A Human Monoclonal Antibody Blocking SARS-CoV-2 Infection

Rutuja Survase[1], Anjum Saudagar[2], Runali bharti[3], Renuka R. Deshpande[4]
[1,2,3] *B.Pharmacy third Year, Latur College of Pharmacy Hasegaon, Tq. Ausa, Dist. Latur-413512, Maharashtra, India*
[4]*Department of Pharmaceutics, Latur College of Pharmacy Hasegaon, Tq. Ausa, Dist. Latur-413512, Maharashtra, India*

***Abstract*- The emergence of the novel human coronavirus SARS-CoV-2 in Wuhan, China has caused a worldwide epidemic of respiratory disease (COVID-19). Vaccines and targeted therapeutics for treatment of this disease are currently lacking. Here we report a human monoclonal antibody that neutralizes SARS-CoV-2 (and SARS- CoV). This cross-neutralizing antibody targets a communal epitope on these viruses and offers potential for prevention and treatment of COVID-19.**

***Index terms*- COVID-19, monoclonal antibody**

ANTIBODIES

Antibodies are proteins that are produced by certain cells of the immune system known as B cells. They are able to bind to "foreign" material that tries to invade the body, such as pathogens, and directly neutralize them or trigger an immune response. This is achieved by binding of the antibody to an antigen, a specific molecule present on the pathogen.

Scientists are now able to create antibodies that target one specific antigen. These are known as monoclonal antibodies and they can be produced in large quantities in a laboratory setting. They can be utilized for a variety of different purposes, such as diagnostic tests and targeted treatment. There are several types of monoclonal antibody. A "human" monoclonal antibody is one that is entirely derived from a human source.

SARS-CoV-2 and SARS-CoV both belong to the Sarbecovirus subgenus of the Coronaviridae family. The trimeric spike (S) glycoproteins present on the viral surface enable entry of the virus into host cells by binding to a receptor protein known as the human angiotensin converting enzyme (ACE2). Here, a "syringe" like mechanism enables injection of the viral genetic material into the cell, which is then replicated.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the coronavirus induced disease 19 (COVID-19) that emerged in China late 2019 and causing a pandemic1. As of 19 April 2020, 2,241,778 cases have been reported worldwide, of which 152,551 (6.8%) succumbed to the infection2. SARS-CoV-2 belongs to the Sarbecovirus subgenus (genus Betacoronavirus, family Coronaviridae)3 together with SARS-CoV that emerged in 2002 causing ~8000 infections with a lethality of 10%. Both viruses crossed species barriers from an animal reservoir and can cause a life-threatening respiratory illness in humans. Presently, no approved targeted therapeutics are available for COVID-19. Monoclonal antibodies targeting vulnerable sites on viral surface proteins are increasingly recognized as a promising class of drugs against infectious diseases and have shown therapeutic efficacy for a number of viruses4, 5.

Coronavirus-neutralizing antibodies primarily target the trimeric spike (S) glycoproteins on the viral surface that mediate entry into host cells. The S protein has two functional subunits that mediate cell attachment (the S1 subunit, existing of four core domains S1A through S1D) and fusion of the viral and cellular membrane (the S2 subunit). Potent neutralizing antibodies often target the receptor interaction site in S1, disabling receptor interactions6, 7, 8, 9, 10, 11. The spike proteins of SARS-CoV-2 (SARS2-S; 1273 residues, strain Wuhan-Hu-1) and SARS-CoV (SARS-S, 1255

residues, strain Urbani) are 77.5% identical by primary amino acid sequence, are structurally very similar12, 13, 14, 15 and commonly bind the human angiotensin converting enzyme 2 (ACE2) protein as a host receptor 1, 16 through their S1B domain. Receptor interaction is known to trigger irreversible conformational changes in coronavirus spike proteins enabling membrane fusion17.

The spike proteins of SARS-CoV-2 and SARS-CoV are very similar on a structural level, sharing 77.5% of their amino acid sequence. Blocking the initial binding of the virus to the ACE2 receptor via the spike protein is one potential treatment avenue. Here's where monoclonal antibodies could help.

Monoclonal antibodies that target vulnerable sites on viral surface proteins are an emerging approach for treating infectious diseases. In this study by Wang et al., a human antibody known as 47D11 was found to bind to SARS-CoV-2 and SARS-CoV, and to potently inhibit the virus' infection of Vero cells, a type of cell line.

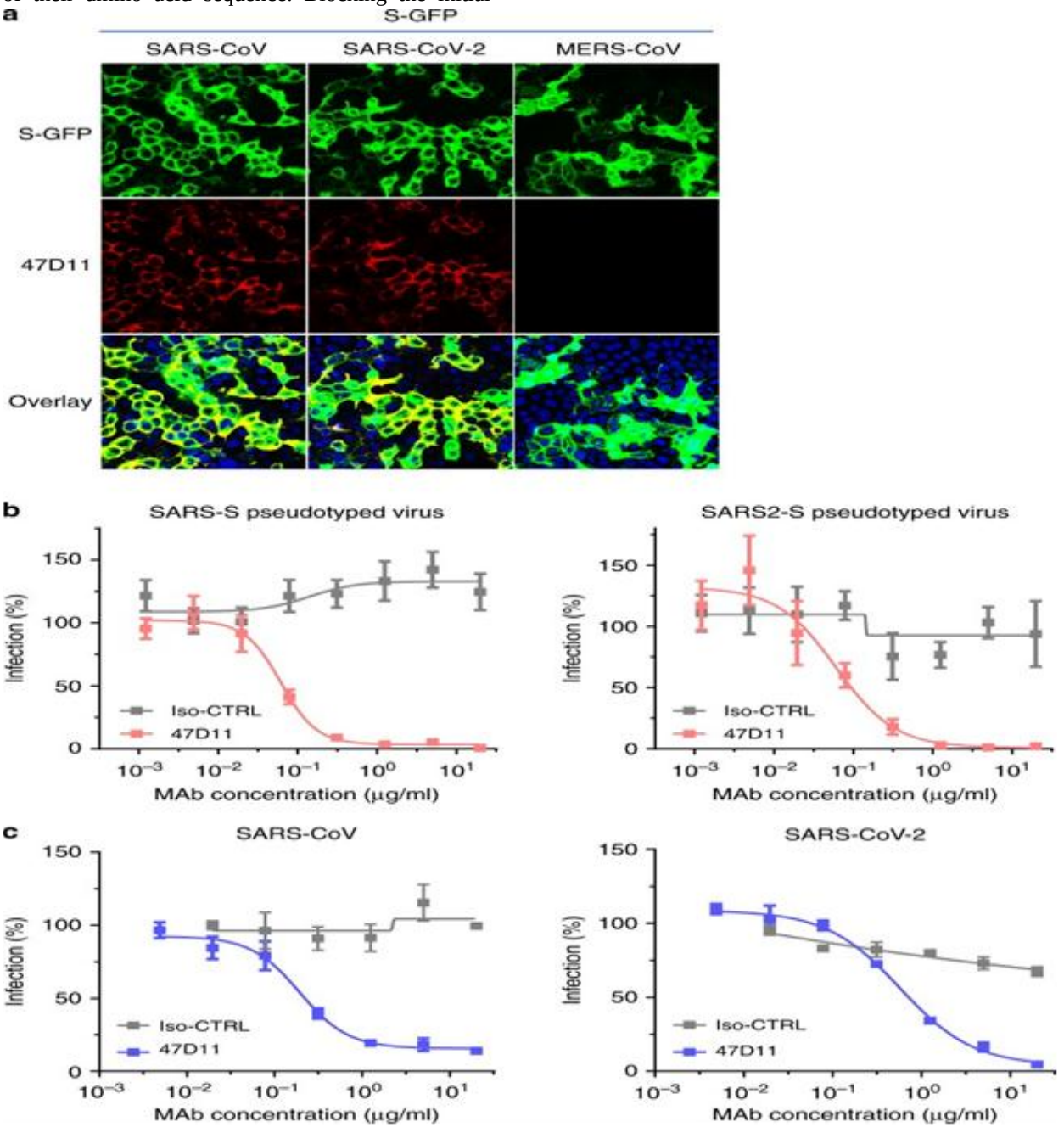

Fig. 1. 47D11 neutralizes SARS-CoV and SARS-CoV-2. a Binding of 47D11 to HEK-293T cells expressing GFP-tagged spike proteins of SARS-CoV and SARS-CoV-2 detected by immunofluorescence assay. The human mAb 7.7G6 targeting the MERS-CoV S1B spike domain was taken along as a negative control, cell nuclei in the overlay images are visualized with DAPI. b Antibody-mediated neutralization of infection of luciferase-encoding VSV particles pseudotyped with spike proteins of SARS-CoV and SARS-CoV-2. Pseudotyped VSV particles pre-incubated with antibodies at indicated concentrations (see Methods) were used to infect VeroE6 cells and luciferase activities in cell lysates were determined at 24 h post transduction to calculate infection (%) relative to non-antibody-treated controls. The average ± SD from at least three independent experiments with technical triplicates is shown. Iso-CTRL: an anti-Strep-tag human monoclonal antibody was used as an antibody isotype control. c Antibody-mediated neutralization of SARS-CoV and SARS-CoV-2 infection on VeroE6 cells. The experiment was performed with triplicate samples, the average ± SD is shown. Source data are provided as a Source Data file.

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The neutralizing 47D11 mAb binds SARS1-S and SARS2-S RBD without eliminating receptor interaction.:-
A ELISA-binding curves of 47D11 to Secto (upper panel) or S1A and S1B (RBD: receptor-binding domain) (lower panel) of SARS-S and SARS2-S coated at equimolar concentrations. The average ± SD from two independent experiments with technical duplicates is shown. b Interference of antibodies with binding of the S-S1B of SARS-CoV and SARS-CoV-2 to cell surface ACE2-GFP analyzed by flow cytometry. Prior to cell binding, S1B was mixed with mAb (mAbs 47D11, 35F4, 43C6, 7.7G6, in H2L2 format) with indicated specificity in a mAb:S1B molar ratio of 8:1 (see Supplementary Fig. 3 for an extensive analysis using different mAb:S1B molar ratio's). Cells are analyzed for (ACE2-)GFP expression (x axis) and S1B binding (y axis). Percentages of cells that scored negative, single positive, or double positive are shown in each quadrant. Experiment was done twice, a representative experiment is shown. c Divergence in surface residues in S1B of SARS-CoV and SARS-CoV-2. Upper panel: Structure of the SARS-CoV spike protein S1B RBD in complex with human ACE2 receptor (PDB: 2AJF)24. ACE2 (wheat color) is visualized in ribbon presentation. The S1B core domain (blue) and subdomain (orange) are displayed in surface presentation using PyMOL, and are visualized with the same colors in the linear diagram of the spike protein above, with positions of the S1 and S2 subunits, the S ectodomain (Secto), the S1 domains S1A-D and the transmembrane domain (TM) indicated. Lower panel: similar as panel above with surface residues on S1B of SARS-CoV that are at variance with SARS-CoV-2 colorored in white. Source data are provided as a Source Data file.

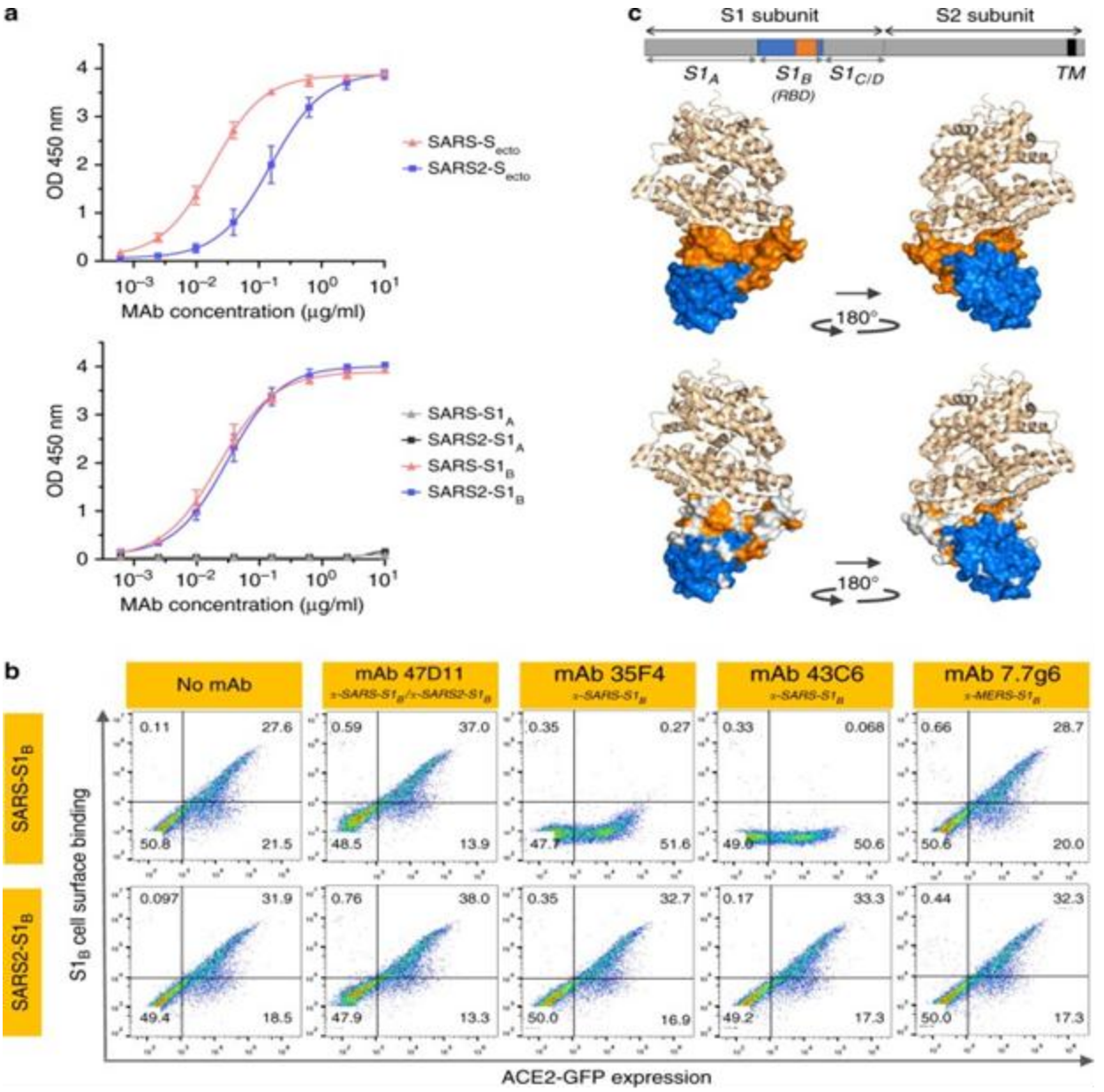

Fig. 2. The neutralizing 47D11 mAb binds SARS1-S and SARS2-S RBD without eliminating receptor interaction. a ELISA-binding curves of 47D11 to Secto (upper panel) or S1A and S1B (RBD: receptor-binding domain) (lower panel) of SARS-S and SARS2-S coated at equimolar concentrations. The average ± SD from two independent experiments with technical duplicates is shown. b Interference of antibodies with binding of the S-S1B of SARS-CoV and SARS-CoV-2 to cell surface ACE2-GFP analyzed by flow cytometry. Prior to cell binding, S1B was mixed with mAb (mAbs 47D11, 35F4, 43C6, 7.7G6, in H2L2 format) with indicated specificity in a mAb:S1B molar ratio of 8:1 (see Supplementary Fig. 3 for an extensive analysis using different mAb:S1B molar ratio's). Cells are analyzed for (ACE2-)GFP expression (x axis) and S1B binding (y axis). Percentages of cells that scored negative, single positive, or double positive are shown in each quadrant. Experiment was done twice, a representative experiment is shown. c Divergence in surface residues in S1B of SARS-CoV and SARS-CoV-2. Upper panel: Structure of the SARS-CoV spike protein S1B RBD in complex with human ACE2 receptor (PDB: 2AJF). ACE2 (wheat color) is visualized in ribbon presentation. The S1B core domain (blue) and subdomain (orange) are displayed in surface presentation using PyMOL, and are

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An unexpected mechanism of action.:-

The antibody was discovered by Wang and colleagues using an ELISA-(cross)reactivity approach, assessing antibody-containing supernatant derived from transgenic mice. Upon discovery that the molecule 47D11 displayed ELISA-cross-reactivity with the SARS spike protein subunits from both SARS-CoV and SARS-CoV2, it was reformatted and expressed as a fully human IgG1 isotope antibody for further study.

How exactly does 47D11 neutralize coronavirus? The scientists aren't too sure yet. However, it appears that it's not by preventing the S protein from binding to ACE2, interestingly.

The authors say in the preprint: "Our data show that 47D11 neutralizes SARS-CoV and SARS-CoV-2 through a yet unknown mechanism that is different from 86 receptor binding interference. Alternative mechanisms of coronavirus neutralization by receptor binding domain-targeting antibodies have been reported including spike inactivation through antibody-induced destabilization of its prefusion structure, which may also apply for 47D11."

They continue: "47D11 binds a conserved epitope on the spike receptor binding domain explaining its ability to cross-neutralize SARS-CoV and SARS-CoV-2, using a mechanism that is independent of receptor binding inhibition."

Conflict of interest statement

A patent application has been filed on 12 March 2020 on monoclonal antibodies targeting SARS-CoV-2 (United Kingdom patent application no. 2003632.3; patent applicants: Utrecht University, Erasmus Medical Center and Harbour BioMed). F.G., D.D., and R.v.H. are non-substantial interest shareholders in Harbour Biomed and were part of the team that generated the mice.

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Rutuja survase
Student of B.pharmacy 3rd year, Latur College of Pharmacy, Hasegaon.

Anjum f.saudagar
Student of B.pharmacy 3rd year, Latur College of Pharmacy, Hasegaon.

Runali ram bharti
Student of B.pharmacy 3rd year, Latur College of Pharmacy, Hasegaon.

Renuka R. Deshpande,
Assistant Professor, Department of Pharmaceutics, Latur College of Pharmacy, Hasegaon