Development of Bioluminescent Cell-based Assay Platforms for Quantitative Measurement of ADCC/ADCP Activities for SARS-CoV-2 Antibodies

Jun Wang, Pete Stecha, Jim Hartnett, Frank Fan, Mei Cong and Zhijie Jey Cheng
Promega Corporation, 2800 Woods Hollow Rd, Madison, WI, 53711

1. Introduction

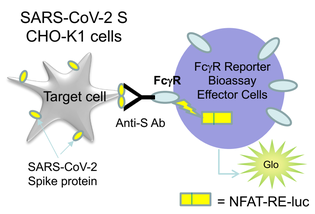
SARS-CoV-2 is a newly emerged coronavirus responsible for the current COVID-19 pandemic. SARS-CoV-2 relies on its surface spike protein to bind to human host cell receptor angiotensin-converting enzyme 2 (ACE2) which is a step critical for viral entry, and thus spike protein has been the main target of antiviral mAb therapy and vaccine development. However, the mechanisms of anti-spike neutralizing antibodies are still not fully understood. Besides neutralization by blocking its interaction with ACE2, anti-spike antibodies may have additional anti-viral activities mediated by Fc domain, including antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). Therefore, it is very important to develop quantitative methods to help understand how anti-spike antibodies play protective and potential pathogenic roles to guide drug design and clinical development.

Previously we developed two cell-based assay platforms for quantitative measurement of antibody Fc functions: FcγR reporter bioassays using engineered reporter effector cell lines to measure antibody ADCC and ADCP activities mediated by activating FcγRs (FcγRI, FcγRIIa, FcγRIIIa), and an improved PBMC ADCC cytotoxicity assay using primary PBMC and engineered HiBiT-target cells. To specifically measure the Fc functions for SARS-CoV-2 spike antibodies, we further developed two engineered Spike protein-expressing stable cell lines. A panel of commercial recombinant human or chimeric anti-spike antibodies were selected and evaluated in the study, with some of their sequences originally derived from patients recovered from COVID-19 infection. Four anti-spike antibodies including three anti-S1 Ab and an anti-S2 Ab showed positive ADCC and ADCP reporter activities when a CHO-K1 cell line stably expressing SARS-CoV-2 spike protein was used as target cells in reporter-based ADCC and ADCP assays. Similar results and Fc functions were confirmed for both anti-S1 and anti-S2 Abs in PBMC ADCC assay using a CHO-K1 cells stably expressing SARS-CoV-2 spike protein and a HiBiT protein to serve as an indication of target cell lysis when being released and detected in the medium after ADCC occurs. Our results demonstrate that these two ADCC assay platforms combined with engineered spike-expressing target cells can potentially serve as valuable tools to help understand Fc-mediated effector functions for anti-SARS-CoV-2 spike antibodies in therapeutic drug development and also from the patient's samples after vaccine administration.

2. Development of Two ADCC/ADCP Assay Platforms

A. FcγR Reporter Bioassays using engineered reporter effector cells and engineered target cells stably expressing SARS-CoV-2 Spike protein

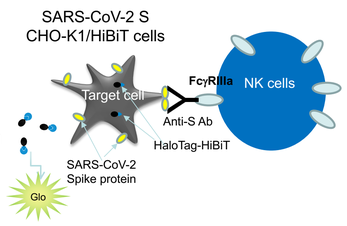
1) Anti-spike antibody binds to S protein on target cells and FcγR* on FcγR Reporter Bioassay Effector Cells simultaneously.
2) It leads to the activation of FcγR receptor and luciferase activation in the reporter effector Cells.



*ADCC Reporter Bioassay: FcγRIIIa
*ADCP THP-1 Reporter Bioassay: FcγRIIa, FcγRI

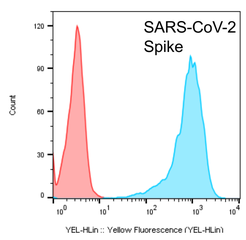
B. PBMC ADCC Assay using primary PBMC and engineered target cells stably expressing SARS-CoV-2 Spike protein and HaloTag-HiBiT

1) Anti-spike antibody binds to S protein on target cells and FcγRIIIa on NK cells simultaneously.
2) When ADCC occurs, the lysis of target cells releases HiBiT protein into the medium which can be detected by NanoLuc HiBiT Extracellular Detection reagent.



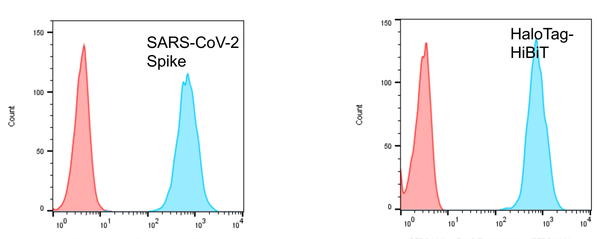
3. Development of Target Cell Lines Stably Expressing SARS-CoV-2 Spike Protein

A. SARS-CoV-2 Spike CHO-K1 Cells



SARS-CoV-2 Spike CHO-K1 Cells were labeled with anti-SARS-CoV-2-S protein antibody (Sino Biological, cat# 40150-D001)

B. SARS-CoV-2 Spike CHO-K1 (HaloTag-HiBiT) Cells



SARS-CoV-2 Spike CHO-K1 (HaloTag-HiBiT) Cells were labeled with left: anti-SARS-CoV-2-S protein antibody (Sino Biological, cat# 40150-D001) or right: Janelia Fluor® 646 HaloTag Ligand (Promega, cat# GA1120)

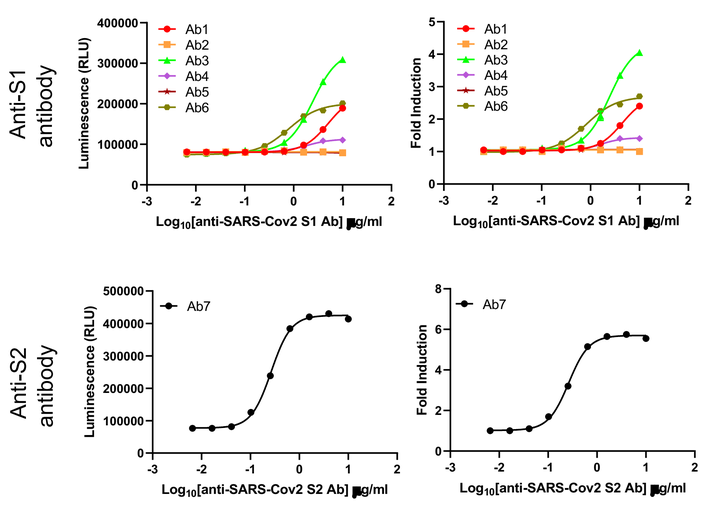
4. Commercial SARS-CoV-2 Antibodies Evaluated in the Study

- Seven commercial antibodies were selected in the study.
- Ab1-6 target S1 subunit or target RBD directly, while Ab7 targets S2 subunit.
- Ab1, 3, 4, 5, 6 are reported to have neutralization activities by the vendor or by the reference paper.
- Ab1-6 are human IgG1, and Ab7 is a chimeric Ab.

Test Ab	anti-SARS-CoV-2 S Ab	vendor	CAT#	IgG isotype	Specificity	Neutralization Activity	Reference
1	Clone 105-9	Biolegend	938501	Human IgG1	S1	Nab, ND50: 0.2-0.8 µg/mL	1
2	Clone 415-6	Biolegend	938601	Human IgG1	RBD	No	1
3	Clone 414-1	Biolegend	938701	Human IgG1	RBD	Nab, ND50: 0.03-0.12 µg/mL	1
4	Clone 414-2	Active motif	91349	Human IgG1	RBD	Nab, ND50 =28.58 nM	1
5	Clone CR3022	Absolute Antibody	Ab01680	Human IgG1	RBD	Nab	2
6	anti-Spike Ab	ACRO Biosystems	SAD-S35	Human IgG1	RBD	Nab, IC50 =1.47 µg/mL	NA
7	anti-Spike S2 Ab	Sino Biological	40590-D001	mouse/human IgG1	S2	No	NA

References:
1. Wan J, *et al. bioRxiv*. 2020 doi: https://doi.org/10.1101/2020.05.19.104117
2. Meulen, J *et al*. PLoS Med. 2006 Jul; 3(7): e237 PMID:16796401

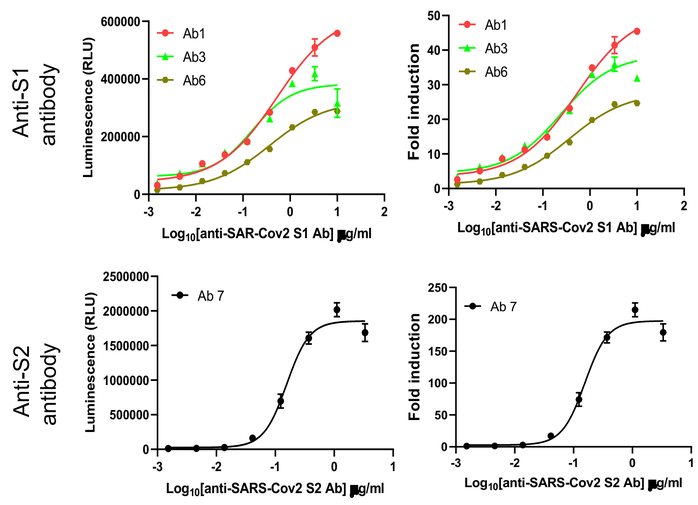
5. ADCC Reporter Activities for anti-SARS-CoV-2 Spike Antibodies



	Ab 1	Ab 2	Ab 3	Ab 4	Ab 5	Ab 6	Ab 7
EC50, mg/ml	~4.4	NA	~2.5	1.7	NA	0.8	0.3
fold	2.7	1.1	4.3	1.4	1.1	2.7	5.7

- Seven anti-SARS-CoV-2 S antibodies were tested in ADCC Reporter Bioassay using SARS-CoV-2 S CHO-K1 target cells.
- Four antibodies, Ab1, 3, 6 and 7 showed positive ADCC reporter activity.

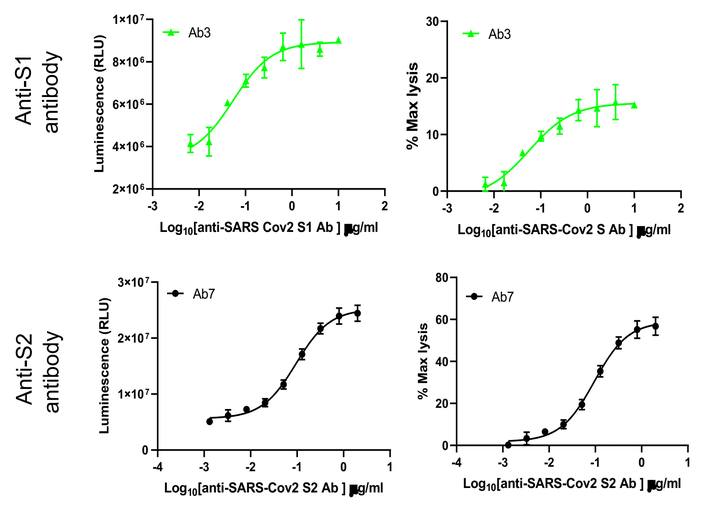
6. ADCP Reporter Activities for anti-SARS-CoV-2 Spike Antibodies



	Ab 1	Ab 3	Ab 6	Ab 7
EC50, mg/ml	~0.53	~0.16	~0.36	0.15
fold	50	40	27	198

- Four anti-SARS-CoV-2 S Antibodies, Ab1, 3, 6 and 7 were tested in ADCP THP-1 Reporter Bioassay using SARS-CoV-2 S CHO-K1 target cells.
- All four antibodies tested showed strong ADCP reporter activity.

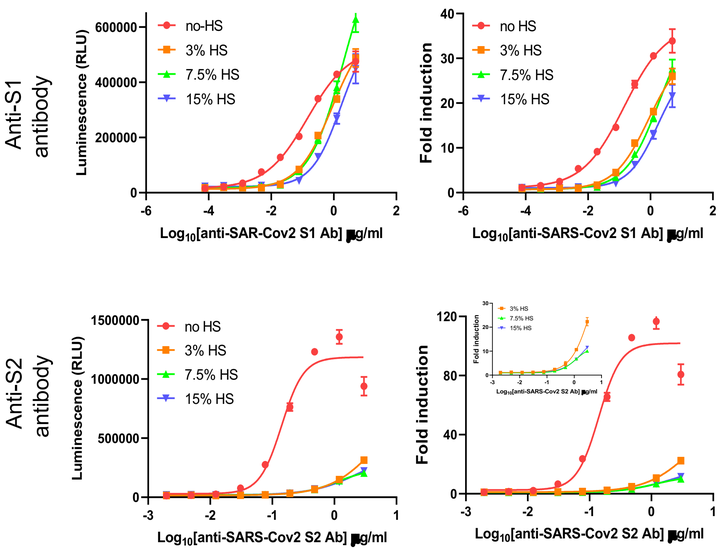
7. PBMC ADCC Activities for anti-SARS-CoV-2 Spike Antibodies



	Ab 3	Ab 7
EC50, µg/ml	0.06	0.1
% Max lysis	16%	59%

- Two anti-SARS-CoV-2 S antibodies, Ab3 and Ab7 were tested in PBMC ADCC assay using primary PBMC and SARS-CoV-2 S CHO-K1 (HaloTag-HiBiT) target cells.
- Both Ab3 and Ab7 showed positive ADCC activity.

8. Testing Human Serum Tolerance for ADCP Reporter Bioassay



Serial titration of anti-SARS-CoV-2 S Antibodies, Ab3 and Ab7 were prepared in assay buffer containing various concentrations of pooled human serum as indicated and tested in ADCP THP-1 Reporter Bioassay using SARS-CoV-2 CHO-K1 target cells.

9. Conclusions

- **We have developed two bioluminescent cell-based assay platforms for quantitively measuring Fc-mediated effector functions for SARS-CoV-2 spike antibodies.**
  1) **FcγR ADCC/ADCP Reporter Bioassays using engineered reporter effector cells and engineered target cells stably expressing SARS-CoV-2 Spike protein**
  2) **PBMC ADCC Assay using primary PBMC and engineered target cells stably expressing SARS-CoV-2 Spike protein and HaloTag-HiBiT**
- **Four out of the seven commercial SARS-CoV-2 spike antibodies tested in the study showed positive ADCC and ADCP activities, including three anti-SARS-CoV-2 S1 antibodies which were reported to have neutralization activity, and an anti-SARS-CoV-2 S2 antibody.**
- **The two ADCC/ADCP assay platforms using engineered spike-expressing target cells can be used for:**
  - **Antibody screening and potency determination during anti-SARS-CoV-2 mAb drug development.**
  - **SARS-CoV-2 spike antibodies screening in patient's samples after vaccine administration.**

Corresponding author: jey.cheng@promega.com