Existence of immunological memory response in true sero-negative individuals post COVID-19 molecular diagnosis

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Abstract

Approximately 1-8% of individuals do not develop antibodies following SARS-CoV-2 infection (sero-negatives). One BNT162b2 dose resulted in potent humoral response in 14 sero-negatives and 15 sero-positives, significantly higher than the response of 15 naïve-individuals, to two doses suggesting that COVID-19 provoked a memory response in individuals without detectable antibodies.

Introduction

Since the COVID-19 vaccination campaign was initiated on December 2020 approximately 62.5% of the world population has been vaccinated with at least one of the available vaccines (https://www.worldometers.info/coronavirus/). Interestingly, despite high antibody response within one to four weeks following BNT162b2 mRNA vaccine, significant waning of antibody levels was observed over a six-month period [1] concomitantly to a significant reduction in vaccine efficacy [2]. To date more than 430 million people were infected with SARS-CoV-2 and approximately 6 million died (https://www.worldometers.info/coronavirus/). Several studies have shown that SARS-CoV-2 re-infection is a rare event [3, 4] and therefore COVID-19 recovered individuals have a significantly lower risk for re-infection, hospitalization and death [4]. Data from COVID-19 convalescent patients suggest that despite reduced antibody responses, most will maintain detectable anti-RBD IgG and neutralizing titres for at least one year following infection [5] . Several studies demonstrated that a small fraction (1-8%) of convalescent patients do not develop SARS-CoV-2 IgG antibodies (sero-negative) [6-9]. It is unclear whether these patients do not develop antibodies, or have an undetectable response. As a consequence, it is unknown whether these patients require vaccination, and whether the vaccine will provoke a humoral antibody response, greater than natural infection or vaccination in naïve individuals. Here, we investigated the humoral response following vaccination in SARS-CoV-2 convalescent patients in whom no evidence of antibodies production was ever detected post molecular diagnosis.

Methods:

Cohorts

Samples obtained from two different ongoing study cohorts were included in this study (Table 1 and Supplementary Figure 1 and Table 1) :

1. A cohort of convalescent individuals, with a positive PCR, with symptomatic or asymptomatic history of COVID-19 infection. This cohort is being followed by serologic tests monthly. From this cohort, we included samples with serological data before and after they were vaccinated with the following characteristics:
    a. Fourteen PCR positive individuals with a negative IgG antibody test taken 2-6 weeks following diagnosis were termed "True sero-negatives". The IgG antibodies against SARS-CoV-2 were negative in all these sero-negative individuals all along their follow up including immediately before vaccination, suggesting that these persons are truly sero-negatives. Serum sample was taken from all subjects 1-5 weeks following the first BNT162b2 vaccine dose. Three individuals underwent a second vaccine dose and sera was obtained one week later (see table 1 for further details).
    b. Fifteen age and gender matched controls convalescent individuals with a positive IgG antibody test taken 2-8 weeks following diagnosis were termed "sero-positives". Serum sample was taken from all subjects 1-5 weeks following the first BNT162b2 vaccine dose (see supplementary table 1 for further details).

2. A cohort of 15 age and gender matched controls naïve Health Care Workers (HCW) from Sheba Medical Center, who received two BNT162b2 vaccine doses three weeks apart were termed "naïve". Samples were obtained immediately before vaccination, three weeks after the first vaccine dose and 1-2 weeks after the second vaccine dose. All HCW participating did not report any co-morbidities (see supplementary table 1 for further details).

Ethical statement

The protocol was approved by the Institutional Review Board of the Sheba Medical Center. Written informed consent was obtained from all participants.

PCR testing

For quantitative RealTime-PCR (qRT-PCR), nasopharyngeal swabs were placed in 3mL of universal transport medium (UTM) or viral transport medium (VTM). Test was performed according to manufacturers' instructions on various platforms: Allplex™ 2019-nCoV (Seegene, S. Korea), NeuMoDx™ SARS-CoV-2 assay (NeuMoDx™ Molecular, Ann Arbor, Michigan), Xpert®, Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA).

Antibody detection

Samples were tested using the SARS-CoV-2 RBD IgG assay (Beckman-Coulter, CA, U.S.A.) commercial test according to manufacturer instructions. All samples with a sample to cut-off (S/CO) score below 1 were considered negative and equal or above, positive.

SARS-CoV-2 Pseudovirus (psSARS-2) Neutralization Assay

SARS-CoV-2 Pseudo-virus (psSARS-2) Neutralization Assay was performed as described [1, 10] using a propagation-competent VSV-spike kindly provided by Gert

Zimmer, University of Bern, Switzerland. Following titration, 100 focus forming units (ffu) of psSARS-2 Wuhan-Hu-1 strain were incubated with 2-fold serial dilution of heat inactivated (56°C for 30 min) tested sera. After incubation for 60 min at 37°C, virus/serum mixture was transferred to Vero E6 cells that had been grown to confluency in 96-well plates and incubated for 90 min at 37°C. After the addition of 1% methyl cellulose in Dulbecco's Modified Eagle's Medium (DMEM) with 2% of fetal bovine serum (FBS) plates were incubated for 24hr and 50% plaque reduction titer was calculated by counting green fluorescent foci using a fluorescence microscope (EVOS M5000, Invitrogen). Sera not capable of reducing viral replication by 50% at 1 to 8 or below were considered non-neutralizing.

Statistical Methods

Plots of IgG and neutralizing antibodies and Geometric Mean Titers (GMT) with confidence interval (CI) of 95% were performed using GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA). Significant differences between the groups were analyzed using Mann-Whitney statistical test.

Results:

Fourteen PCR-positive convalescent individuals who were repeatedly IgG sero-negative for SARS-CoV-2 (Table 1) were tested for IgG and neutralizing antibodies response 1-5 weeks following the first BNT162b2 vaccine dose . Three weeks later, three of the sero-negatives received the second BNT162b2 vaccine dose. Following the first vaccine dose, a significant induction with geometric mean titers (GMT) of 17.44 (95%CI 9.77-31.14) and 1351(95%CI 220.3-8286) in IgG and neutralizing levels, respectively, was detected in all except one individual. In this individual the second vaccine dose elicited significant induction in antibody levels (Figure 1). As match-controls, we evaluated one and two dose vaccination

effects on 15 sero-positive convalescent and 15 naïve individuals, respectively. One vaccine dose given to sero-positive convalescent individuals, rapidly triggered high IgG and neutralizing antibody response with GMT of 40.45 (95%CI 33.05-49.52) and 13,839 (95%CI 10127-18911), respectively, significantly higher than convalescent sero-negatives (p=0.0008 and <0.0001 for IgG and neutralizing antibodies, respectively) . Naïve persons developed low IgG and neutralizing levels with GMT of 5.2 (95%CI 2.97-9.13) and 33.5 (95%CI 17.97-62.52), respectively, three weeks following the first vaccine dose, significantly lower than convalescent sero-negatives (p=0.0026 and 0.0003 for IgG and neutralizing antibodies, respectively). The second vaccine dose elicited significant induction in IgG and neutralizing antibodies to GMT of 38.1 (95%CI 32.31-44.86) and 851 (95%CI 363.2-1995), respectively (Figure 1).

Discussion:

It was previously shown that one mRNA vaccine-dose elicited rapid immune-responses in seropositive participants as compared to low response in naïve individuals [11, 12]. However, no information is available regarding the response of convalescent individuals who did not have detectable antibodies. In this study we demonstrate that infection with SARS-CoV-2 produces an immunological memory which may be undetectable, however, can be significantly induced upon repeated exposure. This phenomenon is different from hepatitis B infection in whom small percentage of infected individuals do not produce antibodies and continue to chronic infection, and similar proportion do not produce antibodies in response to repeated doses of the vaccine [13]. The identification of one true-seronegative COVID-19 convalescent obese individual who did not develop antibodies following the first vaccine dose may suggest that some seronegative individuals with underlying morbidities may

immunologically behave as COVID-19 naïve patients and therefore may not have developed immune memory. Future studies should investigate larger cohorts to refine the memory response in the true-seronegative COVID-19 infected population.

The high neutralizing and anti RBD antibody levels detected in sero-positive individuals after one vaccine dose suggests that a second dose to convalescent individuals may not be necessary in order to reach high levels of antibody and neutralizing titres. Our results, in addition to accumulating evidence of high and continuous vaccine efficacy in one vaccine dose COVID-19 convalescent recipients [14], prompted Israel MOH to require only one vaccine dose from previously SARS-CoV-2 infected individuals. Since anti N antibodies assays are not routinely available and their levels are significantly reduced over time [15], a major issue, is the identification of convalescent individuals without a positive RT-PCR. This study demonstrates that significant induction in IgG levels several days after one vaccine dose can point to prior infection and diagnose convalescent individuals

Our study limitations include the lack of T cell response and the small cohort evaluated, however the use of both naïve and sero-positive convalescent individuals as controls allowed us to distinguish between real naïve and memory-based responses.

Our study also shows that memory response following infection is sustainable for months as our vaccine trigger was performed up to 12 months after their infection. This highlights that we should exercise caution when interpreting protection from infection based only on antibody levels and that, perhaps, immune protection is easier to achieve than anticipated.

NOTES

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Declaration of interests

Dr. Regev-Yochay reports grants from Pfizer, outside the submitted work. Dr. LUSTIG reports grants from Pfizer on a non related subject (TBE seroprevalence), outside the submitted work. None of the other authors has any conflicts.

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Table 1: Characteristics of infected seronegative individuals included in this report

Number	Gender	Age	Symptoms of COVID-19	Ct value at diagnosis^	Time from diagnosis to first negative serology (Weeks)	Time from diagnosis to first vaccine dose(weeks)	Time from first vaccine dose to serology after first dose (weeks)	Co- morbidities	IgG / Neutralizing abs. Response following infection *	IgG / Neutralizing antibodies Response following first vaccine dose#
1	F	28	Mild	34	3	24	2	None	0.44/NA	29.75/NA
2	F	42	Mild	37	4	20	3	None	0.25/<8	19.07/4096
3	F	41	Mild	23	4	20	3	None	0.27/NA	16.61/NA
4	M	51	Asymptomatic	37	1	20	3	None	0.68/NA	21.98/8192
5	F	56	Mild	Positive	2	19	1	None	0.47/NA	44.09/4096
6	M	38	Mild	23	2	17	1	None	0.64/NA	32.2/4096
7	F	38	Mild	26	2	16	5	None	0.63/NA	12.27/1024
8	F	26	Mild	Positive	5	15	3	None	0.03/NA	13.04/512
9	M	55	Mild	25	2	14	5	None	0.26/NA	28.53/NA
10	M	38	Mild	Positive	2	14	5	None	0.03/NA	39.29/NA
11	F	38	Mild	25	6	7	2	None	0.8/NA	NA/8192
12	M	59	Mild	Positive	3	4	2	None	0.6/NA	39.62/8192
13	M	30	Mild	32	5	50	1	None	0.87/<8	3.9/512
14	M	50	Mild	31	4	46	2	Morbid Obesity (BMI=41)	0.4/<8	1.7/0

*IgG antibody response: Negative:<1; Pos: ≥1.

#Neutralizing antibody response: Negative:<16; Pos: ≥16

^In case a Ct value was not available, a positive PCR result was confirmed with the diagnostic lab and result was presented as "positive".

NA stands for cases where no sample was available.

Figure 1: Neutralizing and IgG antibody response in naïve and SARS-CoV-2 previously infected seronegative and seropositive individuals.

Sera obtained from 14 SARS CoV-2 previously infected seronegative (infected seronegatives), 15 SARS CoV-2 previously infected seropositive (infected seropositives) and 15 naïve (naïve) individuals, were subjected to pseudo-neutralization (upper panel) and RBD-IgG antibodies binding (lower panel) assays. Sera was obtained: 1. 2-7 and 2-8 weeks after infection for seronegative and seropositive individuals, respectively (after infection) and immediately before vaccination for naïve individuals (pre dose I), 2. 1-5 weeks after the first vaccine dose for seronegative and seropositive individuals and 3 weeks following the first vaccine dose in naïve individuals (After first dose) and 3. 1-2 weeks following the second vaccine dose for seronegative and naïve individuals, (after second dose). The dashed line indicates cut off titer. Solid lines and numbers indicate the geometric mean titer, and I bars show the 95% confidence interval. Black dot indicates a SARS-CoV-2 previously infected seronegative individual with low humoral response.