Evaluation the correlation between anti-spike protein IgG antibody titers against SARS-CoV-2 Prototype and different Omicron variants by ELISA and neutralization activity

Xiaoling Yu[1], Yan Liu[2], Qiuyu Ding[1], Xiangrong Qi[1], Huihui Wei[1], Yang Zhang[1], Peiyao Li[1], Jun Liu[1], Yuxue Liu[1], Jing Lv[1], and Hao Liu[3]

[1]Gobond Testing Technology Beijing Co Ltd
[2]National Institutes for Food and Drug Control
[3]Peking University Hospital of Stomatology

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Abstract

Evaluating the IgG titers targeting the S protein of SARS-CoV-2 by ELISA assays have been used in the development of several anti-SARS-CoV-2 vaccines in China. It is unclear whether the IgG levels against S protein tested by ELISA can monitor immune evasion against different Omicron variants. In the study, 88 recipients immunized with three doses of a COVID-19-inactivated vaccine were enrolled, whose serum samples were collected within 2 months after the third immunization. The IgG antibody levels were detected by using three commercial ELISA kits, which coated the S protein of the prototype, Omicron BA.1 and BA.5 variants respectively. The neutralizing activities of sera against the pseudotyped Omicron variant, prototype was determined. We also analyzed the correlation between the IgG titers with the neutralizing antibodies. The results showed that, after the third dose of the homologous inactivated vaccine, the neutralizing activity against the Omicron variant BA.1(GMT, 60) and BA. 5(GMT, 42) were decreased significantly compared with the prototype (GMT, 331) respectively ($P<0.05$). However, the IgG titers against the S of Omicron BA.1(GMT, 2334) and BA.5(GMT, 2447) variants showed no significant difference with the prototype (GMT, 2797). Our results showed different correlation levels between anti-Spike protein of SARS-CoV-2 IgG titers and neutralizing antibodies, against SARS-CoV-2 prototype, Omicron variant BA.1 and BA.5 In summary, our result highlight that the Omicron variant BA.1 and BA.5 escape vaccine-induced immunity by neutralization activity test, and IgG titers against the SARS-CoV-2 Spike protein can not predict the viral immune evasion against different Omicron variants.

Introduction:

Serological assays are important tools in the monitor of infectious diseases and the detection of immunity induced by vaccine. In current COVID-19 pandemic, to better understand whether current immunity induced vaccine and pre-infection is effective against different Omicron variants, developing countries have faced crucial challenges to develop validated, standardized serological assays to assess the antibody response against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The neutralizing antibody assays based on live viral particles which detected and quantified in a neutralization assay is considered as the reference gold standard, such as microneutralization assay (1) or plaque-reduction neutralization test (2). Neutralizing assays based on pseudo-neutralization assay is also widely used in the evaluation of immune efficacy in development of vaccines and antibody drugs (3,4), or the screening of recovered patients for plasma therapy (5).

However, these serological assays, which assessing the antibodies that inhibit infection of cultured cells, is labor-intensive or restricted to biosafety level 3 laboratories. To overcome these limitations, standardized

enzyme-linked immunosorbent assay (ELISA) based on recombinant SARS-CoV-2 antigens have developed, including spike (S) protein, S1 domain and receptor-binding domain (RBD) (6,7). Evaluating the IgG titers targeting the S protein of SARS-CoV-2 by ELISA assays have been used in the development of several anti-SARS-CoV-2 vaccines in China. It is unclear whether the IgG levels against S protein tested by ELISA can monitor immune evasion against different Omicron variants.

In current study, we have first determined IgG antibody levels of the serum from donors immunized with inactivated COVID-19 vaccine, against the S protein of the prototype, Omicron BA.1 and BA.5 variants. Meanwhile, we have assessed the neutralizing antibody levels with the same serum based on pseudovirus system. We have also investigated the level of correlation between standardized ELISA for the detection of anti-SARS-CoV-2 S IgG and neutralization activity by pseudo-neutralization assay.

Materials and Methods:

Serum samples

We recruited 88 volunteers who had received three doses of a homologous inactivation-based vaccine. Informed consent forms were signed and ethical approval was obtained from Beijing YouAn Hospital, Capital Medical University (#LL-2021-159-K). Serum samples were collected within 2 months after the third immunization at the Beijing YouAn Hospital.

Enzyme-linked assay (ELISA) test

For the detection of SARS-CoV-2 IgG antibodies, three commercial ELISA kits coated with recombinant S antigen of SARS-CoV-2 prototype, BA1 and BA.5 variant separately were used, namely the human Anti-SARS-CoV-2(Prototype)Spike protein IgG Antibody detection Kit, Human Anti-SARS-CoV-2(BA.1)Spike protein IgG Antibody detection Kit and Human Anti-SARS-CoV-2(BA.5)Spike protein IgG (Zhongyan Guobang (Beijing) Technology Co., LTD).

According to the instructions of the kits, all samples were diluted from 1:400-1:51200 with 2-fold dilutions. The diluted serum (100 μl/well) were added to each well pre-coated with recombinant S antigen and incubated for 1 h at 37°C. After washing, 100 μl HRP-anti-Human IgG working fluid was added to the corresponding wells, and plates were incubated for 45min at 37°C. After washing, 100 μl of Substrate Solution was added to each well. The reaction was stopped by adding Stop Solution at 50 μl/well, and the intensity of the Absorbance was measured at 450 nm˜630nm.

Production and titration of SARS-CoV-2 pseudovirus

A pseudovirus strain of SARS-Cov-2 Omicron BA.1 and BA.5 variants were constructed with more than 32 mutations in the S protein. Pseudoviruses of the prototype was also constructed. The SARS-CoV-2 pseudovirus was prepared by transfecting 293T cells with S protein expression plasmids. And G*ΔG-VSV (Kerafast, Boston, MA) was added by providing genomes of VSV. Following collection and filtering, the pseudotyped viruses were titered on Vero cells (8). Briefly described, the pseudotyped viruses were diluted from 1:10 to 1:7290 by three-fold serial dilutions, and mixed with Vero cells ($2x10^4$ / well) in 96-well plates. The mixture was incubated at 37°C, 5% CO_2 for 24 h. The chemiluminescence signals were collected using the Britelite plus reporter gene assay system (PerkinElmer, Waltham, MA). The 50% tissue culture infective dose (TCID50) was calculated using the Reed-Muench method.

SARS-CoV-2 pseudovirus neutralization assay

The neutralizing activity of serum samples to pseudotyped SARS-CoV-2 variants was detected by chemiluminescence method (8). Firstly, serum samples were performed by 3-fold serial dilutions ranging from *1:10* to *1:7290.* SARS-CoV-2 pseudovirus (650TCID50/ well) were mixed with diluted serum in 96-well plates and incubated at 37°C, 5%CO_2 for 1 h. Subsequently, the Vero cells ($2x10^4$/ well) were added and cultured for 24 h. The expression level of luciferase was detected by the chemiluminescence signals, and the relative luminescence unit (RLU) values was determined. The 50% neutralization dilution (ND50) level was calculated using the Reed-Muench method.

Statistical analysis

Data were plotted using GraphPad Prism 8 (GraphPad, San Diego, CA). T test and unpaired t test with Welch's correction were used for statistical analysis. Spearman non-parametric test was performed to evaluated the correlation between different test assays. Significance thresholds: * P <0.05, ** P < 0.01, ***P < 0.001, **** P < 0.0001, NS, not significant.

Result:

A total of 88 volunteers aged 27.0+-5.0 years old were recruited, including 20 males and 68 females. Among them, 29 participants had accepted vaccine A and 59 participants had received vaccine B (Supplementary Table 1). All the sera samples were collected within 2 months, from whom were immunized with the inactivated vaccine as boosters post two-dose inactivated immunization (Supplementary Table 1). The pseudotyped strains of SARS-Cov-2 Prototype, Omicron BA.1 and BA. 5 variants were constructed. (Figure 1A)

The geometric mean titers (GMT) of the serum pseudovirus neutralization test for Omicron BA.1 and BA.5 variants were 60 and 42, which were decreased by 6.2-fold and 8.0-fold compared with the prototype strain respectively (GMT, 331) (Supplementary Table1, Figure 1C). The overall comparison of neutralizing titres against each variant showed significant differences between the prototype and Omicron variants is shown in Figure 1C. The neutralization activities against the prototype, Omicron BA.1 and BA.5 variants in serum from participants that had received vaccine A were similar to those of participants that had received vaccine B (Supplementary Figure 1B).

Using ELISA test, the GMT of IgG tiers against the S of the prototype, Omicron BA.1 and BA.5 variants were 2797, 2334 and 2447 respectively (Supplementary Table 1), which showed no significant difference between the prototype and Omicron variants (Figure 1B). In addition, the IgG titers against Omicron BA.1 and BA.5 variants in serum from participants that had received vaccine A, were similar to those of participants that had received vaccine B, as well as the prototype (Supplementary Figure 1A).

The neutralization-positive rates of serum against the pseudovirus strains of SARS-Cov-2 Prototype, Omicron BA.1 and BA.5 variant were 100% (88/88), 95.45% (84/88) and 92.04% (81/88) respectively (Table 1). And the IgG binding positive rates to S of SARS-Cov-2 Prototype, Omicron BA.1 and BA.5 variant were all 100% (Table 1). All 88 serum samples testing positive by ELISA were also anti S of SARS-Cov-2 prototype positive by pseudovirus-based neutralization assay, corresponding to a positive agreement rate of 100% (88/88). For SARS-Cov-2 Omicron variants BA.1 and BA.5, the positive agreement rates of the two test methods were 95.45% (84/88) and 92.05% (81/88), respectively.

We also assessed the correlation between neutralizing antibody titers and IgG titers by using Pearson correlation analysis. The strongest correlation was observed between IgG binding activities to S of prototype by ELISA and the titers of neutralizing antibodies (r_s=*0.8238* , p<*0.0001*) (Figure 2). The analysis also revealed statistically significant association between the IgG titers of anti S of SARS-Cov-2 Omicron BA.1 variant and neutralizing antibody titers with ND50 values (r_s=*0.7000* , p<*0.0001*). (Figure 2). A minor correlation was found between two methods in SARS-Cov-2 Omicron BA.5 variant (r_s=*0.5499* ,p<*0.0001*).

Discussion:

The continuous emergence of new variants of SARS-CoV-2 has become the most concern in current COVID-19 pandemic, which has caused successive global waves of infection, and poses great challenges to vaccine research and development. Up to now, Omicron has become the dominating variant due to its high transmissibility and immune evasion (9). It is important to understand whether current immunity induced by vaccine and pre-infection is effective against different Omicron variants. Previous studies showed that three or four doses of parental mRNA vaccine did not elicit robust neutralization against BA.4/5 (10). Recent studies showed that the SARS-CoV-2 omicron variant has less sensitive to neutralizing antibody responses induced by vaccination and previous infection than previous variants (11,12).In the study, we determined

IgG antibody levels and neutralizing antibody levels of the serum from donors immunized with inactivated COVID-19 vaccine.

After the third dose of the homologous inactivated vaccine in 88 young population, the neutralizing activity against the Omicron BA.1 and BA.5 variant were decreased significantly compared with the prototype strain respectively (P <0.05). However, the IgG titers against the S of the prototype, Omicron BA.1 and BA. 5 variants detected by ELISA, showed no significant difference between the prototype and Omicron variants. It may consider that a part of IgG binding to S of different Omicron variants has no neutralization potency.

Some study demonstrated strong correlation of quantitative test with neutralization testing. A study showed that Anti-RBD IgG plasma concentration significantly correlated with the plasma/serum VN activity against SARS-CoV-2 in vitro (r_s=0.888,p <0.0001) (7). The anti-SARS-CoV-2 S1 IgG was also analyzed in other study, indicating a high correlation with the titers of neutralizing antibodies (r_s=0.819,p <0.0001) (13). In our study, we analyzed the correlation of anti- SARS-CoV-2 S IgG titers and neutralizing activities based on pseudovirus. The results showed different levels of correlation of IgG titers by ELISA and neutralizing antibodies, against SARS-CoV-2 prototype, Omicron variant BA.1 and BA.5, which indicated that determination of IgG titers against the SARS-CoV-2 Spike protein can not predict the viral immune evasion against different Omicron variants.

However, there are some limitations in the study. First, the number of serum samples is limited, and the results need to be conformed in a large sample size in further study. Second, the neutralization activity test was on the basis of a pseudotyped virus system. Further studies based on live viral particles need to be carried out.

In summary, we demonstrated that the Omicron variant BA.1 and BA.5 escapes vaccine-induced immunity by neutralization activity test, and IgG titers against the SARS-CoV-2 Spike protein can not predict the viral immune evasion against different Omicron variants.

Declaration of interest

The authors declare no competing interests.

Autor Contributions

H L and J.L. had the concept, designed the study, and made critical revision of the manuscript for important intellectual content. X.Y. made statistical analysis and drafted the manuscript. Q.D., J.L and Y.L. performed the ELISA test. H.W., Y Z. and P.L. performed the neutralizations experiments. Y.L. and X.Q. helped to acquire and analyze data.

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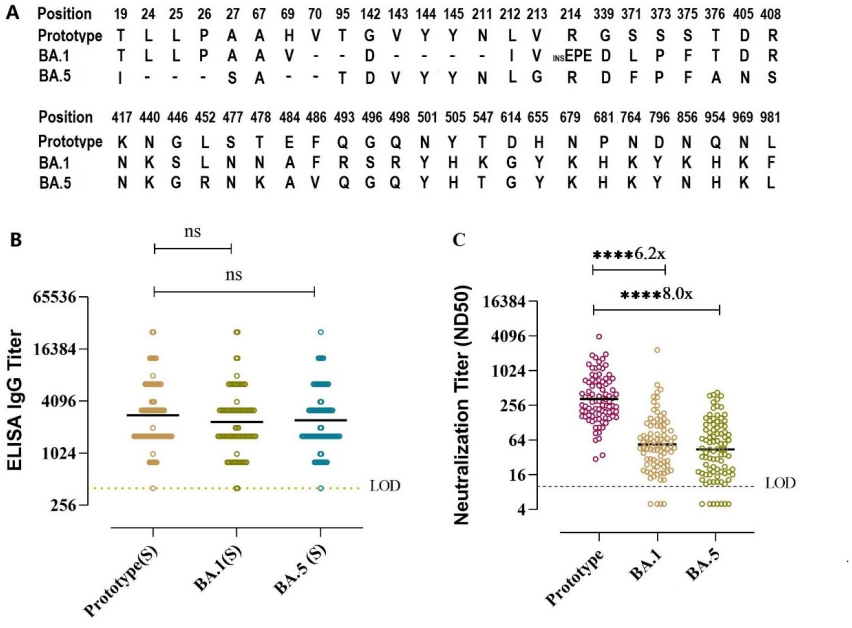
Figure Legend:

Figure 1



A. Spike protein mutation sites of SARS-CoV-2 Prototype (Genebank ID: MN908947), Omicron variants. BA.1 and BA.5. **B.**The IgG titers against S protein of SARS-Cov-2 prototype, Omicron variants BA.1 and BA.5. The limitation of the detection (LOD) was*1:400.* The titers below the LOD were set to 200.**C.** The neutralizing antibody titers against Prototype, and Omicron variants BA.1 and BA.5 in individuals post the 3rd booster vaccination within 2 months. The limitation of the detection (LOD) was *1:10.* The titers below

the LOD were set to 5.P -values were obtained by the unpaired t test, *** $P < 0.001$, **** $P < 0.0001$, ns, not significant.

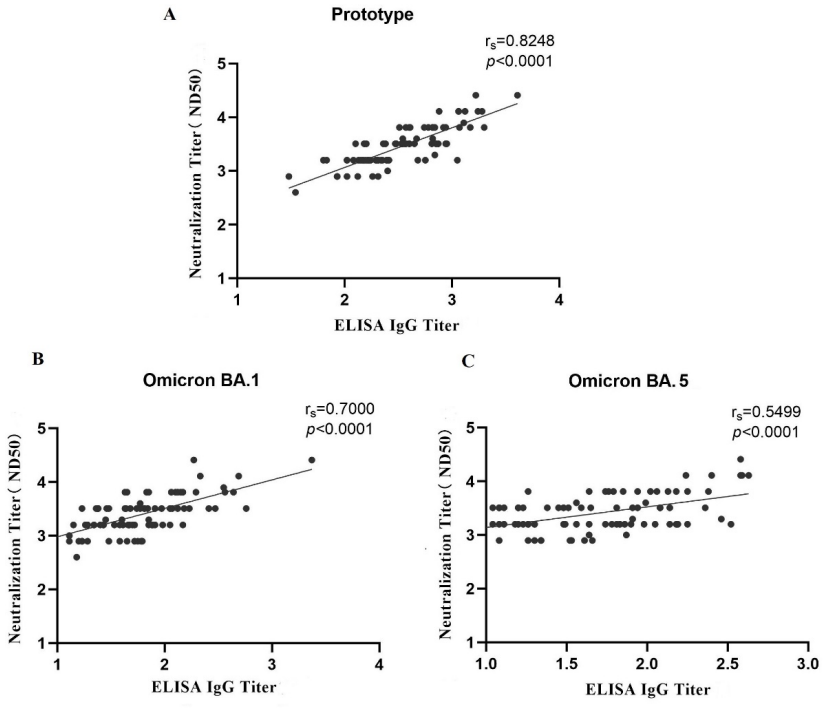


Figure 2 Correlation between neutralization antibody titers and IgG titers by ELISA. Anti-SARS-CoV-2 S IgG was determined using ELISA and neutralization antibody titers were determined by pseudovirus-based neutralization assay. A line of best fit was estimated by linear regression using log-transformed values for the IgG titers and neutralizaiton antibody titers. **A.** The correlation analysis between IgG titers against S protein of SARS-Cov-2 prototype and neutralization antibody titers against pseutyped SARS-Cov-2 prototype.**B.** The correlation analysis between IgG titers against S protein of SARS-Cov-2 Omicron BA.1 and neutralization antibody titers against pseutyped SARS-Cov-2 Omicron BA.1. **C.** The correlation analysis between IgG titers against S protein of SARS-Cov-2 Omicron BA.5 and neutralization antibody titers against pseutyped SARS-Cov-2 Omicron BA.5.r_s , Spearman rank-order correlation coefficient.

Table 1 Agreement between IgG titers by ELISA and neutralization titers using pseudotyped SARS CoV-2 variants in 88 sera samples immunized with three doses of COVID-19-inactivated vaccine

		Pseudovirus neutral-ization assay	**Pseudovirus neutral-ization assay**	**Pseudovirus neutral-ization assay**	**Pseudovirus neutral-ization assay**	**Pseudovirus neutral-ization assay**	**Pseudovirus neutral-ization assay**
		Prototype Positive n (%)	**Prototype Negative n (%)**	**BA.1 Positive n (%)**	**BA.1 Negative n (%)**	**BA.5 Positive n (%)**	**BA.5 Negative n (%)**
Anti-SARS-Cov-2 IgG (ELISA)	**Positive n (%)**	88(100%)	0(0%)	84(95.45%)	4(4.55%)	81(92.05%)	7(7.95%)

	Pseudovirus neutralization assay	Pseudovirus neutralization assay	Pseudovirus neutralization assay	Pseudovirus neutralization assay	Pseudovirus neutralization assay	Pseudovirus neutralization assay
Negative n (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)