Anatomy of Omicron neutralizing antibodies in COVID-19 mRNA vaccinees

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Abstract

SARS-CoV-2 vaccines, administered to billions of people worldwide, are mitigating the effects of the COVID-19 pandemic, however little is known about the molecular basis of antibody cross-protection to emerging variants, such as Omicron (B.1.1.529), and other coronaviruses. To answer this question, 276 neutralizing monoclonal antibodies (nAbs), previously isolated from seronegative and seropositive donors vaccinated with BNT162b2 mRNA vaccine1, were tested for neutralization against the Omicron variant and SARS-CoV-1 virus. Cross-neutralizing antibodies were isolated from 100% of seropositive and 20% of seronegative vaccinees. Only 14.2% and 4.0% of tested antibodies neutralized the Omicron variant and SARS-CoV-1 respectively. These nAbs recognized mainly the SARS-CoV-2 receptor binding domain (RBD) and targeted class 3 and class 4 epitope regions on the SARS-CoV-2 spike protein. Antibodies targeting class 1/2 epitope regions only rarely showed cross-neutralization activity. Cross-protective antibodies derived from a variety of germlines, the most frequents of which were the IGHV1-58;IGHJ3-1 and IGHV1-69;IGHV4-1. Only 15.6% and 7.8% of predominant gene-derived nAbs elicited against the original Wuhan virus cross-neutralized Omicron and SARS-CoV-1 respectively. Our data provide evidence of the presence of cross-neutralizing antibodies induced by vaccination and map conserved epitopes on the S protein that can inform vaccine design.

Introduction

Since its first appearance in December 2019, more than 370 million cases of SARS-CoV-2 infections were reported worldwide, with over 5.6 million deaths. Effective vaccines against the virus that first appeared in Wuhan, China, have been developed with unprecedented speed. However, their ability to contain the global pandemic has been compromised by the inability to timely deliver vaccines to low-income countries and by the appearance of several antigenic variants which escaped the natural and vaccine-induced immunity[2–4]. The main variants that emerged so far, and listed as variants of concern (VoCs), are named B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta) and B.1.1.529 (Omicron)[5,6]. The latter one showed to be the most efficient in spreading into partially immune populations and in few months from its appearance have conquered most regions of the world[7,8]. The Omicron variant contains 37 mutations in the spike (S) protein, with up to 15 mutations in the receptor binding domain (RBD), the primary target for neutralizing antibodies (nAbs). Several reports have shown that the unprecedented number of mutations carried on the Omicron S protein drastically reduce up to 40-fold the neutralizing efficacy of sera from infected and vaccinated people and that this VoC can escape more than 85% of nAbs described in literature, including several antibodies approved for clinical use by regulatory agencies[9–17]. While serum activity and neutralization efficacy of selected mAbs against Omicron have been extensively reported, the functional and genetic anatomy of nAbs elicited in naïve (seronegative) and convalescent (seropositive) people immunized with the BNT162b2 mRNA vaccine remains to be explored. Taking advantage of our previous work[1], we tested 276 human monoclonal antibodies able to neutralize the original SARS-CoV-2 virus isolated in Wuhan, for their ability to neutralize the Omicron variant and the distantly related SARS-CoV-1 virus. Our work unravels the genetic signature of cross-

protective antibodies and mapped conserved sites of pathogen vulnerability on the S protein that can be used to design the next generation of COVID-19 vaccines.

Results

Distribution of Omicron mutations on RBD immunodominant sites

The SARS-CoV-2 B.1.1.529 (Omicron) variant harbors 37 mutated residues in the spike (S) glycoprotein (Fig. 1a). The receptor binding domain (RBD) and N terminal domain (NTD) immunodominant sites are both highly mutated, carrying 15 and 11 mutations respectively (Fig. 1c)[13]. The NTD presents 3 substitutions (27%), A67V, T95I and G142D, 5 deleted residues (46%), Δ69-70 and Δ143-145, and 3 inserted residues (27%), ins214EPE. Conversely, the RBD shows only substituted residues. Within the RBD, the receptor binding motif (RBM), spanning from residues S438 to Y508[18], is the most mutated region containing 10/15 (67%) of all RBD mutations (N440K, G446S, S477N, T478K, E484A, Q493R, Q496S, Q498R, N501Y and Y505H). Impacted residues in the RBM overlap with the epitopes of Class 1 and Class 2 neutralizing antibodies (nAbs), like J08[19,20], that target epitopes spanning from the left shoulder, through the neck and upper part of right shoulder of the S protein (Fig. 1b-d)[21,22]. Class 3 and 4 cluster of antibodies target the lower portion of the RBD, and their epitopes are located on the right and left flanks of this domain. Class 3 nAbs, like S309[23], target the right flank of the RBD where only 2/15 (13%) mutations (G339D and 440K) are found (Fig. 1d). Class 4 mAbs, like CR3022[24], are directed towards the left flank of the RBD which shows 3/15 (20%) mutations (S371L, S373P and S375F) (Fig. 1b).

Omicron effects on vaccine-induced nAbs

To understand the impact of SARS-CoV-2 Omicron on the antibody response, we evaluated the neutralization activity of 276 nAbs previously isolated from seronegative (n=52) and seropositive (n=224) donors immunized with the BNT162b2 mRNA vaccine (Fig. 2)[1]. While Omicron cross-neutralizing nAbs were identified in all seropositives, these antibodies were found only in one out of five seronegatives (20%) (Extended Data Table 1). Only 1 out of 52 nAbs from seronegatives (1.9%) neutralized Omicron with a medium-low neutralization potency (Fig. 2a and c). Since only 1 nAb was identified from this group no statistical difference in the geometric mean (GM) 100% inhibitory concentration (IC_{100}) between the Wuhan and Omicron viruses was found in this group. Conversely, 38 out 224 nAbs from seropositives (16.9%) were able to neutralize Omicron. These nAbs showed a 3.16-fold decreased neutralization potency compared to the Wuhan virus showing a GM-IC_{100} of 719.8 ng/mL (Fig. 2b-c). Overall, 39 Omicron nAbs were identified of which 38 (97.4%) targeted the S protein RBD and 1 (1.6%) recognized the NTD (Extended Data Fig. 2b, left bar). None of the 39 identified nAbs showed an extreme neutralization potency (IC_{100} below 10 ng/mL) against Omicron. To identify immunodominant sites on the Omicron S protein a flow cytometry-based competition assay was performed. In our previous study, we found that

215/276 (77.9%) nAbs bound to the S protein RBD and the majority of these antibodies were competing with J08, which epitope spans between Class 1 and Class 2 regions, and S309, which target the Class 3 region[1]. In this work all 215 RBD targeting-nAbs were additionally tested by competition assay against Class 4 targeting mAb CR3022. Class 1/2, Class 3 and Not-competing nAbs were found in both seronegatives and seropositives, while Class 4 competing nAbs were found exclusively in seropositives (Extended Data Fig. 2a; Extended Data Table 2). In both groups, Class1/2 competing nAbs were the most abundant constituting 70.3% (n=26) and 64.0% (m=114) of all antibodies isolated from seronegatives and seropositives respectively (Extended Data Fig. 2a; Extended Data Table 2). The second most abundant group of mAbs were Not-competing (n=4; 10.8%) and Class 3-competing nAbs (n=47; 27.0%) for seronegatives and seropositives respectively. Finally, only 7 (3.4%) Class 4 competing nAbs were found exclusively in seropositives (Extended Data Fig. 2a; Extended Data Table 2). From the 215 tested nAbs, we found that only 20 out of 140 (14%) Class 1/2 targeting nAbs were able to neutralize Omicron, while up to 29% percent (15/51) of Class 3 nAbs showed neutralization activity against this variant (Fig. 2d-e). Finally, 14% (1/7) Class 4-competing nAbs were able to neutralize the Omicron virus (Fig. 2f). Among the 39 RBD-targeting Omicron nAbs, the majority recognized the Class 1/2 region (n=20; 51.3%) followed by Class 3 (n=15; 38.5%), while nAbs targeting Class 4 region (n=2; 5.1%) and Not-competing (n=2; 5.1%) were the least represented (Extended Data Fig. 2C, left bar).

Antibody cross-protection to SARS-CoV-1

We investigated the ability of COVID-19 mRNA vaccine elicited nAbs to neutralize the ancestral SARS-CoV-1 (SARS1) virus, using lentiviral vector derived pseudoparticles, to identify immunodominant sites of cross-protection across different coronaviruses[25]. All 276 previously identified nAbs were tested for their ability to bind the SARS1 S protein (Extended Data Fig. 1). Four out of five seropositives (80%) and one out of five seronegatives (20%) showed nAbs able to bind the SARS1 S protein (Fig. 3a; Extended Data Table 1). Of the 52 nAbs isolated from seronegatives 3 (7.5%) recognized SARS1 S protein, while 16 out of 224 nAbs (14.8%) from seropositives were able to bind this antigen (Figure 3a). When nAbs were tested for their neutralization activity against SARS1, only 1 (1.9%) and 10 (4.5%) nAbs where found from seronegatives and seropositives respectively (Fig. 3b). All SARS1 cross-neutralizing nAbs recognized the SARS-CoV-2 S protein RBD (Extended Data Fig. 2b, right bar). Class 4 targeting nAbs able to cross-neutralize SARS1 were overall the most frequent, followed by Class 3 binding nAbs (Fig. 3c). While Class 4 were the most frequent, Class 3 targeting nAbs showed the highest neutralization potency (Fig. 3c-d). None of the 140 Class 1/2 region targeting nAbs were able to cross-neutralize SARS1. Among the 11 SARS1 cross neutralizing nAbs, the majority recognized the Class 3 region (n=6; 54.5%), followed by Class 4 (n=4; 36.4%) and Not-competing (n=1; 9.1%) nAbs (Extended Data Fig. 2c, right bar).

Antibody repertoire to Omicron and SARS1

To investigate the genetic basis of antibody cross-protection against Omicron and SARS1, we interrogated the functional antibody repertoire. Previously, we identified five predominant germlines

shared among seronegatives and seropositives (IGHV1-2;IGHJ6-1, IGHV1-69;IGHJ4-1, IGHV3-30;IGHJ6-1, IGHV3-53;IGHJ6-1 and IGHV3-66;IGHJ4-1), and one rearrangement (IGHV2-5;IGHJ4-1), that encoded for potently neutralizing antibodies able to protect against all VoC from alpha (B.1.1.7) to delta (B.1.617.2), found exclusively in seropositives (Fig. 4a, top panel)[1]. Cross-protective nAbs against Omicron and SARS1 use a variety of rearrangements (Fig. 4a, middle ant bottom panels). The most frequent germline among Omicron nAbs was the non-predominant IGHV1-58;IGHJ3-1 (total n=11) found in both seronegatives and seropositives (Fig. 4a, top panel). Only 15.6% (n=10) of predominant gene derived nAbs were able to neutralize Omicron, while up to 64.0% (n=7) of IGHV1-58;IGHJ3-1 encoding nAbs protected from this variant (Fig. 4b-h). Two clonal families (Clone ID27: 3 members; Clone ID77: 2 members) were found among IGHV1-58;IGHJ3-1 encoding nAbs while the majority were orphan sequences (Extended Data Fig. 3a). All IGHV1-58;IGHJ3-1 nAbs bound the RBD and targeted Class 1/2 region (Extended Data Fig. 3b-c). Interestingly, all IGHV1-58;IGHJ3-1 nAbs had a heavy chain complementary determining region 3 (CDRH3) length of 16 amino acid and an average V gene mutation level of 3% (Fig. 3j). Finally, we observed that all IGHV1-58;IGHJ3-1 antibodies able to neutralize Omicron paired with the light chain germline IGKV3-20;IGKJ1-1 (Extended Data Fig. 3d). As for SARS1 cross-neutralizing antibodies, only 7.8% (n=5) of the previously identified predominant germlines showed protection (Fig. 4i). Despite this observation, the most abundant germline used by these nAbs was the predominant IGHV1-69;IGHJ4-1 (n=4; 22% of all antibodies derived from this germline) which was found in both seronegatives and seropositives and showed medium-high 50% neutralization dose (ND_{50}) (Fig. 4a, top panel; Fig. 4i). Of the 18 nAbs derived from this germline, 5 belonged to two distinct clonal families (Clone ID47: 3 members and Clone ID85: 2 members) while the remaining 13 (72.2%) were orphan sequences (Extended Data Fig. 3e). IGHV1-69;IGHJ4-1 derived nAbs showed to be able to recognize both NTD and RBD, and to target Class 1/2 and Class 3 regions on this latter domain (Extended Data Fig. 3f-g). Conversely, IGHV1-69;IGHJ4-1 SARS1 nAbs, showed to bind only the RBD and to target exclusively Class 3 epitope region (Extended Data Fig. 3f-g). IGHV1-69;IGHJ4-1 nAbs showed to use an heterogenous CDRH3 length with SARS1 nAbs spanning from 11-12 amino acid (Fig. 4k, left panel). As for the V gene mutation levels, IGHV1-69;IGHJ4-1 nAbs spanned from 0.69-5.6% with SARS1 nAbs averaging 3.4% (Fig. 4k, right panel). Finally, nAbs derived from the IGHV1-69;IGHJ4-1 showed to accommodate several light chains germlines, while SARS1 nAbs paired exclusively with IGKV3-20;IGKJ1-1 light chains (Extended Data Fig. 3h).

Discussion

In this work we deeply characterized an extensive panel of vaccine elicited-neutralizing human monoclonal antibodies against the heavily mutated Omicron variant and the distantly related SARS-CoV-1 virus to understand the degree of cross-protection and to map conserved regions on the S protein. We found that only 14.2% and 4.0% of our antibody panel was able to neutralize the Omicron VoC and the SARS-CoV-1 virus respectively. Remarkably, from the group of seronegative people vaccinated with two doses of the BNT162b2 mRNA vaccine, we isolated only 1 of the 39 nAbs against Omicron while none of their antibodies was able to neutralize SARS-CoV-1. Cross-neutralizing antibodies were isolated almost

exclusively from people that were vaccinated after infection highlighting once again the broad cross-protection conferred by hybrid immunity[1,26]. Despite these results, we observed that antibody germlines mainly involved in Omicron and SARS-CoV-1 cross-protection (IGHV1-58;IGHJ3-1 and IGHV1-69;IGHJ4-1) were used in both seronegative and seropositive vaccinees suggesting that a third booster dose in naïve people could enhance germline-maturation and induce a more broad and persistent antibody response[27]. Furthermore, a third booster dose could drive affinity maturation of poorly cross-reactive but predominant RBD-targeting B cell germlines elicited following SARS-CoV-2 Wuhan infection or vaccination, which were shown to persist for up to 6 months in the draining lymph nodes of vaccinated individuals[28]. These data suggest that primary immunization with two doses of vaccine in naïve people is not sufficient to elicit a meaningful proportion of cross-neutralizing antibodies, and that this requires a secondary immune response that can be obtained by vaccinating previously infected people or by providing a third booster dose[29]. Further studies will be necessary to understand whether a booster dose in naïve people can elicit a hybrid immunity-like antibody response and to define the molecular basis of cross-protection in this population. In addition to this observation, we herein defined the epitope regions that mediate cross-protection with Omicron and SARS-CoV-1. Indeed, we observed that, while the majority of nAbs against SARS-CoV-2 Wuhan virus recognize the tip of the receptor binding domain, which comprise antigenic Classes 1 and 2, the larger proportion of cross-neutralizing antibodies map in the lower portion of the RBD which comprises exclusively antigenic Classes 3 and 4. This information can support the design of next-generation COVID-19 vaccines broadly protective against current and future variants.

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Methods

Transcriptionally active PCR expression of neutralizing antibodies

The transcriptionally active PCR (TAP) expression of neutralizing antibodies (nAbs) was performed as previously described[1,20]. Antibodies heavy and light chain vectors were initially digested using restriction enzymes AgeI, SalI and XhoI. PCR II products were ligated using the Gibson Assembly NEB into 25 ng of respective human Igγ1, Igκ and Igλ expression vectors[30,31]. TAP reaction was performed using 5 µl of Q5 polymerase (NEB), 5 µl of GC Enhancer (NEB), 5 µl of 5X buffer, 10 mM of dNTPs, 0.125 µl of forward/reverse primers and 3 µl of ligation product, using the following cycles: 98 °C for 2 min, 35 cycles 98 °C for 10 s, 61 °C for 20 s, 72 °C for 1 min and 72 °C for 5 min. TAP products were purified, quantified using the Qubit Fluorometric Quantitation assay (Invitrogen), and used for transient transfection in Expi293F cell line following manufacturer's instructions.

SARS-CoV-2 authentic viruses neutralization assay

All SARS-CoV-2 authentic virus neutralization assays were performed in the biosafety level 3 (BSL3) laboratories at Toscana Life Sciences in Siena (Italy) and Vismederi Srl, Siena (Italy). BSL3 laboratories are approved by a Certified Biosafety Professional and are inspected every year by local authorities. To evaluate the neutralization activity of identified nAbs against SARS-CoV-2 and B.1.1.529 (Omciron) VoC a cytopathic effect-based microneutralization assay (CPE-MN) was performed[1,20]. Briefly, nAbs were co-incubated with a SARS-CoV-2 viral solution containing 100 median Tissue Culture Infectious Dose (100 $TCID_{50}$) of virus for 1 hour at 37°C, 5% CO_2. The mixture was then added to the wells of a 96-well plate containing a sub-confluent Vero E6 cell monolayer. Plates were incubated for 3-4 days at 37°C in a humidified environment with 5% CO_2, then examined for CPE by means of an inverted optical microscope by two independent operators. All nAbs were tested at a starting dilution of 1:8, diluted step 1:2, and the IC_{100} evaluated based on their initial concentration. Technical duplicates for each experiment were performed. In each plate positive and negative control were used as previously described[1,20].

SARS-CoV-2 virus variants CPE-MN neutralization assay

The SARS-CoV-2 Omicron (B.1.1.529) virus used to perform the CPE-MN neutralization assay was supplied and sequenced by the NRC UZ/KU Leuven (Leuven, Belgium). Sequence was deposited on

GISAID with the following ID: EPI_ISL_6794907.

SARS-CoV-2 S protein competition assay

Competitive Flow cytometry-based assay was performed to characterize nAbs binding profiles to SARS-CoV-2 S-protein as previously described[1]. Briefly, magnetic beads (Dynabeads His-Tag, Invitrogen) were covered with His-tagged S-proteins, following manufacturers' instructions. Then, 40 mg/mL of beads-bound-S-protein were incubated with unlabeled nAbs for 40 minutes at RT. Following incubation, samples were washed with PBS and incubated with fluorescently labeled Class 1/2 (J08-A647), Class 3 (S309-A488) or Class 4 (CR3022-A647) S-protein nAbs binders. Antibodies labelling was performed using Alexa Fluor NHS Ester kit (Thermo Scientific). Following 40 minutes of incubation at RT, beads-antibodies mix was washed with PBS, resuspended in 150 µL of PBS-BSA 1% and acquired using BD LSR II flow cytometer (Becton Dickinson). Results were analyzed using FlowJo™ Software (version 10). Beads with or without S-protein incubated with labeled antibodies were used as positive and negative controls respectively.

SARS-CoV-1 S protein binding assay

Expi293F cells (Thermo Fisher) were transiently transfected with SARS-CoV-1 S-protein expression vectors (pcDNA3.3_CoV1_D28) using Expifectamine Enhancer according to the manufacturer's protocol (Thermo Fisher). Two days later, to exclude dead cells from analysis, Expi293F were harvested, dispensed into a 96-well plate ($3x10^5$ cell/well), and stained for 30 minutes at room temperature (RT) with Live/Dead Fixable Aqua reagent (Invitrogen; Thermo Scientific) diluted 1:500. Following Live/Dead staining, cells were washed with PBS and incubated with nAbs candidates for 40 minutes at RT. Next, to identify the SARS-CoV-1 S protein mAbs binders, cells were washed and stained with the Alexa Fluor 488-labelled secondary antibody Goat anti-Human IgG (H+L) secondary antibody (Invitrogen) diluted 1:500. After 40 minutes of incubation, labeled cells were washed, resuspended in 150 µL of PBS and analyzed using the BD LSR II flow cytometer (Becton Dickinson). Cells incubated with the SARS-CoV-1 nAb binder (S309) or incubated only with the secondary antibody were used as positive and negative controls respectively. Data were analyzed with FlowJo™ Software (version 10).

HEK293TN- hACE2 cell line generation

HEK293TN- hACE2 cell line was generated by lentiviral transduction of HEK293TN (System Bioscience) cells as described in Notarbartolo S. et al.[32]. Lentiviral vectors were produced following a standard procedure based on calcium phosphate co-transfection with 3rd generation helper and transfer plasmids. The transfer vector pLENTI_hACE2_HygR was obtained by cloning of hACE2 from pcDNA3.1-hACE2 (a

gift from Fang Li, Addgene #145033) into pLenti-CMV-GFP-Hygro (a gift from Eric Campeau & Paul Kaufman, Addgene #17446). pLENTI_hACE2_HygR is now made available through Addgene (Addgene #155296). HEK293TN-hACE2 cells were maintained in DMEM, supplemented with 10% FBS, 1% glutamine, 1% penicillin/streptomycin and 250 µg/ml Hygromicin (GIBCO).

Production of SARS-CoV-1 pseudoparticles

SARS-CoV1 lentiviral pseudotype particles were generated as described in Conforti et al. for SARS-CoV-2[33]. SARS-CoV1 SPIKE plasmid pcDNA3.3_CoV1_D28 is a gift from a gift from David Nemazee (Addgene plasmid # 170447).

SARS-CoV-1 neutralization assay

For neutralization assay, HEK293TN-hACE2 cells were plated in white 96-well plates in complete DMEM medium. 24h later, cells were infected with 0.1 MOI of SARS-CoV-1 pseudoparticles that were previously incubated with serial dilution of purified or not purified (cell supernatant) mAb . In particular, a 7-point dose-response curve (plus PBS as untreated control), was obtained by diluting mAb or supernatant respectively five-fold and three-fold. Thereafter, nAbs of each dose-response curve point was added to the medium containing SARS-CoV-1 pseudoparticles adjusted to contain 0.1 MOI. After incubation for 1h at 37°C, 50 µl of mAb/SARS-CoV-1 pseudoparticles mixture was added to each well and plates were incubated for 24h at 37°C. Each point was assayed in technical triplicates. After 24h of incubation cell infection was measured by luciferase assay using Bright-Glo™ Luciferase System (Promega) and Infinite F200 plate reader (Tecan) was used to read luminescence. Obtained relative light units (RLUs) were normalized to controls and dose response curve were generated by nonlinear regression curve fitting with GraphPad Prism to calculate Neutralization Dose 50 (ND_{50}).

Functional repertoire analyses

nAbs VH and VL sequence reads were manually curated and retrieved using CLC sequence viewer (Qiagen). Aberrant sequences were removed from the data set. Analyzed reads were saved in FASTA format and the repertoire analyses was performed using Cloanalyst (http://www.bu.edu/computationalimmunology/research/software/)[34,35].

Statistical analysis

Statistical analysis was assessed with GraphPad Prism Version 8.0.2 (GraphPad Software, Inc., San Diego, CA). Nonparametric Mann-Whitney t test was used to evaluate statistical significance between the two groups analyzed in this study. Statistical significance was shown as * for values ≤ 0.05, ** for values ≤ 0.01, *** for values ≤ 0.001, and **** for values ≤ 0.0001.

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Declarations

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Author contributions

E.A. and R.R. conceived the study. I.P. performed binding and competition assays. N.M. expressed monoclonal antibodies. P.P. and E.A. performed repertoire analyses. E.P. and V.A. produced and purified SARS-CoV-2 constructs. E.A., I.P., G.Pie., G.Pic., L.B. and G.G. performed live SARS-CoV-2 neutralization assays. P.M. isolated SARS-CoV-2 B.1.1.529 (Omicron) virus. M.L. propagated SARS-CoV-2 viruses. S.M. and L.D. performed SARS-CoV-1 pseudotype neutralization assays. C.D.S. supported day-to-day laboratory activities and management. E.A. and R.R. wrote the manuscript. E.A., I.P., L.D., S.M., G.Pie., N.M., E.P., V.A., P.P., L.B., G.G., M.L., P.M., C.D.S., G.Pic., C.S., R.D.F., E.M. and R.R. undertook the final revision of the manuscript. E.A., C.S., R.D.F., E.M. and R.R. coordinated the project.

Competing interests

R.R. is an employee of GSK group of companies. E.A., I.P., N.M., P.P., E.P., V.A., C.D.S., C.S. and R.R. are listed as inventors of full-length human monoclonal antibodies described in Italian patent applications n. 102020000015754 filed on June 30th 2020, 102020000018955 filed on August 3rd 2020 and 102020000029969 filed on 4th of December 2020, and the international patent system number PCT/IB2021/055755 filed on the 28th of June 2021. All patents were submitted by Fondazione Toscana Life Sciences, Siena, Italy. R.D.F. is a consultant for Moderna on activities not related to SARS-CoV-2. Remaining authors have no competing interests to declare.

Additional information

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Data availability

Source data are provided with this paper. All data supporting the findings in this study are available within the article or can be obtained from the corresponding author upon request.

Figures

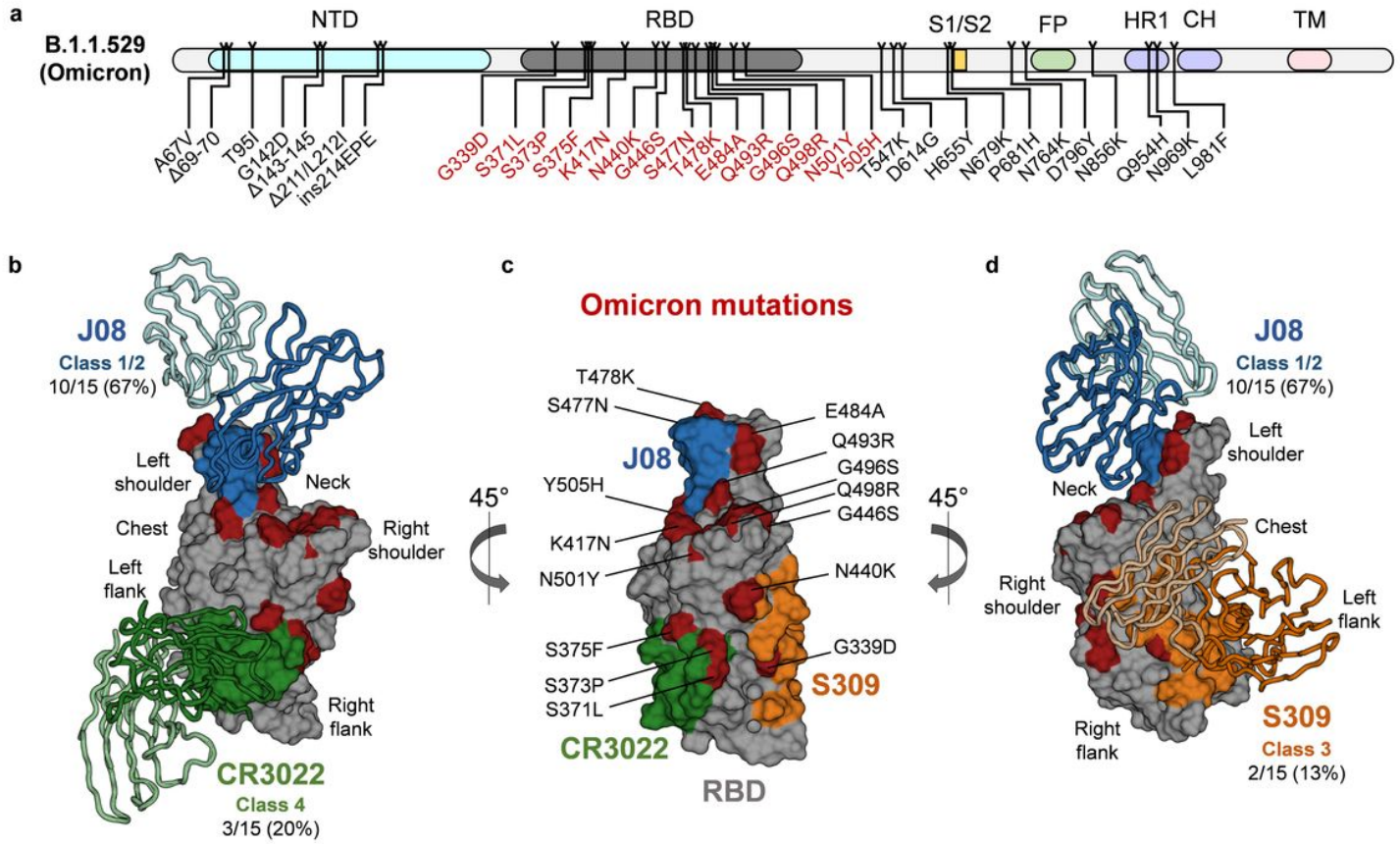

Figure 1

Distribution of Omicron mutations on SARS-CoV-2 RBD. a, The graph shows the Omicron S gene mutations based on the viral strain GISAID accession ID: EPI_ISL_6794907. NTD, RBD, S1/S2 cleavage site, fusion peptide (FP), heptad repeat 1 (HP1), center helix (CH) and transmembrane domain (TM), are shown in light cyan, dark gray, yellow, light green, light violet and light pink respectively. **b-d**, Central panel shows the RBD harboring all 15 Omicron mutations (highlighted in dark red). J08 (representing Class 1/2 nAbs), S309 (representing Class 3 nAbs) and CR3022 (representing Class 4 nAbs) epitopes are highlighted in light blue, orange and green respectively (**c**). Left panel shows RBD rotated by 45 degrees on the right and the distribution of Omicron mutations on Class 1/2 and Class 4 epitope regions are shown (**b**). Right panel shows RBD rotated by 45 degrees on the left and the distribution of Omicron mutations on Class 1/2 and Class 3 epitope regions are shown (**d**).

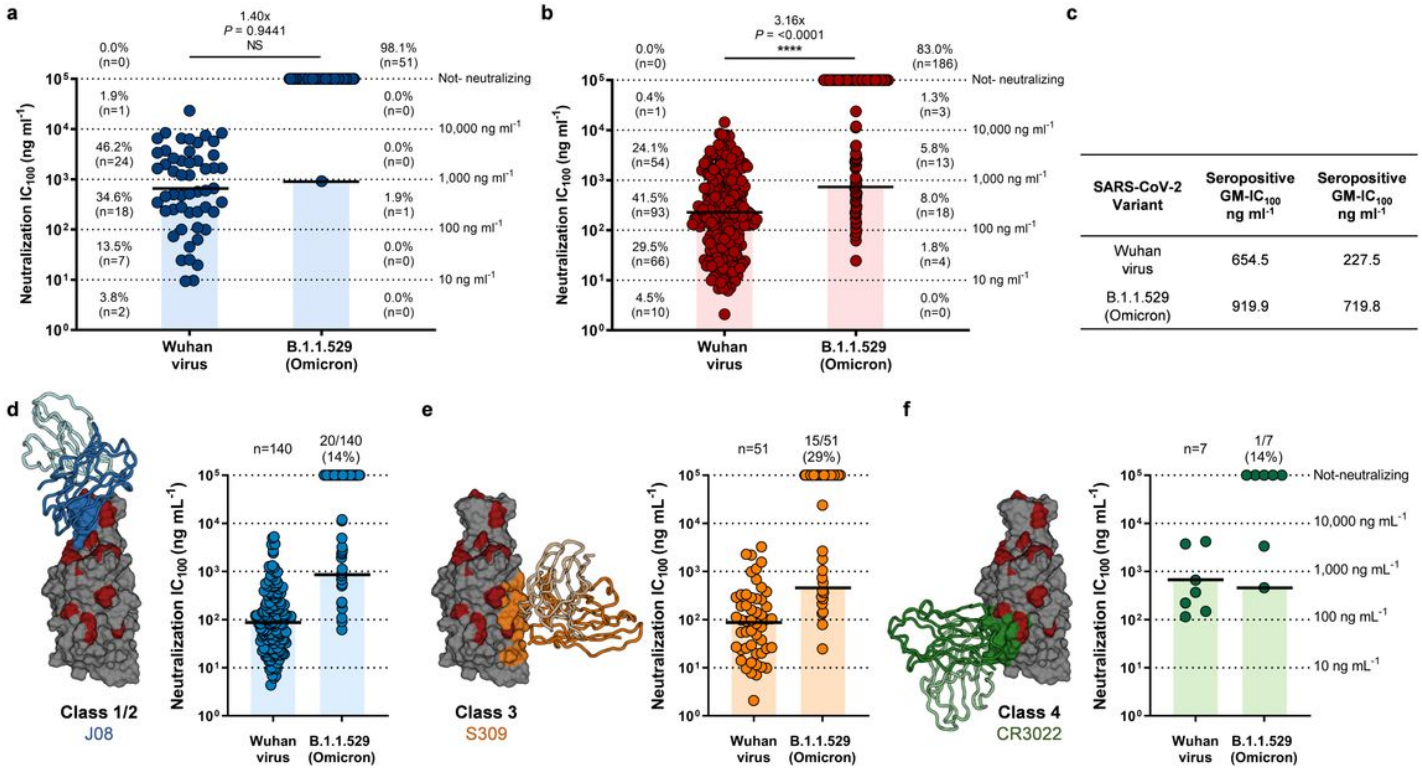

Figure 2

Functional characterization of Omicron nAbs. a-b, Dot charts show the neutralization potency, reported as IC_{100} (ng ml^{-1}), of nAbs tested against the original SARS-CoV-2 virus first detected in Wuhan and the B.1.1.529 (Omicron) VoC for seronegatives (**a**) and seropositives (**b**). The number and percentage of nAbs from individuals who were seronegative versus seropositive, fold change, neutralization IC_{100} geometric mean (black lines, blue and red bars) and statistical significance are denoted on each graph. Technical duplicates were performed for each experiment. A non-parametric Mann–Whitney t-test was used to evaluate statistical significances between groups. Two-tailed P value significances are shown as *P < 0.05, **P < 0.01, ***P < 0.001. NS, not significant. **c**, The table shows the IC_{100} geometric mean (GM) of all nAbs pulled together from each group against the two tested viruses. **d-f**, Dot charts show the distribution of Class 1/2 (**d**), Class 3 (**e**) and Class 4 (**f**) nAbs against the original SARS-CoV-2 virus first detected in Wuhan and the B.1.1.529 (Omicron) VoC. The number and percentage of nAbs and neutralization IC_{100} geometric mean (black lines, light blue, orange and green bars) are denoted on each graph.

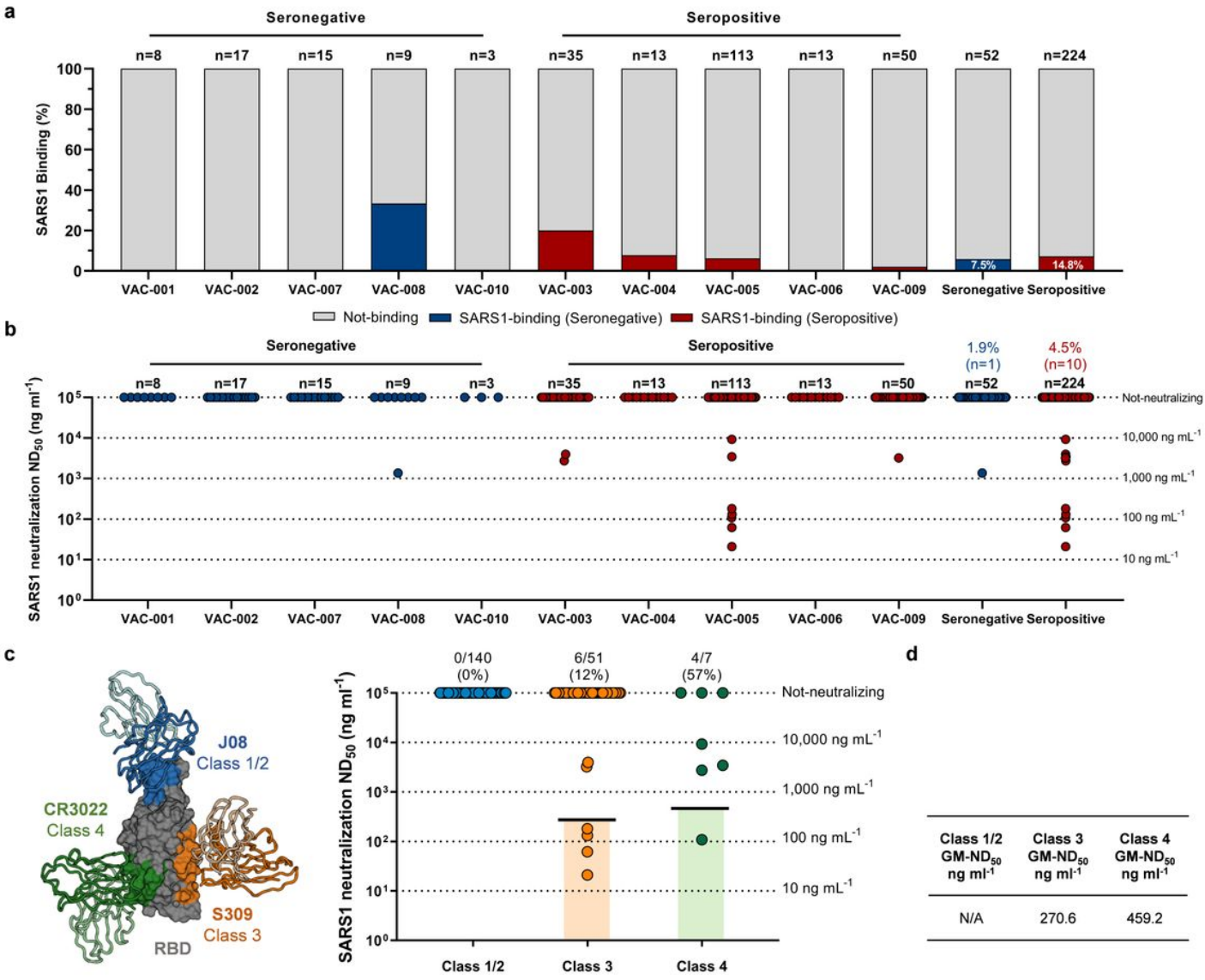

Figure 3

Functional characterization of SARS1 nAbs. a, The bar graph shows the percentage of not-binding antibodies (grey), SARS1-binding nAbs from individuals who were seronegative (dark blue) and SARS1-binding nAbs for individuals who were seropositive (dark red). The total number (n) of antibodies tested per individual is shown on the top of each bar. **b**, Dot chart shows the neutralization potency, reported as ND_{50} (ng ml^{-1}), of nAbs isolated from seronegatives and seropositives against SARS1. The number and percentage of nAbs from individuals who were seronegative and seropositive and neutralization ND_{50} (ng ml^{-1}) ranges (black dotted lines) are denoted on the graph. **c-d**, dot chart shows Class 1/2, Class 3 and Class4 distribution of SARS1 nAbs (**c**). Neutralization potencies, reported as ND_{50} (ng ml^{-1}), and geometric mean (black line) are denoted on the graph. The table shows the ND_{50} (ng ml^{-1}) geometric mean (GM) of all nAbs pulled together from each group against SARS1. Technical triplicates were performed for each experiment (**d**).

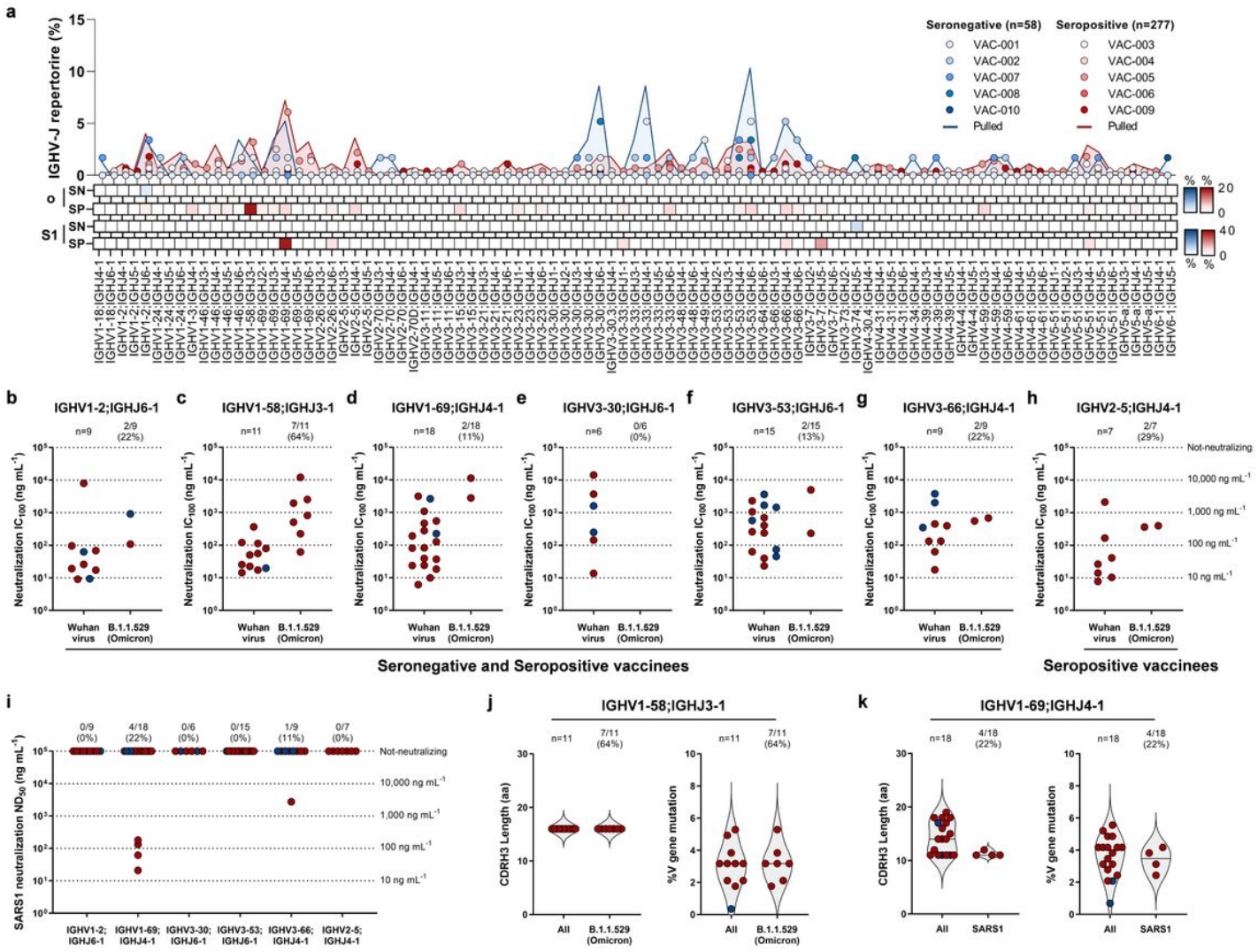

Figure 4

Genetic characterization of Omicron and SARS1 nAbs. a, The graph shows the IGHV-J rearrangement frequencies between vaccinees who were seronegative (SN) and seropositive (SP) (top), and the frequency within Omicron (o) (middle) and SARS1 (S1) (bottom) nAbs. **b–i**, The graphs show the neutralization potency (IC_{100}) of IGHV1-2;IGHJ6-1 (**b**), IGHV1-58;IGHJ3-1 (**c**), IGHV1-69;IGHJ4-1 (**d**), IGHV3-30;IGHJ6-1 (**e**), IGHV3-53;IGHJ6-1 (**f**), IGHV3-66;IGHJ4-1 (**g**) and IGHV2-5;IGHJ4-1 (**h**) gene derived nAbs, against the original SARS-CoV-2 virus first detected in Wuhan and the B.1.1.529 (Omicron) VoC. The graph shows the neutralization potency (ND_{50}) of predominant gene derived nAbs against SARS1 (**i**). Numbers and percentages of nAbs tested for each germline and neutralization ranges (black dotted lines) are denoted on each graph. **j-k**, violin plots show differences in the aminoacidic (aa) CDRH3 length and percentage of V gene somatic mutations for all IGHV1-58;IGHJ3-1 (**j**) and IGHV1-69;IGHJ4-1 (**k**) compared to Omicron and SARS1 nAbs respectively.

Supplementary Files

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