Human serum from SARS-CoV-2 vaccinated and COVID-19 patients shows reduced binding to the RBD of SARS-CoV-2 Omicron variant in comparison to the original Wuhan strain and the Beta and Delta variants

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Abstract

Background

The ongoing COVID-19 pandemic is caused by the beta coronavirus SARS-CoV-2. COVID-19 manifests itself from mild or even asymptomatic infections to severe forms of life-threatening pneumonia. At the end of November 2021, yet another novel SARS-CoV-2 variant named B.1.1.529 or Omicron was discovered and classified as a variant of concern (VoC) by the WHO. Omicron shows significantly more mutations in the amino acid (aa) sequence of its spike protein than any previous variant, with the majority of those concentrated in the receptor binding domain (RBD). In this work, the binding of the Omicron RBD to the human ACE2 receptor was experimentally analyzed in comparison to the original Wuhan SARS-CoV-2 virus, and the Beta and Delta variants. Moreover, we compared the ability of human sera from COVID-19 convalescent donors and persons fully vaccinated with BNT162b2 (Corminaty) or Ad26.COV2.S (Janssen COVID-19 vaccine) as well as individuals who had boost vaccine doses with BNT162b2 or mRNA-1273 (Spikevax) to bind the different RBDs variants.

Methods

The Omicron RBD with 15 aa mutations compared to the original Wuhan strain was produced baculovirus-free in insect cells. Binding of the produced Omicron RBD to hACE was analyzed by ELISA. Sera from 27 COVID-19 patients, of whom 21 were fully vaccinated and 16 booster recipients were titrated on the original Wuhan strain, Beta, Delta and Omicron RBD and compared to the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human) using the original Wuhan strain as reference.

Results

The Omicron RBD showed a slightly reduced binding to ACE2 compared to the other RBDs. The serum of COVID-19 patients, BNT162b2 vaccinated and boost vaccinated persons showed a reduced binding to Omicron RBD in comparison to the original Wuhan strain, Beta und Delta RBDs. In this assay, the boost vaccination did not improve the RBD binding when compared to the BNT162b2 fully vaccinated group. The RBD binding of the Ad26.COV2.S serum group was lower at all compared to the other groups.

Conclusions

The reduced binding of human sera to Omicron RBD provides first hints that the current vaccinations using BNT162b2, mRNA-1273 and Ad26.COV2.S may be less efficient in preventing infections with the Omicron variant.

Introduction

SARS-CoV-2 is the etiological agent of the severe pneumonia COVID-19 (coronavirus disease 2019) [1,2]. A new variant B.1.1.529 of the betacoranavirus SARS-CoV-2 was identified in late November 2021 and has rapidly been classified as a variant of concern (VOC) by the WHO and named Omicron [3]. The Omicron variant shows a high number of mutations in the SARS-CoV-2 spike protein in comparison to the previously described VOCs Alpha [4], Beta (B.1.351) [5], Gamma (P.1) [6] and the currently dominating Delta variant (B.1.617.2) [7]. The first sequenced Omicron variant (GISAID accession ID EPI_ISL_6913995, collection date 2021-11-08, South Africa) contains a total of 36 mutations compared to the original Wuhan strain and include 29 amino acid (aa) changes, six aa deletions and one aa insertion. Fifteen of these mutations are located in the N-terminal receptor binding domain (RBD) of the spike protein which binds to the human zinc peptidase angiotensin-converting enzyme 2 (ACE2) for cell entry [8,9].

Importantly, the RBD is targeted by more than 90% of neutralizing serum antibodies, making it the most relevant target for SARS-CoV-2 neutralization [10,11]. Consequently, the majority of therapeutic antibodies for the treatment of COVID-19 are designed to interact with this part of the SARS-CoV-2 spike protein [12,13]. This indicates that the omicron variant may bind with a different affinity to the cell receptor, altering its propagation characteristics, escape the immune recognition by antibodies, facilitating viral spread in a seropositive population, or both.

While initial studies have shown a severe reduction in serum neutralizing capacity of vaccinated and convalescent patients against the omicron variant [14], it is unclear to which extent the RBD domain mutations contribute to this loss in neutralization activity. Additionally, while several mutations present in Omicron are predicted to increase ACE2 binding affinity, whereas others are predicted to reduce its affinity [15].

In this work, the binding of ACE2 to the new Omicron RBD was determined in comparison to the original Wuhan strain and the Beta and Delta variants in a biological experimental model. The humoral immune response is essential for the anti-viral defense. Therefore, the binding of human sera from COVID-19 hospitalized patients or fully vaccinated persons with BNT162b2 or Ad26.COV2.S as well as boost vaccinated persons on the RBD of the original Wuhan strain, Beta, Delta and Omicron variant was analyzed.

Methods

Serum samples

Blood samples were obtained from hospitalized patients with severe symptoms from the second (pre-alpha) and third (alpha variant) pandemic wave in Croatia or from vaccinated people as indicated. While all voluntary donors were informed about the project and gave their consent for the study, consent requirement was waived by the ethical committee in Rijeka for patients in intensive care where sampling was a part of routine diagnostics. The sampling was performed in accordance with the Declaration of Helsinki. The donors included adults of both sex. The first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136) was used as positive control serum and pre-pandemic negative control sera were provided by the LADR Braunschweig. Approval was given from the ethical committee of the Technische Universität Braunschweig (Ethik-Kommission der Fakultät 2 der TU Braunschweig, approval number FV-2020-02). The study in Croatia was approved by the Ethics committee of the Rijeka Clinical Hospital Center (2170-29-02/1-20-2). The study in Italy was performed under the approval of the Institutional Review Board of Policlinico San Matteo (protocol number P_20200029440). Details about study participants are shown in Table 1.

		n (female/ male)	***Mean age (range)***	***Time point of sampling***
Patients	severe symptoms, hospitalized	27 (7/20)	65 (39-86)	7-25 days after symptom onset (mean 12 days)
Vaccinated persons	2xBNT162b2	15 (4/11)	36 (25-61)	7-43 days after 2nd dose (mean 16 days)
	1xAd26.COV2.S	6 (2/4)	35 (24-40)	14-33 days after 1st dose (mean 25 days)
	BNT162b2 or mRNA-1273	16 (7/9)	39 (24-64)	5-49 days after 3rd dose (mean 17 days)

Table 1: Used human serum samples in this study.

Construction of the expression vectors

All sequences of the RBD variants (319-541 aa of GenBank: MN908947) were inserted in a *Nco*I/*Not*I compatible variant of the OpiE2 expression vector [16] containing a N-terminal signal peptide of the mouse Ig heavy chain and a C-terminal 6xHis-tag. Single point mutations to generate the Beta and Delta variants of RBD were inserted into the original Wuhan strain through site-directed mutagenesis using overlapping primers according to Zheng et al. with slight modifications: S7 fusion polymerase (Mobidiag) with the provided

GC buffer and 3% dimethyl sulfoxide was used for the amplification reaction. The RBD omicron variant was ordered as GeneString from GeneArt (Thermo Fisher) according to EPI_ISL_6590608 (partial RBD Sanger sequencing from Hong Kong), EPI_ISL_6640916, EPI_ISL_6640919 and EPI_ISL_6640917 including Q493K which was corrected later to Q493R. Table 2 gives an overview about the used variants.

RBD wt	Original Wuhan	-
RBD beta	B.1.351	K417N, E484K, N501Y
RBD delta	B.1.617.2	L452R, T478K
RBD omicron	B.1.1.529	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H

Table 2: RBD variants used in this study (319-541 of GenBank: MN908947)

Expression and purification of the RBD variants

The different RBD variants were produced in the baculovirus-free High Five cell system [17] and purified as described before [18]. Briefly, High Five cells (Thermo Fisher Scientific) were cultivated at 27°C, 110- 115 rpm in EX-CELL 405 media (Sigma Aldrich) at a cell density between 0.3– 5.5x10^6 cells/mL. On the day of transfection, cells were centrifuged and resuspended in fresh media to a density of 4x10^6 cells/mL before 4 µg expression plasmid/mL and 16 µg/mL of linear PEI 40 kDa (Polysciences) was pipetted directly into the cell suspension. After 4- 24 h, cells were supplemented with fresh media to dilute the cells ~1x10^6 cells/mL and 48 h after transfection, culture volume was doubled. Cell supernatant was harvested four to five days after transfection by a two-step centrifugation (4 min at 180 xg and 20 min at >3,500 xg) and then 0.2 µm filtered for purification. Immobilized Metal Ion Affinity Chromatography (IMAC) His tag purification of insect cell supernatant was performed with a HisTrap excel column (Cytiva) on Äkta system (Cytiva) according to the manufacturer's manual. In a second step, the RBD domains were further purified by size exclusion chromatography (SEC) by 16/600 Superdex 200 kDa pg column (Cytiva).

Expression and purification of ACE2-hFc

The extracellular domain of ACE2 receptor (GenBank NM_021804.3) was produced in pCSE2.6-hFc expression vector in Expi293F cells (Thermo Fisher Scientific) as described before (Bertoglio et al., 2021b). In brief, Expi293F cells were cultivated at 37°C, 110 rpm, and 5% CO_2 in Gibco FreeStyle F17 expression media (Thermo Fisher Scientific) supplemented with 8 mM Glutamine and 0.1% Pluronic F68 (PAN Biotech). For

transfection 1 µg DNA and 5 µg of 40 kDa PEI (Polysciences) per mL transfection volume were diluted separately in 5 transfection volume and than mixed for formation of complexes (20- 30 min). Afterwards PEI:DNA complexes were added to 1.5- 2×10^6 cells/mL. Forty-eight hours later, the culture volume was doubled by feeding HyClone SFM4Transfx-293 media (GE Healthcare) supplemented with 8 mM Glutamine and HyClone Boost 6 supplement (GE Healthcare) with 10% of the end volume. One week after transfection, supernatant was harvested by 15 min centrifugation at 1,500 ×g. Purification was performed on 1 mL HiTrap Fibro PrismA (Cytiva) column on Äkta go (Cytiva) according to the manufacturer's manual.

ACE2 titration ELISA on RBD

ACE2 binding to the produced RBD25 variant antigens was analyzed in ELISA in triplicates where 300 ng RBD per well was immobilized on a Costar High binding 96 well plate (Corning, Costar) at RT for 1 h. Next, the wells were blocked by 330 µL 2% MPBST (2% (w/v) milk powder in PBS; 0.05% Tween20) for 1 h at RT and then washed 3 times with H_2O and 0.05% Tween20 (BioTek Instruments, EL405). ACE2-hFc was titrated from 0.01 mg/mL down to 1 ng/mL and incubated 1 h at RT prior to another 3x times washing step. Detection was performed by goat-anti-hIgG(Fc) conjugated with HRP (1:70,000, A0170, Sigma) and visualized with tetramethylbenzidine (TMB) substrate (20 parts TMB solution A (30 mM potassium citrate; 1% (w/v) citric acid (pH 4.1)) and 1 part TMB solution B (10 mM TMB; 10% (v/v) acetone; 90% (v/v) ethanol; 80 mM H_2O_2 (30%)) were mixed). After addition of 1 N H_2SO_4 to stop the reaction, absorbance at 450 nm with a 620 nm reference wavelength was measured in an ELISA plate reader (BioTek Instruments, Epoch). EC_{50} were calculated with OriginPro Version 9.1, fitting to a five-parameter logistic curve.

Serum titration ELISA

For titration ELISA, sera were diluted 1:100 to 1: 9.19x10^7 in 384 well microtiter plates (Greiner Bio-One) coated with 30 ng/well of the respective RBD variant. In addition, all sera were also tested at the lowest dilution (1:100) for determination of unspecific cross-reactivity on Expi293F cell lysate (30 ng/well), BSA (30 ng/well) and lysozyme (30 ng/well). IgGs in the sera were detected using goat-anti-hIgG(Fc)-HRP (1:70,000, A0170, Sigma). 384-well liquid handling was performed with a Precision XS microplate sample processor (BioTek), EL406 washer dispenser (BioTek) and BioStack Microplate stacker (BioTek). OD450 nm-620 nm was measured in an ELISA plate reader (BioTek Instruments, Epoch)

and its software Gen5 version 3.03 was used to calculate EC_{50} values, further expressed as relative potency towards an internal calibrant for which the Binding Antibody Unit (BAU) was calculated using the WHO International Standard 20/136 in relation to the original Wuhan strain RBD25. The graphics were created by GraphPad Prism 9.1. Significance was calculated by pairwise non-parametric multiple comparison ANOVA (Friedrich's test), using the Wuhan wt RBD values as reference value.

Results

The Omicron RBD shows a slightly reduced binding to ACE2

The RBD of the original Wuhan strain, the Delta variant and the Omicron variant were produced in insect cells and purified by IMAC and SEC. The quality of the recombinant proteins was analyzed by SDS-PAGE (Supplementary Figure 1). The production yield of Omicron RBD was tendentially higher than the production yield of Wuhan RBD. All RBDs were immobilized on plates and binding of soluble receptor ACE2 was analyzed by ELISA (Figure 1). The Omicron RBD showed a slightly reduced binding to ACE2 (EC_{50} 150 ng/mL) compared to the Wuhan strain RBD (EC_{50} 120 ng/mL) and more to Beta (EC_{50} 89 ng/mL) and Delta RBD (EC_{50} 89 ng/mL). Similar results were observed when soluble RBDs were added to plate-coated ACE2 (data not shown).

Human sera of COVID-19 patients and vaccinated persons show a reduced binding to Omicron RBD

The binding of human sera from hospitalized COVID-19 patients (Figure 2A), from people fully vaccinated with BNT162b2 (Corminaty) (7-43 days after second immunization) (Figure 2B), Ad26.COV2.S (Janssen COVID-19 Vaccine) (14-33 days after immunization) (Figure 2C) or from mRNA vaccine boost-vaccinated persons (5-49 days after boost vaccination, first immunization 2xBNT162b2 or 1xAd26.COV2.S) (Figure 2D) was analyzed by ELISA on Wuhan wt, Delta, Beta and Omicron RBD. A direct comparison of the binding to Omicron RBD of all four serum groups is given in Figure 2E.

The sera of COVID-19 patients and BNT162b2 showed a highly significant reduction in binding to Omicron RBD in a non-parametric pairwise analysis. This reduction was more pronounced than the one observed against Beta or Delta RBD binding assays. In the booster group the binding to original Wuhan strain, Beta and Delta RBDs was nearly equal, but significantly reduced to Omicron RBD. Ad26.COV2.S group showed very low binding to all RBDs tested, suggesting a clearly weak immunogenicity of this vaccine formulation.

An increase in binding to the Omicron RBD variant after boost immunization was not observed.

Discussion

RBD-ACE2 interaction is a prerequisite for SARS-CoV-2 viral entry [8,9,19]. The binding of the Omicron RBD to the ACE2 receptor appears to be slightly reduced in our setting compared to the currently dominant Delta variant. Several Omicron RBD mutations are assumed to increase the binding to ACE2: G339D, S477N, T478K, Q493K, N501Y, others are neutral: S371L, S373P, G446S, E484A, Q493R, Q498R or are assumed to reduce the binding to ACE2: S375F, K417N, G496S, Y505H according to Starr et al. [19]. However, all of these considerations are based on modelling. RBD-ACE2 interaction involving a heavily mutated RBD such as the one of Omicron VOC may deviate from predictions. To our knowledge this is among the first empirical analysis of Omicron RBD binding efficacy to the ACE2 receptor. Surprisingly, the binding of Omicron RBD to human ACE2 was not increased, but rather decreased, especially when compared to Beta and Delta. One has to consider that the decrease in RBD binding does not necessarily translate into reduced infectivity, as infectivity and replication is also defined by proteolytic spike processing, fusion efficacy and RNA replication efficiency. Nevertheless, our results argue that increased binding to the hACE2 receptors may be an unlikely cause of rapid Omicron spread. One has also to consider that we utilized in our work the originally available sequence with a Q493K mutation, whereas Q493R sequences have been published since. According to Starr et al. [19], the K mutation has a higher affinity as the R mutation in *in vitro* binding studies. On the other hand Kumar et al.[15] calculated an increased binding to ACE2 for the Q493R mutation in docking studies. Regardless, future studies will validate our observation.

Importantly, RBD mutations may also lead to immune escape [20]. The humoral immune answer is a key factor for the anti-viral defense [21] and the RBD is the main target of neutralizing antibodies [10,11,22]. The very low RBD binding in the Ad26.COV2.S compared to the COVID-19 patient group or the BNT162b2 vaccinated group is in accordance with the former results[23] but impaired definite conclusions on omicron immune escape upon Ad26.COV2.S vaccination. The reduced binding of sera from COVID-19 patient and BNT162b2 groups to the Omicron RBD was in accordance with current results describing a highly reduced neutralization of the Omicron variant by human sera from vaccinated persons [14,24]. Surprisingly, the boost vaccination did not improve the Omicron RBD binding in this assay, probably because the results were compared to early serum responses upon the 2nd vaccination dose.

The reduced binding of the patient, vaccinated and boost-vaccinated sera to the Omicron RBD is a snapshot of the current situation. According to the sequencing data deposited as GISAID (https://www.gisaid.org/) and the analysis on Outbreak.info [25], the frequency of the 15 aa mutations in the RBD is very dynamic, e.g. K417N and G446S described for the initial Omicron variant is occurs in ~ 40% of all sequenced Omicron variants, S477N and T478K is in ~60%, N501Y is in ~90% of the sequences (status 2021-12-09, 837 sequences). The K417N mutation is a key mutation also in the Beta variant, the T478K mutation in the Delta variant and N501Y in the Alpha, Beta and Gamma variant [26]. All of these variants may contribute to both ACE2 binding efficacy and immune escape. Therefore, Omicron variants with alternative mutations might evolve in the near future and alter the antibody recognition and the binding efficacy.

Declaration of interests

M.S., F.B., S.S., P.H., S.D. and M.H. are inventors on a patent application on blocking antibodies against SARS-CoV-2. S.D. and M.H. are co-founders and shareholders of CORAT Therapeutics GmbH, a company founded for clinical and regulatory development of COR-101, an antibody for the treatment of hospitalized COVID-19 patients.

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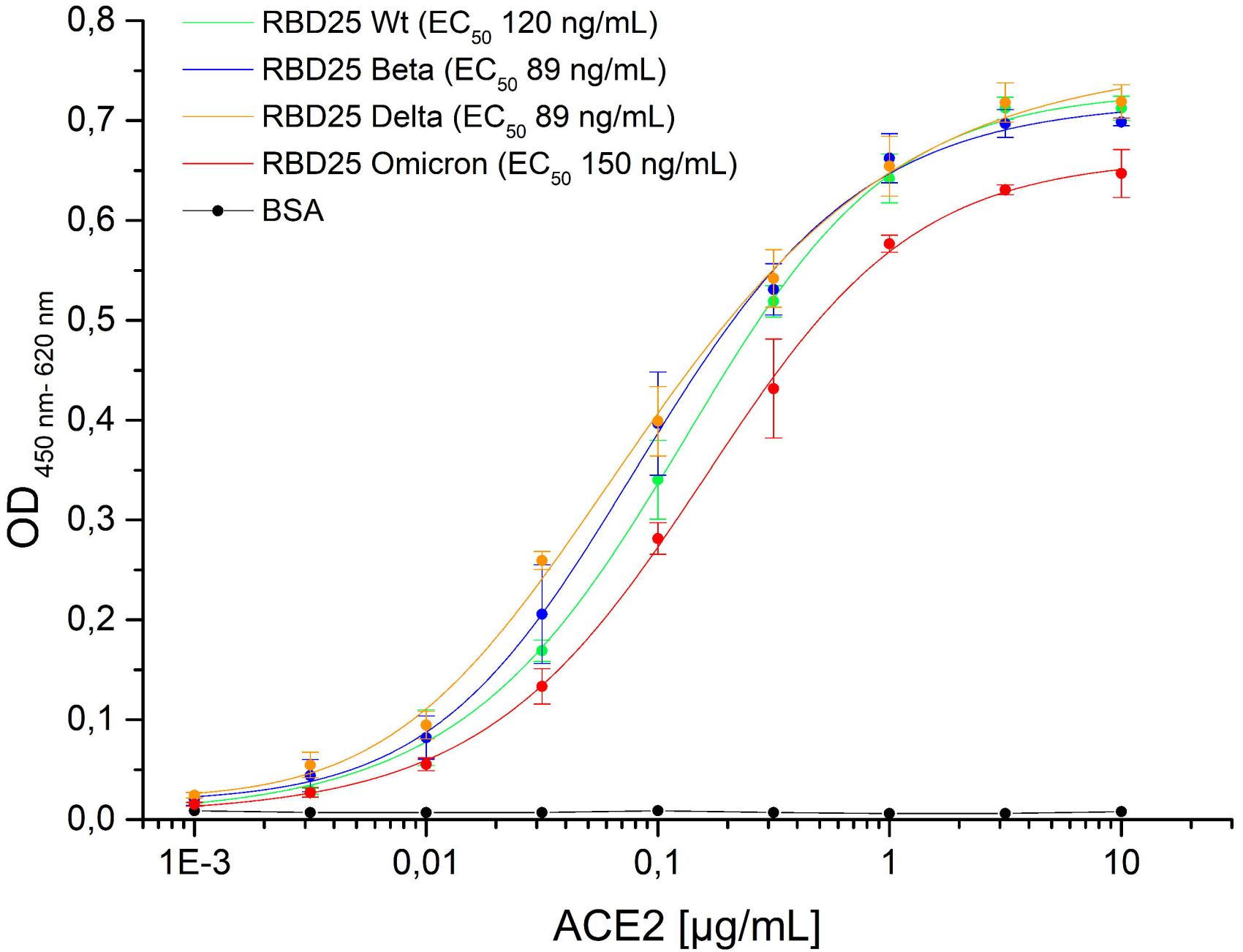
Figure legends

Figure 1: RBD variants binding to human ACE2. 300 ng/well immobilized Wuhan wt, Beta, Delta or Omicron RBD were detected with human ACE (fusion protein with human Fc part) in titration ELISA. BSA was used as negative control. Experiments were performed in triplicates and mean values are given. EC_{50} were calculated with OriginPro Version 9.1, fitting to a four-parameter logistic curve.

Figure 2: Human serum binding to SARS-CoV-2 Wuhan original strain, Beta, Delta and Omicron RBD. (A) ELISA using sera from hospitalized COVID-19 patients. (B) ELISA using sera from 2xBNT162b2 vaccinated persons (7-52 days after 2nd immunization). (C) ELISA using sera from 1xAd26.COV2.S vaccinated (14-33 days 1st immunization). (D) ELISA using sera from 2xBNT162b2 or Ad26.COV2.S vaccinated and boosted with BNT162b2 or mRNA-1273 (5-49 days after 3rd or in case of Ad26.COV2.S 2nd immunization). (E) ELISA using sera from 2xBNT162b2 or Ad26.COV2.S vaccinated and boosted with BNT162b2 or mRNA-1273 (5-49 days after 3rd or in case of Ad26.COV2.S 2nd immunization) binding to the Omicron variant. This is a rearranged representation of the data presented in (A-E). The ELISAs were performed as single point titrations. EC_{50} were calculated by DataGraph Version 4.7.1, fitting to a four-parameter logistic curve and expressed as relative potency in respect to an internal calibrant, for which the Binding Antibody Unit (BAU) was calculated using the WHO International Standard 20/136 titrated on Wuhan wt as reference. The median values are given in the graphs. The graphics and statistical analysis were made with Graphpad Prism 9.1.


RBD25 Wt (EC50 120 ng/mL)
RBD25 Beta (EC50 89 ng/mL)
RBD25 Delta (EC50 89 ng/mL)
RBD25 Omicron (EC50 150 ng/mL)
BSA
OD 450 nm- 620 nm
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
1E-3
0,01
0,1
1
10
ACE2 [µg/mL]