AS739, AT693 and AU734 antibodies recognize the spike S protein from SARS-CoV-2 by ELISA

Payot, Nina Véronique; Vaudano, Alexandre Pierre; Khatibi, Khatiba; Nemeth, Anthony; Gözlügöl, Ezgi; Chammartin, Nylsa Darlène; Lazzarotto, Célia Sophie Bernadette; Ollivier, Julien; Marques Ferreira Pinto da Fonte, Sara; Jaques, Emma; El Matribi, Zacharie Elie; Berkcan, Serkan; Gil Fraga, Daniel; Poncet, Clément [**and 9 more**]

How to cite

PAYOT, Nina Véronique et al. AS739, AT693 and AU734 antibodies recognize the spike S protein from SARS-CoV-2 by ELISA. In: Antibody reports, 2021, vol. 4, n° 1, p. e279. doi: 10.24450/journals/abrep.2021.e279

This publication URL: https://archive-ouverte.unige.ch/unige:157178

Publication DOI: 10.24450/journals/abrep.2021.e279

AS739, AT693 and AU734 antibodies recognize the spike S protein from SARS-CoV-2 by ELISA

Nina Payot, Alexandre P. Vaudano, Khatiba Khatibi, Anthony Nemeth, Ezgi Gozlugol, Nylsa Chammartin, Célia Lazzarotto, Julien Ollivier, Sara Da Fonte, Emma Jaques, Zacharie El Matribi, Serkan Berkcan, Daniel Gil, Clément Poncet, Maxime Volery, Clément Bindschaedler, Margaux Gosetto, Ezia Oppliger, Marie N. Schmid, Monica Didier, Philippe Hammel, Ali Sassi, Cyril Guilhen

Bachelor in Biomedical Sciences, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies AS739, AT693 and AU734 detect by ELISA the spike S protein from SARS-CoV-2.

Introduction

The spike S glycoprotein (UniProt P0DTC2) mediates the 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). Five recombinant antibodies recognizing the S1 domain of the S protein from SARS-CoV-2 (AS739, AT693, AU197, AU734 and AU753) were tested for their ability to recognize the S protein by ELISA. Three antibodies (AS739, AT693 and AU734) detected the S protein from SARS-CoV-2; two others (AU197 and AU753) did not.

Materials & Methods

Antibodies: ABCD_AS739, ABCD_AT693, ABCD_AU197, ABCD_AU734 and ABCD_AU753 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 portion fused to a rabbit IgG 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 4 days.

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

ABCD	Clone	Epitope	Reference	Yield (mg/L)
AS739	S309	S1/RBD	Pinto *et al*., 2020	100
AT693	BD-23		Cao *et al*., 2020	80
AU197	2B04		Alsoussi *et al*., 2020	30
AU734	2-43		Liu *et al*., 2020	40
AU753	MAb362		Ejemel *et al*., 2020	10

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.*, 2020), 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: The whole procedure was carried out at room temperature. Biotinylated BSA (10 µg/mL) or S protein (10 µg/mL) were incubated in a streptavidin-coated 8-well plate (50 µl/well) (Pierce 15120) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.1% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 h with 50 µl of antibody-containing supernatant diluted in washing buffer as indicated (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma A8275, dilution 1:1000, 50 µl per well) for 30 min. After 5 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.

Results

Antibodies AS739, AT693 and AU734 bound in a concentration-dependent manner to the SARS-CoV-2 spike S protein, but not to the BSA negative control (Fig. 1). AU197 and AU753 did not recognize the S protein by ELISA; for AU753, this is possibly due to the fact that this antibody is poorly produced.

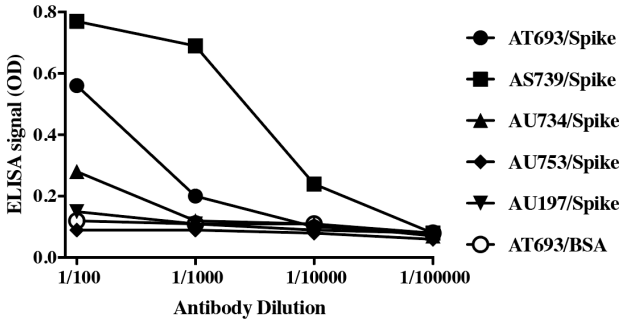

Fig. 1. AS739, AT693 and AU734 bound specifically to the SARS-CoV-2 S protein, but not to the BSA control (shown only for AT693; AS739, AU197, AU734 and AU753 background curves were superimposed), as detected by ELISA.

References

Alsoussi WB, Turner JS, Case JB, *et al*. A potently neutralizing antibody protects mice against SARS-CoV-2 infection. J Immunol. 2020; 205:915-22. PMID: 32591393

Cao Y, Su B, Guo X, *et al*. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. Cell 2020; 182:73-84. PMID: 32425270

Ejemel M, Li Q, Hou S, *et al*. A cross-reactive human IgA monoclonal antibody blocks SARS-CoV-2 spike-ACE2 interaction. Nat Commun. 2020; 11:4198. PMID: 32826914

Liu L, Wang P, Nair MS, *et al.* Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. Nature 2020; 584:450-6. PMID: 32698192

Pinto D, Park Y-J, Beltramello M, *et al.* Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. Nature 2020; 583: 290-5. PMID: 32422645

Wrapp D, Wang N, Corbett KS, *et al.* Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 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

Conflict of interest

The authors declare no conflict of interest.