Materials and Methods

Production of SARS-CoV-2 RBD recombinant protein. – Mammalian protein expression plasmid for SARS-CoV-2 RBD was recently published[1,2]. Briefly, the SARS-CoV-2 RBD plasmid containing signal peptide amino acids 1-14 and RBD amino acids 319-541 with a C-terminal 6X-His tag, was cloned into mammalian expression vector pcDNA 3.1. Recombinant protein was produced using the FreeStyle 293 Expression System (Thermo Fisher Scientific) according to the manufacturer's instruction. Supernatants from transfected cells were harvested on day 5 post-transfection and protein was purified using Ni Sepharose excel affinity resin (Sigma) according to manufacturer guidelines. Protein was concentrated and buffer exchanged to phosphate-buffered saline (PBS) using Amicon Ultra-15 Centrifugal Filter Units (Sigma). SARS-CoV RBD was obtained from Sino Biological (Catalog: 40150-V08B2-100).

Mammalian glycan array analysis. – Glycans were prepared as described previously[3], followed by printing onto NHS-activated glass slides in 300 mM phosphate, pH 8.5 containing 0.005% Tween-20, 6x at a concentration of 10 μM. Following printing, slides were allowed to react at 80% humidity for 30 minutes followed by desiccation overnight. SARS-CoV-2 and SARS-CoV RBDs were incubated as outlined previously[4], in PBS containing 0.05% Tween 20 and 1% BSA, for 1 h at room temperature in a dark humid chamber, followed by incubation with 5 μg/ml anti-His antibody (Anti-His-tag mAb-Alexa Fluor® 647 from MBL Code No. D291-A64). Slide were washed between primary and secondary incubations by successive immersion in PBS containing 0.05% Tween 20 (4x) followed by PBS (4x) and then H_2O (4x). Slides were dried by microcentrifugation followed by imaging by microarray scanner (Scan Array Express, PerkinElmer Lifer Sciences) and image analysis using Imagene software (BioDiscovery).

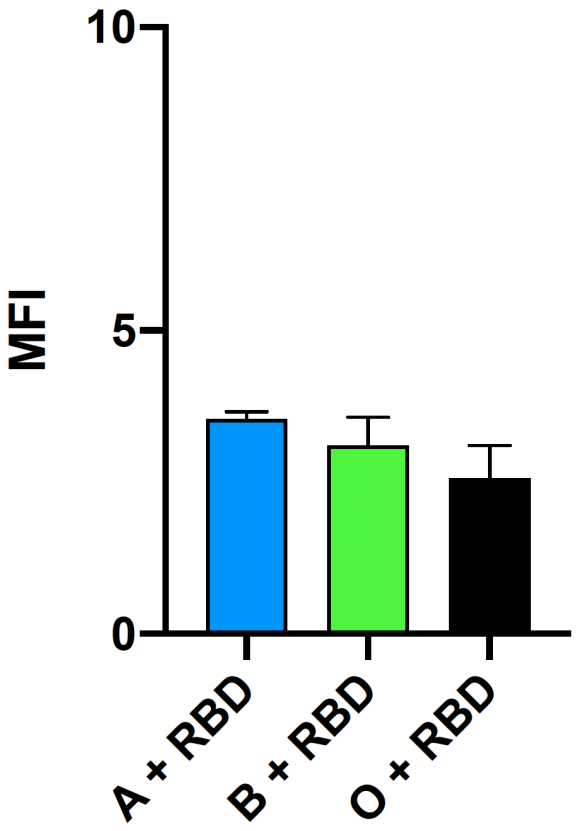
Red blood cell and HEK293 T cell analysis with flow cytometry. – Donor-derived reagent red blood cells (Immucor, Norcross, GA) were provided by the Emory University Hospital blood bank. Prior to staining, RBCs were resuspended and washed twice in FACS buffer (PBS with 2% BSA) at 4 °C. 1 μl packed RBCs were incubated with SARS-CoV-2 RBD for 1h followed by incubation with anti-His (MBL Cat No. D291-A64) at 4 °C for 1 h. After incubation cells were washed twice and resuspended in FACS buffer for flow cytometry analysis (BD Biosciences). 1 μl packed RBCs were incubated with anti-Blood Group A antibody (abcam Cat No. ab2521) followed by incubation anti-mouse IgM Antibody (BioLegend Cat No. 406525) as a positive control for RBC binding. Adherent lentivirus-transduced ACE-2 expressing HEK 293T cells

(293T-ACE2)[5] were grown in complete DMEM supplemented with 10% heat inactivated FBS and no antibiotics. To measure binding by of recombinant the SARS-CoV-2 RBD to HEK 293T-ACE2, cells were mechanically disrupted in phosphate buffered saline by tapping and serological pipetting (to preserve surface receptor expression) before filtration to a single cell suspension using a 70 μm filter. $2x10^6$ 293T-ACE2 or 293T cells were incubated for 1 h with RBD followed by detection with anti-His secondary reagent. For each cell type a secondary alone condition was similarly evaluated. Flow cytmeric analysis was accomplished using a FACSCalibur followed by analysis using FlowJo v.10.

REFERENCES:

1. Amanat, F*., et al.* A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat. Med.* (2020).
2. Stadlbauer, D*., et al.* SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol* **57**, e100 (2020).
3. Blixt, O*., et al.* Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proc Natl Acad Sci U S A* **101**, 17033-17038 (2004).
4. Stowell, S.R*., et al.* Innate immune lectins kill bacteria expressing blood group antigen. *Nat. Med.* **16**, 295-301 (2010).
5. Crawford, K.H.D*., et al.* Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. *Viruses* **12**(2020).

Wu et al. Supplemental Figure 1



Supplemental Figure 1: Quantification of SARS-CoV-2 RBD binding to human RBCs. Mean fluorescence intensity (MFI) of RBD binding to A (A + RBD), B (B + RBD) and O (O + RBD) RBCs as measured by flow cytometric analysis. Error bars represent mean ± standard deviation (SD). Data representative of 3 individual experiments. Statistics were generated by a one-way ANOVA analysis with a post hoc Tukey's multiple comparison. No significant difference in binding was found between each group.