RB579, RB580, RB581, RB582, RB583, RB584 and RB585 antibodies recognize a peptide of the SARS-CoV-2 E protein by ELISA

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Abstract

The recombinant antibodies RB579, RB580, RB581, RB582, RB583, RB584 and RB585 detect by ELISA a synthetic peptide from the SARS-CoV-2 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 seven recombinant antibodies (RB579, RB580, RB581, RB582, RB583, RB584 and RB585) to detect by ELISA a fragment of the C-terminal domain of the SARS-CoV-2 E protein (UniProt P0DTC4).

Materials & Methods

Antibodies: ABCD_RB579, ABCD_RB580, ABCD_RB581, ABCD_RB582, ABCD_RB583, ABCD_RB584 and ABCD_RB585 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 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 (50-100 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against an N-biotinylated synthetic peptide, corresponding to the last 31 C-terminal residues (NIVNVSLVKPSFYVYSRVKNLNSSRVPDLLV). 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 (DSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ) 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 H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RB579, RB582, RB583, RB584 and, to a lesser extent, RB580, RB581 and RB585 bound in a concentration-dependent manner to the SARS-CoV-2 E peptide against which they were raised. 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.
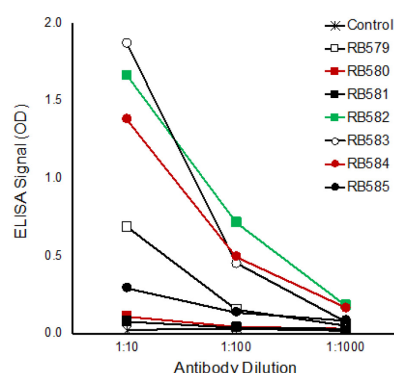

Fig. 1. Specific binding of RB antibodies to the target E peptide (ratio signal:background >2), but not to the control peptide (shown only for RB583; all other background curves are superimposed), as detected by ELISA.

References

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Conflict of interest

Pierre Cosson and Vanessa C. Lima are editors of the Antibody Reports journal.