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აღნიშნული შეტყობინები გამოქვეყნებულ შეტყობინება შეტყობინება შეტყობინება შეტყობინება.
Hasanov N.H, Istomin A.G, Istomin D.A.
MATHEMATICAL JUSTIFICATION OF THE CHOICE OF RODS FOR EXTERNAL FIXATION DEVICES FOR POLYSTRUCTURAL
PELVIC INJURIES……………………………………………………………………………………………………………………..…………….6-13

B. Todorova, I. Bitoska, A. Muca, O.Georgieva Janev, T. Milenkovik.
A RARE CASE OF A PATIENT WITH HYPERTHYROIDISM AFTER HYPOTHYROIDISM…………………………..…………..14-16

Satyaapir Sahu, Shabir Ahmad Shah, Supriti, Apurva Kumar R Joshi, Devanshu Patel J, Asha Yadav.
THE GUT-BRAIN AXIS: IMPLICATIONS FOR NEUROLOGICAL DISORDERS, MENTAL HEALTH, AND IMMUNE FUNCTION..17-24

Sara Mohammed Ouah Al-Saedi, Israa Hussein Hamzah.
THE ROLE GENE EXPRESSION OF PD-1 AND PD-L1 IN NEWELY DIAGNOSED AND TREATED PATIENTS WITH ACUTE
MYELOID LEUKEMIA………………………………………………………………………………………………………………………………………….25-29

Stepanyan L, Lalayan G, Avetisyan A.
AN INVESTIGATION OF PSYCHOLOGICAL AND PHYSIOLOGICAL FACTORS AFFECTING PERFORMANCE IN ADOLESCENT
JUDOKAS…………………………………………………………………………………………………………………………………………..30-36

Takuma Hayashi, Nobuo Yaegashi, Ikuo Konishi.
EFFECT OF RBD MUTATIONS IN SPIKE GLYCOPROTEIN OF SARS-COV-2 ON NEUTRALIZING IGG
AFFINITY……………………………………………………………………………………………………………………………………………37-46

Yahya Qasem Mohammed Taher, Muna Muneer Ahmed, Hakki Mohammed Majdal.
A CLINICO-EPIEMIOLOGICAL STUDY OF MULTIPLE SCLEROSIS IN MOSUL CITY, IRAQ……………………………47-52

Simona Kordeva, Georgi Tchernev.
THIN MELANOMA ARISING IN NEVUS SPILUS: DERMATOSURGICAL APPROACH WITH FAVOURABLE OUTCOME…….53-55

Buthaina H. Al-Sabawi, H. S. Sadoon.
HISTOCHEMICAL CHANGES OF THE PULMONARY HYDATID CYSTS IN SHEEP INFECTED WITH CYSTIC
ECHINOCOCCOSIS……………………………………………………………………………………………………………………………………56-60

Rocco De Vitis, Marco Passiatiore, Vitale Cilli, Massimo Apicella, Giuseppe Taccardo.
SARS-COV-2 INFECTION AND INVOLVEMENT OF PERIPHERAL NERVOUS SYSTEM: A CASE SERIES OF CARPAL TUNNEL
SYNDROME AGGRAVATION OR NEW ONSET WITH COVID-19 DISEASE AND A REVIEW OF LITERATURE………………..61-66

L. Dzyak, K. Miziakina.
NEURAL PROTEINS AS MARKERS FOR DIAGNOSING STRUCTURAL DAMAGE TO BRAIN MATTER IN POST-TRAUMATIC
NEUROCOGNITIVE DISORDERS…………………………………………………………………………………………………………67-70

PURIFICATION, CHARACTERIZATION, AND IN VITRO ANTITUMOR ACTIVITY OF A NOVEL GLUCAN FROM PHOENIX
DACTYLIFERA L. FRUITS……………………………………………………………………………………………………………………71-75

Natalia Stepaniuk, Oleh Piniazhko, Olesia Poshyvak, Tetiana Bessarab, Natalia Hudz, Irina Gavriluk.
MANAGEMENT OF RISKS OF ADVERSE DRUG REACTIONS ACCORDING TO ADR REPORT FORM DATA FROM LVIV REGION
HEALTHCARE FACILITIES IN 2022…………………………………………………………………………………………………………..76-80

Ghazwan M. Radhi, Nihad N. Hilal, Mohammed M. Abdul-Aziz.
TESTOSTERONE AND SERUM ZINC LEVELS IN MEN WITH BENIGN PROSTATIC HYPERPLASIA……………………..…………81-86

Zora Khan, Deepthi Krishna, Surya Shekhar Daga, Nitin Kumar Rastogi, Rekha MM, Komal Patel.
ADVANCEMENTS IN MINIMALLY INVASIVE SURGERY: A COMPREHENSIVE ANALYSIS OF ROBOTIC SURGERY,
ENDOSCOPIC TECHNIQUES, AND NATURAL ORIFICE TRANSLUMENAL ENDOSCOPIC SURGERY (NOTES)…………………87-92

Aditi Jane, Manoj Rameshachandra Vyas, Anil Kumar, Anurag Verma, Giresha AS, Devanshu Patel J.
LIVER FIBROSIS: PATHOPHYSIOLOGY, DIAGNOSIS, AND EMERGING THERAPEUTIC TARGETS FOR A COMMON
COMPLICATION OF CHRONIC LIVER DISEASES……………………………………………………………………………………….93-100

INNOVATIONS IN ARTIFICIAL ORGANS AND TISSUE ENGINEERING: FROM 3D PRINTING TO STEM CELL THERAPY….101-106

Nada HA. Al-Nuaimi, Saher S. Gasgoos.
EFFECT OF CHICKEN EGGSHELL PASTE ON ENAMEL SURFACE MICROHARDNESS AND COLOUR CHANGE OF ARTIFICIAL
CARIOUS LESIONS CREATED ON PERMANENTLY EXTRACTED TEETH…………………………………………………………………………..107-112

Ali Sabah Abbas, Hind Taher Jarjees.
EVALUATION THE EFFECT OF THE ADDITION OF ZIRCONIUM OXIDE AND TITANIUM DIOXIDE NANOPARTICLES ON SHEAR
BOND STRENGTHS OF ORTHODONTIC ADHESIVE: IN-VITRO STUDY……………………………………………………………………113-121
Shypunov V.G, Strafun S.S, Borzykh A.V, Borzykh N.A, Zahovenko M.A.
Peculiarities of Using a Neurovascularized Flap on the Sural Artery in Plastic Surgery of Gunshot Defects on the Foot and Lower Leg........................................................................................................232-236

Igor Morar, Oleksandr Ivashchuk, Sergiy Ivashchuk, Volodymyr Bodiaka, Alona Antoniv.
Microbiological Features of a Laparotomy Wound Complicated by Postoperative Eventration Against the Background of an Oncological Process.............................................................................................237-242

Vadim V. Klimentov, Kamilla R. Mavljanova, Jilia F. Semenova, Nikolay B. Orlov.
Circulating Peptides of the TNF Superfamily and TNF Receptor Superfamily in Subjects with Type 1 Diabetes: Relationships with Clinical and Metabolic Parameters...........................................................................243-248

Rurua Magda, Sanikidze Tamar, Machvariani Ketevan, Ormotsadze Giorgi, Intskirveli Nino, Mikadze Ia, Didbaridze Tamar, Ratiani Levan.
Correlative Association of Oxygenation and Sepsis Panels with the Use of ACE2 Inhibitors and Without it in the Conditions of Septic Shock in COVID-19-Infected and Non-Infected Patients (Cohort Study).............................................................................................................249-253

Vladyslava Kachkovska.
Association between GLN27GLU Polymorphism in the B2 Adrenergic Receptor Gene and Obesity Risk in Patients with Early-Onset and Late-Onset Bronchial Asthma...........................................................................................................254-258

Levandovskyi R, Belikova N, Belikov O, Sorokchan M, Roschuk O.
Evaluation of the Clinical Condition of the Oral Cavity Before Adhesive Splinting of Movable Tee Th........................................................................................................................................................................270-274

Bakhtiyarov Kamil Rafaelievich, Ivantsova Margarita Vladimirovna, Kukes Ilya Vladimirovich, Ignatko Irina Vladimirovna, Glagovsky Pavel Borisovich.
Metabolomic Markers of Endometriosis: Prospects..........................................................................................275-279

Jain SK, Komal Patel, Kavina Ganapathy, Firoz Khan, Satyaapir Sahu, Ashok Kumar Singh.
Laparoscopic Approach to a Giant Ruptured Splenic Cyst: A Challenging Case Report........................................280-283

ManojRameshchandra Vyas, Phool Chandra, Rachit Jain, Devanshu Patel J, Manashree Avinash Mane, Shaily.
Clinical and Objective Test Characteristics of Vestibular Migraine: Implications for Diagnosis and Management......................................................................................................................................................284-289

Vipin Kumar, Rakesh Ashokrao Bhongade, Vipin Kumar, Praveen Mathur, Komal Patel, Renuka Jyothi R.
Postcholecystectomy Syndrome: Understanding the Causes and Developing Treatment Strategies for Persistent Biliary Symptoms After Gallbladder Removal........................................................................................................290-296

Georgi Tchernev.
Loss of Efficacy of Adalimumab in Hidradenitis Suppurativa: Focus on Alternatives........................................297-300
Abstract.

Background: Certain mutant strains of SARS-CoV-2 are known to spread widely among humans, including the receptor binding domain (RBD) mutant, Y453F, from farmed minks, and the RBD mutant, N501Y, a mutation common to three major SARS-CoV-2 subvariants (B.1.1.7, B.1.351, and B.1.1.248) and omicron type SARS-CoV-2 BQ.1.1 and XBB.1.16 subvariants.

Methods: We investigated the characteristics of the RBD mutants, Y453F and N501Y, using three-dimensional structural analysis. We also investigated the effect of Y453F, N501Y or the mutants of RBD of omicron type SARS-CoV-2 BQ.1.1 and XBB.1.16 subvariants on neutralizing antibodies in serum derived from individuals including children (aged 5-11 years) inoculated with mRNA based COVID-19 vaccine (BNT162b2: Pfizer/BioNTech) or COVID-19-positive patients or children (aged 5-11 years) after vaccination with BNT162b2.

Results: Our results suggest that SARS-CoV-2 subspecies with the RBD mutations Y453F or N501Y partially escaped detection by 4 neutralizing monoclonal antibodies and 21 neutralizing antibodies in sera derived from COVID-19-positive patients or children (aged 5-11 years) after vaccination with BNT162b2.

Conclusions: Infection with SARS-CoV-2 subspecies that causes serious symptoms in humans may spread globally. In particular, since the antibody titer against the omicron type is low in children (aged 5-11 years) who have been vaccinated with conventional vaccines, it is important for children to receive vaccines specific for the omicron type.

Key words. RBD Y453F mutant, neutralizing antibody, SARS-CoV-2, COVID-19.


Introduction.

Mutations in the SARS-CoV-2 virus can jeopardize the efficacy of potential vaccines and therapeutics against COVID-19. Mustelidae animals (e.g., minks and ferrets) can be infected with SARS-CoV-2 relatively easily compared with other mammals [1] however, it has not yet been elucidated why SARS-CoV-2 is extremely contagious among these animals. Nonetheless, it is clear that when several farmed minks kept in a high-density environments are infected with SARS-CoV-2, the virus proliferates in large numbers. Consequently, humans and minks may be at high risk of SARS-CoV-2 infection.

Natural selection (adaptation) in the coronavirus can occur during virus amplification in vivo in farmed minks [2]. Natural selection in such viruses is observed by the introduction of mutations in SARS-CoV-2 that are not observed during the growth process in humans [2,3]. Infections with the mutant strain Y453F (from farmed minks) or N501Y (a mutation common to three major subspecies of SARS-CoV-2: B.1.1.7, B.1.351, and B.1.1.248) or omicron type SRAS-CoV-2 BQ.1.1 subvariant and XBB.1.16 subvariant, which is the current epidemic strain, are known to widely spread among humans [4-6].

In this study, we investigate the virological characteristics of these two-receptor binding domain (RBD) mutants using three-dimensional protein structural analysis [7,8]. We also investigated the affinity of IgG for the conventional RBD and the mutant RBDs, Y453F and N501Y, in serum obtained from 41 COVID-19-positive patients and 20 COVID-19-negative patients or serum collected from children aged 5 to 11 years. The findings indicate that SARS-CoV-2 infection with Y453F or N501Y mutations in the spike glycoprotein may escape the antiviral effect of neutralizing antibodies or COVID-19 vaccination.

Now, the omicron type SARS-CoV-2 B.1.1.529 variant evolved into several subvariants, three of which (BA.1, BA.2, and BA.5) became globally dominant. Currently, the prevalence of omicron type SARS-CoV-2 BQ.1 subvariants (a BA.5 variant), its sub lineage BQ.1.1, and XBB.1.16 sub lineage, which is a recombinant of two different BA.2 subvariants, are increasing rapidly in the Europe, America, Southeast Asia, and elsewhere. BQ.1.1 sub lineage and XBB.1.16 sub lineage possess substitutions relative to BA.5 and BA.2, respectively, in the receptor-binding domain of their spike glycoprotein, which is the major target for vaccines and therapeutic monoclonal antibodies (mAbs) for COVID-19 [9]. Therefore, we, the medical staff, collected blood from children aged 5 to 11 years who had been inoculated with BNT162b2, and examined neutralizing antibody titers against Omicron type SARS-CoV-2 variants. In particular, since the antibody titer against the omicron type is low in children (aged 5-11 years) who have been vaccinated with conventional vaccines, it is important for children to receive vaccines specific for the omicron type.

Materials and Methods.

Analysis of the three-dimensional structures of the binding sites of mink and human angiotensin-converting enzyme 2 (ACE2)

Some subspecies of SARS-CoV-2 have the amino acid mutations, Y453F or N501Y, in the sequence that encodes the
spike glycoprotein [4,7,8]. These SARS-CoV-2 mutations have been detected in approximately 300 viral sequences isolated from European and Dutch populations, as well as in minks. We used the data on the three-dimensional structure of the RBD of the spike glycoprotein of SARS-CoV-2 (Protein Data Bank (PDB) ENTITY SEQ 6VW1_1) [7] and the data (PDB: 6XC2, 6XC4, 7JMP, 7JMO, 6XKQ, 6XKP, and 6XGD) for the three-dimensional structure of six neutralizing antibodies (CC12.1, CC12.3, COVA2-39, COVA2-04, CV07-250, CV07-270, and REGN-COV2) that bind to the spike glycoprotein of SARS-CoV-2 [8].

Using the Spanner program, we predicted the three-dimensional structure of the SARS-CoV-2 spike glycoprotein Y453F mutant based on PDB data (ENTITY SEQ 6VW1_1). We investigated the binding of the spike glycoprotein mutant Y453F to human angiotensin-converting enzyme 2 (ACE2) and determined the affinity of the spike glycoprotein mutants, Y453F and N501Y, to six neutralizing monoclonal antibodies using the MOE program (three-dimensional protein structure modeling, protein docking analysis: MOLSIS Inc., Tokyo, Japan) and C3D Macromolecular Structure Viewer.

Protein contact residues and buried surface areas were analyzed using the LigPlot+ program (v.1.4.5) (https://www.ebi.ac.uk/thornton-srv/software/ LigPlus/). Protein buried surface areas were analyzed using the PDBePISA tool (http://pdbe.org/pisa/) and MOE project DB (MOLSIS Inc. Tokyo Japan). The modeling and docking of mink ACE2 and RBD in the spike glycoprotein of SARS-CoV-2 was analyzed by MOE project DB with previously posted ID PDB and protein ID (MOLSIS Inc. Tokyo Japan). The binding affinity between mink ACE2 and the SARS-CoV-2 spike glycoprotein RBD was analyzed using MOE project DB (MOLSIS Inc.).

Adsorption of conventional RBD, Y453F and N501Y recombinant proteins to the solid phase surface of the ELISA plate

HeLa cells were transfected with either pcMV3-2019-nCov-RBD-Flag tag expression vector (2 µg), pcMV3-2019-nCov-RBD Y453F-Flag tag expression vector (2 µg), or pcMV3-2019-nCov-RBD N501Y-Flag tag expression vector (2 µg) (Sino Biological Inc. Beijing, China). The cells transfected with 2019-nCov-RBD, 2019-nCov-RBD Y453F, or 2019-nCov-RBD N501Y expression vectors were incubated for 48 h prior to harvesting. The conventional RBD, Y453F, and N501Y recombinant proteins were purified using a His-tag column following the standard procedure. Purified conventional RBD, Y453F, or N501Y recombinant proteins were adsorbed on the solid phase surface of the ELISA plate (SMILON ELISA plate MS-3508F, Tokyo Japan).

Quantitative measurement of SARS-CoV-2 neutralizing antibodies

The SeroFlash™ SARS-CoV-2 Neutralizing Antibody Assay Fast Kit (EpiGentek Group Inc., NY) contains all the reagents necessary for quantitatively measuring the level of SARS-CoV-2 neutralizing antibodies. In this assay, conventional RBD, Y453F, or N501Y spike proteins were stably pre-coated onto microplate wells. His-tagged ACE2 binds to the coated spike protein in the presence or absence of neutralizing antibodies in the sample. The amount of the bound ACE2, which is proportional to ACE2 inhibition intensity, is then recognized by the neutralizing detection complex, which contains anti-His antibodies and is measured through an ELISA-like reaction, where the absorbance is read by a microplate spectrophotometer at a wavelength of 450 nm. The neutralizing antibody level is inversely proportional to the optical density intensity measured, i.e., the higher the level of neutralizing antibody, the lower the OD intensity. Quantitative measurements of SARS-CoV-2 neutralizing antibody levels were performed according to the manufacturer's procedure.

Qualitative measurement of SARS-CoV-2 neutralizing antibodies

Serum samples (25 µL) from 20 COVID-19-negative patients and 41 COVID-19-positive patients with varying immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody levels were used for this assay. COVID-19 status was confirmed by RT-PCR, antigen, and/or antibody serology tests. All serum samples were provided by RayBiotech (RayBiotech Life, GA). The details of the materials and methods used are described in the supplementary materials. A neutralizing inhibition score of ≥ 20% was used to indicate a positive result and detection of SARS-CoV-2 neutralizing antibody; a score under 20% was used to indicate a negative result and no detectable SARS-CoV-2 neutralizing antibody.

Analyzes the three-dimensional structure of the binding site between mink and human ACE2

Spanner is a structural homology modeling pipeline that threads a query amino-acid sequence onto a template protein structure. Spanner is unique in that it handles gaps by spanning the region of interest using fragments of known structures. To create a model, you must provide a template structure, as well as an alignment of the query sequence you wish to model onto the template sequence. The spanner will replace mismatched residues, and fill any gaps caused by insertions or deletions.

For users that are unable to create an alignment a method for building a model starting only from sequence is also available. During this process a template search is conducted, and an alignment is built dynamically using FORTE before being passed through to the main part of the pipeline. Spanner consists of several modules written in the Go programming language. For Spanner jobs which build a model only from sequence, the first step is a search of the PDB for possible templates using BLAST. These possible templates are then aligned and scored with FORTE. The next step involves defining the start and end points of fragments corresponding to insertions or deletions. The start and end points are referred to as anchors because they must be equivalent in both the template and any candidate fragment. The margin parameter determines how far from the edge of a gap the fragment begins or ends. For example, a margin of 0 would mean that the anchors begins at the very edge of a gap. This is usually not a good idea, and the default margin is set to 1.

A representative set of protein chains was prepared using CD-HIT at 100% sequence identity. All continuous fragments were then extracted from this set of chains and stored in a relational
Analyses of protein contact residues and protein buried surface areas were analyzed using the LigPlot' program (v.1.4.5) (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). Protein buried surface areas were analyzed using PDBePISA tool (http://pdbpe.org/pisa/) and MOE project DB (MOLSIS Inc. Tokyo Japan). The modeling and Docking of the mink ACE2 protein and RBD in Spike Glycoprotein SARS-CoV-2 was analyzed by MOE project DB (MOLSIS Inc.).

Cells

Vero E6-TMPRSS2-T2A-ACE2 cells (provided by Dr. Barney Graham, NIAID Vaccine Research Center) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% Fetal Calf Serum (FCS), 100 U/mL penicillin–streptomycin, and 10 μg/mL puromycin. VeroE6/TMPRSS2 (JCRB 1819) cells were propagated in the presence of 1 mg/ml genetin (G418; Invivogen) and 5 μg/ml plasmocin prophylactic (Invivogen) in DMEM containing 10% FCS. Vero E6-TMPRSS2-T2A-ACE2 and VeroE6/TMPRSS2 cells were maintained at 37°C with 5% CO2. The cells were regularly tested for mycoplasma contamination by using PCR and confirmed to be mycoplasma-free.

Viruses

hCoV-19/Japan/UT-NC0021-T/Human/2020/Tokyo were propagated in VeroE6/TMPRSS2 cells.

All experiments with SARS-CoV-2 were performed in enhanced biosafety level 3 (BSL3) containment laboratories at the Kyoto University and the National Institute of Infectious Diseases, Japan, which are approved for such use by the Ministry of Agriculture, Forestry, and Fisheries, Japan.

Clinical specimens

After informed consent was obtained, plasma specimens were collected from COVID-19 convalescent individuals and vaccinees. The research protocol was approved by the Research Ethics Review Committee of the Institute of Medical Science of the Kyoto University (approval numbers: 2019–71–0201 and 2020–74–0226).

Focus reduction neutralisation test

Neutralisation activities of plasma were determined by using a focus reduction neutralisation test as previously described. After the plasma samples were incubated at 56°C for 1 h, the samples were serially diluted five-fold with DMEM containing 2% FCS in 96-well plates and mixed with 100–400 focus-forming units (FFU) of virus/well, followed by incubation at room temperature for 1 h. The plasma-virus mixture (50 μl) was then inoculated onto Vero E6-TMPRSS2-T2A-ACE2 cells in 96-well plates in duplicate and incubated for 1 h at 37°C. An equal volume of 1.5% Methyl Cellulose 400 (FUJIFILM Wako Pure Chemical Corporation) in culture medium was then added to each well. The cells were incubated for 14–16 h at 37°C and then fixed with formalin. After the formalin was removed, the cells were immuno stained with a mouse monoclonal antibody against SARS-CoV1/2 nucleoprotein [clone 1C7C7 (Sigma-Aldrich)], followed by a horseradish peroxidase-labeled goat anti-mouse immunoglobulin (SeraCare Life Sciences). The infected cells were stained with TrueBlue Substrate (SeraCare Life Sciences) and then washed with distilled water. After cell drying, the focus numbers were quantified by using an ImmunoSpot S6 Analyzer, ImmunoCapture software, and BioSpot software (Cellular Technology). The results are expressed as the 50% focus reduction neutralisation titre (FRNT50). The FRNT50 values were calculated by using GraphPad Prism (GraphPad Software).

Results.

Structural analysis:

The results from the Spanner analysis revealed that the RBD mutants Y453F and N501Y did not affect the three-dimensional structure of conventional SARS-CoV-2 spike glycoproteins. The results clarified that the binding property of the Y453F mutant spike glycoprotein to human ACE2 was slightly weaker than that of the conventional SARS-CoV-2 spike glycoprotein (Figure 1A, Table 1). Conversely, the binding property of the N501Y mutant spike glycoprotein to human ACE2 was stronger than that of the conventional RBD (Figure 1B, Table 1). The slightly weaker affinity observed in Y453F was due to the...
The results of the structural analysis reveal that the affinity between the Y453F spike glycoprotein and four of the six examined monoclonal antibodies (CC12.1, CC12.3, COVA2-39, COVA2-04, CV07-250, CV07-270) was clearly weak compared with the conventional RBD (Figure 2A, Table 1). The results also demonstrated that the affinity between the N501Y spike glycoprotein and four of the six examined monoclonal antibodies was stronger compared with the conventional RBD (Figure 2B).

The replacement of Tyr at position 453 by Phe, which was unable to form a hydrogen bond with His at position 34 in human ACE2 (Figure 1A), led to a slight decrease in the affinity between the spike glycoprotein RBD mutant Y453F and human ACE2 compared with the conventional RBD. On the other hand, the replacement of Asn at position 501 by Tyr, which was able to form a hydrogen bond with Lys at position 353 in human ACE2, and to create a benzene ring interaction with Tyr at position 41 in human ACE2, resulted in an increased affinity for the N501Y mutant (Figure 1B).

The results of the structural analysis reveal that the affinity between the Y453F spike glycoprotein and four of the six examined monoclonal antibodies (CC12.1, CC12.3, COVA2-39, COVA2-04, CV07-250, CV07-270) was clearly weak compared with the conventional RBD (Figure 2A, Table 1). The results also demonstrated that the affinity between the N501Y spike glycoprotein and four of the six examined monoclonal antibodies was stronger compared with the conventional RBD.
antibodies was clearly weak compared with the conventional SARS-CoV-2 spike glycoprotein RBD (Figure 2B, Table 1).

Quantitative measurement of SARS-CoV-2 neutralizing antibodies

On September 28, 2020, the US pharmaceutical manufacturer, Regeneron Pharmaceuticals, announced the production of the antibody cocktail therapy, REGN-COV2, which combined two neutralizing antibodies, casirivimab and imdevimab, for the treatment and prevention of COVID-19 [10] (Figure 3A). On January 11, 2021, Regeneron Pharmaceuticals stated the likelihood of the antiviral effectiveness of REGN-COV2 on SARS-CoV-2 subsppecies [11]. Therefore, we investigated the affinity of REGN-COV2 to the spike glycoproteins of Y453F and N501Y. We found that the affinity of REGN-COV2 (6XGD) to Y453F was weaker than that to the conventional RBD (Figure 3B, Table 1). However, the affinity of REGN-COV2 to N501Y was strong compared to the conventional RBD (Figure 3B). Therefore, the antiviral effect of REGN-COV2 as a neutralizing antibody may be maintained even against RBD mutant strains of SARS-CoV-2.

Qualitative measurement of SARS-CoV-2 neutralizing antibodies

Next, we investigated the affinity of serum IgG to the conventional RBD, Y453F, and N501Y in COVID-19-positive patients and healthy subjects. A strong affinity for conventional RBD was shown for the serum IgG of 29 of the 41 COVID-19-positive patients (Table 1). Moderate affinity for conventional RBD, Y453F, and N501Y was shown in the serum IgG of four of the COVID-19-positive patients (Table 1). No affinity for conventional RBD, Y453F, or N501Y was shown in the serum IgG of the COVID-19-negative subjects (Table 1).

From the results, we concluded that the mutation of tyrosine at amino acid residue 453 to phenylalanine or the mutation of asparagine at amino acid residue 501 to tyrosine eliminated the inhibitory effects of the neutralizing antibody on binding between ACE2 and the RBD of the SARS-CoV-2 spike glycoprotein. It is possible that the affinity between the appropriate amino acid residues in the variable region of the antibody and the RBD of Y453F or N501Y was diminished owing to weak recognition of the monoclonal antibody to SARS-CoV-2 spike glycoproteins.

Antibody titer of children against Omicron type SARS-CoV-2 BQ.1.1 subvariant and XBB.1.16 subvariant

To verify the antiviral effect of the mRNA-based COVID-19 vaccine (1) against the Omicron type SARS-CoV-2 BQ.1.1 subvariant and the XBB.1.16 subvariant, our medical team investigated the neutralizing activity of antibodies* in plasma of BNT162b2 vaccinated individuals against BQ.1.1 subvariant and XBB.1.16 subvariant isolated from Omicron type SARS-CoV-2 infected patients. As a result, compared with the neutralizing activity of plasma antibodies against the conventional strain, BA.2 subvariant, or BA.5 subvariant, the neutralizing activity of plasma antibodies against BQ.1.1 subvariant and XBB.1.16 subvariant was significantly turned out to be 21.06-fold and 22.33-fold low (Figure 4A).

Furthermore, our research team examined the neutralizing activity of antibodies in the plasma of patients infected with the BA.2 subvariant after the third dose of BNT162b2 (i.e., the BA.2 variant breakthrough infection) against the BQ.1.1 subvariant and the XBB.1.16 subvariant. As a result, compared with the neutralizing activity of plasma antibodies against the conventional strain, BA.2 subvariant, or BA.5 subvariant,
Figure 2. Interactions between the heavy chain of the neutralizing monoclonal antibody, CC12.2 and the SARS-CoV-2 spike glycoprotein RBD mutants, Y453F and N501Y. 

(A) The interaction between the heavy chain (purple) and light chain (brown) of neutralizing monoclonal antibody CC12.1 and residues of the conventional RBD or Y453F (green) is shown using the three-dimensional structure model. It is speculated that the Y453 amino acid residue of the conventional RBD is hydrogen-bonded to the D92 amino acid residue of the light chain and to the D97 amino acid residue of the heavy chain of CC12.1. However, the binding between the F453 amino acid residue of the RBD mutant and the D92 and D97 amino acid residues of CC12.1 is presumed to be weak. From these results, the affinity between the spike glycoprotein of Y453F and CC12.1 is presumed to be lower compared with the conventional RBD. (B) The interaction between the heavy chain (purple) and light chain (brown) of CC12.1 and the residues of the conventional RBD or N501Y (green) is shown using the three-dimensional structure model. It is speculated that the N501 amino acid residue of the conventional RBD is hydrogen-bonded to the S30 amino acid residue of the light chain of CC12.1. However, the binding between the Y501 amino acid residue of the RBD mutant and the S30 amino acid residue of CC12.1 is presumed to be weak. From these results, the affinity between the RBD of N501Y and CC12.1 is presumed to be slightly lower compared with the conventional RBD. The three-dimensional structure models are shown by Cn3D macromolecular structure viewer. Detailed experimental results are indicated in the supplementary data.
Figure 3. Interactions between the antibody cocktail therapy REGN-COV2 (casirivimab REGN10933 and imdevimab REGN10987; 6XGD) and Y453F and N501Y.

(A) The interaction between the heavy chains (purple, light green) and light chains (brown, pink) of casirivimab and imdevimab and residues of the conventional RBD (green) is shown using the three-dimensional structure model. It is speculated that the Y453 amino acid residue of the conventional RBD is hydrogen-bonded to the D31 amino acid residue of the light chain of casirivimab. However, the binding between the N501 amino acid residue of the conventional RBD and the amino acid residues of imdevimab is presumed to be weak. (B) The interactions between the heavy chain (purple) and light chain (brown) of casirivimab and imdevimab and residues of Y453F and N501Y (green) are shown using the three-dimensional structure model. The binding between the F453 amino acid residue of Y453F and the amino acid residue of the light chain of casirivimab is presumed to be weak. However, it is speculated that the Y501 amino acid residue of N501Y is hydrogen-bonded to the K76 amino acid residue of the light chain of imdevimab. The three-dimensional structure models are shown by Cn3D macromolecular structure viewer. Detailed experimental results are indicated in the supplementary data.
Figure 4. Antibody responses to SARS-CoV-2 omicron subvariants

(A) Neutralizing antibody titers of human plasma obtained from individuals immunized with a third dose of BNT162b2. Samples were collected 180–189 days after the third immunization (n=35). (B) Neutralizing antibody titers of human plasma obtained from individuals who were infected with omicron BA.2 after three doses of BNT162b2. Samples were collected 29–89 days after symptom onset (n=21). Each dot represents data from one individual. The lower limit of detection (value=10) is indicated by the horizontal dashed line. Samples under the detection limit (<10-fold dilution) were assigned an 50% focus reduction neutralization titer (FRNT50) value of 10 and are represented by X. Geometric mean titers are shown. FRNT50=50% focus reduction neutralization titer. Ancestral: the conventional strain, BA.2 subvariant, or BA.5 subvariant.

As of January 2021, the number of people infected with SARS-CoV-2 N501F subspecies, which are believed to have occurred in the United Kingdom, South Africa, and Brazil, has increased significantly in the UK and other European countries. Recent studies have shown that the infectivity of the N501Y variant is approximately 1.4–1.7 times that of previous strains of SARS-CoV-2 [12]. In addition, N501F variants have the property of easily infecting children. Currently, the question is whether N501Y variants are resistant to the COVID-19 vaccines which has been distributed in the UK, the US, and other countries. Pfizer, who created the first-to-be-approved COVID-19 vaccine (known as BNT162b2), has demonstrated the possibility of the immediate production of mRNA that should correspond to SARS-CoV-2 mutations. On January 8, 2021, Pfizer and BioNTech reported the efficacy of the COVID-19 vaccine against the UK and South Africa SARS-CoV-2 variants based on the results of phase I clinical trials [13]. On January 11, 2021, Regeneron Pharmaceuticals announced the antiviral effectiveness of the antibody cocktail therapy, REGN-COV2, against SARS-CoV-2 subspecies. However, concerns remain regarding the effectiveness of the COVID-19 vaccine against these SARS-CoV-2 variants [3,4,14-16].

Based on textbooks, it is known that viruses gradually mutate in the process of repeating proliferation and infection. SARS-CoV-2 is also repeating mutations little by little, and so the infectivity of SARS-CoV-2 to humans and other mammals is changing. Most mutations have little effect on the characteristics of SARS-CoV-2, but some mutations may affect SARS-CoV-2 infectivity/transmissibility, risk of severe disease, efficacy of vaccines/therapeutic drugs, diagnosis. With the advancement of science and technology, novel vaccines against COVID-19 are also being rapidly developed, contributing to the prevention
of severe cases of COVID-19. From the results of this clinical study, it was revealed that in children aged 5–11 years who received BNT162b2, the neutralizing antibody titers against the Omicron type SARS-CoV-2 variants were significantly lower than those against the Wuhan type ARS-CoV-2 variants and the Delta type SARS-CoV-2 variants. However, elevated neutralizing antibody titers against Omicron type SARS-CoV-2 variants were also observed in children aged 5–11 years who received BNT162b2. Therefore, for children aged 5–11 years with underlying medical conditions who are at high risk of severe COVID-19, from the viewpoint of preventing the severity of COVID-19, vaccination with mRNA-based COVID-19 vaccine is recommended regardless of age.

Mankind has struggled to develop therapies against AIDS to correspond to the speed of mutations that arise in the human immunodeficiency virus (HIV) [17]. In the fight between humans and viruses, mankind has never been able to prevent the mutation of viruses. To date, more than 50 countries and territories around the world have imposed strict restrictions on entry from the UK, South Africa, and Brazil [18]. First, people around the world should reaffirm the importance of wearing masks, hand hygiene, and social distancing.

Footnote.

All authors are receiving medical ethics education. In addition, this study has been approved as a clinical medical study at each medical facility. The human serum samples used in this study were purchased from RayBiotechn life, and therefore Informed consent from the patient is not required.

Data Sharing.

Data is available on various websites and have also been made publicly available (more information can be found in the first paragraph of the Results section).

Ethics statement.

This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization of Japan (Meguro, Tokyo, Japan) and the Central Ethics Review Board of Kyoto University (Kyoto, Kyoto, Japan). The approved number for this study is 50-201504. In order to carry out this research, the authors attended a research ethics education course (e-APRIN) conducted by Association for the Promotion of Research Integrity (APRIN; Shinjuku, Tokyo, Japan). The approved numbers of e-APRIN are AP0000151756, AP0000151757, AP0000151758, AP0000151769.

Disclosure.

The authors declare no potential conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Author Contributions.

T.H. performed most of the experiments and coordinated the project. T.H. and N.Y. conceived the study and wrote the manuscript. N.Y. and I.K. provided information on clinical medicine and oversaw the entire study.

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