The RB596 antibody recognizes a linear epitope from the spike S protein from SARS-CoV-2

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Abstract

The recombinant antibody RB596 recognizes a linear epitope (residues 973-984, ISSVLNDILSRL) from the SARS-CoV-2 spike S protein.

Introduction

The spike (S) glycoprotein from SARS-CoV-2 (UniProt P0DTC2) mediates the cell entry of the virus into the host cell through the binding to ACE2 (Yan *et al.*, 2020). Therefore, antibodies targeting this protein are of great interest. Here we used a PNA-peptide microarray (Farrera *et al.*, 2020) to characterize the binding site of the RB596 recombinant antibody.

Materials & Methods

Antibodies: ABCD_RB596 antibody (ABCD nomenclature, https://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as a mini-antibody with the antigen-binding VHH portion fused to a mouse IgG2A Fc (Hammel *et al.*, 2020a). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the VHH-Fc. Supernatant (100 mg/L) was collected after 4 days.

Microarray epitope mapping: The microarray experiment was carried out as previously described (Farrera *et al.*, 2020) using the same PNA-peptide library and the same procedures. The only difference was the use of a goat-anti-mouse IgG antibody, Cy3 conjugate (Merck, AP124C) as a secondary antibody at 1:500 dilution factor. All mouse-Fc antibodies tested on that library show two strong signals on the epitopes 1033-1044 and 1057-1068. For that reason, the microarray obtained from the mouse-Fc recombinant antibody AS702 (Hammel *et al.*, 2020b) was used as a control to determine the non-specific binding coming from the common part of the antibody and/or secondary antibody.

Binding characterization by Biolayer interferometry: A BLItz instrument was used with ForteBio's Streptavidin (SA) biosensors (ForteBio, 18-5019). An adapted method (Shah and Duncan, 2014) was used with the following steps: (1) 30 seconds Baseline (PBS-t); (2) 120 seconds Immobilization of the biotinylated peptide (5 µM in PBS-t); (3) 30 seconds Blocking with PBS-t + 0.5% BSA; (4) 30 seconds Baseline (PBS-t); (5) 120 seconds Association (compound in PBS-t); (6) 120 seconds Dissociation (PBS-t). K_D was measured using four serial dilutions with the BLItz Pro™ software.

Results

Microarray analysis of the RB596 recombinant antibody revealed that it recognizes the peptide ISSVLNDILSRL (973-984) on the spike S protein of SARS-CoV-2 (Fig. 1A). Two other spots were also observed on the microarray; however, they were also observed with the control antibody AS702, and therefore most probably result from unspecific interactions. The binding of RB596 to the 973-984 peptide was further confirmed by BLItz, showing a K_D on the low nanomolar range (Fig. 1B). This peptide belongs to the S2 subunit of the spike protein, and even though it seems to be shielded by the S1 subunit, the dynamics of the protein could allow binding of the antibody at this site (Fig. 1C).

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Conflict of interest

The authors declare no conflict of interest.

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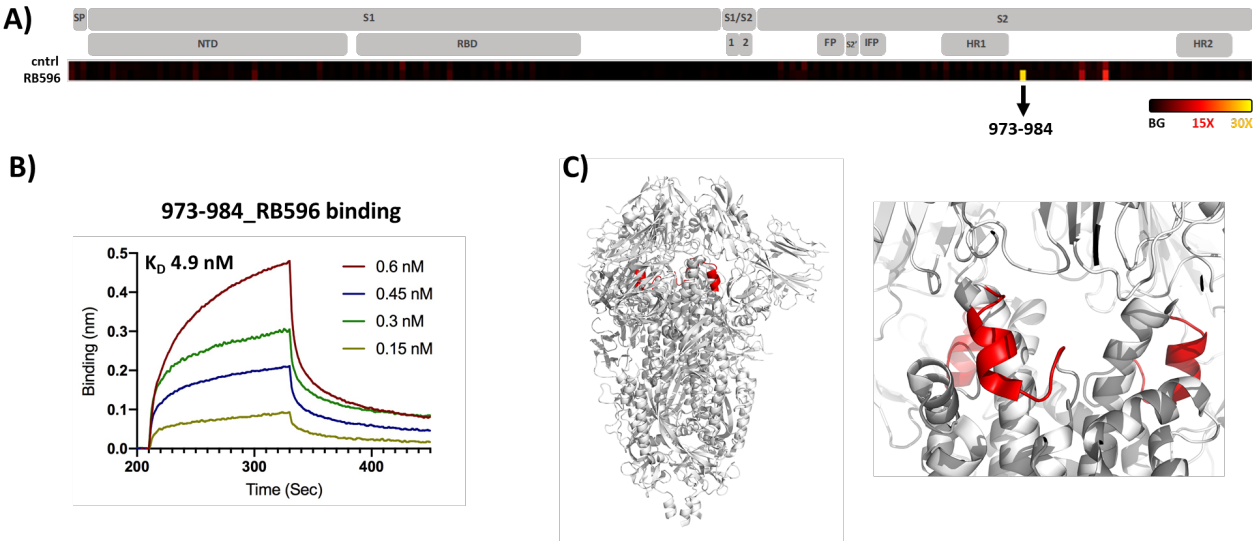

Fig. 1. (A) Domains of the spike S protein (SP = signal peptide, NTD = N-terminal domain, RBD = receptor-binding domain, FP = fusion Peptide, IFP = internal fusion protein, HR1 = heptad repeat 1, HR2 = heptad repeat 2) and heat map of antibody binding to the peptide fragments (black: background intensity; red: 15x background intensity; yellow: 30x background intensity). Sample name is indicated on the left of the heat map. **(B)** Sensogram of the BLItz affinity measurement of the biotinylated peptide 973-984 with the recombinant antibody RB596. **(C)** Localization of the selected epitope (epitope 973-984, in red) on the crystal structure of the trimeric SARS-CoV-2 spike protein (in grey) (PDB ID: 6VXX; Walls *et al.*, 2020).