AH971 and AV865 antibodies label the SARS-CoV-2 nucleocapsid protein in focus-forming assay

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Abstract

AH971 and AV865 antibodies detect the nucleocapsid proteins of severe acute respiratory SARS coronavirus-2 (SARS-CoV-2) in a focus forming assay.

Introduction

The nucleocapsid proteins (N) of SARS-CoV-2 shares 90% amino acid sequence identity with the N protein of SARS-CoV and plays a crucial role in viral RNA replication, assembly, and formation of the ribonucleoprotein complex. The N protein is one of the most abundant structural proteins in virus-infected cells (Yan *et al*., 2022). In this study, we successfully tested the human recombinant antibody AH971 for labeling the SARS-CoV-2 N protein in a focus-forming assay. This antibody was originally developed against the SARS-CoV N protein. As a positive control, we used the recombinant antibody AV865, originally developed against the SARS-CoV-2 N protein and previously validated (under the name JS02) in a focus-forming assay (Puhach *et al*., 2022).

Materials & Methods

Antibodies: ABCD_AH971 and ABCD_AV865 antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies) as mini-antibodies with the antigen-binding scFv portion fused to a human IgG1 Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the anti-SARSCoV antibody CR3009 (for AH971; van den Brink *et al*., 2005) and the anti-SARSCoV2 antibody JS02 (for AV865; Zhang *et al*., 2020), joined by a peptide linker $(GGGGS)_3$. HEK293 suspension cells growing in HEK TF medium (Xell #861-0001, Sartorius) supplemented with 0.1% Pluronic F68 (Sigma #P1300) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~125 mg/L) were collected after 5 days.

Antigen: Vero E6-TMPRSS cells were cultured in DMEM GlutaMAX I medium supplemented with 10% FBS, 1× non-essential amino acids and antibiotics (penicillin–streptomycin). These cells were seeded in 96-well plates (CytoOne) and infected the following day with previously titrated ancestral SARS-CoV-2 strain (GISAID ID: EPI_ISL_414019) or SARS-CoV-2-positive nasopharyngeal swabs (NPS) collected in the outpatient testing center of the Geneva University Hospital with unknown virus titer.

Protocol: NPS samples were serially diluted and applied on a monolayer of cells in duplicates. For ancestral SARS-CoV-2, 50 focus-forming units (ffu) per well were applied instead. After 1 hour at 37 °C, the media were removed, and pre-warmed medium containing 0.85% methylcellulose was overlaid. Plates were incubated at 37 °C for 24 hours and then fixed using 6% paraformaldehyde for 1 hour at room temperature. Cells were permeabilized with 0.1% Triton X-100 and blocked with 1% BSA (Sigma-Aldrich). Samples were then incubated with a primary monoclonal antibody diluted to 0.2 µg per ml for 1 hour at room temperature and then with peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, 109-036-09, diluted to 1:2,000) for 30 minutes at room temperature. Foci were visualized using True Blue HRP substrate (Avantor) and imaged on an Mabtech IRIS. A cluster of adjacent cells expressing viral antigen was identified as foci.

Results

Antibodies AH971 and AV865 detected specifically a signal in SARS-CoV-2-infected cells (Fig. 1). We did not see any differences in the staining with both antibodies. No signal was detected in non-infected cells (Fig. 1).

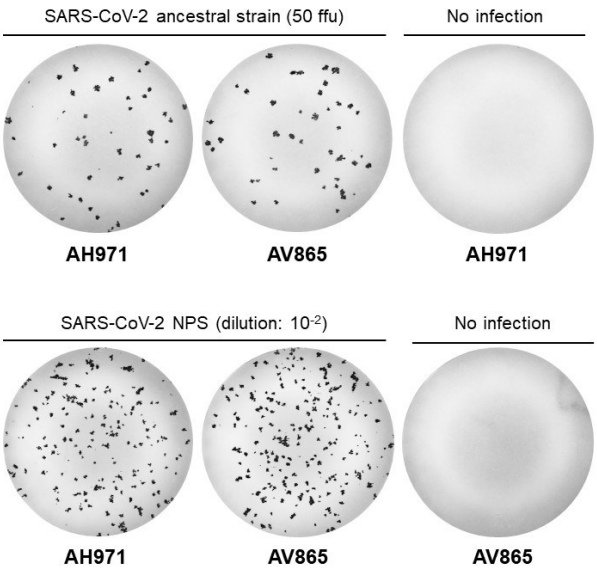

Fig. 1. AH971 and AV865 antibodies successfully labelled virus foci in Vero E6-TMPRSS cells infected with 50 ffu of ancestral SARS-CoV-2 or NPS of SARS-CoV-2 (dilution 10^{-2} is shown). No labelling was seen in uninfected cells.

References

Puhach O, Adea K, Hulo N, Sattonnet P, Genecand C, Iten A, Jacquérioz F, Kaiser L, Vetter P, Eckerle I, Meyer B. Infectious viral load in unvaccinated and vaccinated individuals infected with ancestral, Delta or Omicron SARS-CoV-2. Nat Med. 2022 Jul;28(7):1491-1500. PMID: 35395151.

van den Brink EN, Ter Meulen J, Cox F, Jongeneelen MA, Thijsse A, Throsby M, Marissen WE, Rood PM, Bakker AB, Gelderblom HR, Martina BE, Osterhaus AD, Preiser W, Doerr HW, de Kruif J, Goudsmit J. Molecular and biological characterization of human monoclonal antibodies binding to the spike and nucleocapsid proteins of severe acute respiratory syndrome coronavirus. J Virol. 2005 Feb;79(3):1635-44. PMID: 15650189.

Yan W, Zheng Y, Zeng X, He B, Cheng W. Structural biology of SARS-CoV-2: open the door for novel therapies. Signal Transduct Target Ther. 2022 Jan 27;7(1):26. PMID: 35087058.

Zhang L, Zheng B, Gao X, Zhang L, Pan H, Qiao Y, Suo G, Zhu F. Development of Patient-Derived Human Monoclonal Antibodies Against Nucleocapsid Protein of Severe Acute Respiratory Syndrome Coronavirus 2 for Coronavirus Disease 2019 Diagnosis. Front Immunol. 2020 Nov 13;11:595970. PMID: 33281824.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The study was approved by the Cantonal Ethics Committee at the University Hospital of Geneva (CCER no. 2021-01488). The study was conducted in accordance with Helsinki Declaration as revised in 2013 and all study participants and/or their legal guardians provided informed consent.