Monoclonal antibodies binding data for SARS-CoV-2 proteins

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Data Article

Monoclonal antibodies binding data for SARS-CoV-2 proteins

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\textbf{A B S T R A C T}

SARS-CoV-2 pandemic opens up the curiosity of understanding the coronavirus. This demand for the development of the regent, which can be used for academic and therapeutic applications. The present data provide the biochemical characterisation of synthetically developed monoclonal antibodies for the SARS-CoV-2 proteins. The antibodies from phage-displayed antibody libraries were selected with the SARS-CoV-2 proteins immobilized in microwell plates. The clones which bind to the antigen in Fab-phage ELISA were selected, and a two-point competitive phage ELISA was performed. Antibodies binding kinetic of Igs for SARS-CoV2 proteins further carried with B.L.I. Systematic analysis of binding with different control proteins and purified SARS-CoV-2 ensured the robustness of the antibodies.

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Specifications Table

| Subject                        | Biochemistry |
|-------------------------------|--------------|
| Specific subject area         | Synthetic developed antibodies for the SARS-COV-2 using Phage-displayed Fab and Antibodies characterization |
| Type of data                  | Figures and Table |
| How the data were acquired    | Isolated Fab-phage clones specific for for SARS CoV-2 proteins were used for a two-point competitive phage ELISA. The EC50 values were estimated by fitting the data using standard four-parameter logistic equations in GraphPad Prism (GraphPad Software, La Jolla, CA). Clones with higher bind were used to generate the human IgG backbone. The binding kinetics of antibodies were validated by BLI instrument on an Octet H.T.X. instrument (Sartorius) at 1000 rpm and 25°C. Binding response data were generated by subtracting reference buffer and were globally fitted with a 1:1 binding model using ForteBio's Data Analysis software 9.0. The antibodies were further validated by western and Immunofluorescence Staining on SARS-CoV-2 infected cells. |
| Data format                   | Analyzed and Filtered |
| Description of data collection| SARS-CoV-2 proteins were purified, with or without tags. The proteins were used as the antigen for the synthetic antibodies generated by the Fabs-phage display library. The M.B.P. and G.S.T. tags were used as the negative control during screening and selections. The binding affinity and IgG binding kinetic were estimated for the antibodies. For ELISA binding, purified proteins from SARS were coated, and Fabs-Phage or IgGs were used. The EC50 for antibodies was determined. The binding kinetics were estimated by the Biolayer Interferometry (B.L.I.). The AR2G biosensors were used where proteins were covalently cross-linked, and the association and dissociation of IgG analyzed |
| Data source location         | • Institution: Washington University and The Donnelly Centre, University of Toronto |
|                              | • City/Town/Region: Saint Louis and Toronto |
|                              | • Country: U.S.A. and Canada |
| Data accessibility           | Direct link to datasets: https://data.mendeley.com/datasets/9hns9ss6ny/9 |
|                              | Repository Name: Mendeley Data |
|                              | Data identification number: 10.17632/9hns9ss6ny.9 |
| Related research article     | N. Mishra, J. Teyra, R. Boytz et al., Development of monoclonal antibodies to detect for SARS-CoV-2 proteins, Journal of Molecular Biology, Volume 434, Issue 10, 2022,167583, ISSN 0022-2836, https://doi.org/10.1016/j.jmb.2022.167583. |

Value of the Data

- Antibodies are high-value reagents. People use antibodies in many biological assays like co-localization, identification of cellular markers, and FACS-based techniques to understand the function of different biomolecules. Here we describe the validation of phage-display derived antibodies. Fabs were generated and selected using control proteins, and higher binding to the antigens was selected in the study. Data shown in this study show that Fabs-phage generated during selection has a specific binding for the selected antigens, including several high affinity binders. Biochemical assays coupled with western blot and immunofluorescence analysis further validate these reagents and their usefulness.
• The data in this study will benefit the researchers working in the field of immunology, antibody designing and development, and immune-based study for SARS-CoV2. SARS-CoV2 is a recently identified virus. There are many antibodies for the spick proteins but lack antibodies for other viral proteins, which limits understanding the SARS CoV2 biology and pathogenesis. In this study, we generated antibodies to understand and characterize the host translation shutdown function of NSP1 and the replication function of NSP8 and NSP12. The antibodies could be used for the protein interaction and microscopic to understand the role of SARS-CoV2. We had shown in the study how two different antibodies of NSP1 bind to two different regions in NSP1\(^3\). Using those antibodies, the function of N-terminal and C-terminal NSP1 could be studied. Similarly, for NSP8 and NSP12, we had shown how NSP8 antibodies detected the NSP 8 proteins in western, which could not be stained in SARS-infected cells \(3\). By exploring the information’s using the antibodies, we could understand the structure or conformation of the molecules which will aid in understanding the molecule mechanism of viral biology.

• There are limited antibodies for the SARS virus proteins. Our work was in the direction to generate antibody resources that could be used to understand the biology of SARS-Cov2. We have generated many antibodies in the process. The data show confidence in the quality of antibodies. The information about binding kinetic and application assay could provide a basis for further optimization to develop antibodies with enhanced affinity and specificity. In their current form, these reagents can be used to assess SARS-CoV-2 spread and the presence of specific viral proteins in samples derived from in vitro and in vivo viral infections. Thus, these reagents have the ability to provide a unique window into previously unknown host-pathogen interactions.

1. Data Description

The data presented here for phage-display derived antibodies that recognize distinct proteins from SARS-CoV-2. The Synthetic antibodies were generated using a specific Fab-phage display library and selected for specific clones with high specificity to bind SARS-CoV-2 proteins. Initial selection followed by selective optimization of complementarity-determining regions (CDRs) can provide a facile and rapid means to develop highly specific antibodies. This study highlights the utility and the potential impact of using phage display to generate antibodies as well as methods for evaluation, screening, and characterization of new antibodies Figs 1–4 and Table 1.

![Fig. 1.](image-url) illustrates the schematic workflow of antibodies generational and characterizations. Fab-phage clone libraries were generated in the published paper [1]. The synthetic antigen-binding fragment (Fab) library focused on the human Fab frame’s three heavy-chain C.D.R.s and CDR-L3.

2. Experimental Design, Materials and Methods

2.1. SARS-CoV-2 proteins purification

Glutathione S-transferase (G.S.T.) or maltose–binding protein (M.B.P.) tagged proteins of SARS-CoV-2 were expressed in BL21 (DE3) Escherichia coli cells (Novagen). The bacteria were induced with 0.5 mM of IPTG (isopropyl-β-thiogalactopyranoside) at 0.6 (measured at 600 nm), optical
Fig. 2. Heat map representation of Fab-phage clone numbers and binding signals to their cognate antigen. ELISAs are compared to negative controls (GST or MHT and BSA) and the data were represented as intensities ranging from high (green) to low (yellow).

| Fab Code | IC50 (nM) | Pos | Neg | Number |
|----------|-----------|-----|-----|--------|
| FAB_A_D05 | 730       | 0.63 | 0.15 | 1      |
| FAB_C2_H03 | 113       | 0.94 | 0.12 | 3      |
| FAB_C2_H04 | 739       | 0.93 | 0.13 | 1      |
| FAB_C2_H07 | 257       | 0.9  | 0.12 | 1      |
| FAB_C2_H08 | 102       | 0.91 | 0.18 | 4      |
| FAB_C2_H09 | 163       | 0.95 | 0.14 | 1      |
| FAB_B_A06 | 85        | 0.96 | 0.1  | 3      |
| FAB_B_A02 | 55        | 1.14 | 0.1  | 14     |
| FAB_B_B11 | 55        | 1.08 | 0.11 | 1      |
| FAB_B_B12 | 65        | 0.95 | 0.12 | 2      |
| FAB_B_A12 | 60        | 0.98 | 0.11 | 4      |
| FAB_B_B09 | 177       | 1    | 0.1  | 1      |
| FAB_B_B02 | 29        | 0.96 | 0.1  | 3      |
| FAB_B_B04 | 5.5       | 1.09 | 0.1  | 2      |
| FAB_B_C09 | 46        | 1.04 | 0.11 | 1      |
| FAB_B_D03 | 9.5       | 0.99 | 0.1  | 1      |
| FAB_B_D11 | 23        | 1.06 | 0.1  | 1      |
| FAB_B_E08 | 8.8       | 0.81 | 0.19 | 1      |
| FAB_B_E12 | 1.4       | 0.81 | 0.11 | 1      |
| FAB_B_G10 | 8.5       | 0.85 | 0.16 | 1      |
| FAB_B_H10 | 13        | 0.84 | 0.21 | 1      |
| FAB_B_F10 | 8.4       | 0.85 | 0.11 | 1      |
| FAB_B_E04 | 26        | 0.79 | 0.11 | 16     |
| FAB_B_F05 | 12        | 0.94 | 0.42 | 2      |
| FAB_B_G03 | 1.2       | 0.92 | 0.4  | 1      |
| FAB_B_H05 | 34        | 0.83 | 0.41 | 1      |

Phage ELISA binding of Fab-phage clones IC50, Positive and negative signal of ELISA, and the number of clones for Nsp1, Nsp8, and Nsp12.
Fig. 3. ELISA profile for the selected clone for which Fabs were generated. Out of different Positive Phase Fab clones, some with higher EC50 were selected for the generation of Fab, and later humanized IgGs were generated.
density, and grown for 12–15 h at 18°C. Cells were harvested and lysed using an EmulsiFlex-C5 homogenizer (Avestin), a sonicator, or a cell disruptor apparatus (1.7 kBar) in buffer (25 mM Tris (pH 7.5), 150 mM NaCl, 20 mM imidazole, and 5 mM 2-mercaptoethanol). Lysed cells were centrifugation at 30,000 × g at 4°C for 40 min, and the clear supernatant was filtered using 0.4 uM filter. MBP-fused proteins were purified using an MPTrap HP column (G.E. Healthcare) and ion-exchange chromatography. GST-fused proteins were purified using glutathione Sepharose 4 Fast Flow media (G.E. Healthcare) to the manufacturer’s protocols. Later the purified proteins were run through a size exclusion column before further use.

2.2. Selection and characterization of Fabs

Synthetic Fab-phage clones library was designed by introducing binary Tyr/Ser diversity into the three heavy-chain C.D.R.s (CDR-H1, -H2, and -H3) and the third C.D.R. of the light chain (CDR-L3) [1,2]. The generated library was assed for specificity by ELISAs against the set of antigens from SARS-CoV-2. About 48 Fab-phage clones were amplified per antigen, antibody variable domains of clones were sequenced by PCR amplification, and unique clones were selected. Affinities of the individual antigen-binding clone were estimated by two-point competitive phase ELISA using 200 nM or 50 nM antigen. Diluted phage supernatant in PBS buffer was incubated with antigen for an hour. The mixtures were transferred to an antigen-coated plate. After 15 min the plates were washed and incubated with horseradish peroxidase/anti-M13 antibody conjugate (1:5000 dilution in PBT buffer) T.M.B substrate (K.P.L. Labs, Gaithersburg, MD) was added to develop. For quenching the reaction 0.5 M H2SO4 was used and absorbance was taken at 450 mM. The fraction of phage not bound to solution-phase antigen was determined as the ratio of phage captured was determined from the fraction of phage not bound to the solution-phase antigen. Fab clones showing the lowest signal ratio were used to make IgG format for future characterizations [2].

2.3. IgG proteins purification

The variable domain D.N.A. was subcloned into pSCSta-hlg1 and pSCST1-hk vectors. According to the manufacturer’s instructions, vectors for the heavy and light chains were transfected
into HEK293F cells (Invitrogen, Grand Island, NY) using FectoPro (Polyplus Transfection, NY). Cell cultures were incubated for 4–5 days, the supernatant was collected, and protein-A affinity (~2 mL packed beads per 600 mL culture) (Pierce, ThermoScientific, Rockford, IL) purifications were carried out. IgG proteins were eluted and buffer exchanged on PBS, pH 7.4.

2.4. IgG binding ELISA

2 μg/mL protein or GST/MHT control proteins in PBS pH 7.4 were used to coat 384-well microplates overnight at 4 °C. The plates were blocked with 0.2% B.S.A. for an hour and washed 4 times with 0.05% Tween in PBS (PBT). Serial dilution of IgGs were made in PBT and transferred to the antigen-coated plates and the plates were incubated at room temperature for 30 min, washed with PBST four times, and incubated for 30 min with anti-β-HRP antibody conjugate (1:7500 dilution in PBT). The plate was then used for read. The data fit standard four-parameter logistic equations using GraphPad Prism (GraphPad Software, La Jolla, CA), and EC<sub>50</sub> values were estimated. The analyzed raw data for EC50 are included in the manuscript [3].

2.5. Binding Kinetics

B.L.I. experiments were at 1000 rpm and 25°C using Octet H.T.X. instrument (Sartorius). SARS-CoV-2 antigens (2–20 μg/ml) were covalently captured on AR2G biosensors (Sartorius, 18-5092) with N-hydroxysulfosuccinimide (N.H.S.) esters on the biosensors. The reactions were quenched for 600 s by 1M ethanolamine (pH 8.5) [4,5]. Covalently linked biosensor probes were equilibrated with assay buffer (PBS, 1% B.S.A., 0.05% Tween 20), for association the probes were dipped for 600 s into wells containing serial 3-fold dilutions of IgGs. After association, the probes were transferred back into assay buffer for 600 s of dissociation. The binding responses were analyzed by subtracting data from reference, and data were globally fitted with a 1:1 binding model using ForteBio’s Data Analysis software 9.0. The analyzed raw data for BLI are included in the manuscript [3].

2.6. Western blot analyses

To check the specificity of antibodies, western blots were carried out. 0.3 μg of purified SARS-CoV-2 and Maltose Binding Protein (M.B.P.) was loaded onto a pre-cast 15% SDS-PAGE gel, including a Precision Plus Protein Dual Color Standard (Bio-Rad). After gel electrophoresis (running buffer: 25 mM Tris HCl, 193 mM glylcine, 0.1% sodium dodecyl sulfate (S.D.S.), pH 8.3), the blots were transferred onto nitrocellulose membranes (Bio-Rad) (transfer buffer: 20% methanol, 25 mM Tris HCl, 193 mM glyccine) at 4 °C 2 h. The transferred membrane was blocked in 5% skim milk for 60 min, and the membrane was incubated in synthetic antibody (primary antibody) for 2 h. Secondary Rabbit anti-mouse IgG H.R.P. conjugated (Cruz Biotechnologies) was used for 1 h. They were then washed and incubated with H.R.P. substrate (G.E. Healthcare Life Sciences, Pittsburgh, PA) for 1 min before recording the chemiluminescent signal using a ChemiDoc MP gel imaging system (G.E.).

Ethics Statements

No involvement of human subjects, animal experiments, and data collected from social media platforms had carried out for this work.
CRediT Author Statement

Nawneet Mishra and Joan Teyra: Conceptualization and Investigation of the study, and original draft preparation; Ruthmabel Boytz, Trudy N. Merritt, Lia Cardarelli, Maryna Gorelik, Filip Mihalic, Per Jemth: Investigation; Shane Miersch: Project administration and supervision; Robert Davey, Sachdev S. Sidhu, Daisy W. Leung and Gaya K. Amarasinghe: Conceptualization, supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Monoclonal antibodies binding data for SARS-CoV-2 proteins (Original data) (Mendeley Data).

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