AI334 and AQ806 antibodies recognize the spike S protein from SARS-CoV-2 by ELISA

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Abstract

We tested 10 recombinant antibodies directed against the spike S protein from SARS-CoV-1. Among them, antibodies AI334 and AQ806 detect by ELISA the spike S protein from SARS-CoV-2.

Introduction

The spike (S) glycoprotein (UniProt #P0DTC2) mediates attachment of coronaviruses to the host ACE2 receptor (through the Receptor-Binding Domain [RBD] in the S1 subunit) and fusion with the host cell membrane (through the S2 subunit) (Yan *et al.*, 2020). Here we describe the ability of two recombinant antibodies (AI334 and AQ806) to detect by ELISA the soluble ectodomain of the S protein from SARS-CoV-2.

Materials & Methods

Antibodies: ABCD_AA831, ABCD_AF167, ABCD_AF618, ABCD_AH286, ABCD_AH287, ABCD_AH971, ABCD_AH974, ABCD_AI334, ABCD_AQ601, ABCD_AQ602, and ABCD_AQ806 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv portion fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$ (see Table 1 for clone names and references). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 5 days.

Table 1: Clone number, epitope, reference and production yields for the antibodies used in this study.

ABCD	Clone	Epitope	Reference	Yield (mg/L)
AA831	80R	S1/RBD	Sui *et al.*, 2004	100
AF167	S230.15	S1/RBD	Rockx *et al.*, 2008	80
AF618	S227.14	S1/RBD	Rockx *et al.*, 2008	80
AH286	m396	S1/RBD	Prabakaran *et al.*, 2006	120
AH287	F26G19	S1/RBD	Berry *et al.*, 2004	<5
AH974	CR3013	S1	van den Brink *et al.*, 2005	120
AI334	CR3022	S1	ter Meulen *et al.*, 2006	50
AQ601	3C7	S1/RBD	Coughlin *et al.*, 2007	50
AQ602	4D4	S1	Coughlin *et al.*, 2007	<5
AQ806	VHH-72	S1/RBD	Wrapp *et al.*, 2020a	100
AH971	CR3009	N protein	van den Brink *et al.*, 2005	150

Antigen: The prefusion ectodomain (residues 1-1208) of the SARS-CoV-2 S protein, with a KV->PP substitution at residues 986/987, a RRAR->GSAS substitution at residues 682-685, and C-terminal T4 fibritin trimerization motif, protease cleavage site, TwinStrepTag and 8xHisTag (PDB #6VSB; Wrapp *et al.*, 2020b), was transiently transfected into $25x10^8$ suspension-adapted ExpiCHO cells (Thermo Fisher) using 1.5 mg plasmid DNA and 7.5 mg of PEI MAX (Polysciences) in 500 mL ProCHO5 medium (Lonza). Incubation with agitation was continued at 31°C and 4.5% CO_2 for 5 days. The clarified supernatant was purified in two steps: via a Strep-Tactin XT column (IBA Lifesciences) followed by Superose 6 10/300 GL column (GE Healthcare) to a final concentration of 180 µg/ml in PBS.

Protocol: S protein (10 µg/ml, 50 µl/well in PBS 0.5% (w/v) BSA, 0.1% (w/v) Tween20) was immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of each antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Bio-Rad #170-6516, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

We tested 10 antibodies, originally developed against the SARS-CoV-1 S protein, for detection of the SARS-CoV-2 S protein by ELISA. From these, only two (AI334 and AQ806) bound in a concentration-dependent manner to the SARS-CoV-2 protein (Fig. 1). Two other antibodies, used as negative control (AH917 against the SARS-CoV-1 N protein and RB168 against an amoeba protein), also did not bind the S protein. AI334 and AQ806 antibodies have recently been shown to bind the RBD of the SARS-CoV-2 spike protein (Yuan *et al.*, 2020; Wrapp *et al.*, 2020a).

References

Berry JD, Jones S, Drebot MA, *et al*. Development and characterization of neutralizing monoclonal antibody to the SARS-coronavirus. J Virol Methods. 2004; 120:87-96. PMID:15234813

Coughlin M, Lou G, Martinez O, *et al*. Generation and characterization of human monoclonal neutralizing antibodies with distinct binding and sequence features against SARS coronavirus using XenoMouse. Virology. 2007; 361:93-102. PMID:17161858

Prabakaran P, Gan J, Feng Y, *et al*. Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. J Biol Chem. 2006; 281:15829-36. PMID:16597622.

Rockx B, Corti D, Donaldson E, *et al*. Structural basis for potent cross-neutralizing human monoclonal antibody protection against lethal human and zoonotic severe acute respiratory syndrome coronavirus challenge. J Virol. 2008; 82:3220-35. PMID:18199635

Sui J, Li W, Murakami A, *et al*. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. Proc Natl Acad Sci USA. 2004; 101:2536-41. PMID:14983044

ter Meulen J, van den Brink EN, Poon LL, *et al*. Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. PLoS Med. 2006; 3:e237. PMID:16796401

van den Brink EN, Ter Meulen J, Cox F, *et al*. Molecular and biological characterization of human monoclonal antibodies binding to the spike and nucleocapsid proteins of severe acute respiratory syndrome coronavirus. J Virol. 2005; 79:1635-44. PMID:15650189

Wrapp D, De Vlieger D, Corbett KS, *et al*. Structural basis for potent neutralization of betacoronaviruses by single-domain camelid antibodies. Cell 2020a; pii:S0092-8674(20)30494-3. PMID:32375025

Wrapp D, Wang N, Corbett KS, *et al*. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020b; 367:1260-1263. PMID:32075877

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020; 367:1444-1448. PMID:32132184

Yuan M, Wu NC, Zhu X, *et al*. A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV. Science. 2020. pii:eabb7269. PMID: 32245784

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Conflict of interest

The authors declare no conflict of interest.

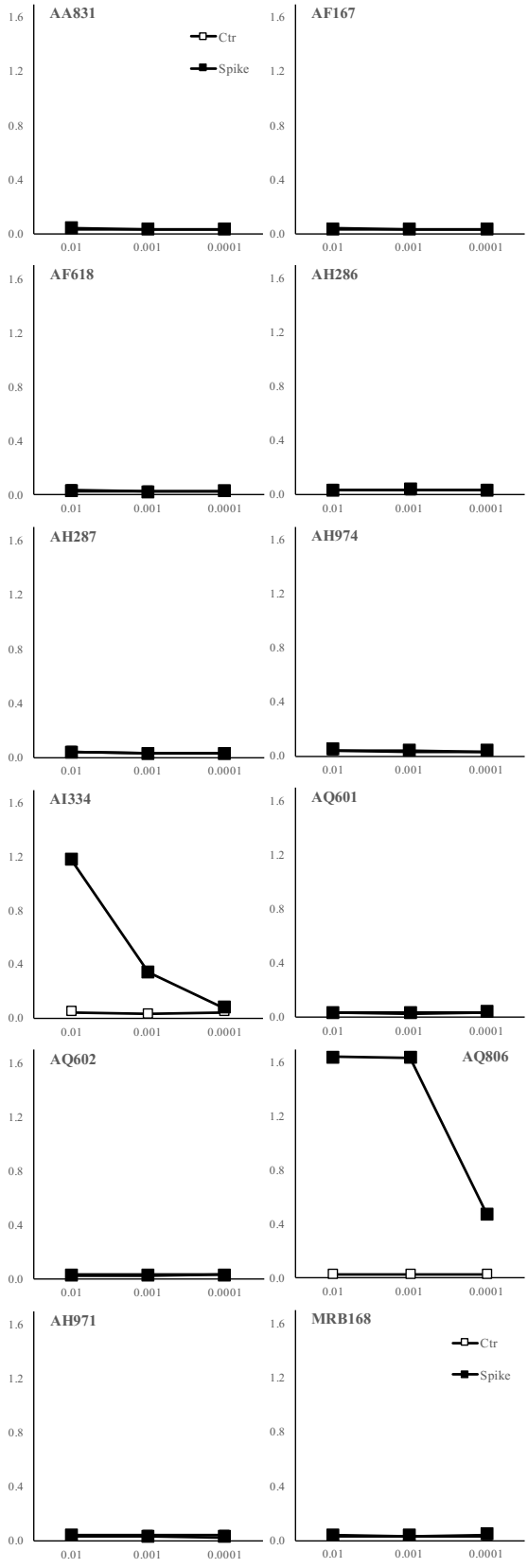

Fig. 1. Specific binding of AI334 and AQ806 antibodies to the SARS-CoV-2 S protein, as detected by ELISA. On the Y axis, ELISA signal (in arbitrary units). On the X axis, the antibody dilution (1:100, 1:1'000 and 1:10'000). 'Spike' refers to the binding to the spike S protein; 'Ctr' refers to the binding to biotinylated BSA.