 9% of Gam-COVID-Vac, 57% of ChAdOx1, and 56% of BBIBP-CorV subjects presented no cellular response. Further correlation matrix analyses indicated that anti-spike IgG and neutralizing antibodies production, and T-cell activation follow the same trend after immunization.

CONCLUSIONS: RNA-based vaccines induced the most robust adaptive immune activation against SARS-CoV-2 by promoting a significantly higher T-cell response, anti-spike IgG and neutralization levels. Vector-based vaccines protected against the virus at a comparable level to convalescent patients.

Key Words:
COVID vaccines, Immune activation, Vaccine comparison, RNA vaccines, Anti-SARS-CoV-2 response.

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has not only greatly challenged nations and individuals in

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the last two years, but also the pharmaceutical industry, especially vaccine development units. This pandemic has set a precedent by deeply modifying the timeline of vaccine development and approval. Strikingly, according to the World Health Organization, up to 13 vaccines are available for human use. All of them were developed following different approaches, showing the plasticity of vaccine technologies. Due to the accelerated approval of the new mRNA-based vaccines, there is a hiatus in comparative studies with earlier generations of vaccines, e.g. inactivated or vector vaccines. It is essential to understand how each of them protects the individual as it directly influences the design of mass vaccination campaigns. In addition, it may also impact current decision-making approaches to booster dosages. It has been shown that vaccination against SARS-CoV-2 with BNT162b1 have had a significant impact in decreasing infection rates and clinical severity, avoiding hospitalization and death six months after the administration of the second dose; however, it has also been observed that this protection decreases over time[1-3]. Most of the studies[4-10] performed on SARS-CoV-2 vaccines' effectiveness focused on the test of one vaccine against control groups to show any potential protection, and in a few cases, the comparison was expanded to two or three vaccines. In an attempt to overcome this issue, Doroftei et al[11] used 19 publically available studies with such comparisons and tried in retrospect to compile the results for a wider view[11]. However, technical and methodological differences between the studies may hinder or alter results. Therefore, there is a lack of head-to-head comparative studies between multiple vaccines. Moreover, plasma from convalescent donors is currently being used as a direct treatment or drug development material for Coronavirus disease 2019 (COVID-19) patients[12]. Controversies may arise when plasma from convalescent donors is not available anymore and plasma from vaccinated volunteers may be used as a replacement[13].

Due to the significant time-shortening of vaccine development and approval, it is of worldwide interest to compare as much data as possible, to establish safe protocols and a robust trust in vaccines. Within Europe, Hungary is the only country where up to 8 COVID-19 vaccines have been registered and approved for use, including vaccines authorized by the European Medicines Agency ChAdOx1 (AZD1222, AstraZeneca), BNT162b2 (Pfizer), and mRNA-1273 (Moderna); but also the Chinese BBIBP-CorV (Sinopharm), and the Russian Gam-COVID-Vac (Sputnik V), which are widely used across Asia and South America.

Vaccines induce the combined activation of humoral (antibodies) and cellular (cytotoxic T-cells) immune response[14]. While antibodies recognize viral proteins, such as the nucleocapsid and spike proteins, activated cytotoxic T-cells eliminate the infected cells[15]. It has been shown that the immunological response after immunization begins to diminish over time[16]. Moreover, some individuals might not even react to immunization. To understand the immunization mechanism of the vaccines against SARS-CoV-2, effectiveness studies are needed. However, in most countries two or three different vaccines have been registered and approved for use, leading to incomplete studies or retrospective comparisons of different studies done under different conditions[1,2,6,11,17]. Therefore, a study comparing five of the most used SARS-CoV-2 vaccines worldwide using the same conditions and population may help to understand and compare the effectiveness of those vaccines. Apart from the reasons listed above, such studies may support understanding of scientific data and the vaccines' outcome, leading to lower levels of misinterpretation of clinical studies and to a potential rise in trust towards vaccines, increasing the percentage of vaccinated citizens[18].

Materials and Methods

Study Design and Participants

95 participants were recruited after obtaining written informed consent. Eligible participants were healthy adults, both males and females, aged 18-65 years, without previous pathologies except for hypertension (Table I). 84 subjects had complete immunization between two and eight weeks prior to blood sampling. 11 subjects were healthy convalescents, evidenced by prior positive polymerase chain reaction (PCR) tests and mild or moderate COVID-19 symptomatology, with no hospitalization. At the time of blood collection, a negative PCR test 2-8 weeks after complete convalesence was produced and presented to this study. Ethical Approval was granted by the Local Ethical Committee National Public Health Center (1943-6/2020/EÜIG) under the code (38175-7/2021EÜIG).

Blood Samples Collection

Whole blood from venipuncture was centrifuged at 1,710 xg at room temperature for 10 min-

Table I. Donor information.

Condition	Number of donors	Percentage of females (%)	Mean age (±SD)	Mean time between second dose and donation (days, ±SD)(%)
COVID-19 healed	11	8 (72.7)	37.71 (±7.45)	Not applicable
Vaccinated BNT162b2	24	20 (83.3)	42 (±12.2)	38 (±19.33)
Vaccinated mRNA-1273	8	6 (75)	48.37 (±8.22)	35.57 (±10.64)
Vaccinated ChAdOx1	22	7 (31.8)	39.6 (±9.2)	54.3 (±24.89)
Vaccinated Gam-COVID-Vac	14	10 (71.4)	44.64 (±12.07)	41.52 (±22.35)
Vaccinated BBIBP-CorV	16	7 (43.8)	41.6 (±16.44)	33.81 (±25.32)

utes, plasma was separated and stored at -20ºC for further analyses. Samples were barcoded to ensure patients' data protection.

Anti-SARS-CoV-2 Antibody Quantification

Plasma from donors was used for antibody quantification by enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. Anti-SARS-CoV-2 nucleocapsid IgG antibodies were measured with the kit recomWell SARS-CoV-2 IgG kit (Cat. No. 7304, Mikrogen Diagnostik, Neuried, Germany); anti-SARS-CoV-2 spike IgG antibodies were quantified with the Anti-SARS-CoV QuantiVac Elisa IgG kit (Cat. No. EI 2606-9601-10G, Euroimmun, Lübeck, Germany); and neutralization antibodies were measured with the cPass™ SARS-COV-2 Neutralization Antibody Kit (Cat. No. L00847-5 GenSript, Leiden, the Netherlands). Samples were measured with an ELISA microplate reader (LT-4000, LabTech International Ltd., East Sussex, UK.) in duplicates and the mean optical density was used for the following analysis.

T-cells Quantification

To isolate plasma, whole blood from donors was collected according to the manufacturer's instructions; QuantiFeron SARS COV-2 Starter pack (Cat.No. 626715, Qiagen, Germantown, MD, USA) and QuantiFERON Control Set (Cat. No. 626015, Qiagen, Germantown, MD, USA). One ml of blood was directly drawn into QFN SARS-CoV-2 blood collection tubes (BCTs) and after labelling, filling, and shaking, samples were immediately transferred to a 37°C water bath and incubated overnight. After incubation, samples were centrifuged for 15 minutes at 2,000 xg, then plasma was harvested carefully. Plasma samples were stored at 4°C until Interferon gamma (IFNγ) was quantified with QuantiFERON ELISA Kit (Cat. No. 626410, Qiagen, Germantown, MD, USA). Results were analyzed using QuantiFERON R&D Analysis RUO Software (Qiagen, Germantown, MD, USA).

Statistical Analysis

The skewness of continuous variables, the shape of their distribution and the equality of their variances were tested using the Shapiro-Wilk test, Q-Q plots, and Brown-Forsythe test for homogeneity of variances, respectively. Taking into account the shape of the distribution of continuous variables and the inequality of variances, means were compared between groups using Brown-Forsythe ANOVA test, followed by Dunnett's T3 post-hoc tests. Spearman's rank-order correlation method was used in the correlation analyses. The rank correlation coefficients were expressed as r_s. Continous variables were presented as mean values ± standard deviation (±SD). Statistical analyses were carried out and the figures were made with GraphPad Prism 9 software (GraphPad, Irvine, CA, USA). The radar chart was created using RAWGraphs 2.0 (RAWGraphs, Milano, Italy). $p < 0.05$ was considered statisticaly significant. In the figures, asterisks denote the significant difference (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

Results

In the convalescent group, anti-nucleocapsid IgG titer was significantly higher compared to the four vaccinated groups: BNT162b1, mRNA-1273 and ChAdOx1 ($p = 0.003$), and Gam-COVID-Vac ($p = 0.004$), but not significantly different from BBIBP-CorV ($p = 0.08$). 20 individuals, corresponding to 20.4% of all subjects, produced anti-nucleocapsid IgG titers above the cutoff level. They were distributed as follows: 90.1% of convalescents (control subjects), 56.3% of BBIBP-

CorV group and 4.5% of Gam-COVID-Vac group. Interestingly, among the vaccinated donors, the mean nucleocapsid antibody level above 24 U/ml was only detectable in subjects from the BBIBP-CorV group (Figure 1).

Mean anti-spike IgG level was not detectable in convalescent groups. Participants who received vector and inactivated vaccines showed a mild response, when compared to the mRNA-based vaccines. The vast majority of donors had positive titers of anti-spike IgG and only 8 of them, representing 8.4%, did not achieve values above the cutoff level. They were distributed as follows: 28.6% of ChAdOx1, 12.5% of BBIBP-CorV, 9.1% of convalescent, and 4.5% of Gam-COVID-Vac. Supreme anti-spike IgG values were detected in BNT162b1 and mRNA-1273 groups achieving significantly higher titers compared to controls ($p < 0.001$ for BNT162b1 and mRNA-1273 *vs.* convalescents), and vector and inactivated vaccines groups ($p < 0.001$ for BNT162b1/mRNA-1273 *vs.* ChAdOx1 and BBIBP-CorV; $p < 0.001$ for BNT162b1 *vs.* Gam-COVID-Vac) (Figure 1B).

Signal inhibition in BNT162b1 group was significantly higher than in COVID control group ($p = 0.001$), ChAdOx1 ($p = 0.008$), vector vaccines, and inactivated groups ($p < 0.001$). Similarly, signal inhibition in mRNA-1273 group was significantly higher than COVID-19 control ($p = 0.001$), ChAdOx1 ($p = 0.01$) as well as vs BBIBP-CorV ($p = 0.001$) (Figure 1C).

Two sets of SARS-CoV-2 specific antigen pools were used to stimulate T-cells that produce IFNγ in the activated state (Figure 2). Ag1 represents $CD4^+$ response, while Ag2 represents $CD4^+$ and $CD8^+$ combined (Figure 2A and Figure 2B, respectively). 28.42% (n = 27) did not respond to Ag1 or Ag2: 55% convalescents, 8% of BNT162b1, 12.5% of mRNA-1273, 9% of Gam-COVID-Vac, 57% of ChAdOx1, and 56% of BBIBP-CorV volunteers. Overall, convalescent, vector, and inactivated vaccines groups showed similarly low responses compared to the mRNA vaccine group.

r_s values reached the value of 0.54 for anti-spike IgG *vs*. Ag1 antigen, and 0.49 for anti-spike IgG *vs*. Ag2 antigen for all donors (Figure 3A). For these groups, $p < 0.0001$ revealed a strong statistically significant correlation, while for anti-nucleocapsid IgG *vs*. Ag1 and Ag2 values (all volunteers) r_s was lower (-0.37 and -0.43, respectively) and no significant correlation was observed ($p = 0.159$ and 0.2590, respectively). To illustrate the correlation among the different groups a further matrix analysis was performed and the results are presented as bubble charts (Figure 3B,C). This time there was no significant correlation for anti-spike IgG *vs*. Ag1 analysis; however, the r_s value was the highest for mRNA-1273 group (0.49) and the lowest for BNT162b1 (0.21). Analysis between anti-spike IgG and Ag2 revealed a significant correlation only for mRNA-1273 group ($p = 0.015$), where the r_s value was the highest (0.83). The lowest r_s value was observed in ChAdOx1 group (0.08).

In summary, to illustrate the humoral and cellular responses of different types of vaccines compared to the potential protection level in the convalescent group, radar charts were created for each condition (Figure 4). The convalescent group achieved superior results only in their anti-nucleocapsid titers. However, the mRNA-based vaccines BNT162b2 and mRNA-1273 evoke robust anti-spike IgG titers, higher neutralization levels and more intense T-cell responses, in comparison to vector or inactivated vaccine groups, as well as convalescents.

Discussion

In this pioneering study, evoked immune responses of five vaccines and convalescence from COVID-19 were evaluated. Vaccines included BNT162b1 (Pfizer), mRNA-1273 (Moderna), ChAdOx1 (AZD1222, AstraZeneca), BBIBP-CorV (Sinopharm), and Gam-COVID-Vac (Sputnik V). BBIBP-CorV is a first-generation type, an inactivated whole virion vaccine developed by the Chinese Academy of Medical Sciences. In this case, the actual infectivity of the virus is demolished by radiation techniques and by chemicals, and therefore, it contains viral nucleocapsid, membrane and spike proteins[19]. Second generation type viral vector-based vaccines are Gam-COVID-Vac and ChAdOx1, which contain non-replicating adenovirus vector as a delivery system for coding S1 protein[20]. Third generation type vaccines developed by Pfizer and Moderna use mRNA technology and lipid nanoparticle delivery system to encode the production of SARS-CoV2 S1 protein[21]. In this study, whole blood from volunteers who had been previously vaccinated with the aforementioned vaccines and COVID-19-recovered patients was used to assess the immunological response. Currently, there are various serological tests that measure the immune response against anti-nucleocapsid antigens, anti-spike S1 and 2

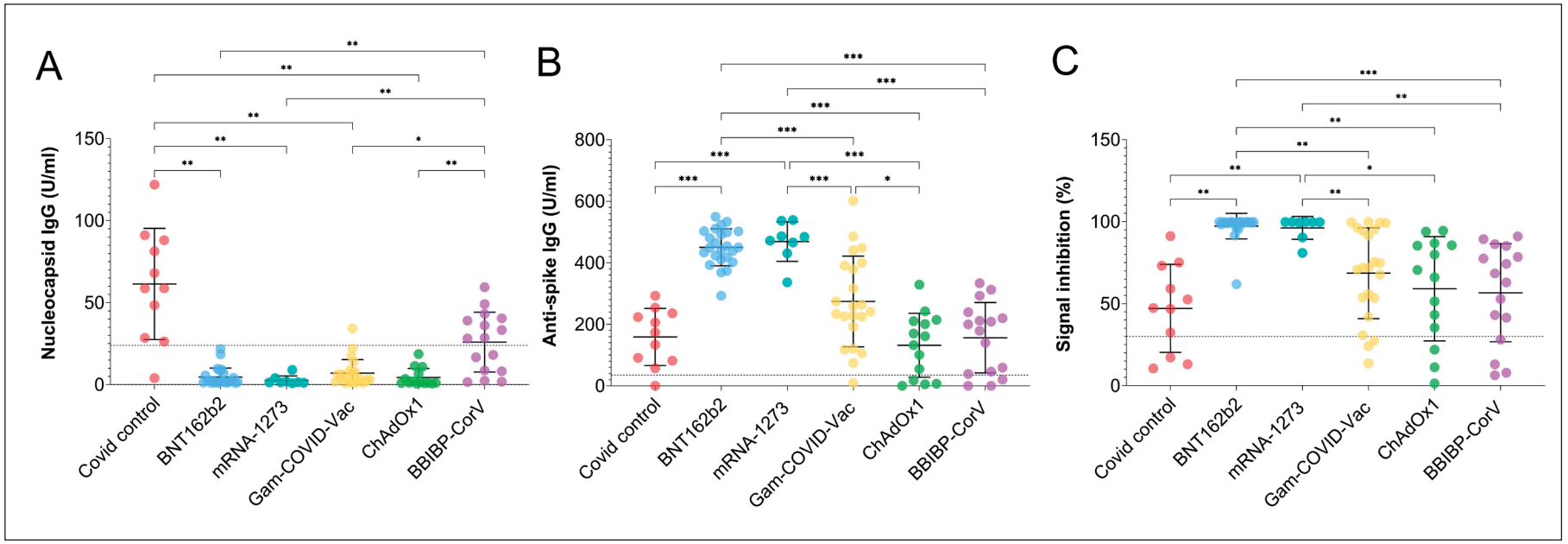

Figure 1. Anti-nucleocapsid IgG (**A**), anti-spike IgG (**B**), antibody titers and signal inhibition in neutralization assay (based on detection of functional immunoglobulins neutralizing the interaction between RBD and hACE2 - **C**), measured in COVID-19 control groups (COVID-19 control: convalescent patients who recovered from COVID-19), and the five vaccine groups (n = 95). Scattered line on Y axis indicates the cutoff levels 24 U/ml (**A**), 35.2 U/ml (**B**) and 30% (**C**), respectively.

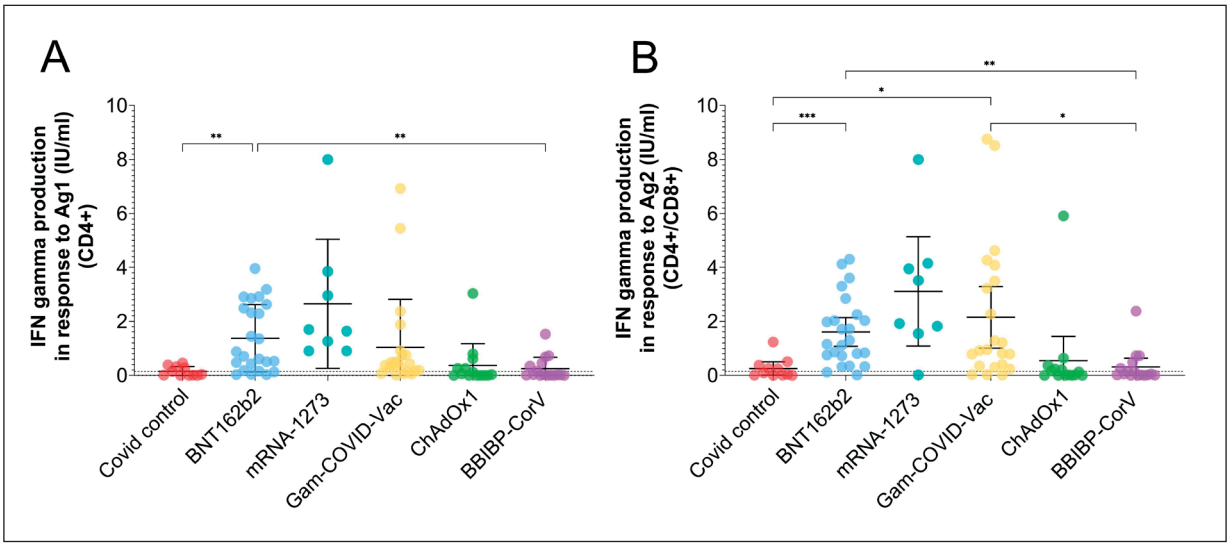

Figure 2. T-cell reaction in response to antigens SARS-CoV-2 Antigen 1 (**A**) and SARS-CoV-2 Antigen 2 (**B**), measured in a control group (COVID-19 control: convalescent patients who recovered from COVID-19); and five vaccine groups (n = 95). The scattered line indicates the cutoff level 0.15 (IU/ml).

proteins, and neutralizing antibody activity[22,23].

S1 protein is the main antigen target of COVID-19 vaccines due to its high antigenicity with the ability to induce humoral and cellular immune response[24]. In this study, it has been observed that both mRNA-based vaccines induced the highest levels of anti-spike antibodies, maintaining similar responses between all the participants. It had been previously identified that BNT162b2 and mRNA-1273 induced both anti-spike S1 when compared to negative controls[4,5]. Inactivated and vector vaccines showed a mild production of anti-spike IgG, similar to the response observed in the convalescent group. The robustness of anti-spike IgG levels was low with high variance, in some cases reaching anti-Spike IgG production comparably higher than mRNA-based vaccination, while others had a complete lack of response. Similar results to ours were obtained by Shrotri et al[6], showing that BNT162b2 induced a significantly stronger response of anti-spike S1 than ChAdOx1[6]. However, previous studies[7-10] where ChAdOx1, Gam-COVID-Vac and BBIBP-CorV were compared to negative control groups, proved that the production of anti-spike antibodies and neutralizing antibodies was induced in all cases after the second dose. Quantification of neutralization levels in our study showed similar results to the anti-spike S1 protein measurements. The production of neutralizing antibodies against SARS-CoV-2 was significantly stronger after vaccination with BNT162b2 and mRNA-1273 when compared to the other vaccines. Moreover, this protection was robust and more equal within the groups of mRNA-based vaccinated volunteers. As expected, previous studies[25] demonstrated that mRNA-based vaccines induced a significant neutralizing antibody response, when compared to negative controls. In this work, it was shown that inactivated and vector vaccines induced a comparable amount of neutralization level against the virus, as for instance the acquired immunity after recovering from COVID-19, which was outperformed by mRNA-based vaccines. Again, for these conditions, the response differs greatly between donors. Subjects that had recovered from COVID-19 had significantly higher levels of anti-nucleocapsid IgG when compared to all the vaccinated groups. However, the robustness of this group was low, as shown from the wide standard deviation, indicating that after suffering from COVID-19, patients may or may not produce these antibodies. As expected, between the vaccinated groups, anti-nucleocapsid antibody production was higher for the volunteers that were vaccinated with BBIBP-CorV when compared to spike protein-based vaccines, as explained in detail in another study[26].

$CD4^+$ and $CD8^+$ T-cell activation was measured after incubation of T-cells with two antigens from SARS-CoV-2. For both antigens, the production of IFNγ indicated that the donors who had been vaccinated with mRNA-based vaccines had more reactive T-cells. Moreover, our data may

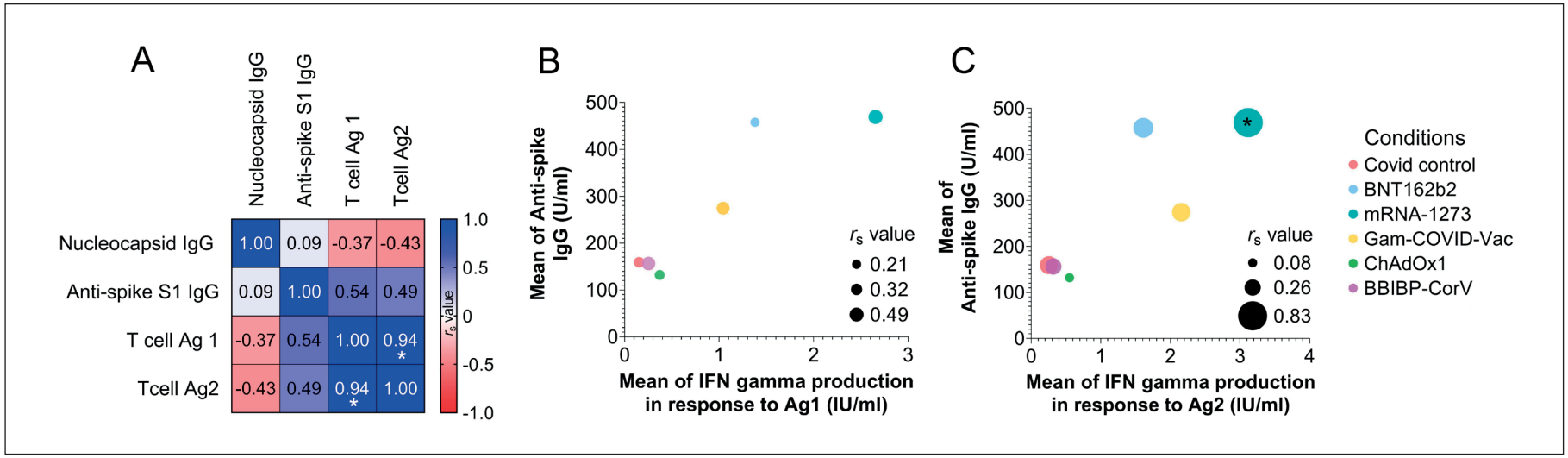

Figure 3. A, Correlation matrix between anti-nucleocapsid, anti-spike IgG values (U/ml), and IFN gamma (IU/ml) concentration after T-cells activation in response to Ag1, Ag2 antigens measured for all volunteers (n = 95). Bubble charts presenting the r_s between anti-spike IgG (U/ml) and Ag1 (**B**) or Ag2 (**C**) measured for COVID-19 control: (patients who recovered from COVID-19), and 5 vaccine groups (n = 95). Bubble sizes reflect the r_s values (correlations bellow 0.39 indicate a very weak or weak correlation. 0.49 and 0.83 Spearman's rank suggest a moderate and very strong correlation, respectively. Colors indicate the different conditions).

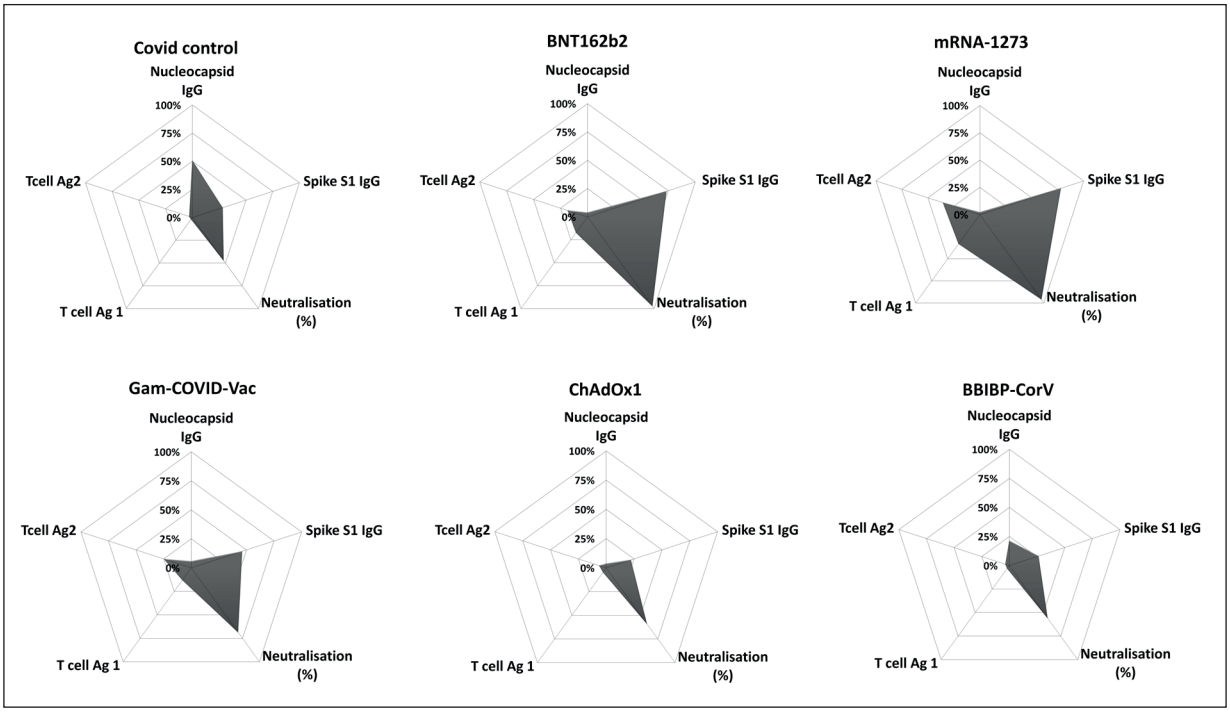

Figure 4. Summary of immune responses to COVID-19 disease and five different vaccines. Radar charts show the percentage values for each measured variable on the same scale to make overall comparisons possible. Stronger immune responses evidenced by larger chart areas are produced by mRNA vaccines, however, with a different pattern to the convalescent group. Note that the inactivated vaccine group is closest to convalescents both in magnitude and pattern.

suggest that mRNA-1273 may induce it slightly higher than BNT162b1, although this difference was not significant for our sample size. Interestingly, it was previously shown[4] that BNT162b2 immunization induced $CD4^+$ and $CD8^+$ T-cells activation and mRNA-1273 presented a significant increase of $CD4^+$ T-cells, but $CD8^+$ was low. Unfortunately, inactivated and vector vaccines did not show a significant response of T-cell activation in our study. However, in contrast to our data, Logunov et al[27] proved the activation of $CD4^+$ and $CD8^+$ T-cells after Gam-COVID-Vac immunization[27]. As was previously shown by Chung et al[28], the key to evoking long-term protection against SARS-CoV-2 infection by mRNA-based vaccines relies on the appropriate stimulation of the adaptive immune system for the development of neutralizing antibodies, and memory T- and B-cells[28]. Upon injection, the mRNA encoding S1 protein gains entry into the dendritic cells, leading to the intensive production of S1 protein. The adjuvants stimulate innate immune cells and generate type I IFN and other cytokines. Co-stimulatory signals, including the presentation of the S1 protein as an antigen, induce the differentiation of naive T-cells into CD4+ (helper) and CD8+ (cytotoxic). CD4+ T cells trigger the generation of memory B-cells and the anti-spike S1 antibodies by plasma cells[28].

In this study, after comparing all the different types of immune responses, a strong relationship between $CD4^+$ and $CD8^+$ T-cell response was observed; as well as a mild correlation between neutralization levels and anti-spike S1 antibodies.

Conclusions

In summary, the present study suggests that protection obtained after the administration of mRNA-based vaccines may be more robust than other vaccinations or even the disease itself by promoting a significantly higher T-cell response, anti-spike IgG and neutralization levels. It was also shown that vector-based vaccines protected against the virus at a comparable level to convalescent patients.

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Authors' Contributions

Conceptualisation: F.J., Z.L., G.K., data curation: O.K.P., M.B., E.F., formal analysis: O.K.P., E.F., I.O.C., Z.L., funding acquisition: Z.L., methodology: I.H., A.H., E.H., A.M., F.F., Z.B., project administration: E.F., Z.L., visualisation: O.K.P, M.B., writing – original draft: I.O.C, E.F., O.K.P., writing– review & editing: Z.L., G.K., M.B., F.J.

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Conflict of Interest

Authors declare no conflict of interest.

Ethics Approval

Ethics approval was granted by the local ethical committee National Public Health Center (1943-6/2020/EÜIG) under the code: 38175-7/2021EÜIG.

Informed Consent

All participants were recruited after obtaining written informed consent.

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