RB571, RB572, RB573, RB574, RB575, RB576, RB577 and RB578 antibodies recognize a fragment of the membrane M protein from SARS-CoV-2 by ELISA

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Abstract

The recombinant antibodies RB571, RB572, RB573, RB574, RB575, RB576, RB577 and RB578 detect by ELISA a synthetic peptide from the SARS-CoV-2 M protein.

Introduction

The SARS-CoV membrane (M) protein is the most abundant structural protein, driving virus assembly and budding into the lumen of the endoplasmic reticulum-Golgi intermediary compartment (ERGIC) via interactions with the E, S and N proteins (Siu *et al.*, 2008). Here we describe the ability of eight recombinant antibodies (RB571, RB572, RB573, RB574, RB575, RB576, RB577 and RB578) to detect by ELISA a C-terminal fragment of the cytosolic domain of the SARS-CoV-2 M protein (UniProt P0DTC5).

Materials & Methods

Antibodies: ABCD_RB571, ABCD_RB572, ABCD_RB573, ABCD_RB574, ABCD_RB575, ABCD_RB576, ABCD_RB577 and ABCD_RB578 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 33 cytosolic C-terminal residues (DSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ). This peptide was also used as antigen for ELISA detection. As a negative control, an N-biotinylated peptide corresponding to the last 31 C-terminal residues (NIVNVSLVKPSFYVYSRVKNLNSSRVPDLLV) of the SARS-CoV-2 E protein (UniProt P0DTC4) 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 RB571, RB572, RB574, RB576, RB577 and, to a lesser extent, RB573, RB575 and RB578 bound in a concentration-dependent manner to the SARS-CoV-2 M peptide against which they were raised, but not against an unrelated peptide (Fig. 1). Note that the peptidic antigen used here is a short cytosolic domain of the M protein; it does not fold into a complex structure, nor contains glycosylation sites (Fung and Liu, 2018). Accordingly, it is reasonable to expect that the RB antibodies will also recognize the full-length protein. Further experiments will be necessary to determine if this is the case, and in which experimental procedures the antibodies can be used.

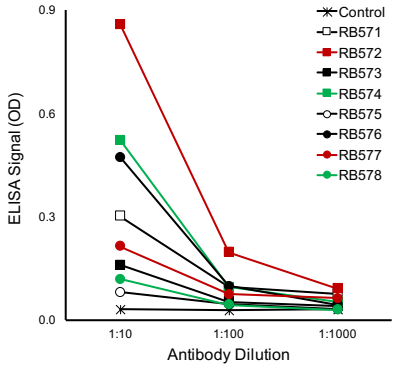

Fig. 1. Specific binding of RB antibodies to the target M peptide (ratio signal:background >2), but not to the control peptide (shown only for RB574; all other background curves are superimposed), as detected by ELISA.

References

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