

RB620 and RB621 antibodies recognize a peptide of the SARS-CoV-2 E protein by ELISA

Philippe Hammel¹, Frederic Zenhausern^{2,3,4}

¹ Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland; ² Center for Applied NanoBioscience and Medicine, The University of Arizona, Phoenix, AZ 85004, USA; ³ Whitespace Enterprise Corporation, 1305 Auto Drive, Tempe, AZ 85284, USA; ⁴ School of Pharmaceutical Sciences, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies RB620 and RB621 detect by ELISA a synthetic peptide from the SARS-CoV-2 envelope E protein.

Introduction

The SARS-CoV envelope (E) protein is a minor structural protein, implicated in viral morphogenesis, assembly and budding. It is a short, integral membrane protein, potentially able to adopt multiple membrane topologies and orientations (Schoeman and Fielding, 2019). Here we describe the ability of the antibodies RB620 and RB621 to detect by ELISA a fragment of the C-terminal domain of the SARS-CoV-2 E protein (UniProt P0DTC4).

Materials & Methods

Antibodies: ABCD_RB620 and ABCD_RB621 (ABCD nomenclature, <https://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (<https://www.unige.ch/medecine/antibodies/>) as a mini-antibodies with the antigen-binding VHH portion fused to a mouse IgG2A Fc. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the VHH-Fc of each antibody. Supernatants (~100 mg/L) were collected after 4 days.

Antigen: The antibodies were raised against an N-biotinylated synthetic peptide, corresponding to the last 31 C-terminal residues (NIVNVSLVKPSFYVYSRVKLNLSRVPDLLV). This peptide was also used as antigen for ELISA detection. As a negative control, an N-biotinylated peptide corresponding to the last 33 C-terminal residues (DSGFAAYSRYRIGNYKLNTDHSSSDNIALLVQ) of the SARS-CoV-2 M protein (UniProt P0DTC5) was used.

Protocol: The whole procedure was carried out at room temperature. Biotinylated peptides at saturating concentration (10 pmol/well) were 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 RB 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 H₂SO₄. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RB620 and RB621 bound in a concentration-dependent manner to the SARS-CoV-2 E peptide against which they were selected. They did not bind to an unrelated peptide (Fig. 1). Note that the peptidic antigen used here is a short segment of the E protein C-terminus. The topology of the E protein is still debated and its C-terminus could also be glycosylated if it is present in the lumen of the ER. This domain presumably folds as a β-strand followed by an α-helix (Li *et al.*, 2014; Surya *et al.*, 2018; Schoeman and Fielding, 2019), and it remains to be established if the three-dimensional structure of the synthetic peptide is similar to that of the full-length E protein. Further experiments will be necessary to determine if and in which experimental procedures each of these antibodies recognizes the full-length E protein expressed in human cells.

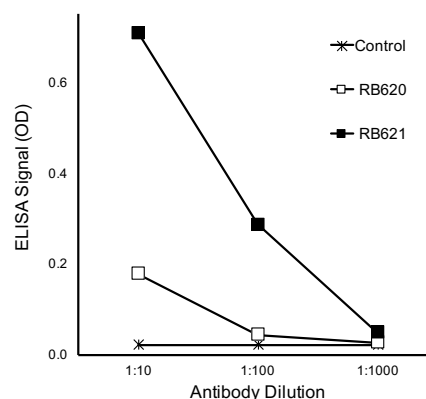


Fig. 1. Specific binding of RB antibodies to the target E peptide (ratio signal:background >2), but not to the control peptide (shown only for RB620; the RB621 background curve was superimposed), as detected by ELISA.

References

- Li Y, Surya W, Claudine S, Torres J. Structure of a conserved Golgi complex-targeting signal in coronavirus envelope proteins. *J Biol Chem.* 2014; 289:12535-49. PMID:24668816
- Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. *Virol J.* 2019; 16:69. PMID:31133031
- Surya W, Li Y, Torres J. Structural model of the SARS coronavirus E channel in LMPG micelles. *Biochim Biophys Acta Biomembr.* 2018; 1860:1309-1317. PMID:29474890

Acknowledgments

This work was co-sponsored by NASA TRISH contract #NNX16AO69A/CAT0001.

Conflict of interest

The authors declare no conflict of interest.