Clinical Application of Antibody Immunity Against SARS-CoV-2: Comprehensive Review on Immunoassay and Immunotherapy

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Abstract

The current COVID-19 global pandemic poses immense challenges to global health, largely due to the difficulty to detect infection in the early stages of the disease, as well as the current lack of effective antiviral therapy. Research and understanding of the human immune system can provide important theoretical and technical support for the clinical diagnosis and treatment of COVID-19, the clinical implementations of which include immunoassays and immunotherapy, which play a crucial role in the fight against the pandemic. This review consolidates the current scientific evidence for immunoassay, which includes multiple methods of detecting antigen and antibody against SARS-CoV-2. We compared the characteristics, advantages and disadvantages, and clinical applications of these three detection techniques. In addition to detecting viral infections, knowledge on the body's immunity against the virus is desirable; thus, the immunotherapy-based neutralizing antibody (nAb) detection methods were discussed. We also gave a brief introduction to the new immunoassay technology such as biosensing. This was followed by an in-depth and extensive review on a variety of immunotherapy methods. It includes convalescent plasma therapy, neutralizing antibody–based treatments targeting different regions of SARS-CoV-2, immunotherapy targeted on the host cell including inhibiting the host cell receptor and cytokine storm, as well as cocktail antibodies, cross-neutralizing antibodies, and immunotherapy based on cross-reactivity between viral epitopes and autoepitopes and autoantibody. Despite the development of various immunological testing methods and antibody therapies, the current global situation of COVID-19 is still tense. We need more efficient detection methods and more reliable antibody therapies. The up-to-date knowledge on therapeutic strategies will likely help clinicians worldwide to protect patients from life-threatening viral infections.

Keywords SARS-CoV-2 · COVID-19 · Antibody · Immunity · Immunoassay · Immunotherapy

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Abbreviations

ANCA	Anti-neutrophilic cytoplasmic antibodies
CLIA	Chemiluminescence immunoassay
COVID-19	Coronavirus disease 2019
CP	Convalescent plasma
ELISA	Enzyme-linked immunosorbent assay
Fc	Fragment crystallizable
PRNT	Plaque reduction neutralization test
HR	Heptad repeat
hrsACE2	Human recombinant soluble ACE2
HSPGs	Heparan sulfate proteoglycans
Ig	Immunoglobin
LFIA	Lateral flow immunochromatographic assays
mAb	Monoclonal antibody
N protein	Nucleocapsid protein
nAb	Neutralizing antibody
NET	Neutrophil extracellular traps

NR	Not reported
NTD	N-terminal domain
pAb	Polyclonal antibody
RBD	Receptor-binding domain
S protein	Spike protein
SARS-CoV-2	Severe acute respiratory syndrome corona-virus 2

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes coronavirus disease 2019 (COVID-19), the contagious disease responsible for the COVID-19 pandemic [1]. SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. It enters human cells by binding the S protein on its surface to the ACE2 receptor of human cells and thus proliferates in large numbers [2]. SARS-CoV-2 infection stimulates antigen-specific antibody responses.

Immunoassays and immunotherapy are the two broad areas of clinical application for antibody immunity. The primary goal of immunoassay is to detect the presence of antibodies, which can be used to monitor immunity and infection in the population, as well as to aid in the development of vaccines. As a result, developing a faster and more accurate antibody detection method is an important way to aid in the fight against the COVID-19 pandemic. Immunotherapy, on the other hand, could reduce mortality by offering a new way to treat patients without targeted drugs.

Therefore, the purpose of this review is to summarize the current scientific evidence for immunoassay and immunotherapy to further provide important theoretical and technical support for the clinical diagnosis and treatment of COVID-19.

Immunoassays

The term immunoassay refers to any assay that, at its core, depends on the binding of antigen and antibody. There are three types of tests available for COVID-19: molecular, antigen, and antibody (serology) testing. Molecular tests like the polymerase chain reaction (PCR) are considered to be the gold standard for diagnosis by amplifying and detecting its genetic material. However, this process can take hours and requires sophisticated lab equipment and technicians, and its detection sensitivity can vary depending on the sample collection handling method [3]. As an alternative, highly sensitive immunological methods that directly detect viral antigens or antibodies (separately or simultaneously) in clinical samples without sample preparation steps are necessary for the accurate diagnosis of COVID-19. These immune assays are fast (even within 15 min) and do not rely on professional technicians, equipment, and facilities.

Antigen assays are immunoassays that detect the specific viral proteins (antigens) using specific antibodies, allowing the capture of the entire virus or its fragments. Antigen tests have been developed for use in the laboratory or at the point of care, the latter being known as rapid antigen detection (RAD). SARS-CoV-2 RAD can detect the virus directly in respiratory samples. Furthermore, antigens are far more stable than RNA, making them less prone to degradation during transport and storage. Therefore, it may be a useful tool for the early diagnosis of the asymptomatic population. Both molecular and antigen tests detect whether a person is currently infected, while antibody (serology) mainly detects whether a person had an infection in the past (IgM can also detect recent or current infection), even if they had no symptoms of the illness. Antigen-specific antibody binding is used in the antibody detection assay to detect IgA, IgM, and IgG in blood, plasma, and serum samples. In a follow-up study of 173 patients [4], the antibody presence rate was < 40% in the first week after onset but quickly increased to 100.0% (IgA), 94.3% (IgM), and 79.8% (IgG) on the 15th day after onset. Therefore, antibody levels lag behind the infection, typically rising significantly in the second and third weeks after initial onset. This feature prevents antibody testing from being used for the timely diagnosis of COVID-19; however, the detection of IgG and IgM is beneficial to the study of the disease course. Thus, combining PCR and antibody detection can make up for their respective shortcomings. At the stage of large-scale vaccination, the significance of antibody detection mainly lies in monitoring the production of antibodies after a vaccine injection, in order to monitor the number of people immunized with the virus in the population and identify asymptomatic infections [5].

Among the four structural proteins of SARS-Cov-2, the most commonly used biomarkers for the detection of COVID-19 are the S and N protein antigens. As an evolutionary conserved and highly immunogenic phosphoprotein, N protein is the most frequently present protein in the SARS-CoV-2 structure. Thus, the SARS-CoV-2 antigen detection kits reported thus far primarily detect N protein [6]. The S protein is also very suitable as a diagnostic antigen because it is the main transmembrane protein of the virus and is highly immunogenic [7]. In addition, the spike protein exhibits amino acid sequence diversity among coronaviruses [8], enabling the specific detection of SARS-CoV-2. The SARS-CoV-2 spike detection enzyme-linked immunosorbent assay (ELISA) test has been developed for the quantitative detection of SARS-CoV-2 spike protein and is based on the solid-phase sandwich enzyme immunoassay. This test contains antibodies specific for the recombinant spike protein and can recognize both the recombinant as well as wild-type spike protein with 67.02 pg/ml sensitivity [9]. Both N and S proteins are highly antigenic

Table 1 Summary of pros and cons of immunoassays

Immunoassay	Main properties	Pros	Cons	Reference
LFIA	Based on the movement of a liquid sample through a polymeric strip with attached molecules that interact with the analyte	• Visually recognizable signal; • Fast, low cost, portable, and easy-to-use; • Long shelf life, no need to refrigerate	• Low sensitivity; • Qualitative or semi-quantitative result; • Good antibody preparation is obligatory;	[14, 102]
CLIA	Labeling antibodies or antigens with chemical luminescent agents, and the luminescent markers were quantitatively or qualitatively detected after the reflection	• High sensitivity and specificity; • Wide range of detection	• Require professional laboratories and large instruments	[13, 103]
ELISA	An enzyme-labeled method of labeling primary or secondary antibody-specific antigens and antibodies with enzymes	• High sensitivity; • High-throughput;	• Time-consuming	[12, 104]
Neutralization test	Virus and antibody are mixed and incubated under appropriate conditions before testing the infection of the virus on the cell	• Can determine whether the antibody can neutralize the virus	• Need sophisticated skills and biosafety laboratory to manipulate the live pathogen; • Time-consuming	[15–17]
Biosensor assays	Combines a biological component with a physicochemical detector	• Fast and sensitive response; • High-throughput;	• Electrode not easy to maintain; • Need advanced skills; • Expensive labels	[104]

and play an important role in inducing a host immune response and pathogenesis. Antibodies against N proteins are longer-lived and occur in greater abundance than antibodies against other viral components [4]. Several studies have been conducted to compare the sensitivity and specificity of the S and N proteins [4, 10, 11]. When Li et al. compared SARS-CoV-2 antigen detection from 20 studies [4], they concluded that the specificities of these two antigens were generally comparable, while antigen detection using the N protein antibodies was more sensitive than that using the S protein antibodies for early infections.

Two common technologies used in antigen detection are lateral flow immunoassay (LFIA) and chemiluminescence immunoassay (CLIA) (Table 1), while antibody detection methods fall into four categories: laboratory tests for ELISA [12], CLIA [13], LFIA [14], and neutralization test [15–17]. The LFIA method detects antigen or antibodies by using colloidal gold test paper, which is simple in design, portable, rapid, and easy to interpret, but is limited to qualitative detection and lacks sensitivity [18]. Both ELISA and CLIA are quantitative methods that involve labeling antibodies or antigens with enzymatic or chemical luminescent agents. In a meta-analysis using 24 articles, Mekonnen et al. [19] evaluated the diagnostic accuracy of serological antibody detection and found that the summary sensitivity/specificity of CLIA, ELISA, and LFIA were 92% (95% CI: 86–95%)/99% (CI: 97–99%), 86% (CI: 82–89%)/99% (CI: 98–100%), and 78% (CI: 71–83%)/98% (95% CI: 96–99%), respectively.

However, none of these three methods can determine whether the antibody has the ability to neutralize the virus.

The presence of neutralizing antibodies (nAbs) is considered a functional correlate of immunity because it provides at least partial resistance to subsequent infections by virus–antigen binding to prevent interaction with host cells [20, 21]. Therefore, comparing new serological assays to virus-neutralizing tests is critical as part of the validation process. The current gold standard method to measure nAb is the conventional virus neutralization test. In the test, virus and nAbs are mixed and incubated under appropriate conditions before testing the infection of the virus on the cell [15–17]. The FDA stated in one of the templates [22] that plaque reduction neutralization test (PRNT) [23] is currently considered to be the gold standard for detecting and measuring nAb titers. Microneutralization assays and focus reduction neutralization testing are also accepted as comparable neutralization comparator methods. At the moment, the neutralization antibody assay is the gold standard for the diagnosis of antibodies and evaluation of the effectiveness of antibodies against COVID-19; however, the neutralization test requires a biosafety level 3 laboratory to manipulate the live pathogen [24] and a longer detection time; thus, it is far less popular than the methods listed above [15, 17] and cannot be used for large-scale screening. Therefore, neutralization assays using pseudovirus [25] and surrogate virus [26] were developed. Results of pseudovirus PRNT prepared by Yang et al. [27] were highly correlated with those obtained using live viruses ($R^2 = 0.6931$, $P < 0.005$). Studies of surrogate viruses by Tan et al. [28] achieved a specificity of 99.93% and sensitivity of 95–100%, and neither pseudoviruses nor surrogate viruses required a biosafety level 3 laboratory [29]. They not only ensure the certainty

of antibody detection but also reduce the limitation of test conditions.

In addition to the abovementioned traditional immunoassays, a wide range of technologies has also been developed to overcome the shortages in the testing area. Biosensing refers to the detection of biomolecules using an analytical device (i.e., biosensor) that combines a biological component with a physicochemical detector. Researchers have recently reported a variety of biosensing strategies such as electrochemical, optical, electrical, mechanical, and piezoelectric biosensors for the detection of pathogens [30, 31]. Among them, field-effect transistors have attracted the attention of scientists due to their miniaturized size, fast and sensitive response, and the potential for parallel sensing. Seo et al. [7] modified a graphene-based FET biosensor device (COVID-19 FET sensor) to detect spike protein on SARS-CoV-2. This device used probe linkers like 1-pyrenebutyric acid N-hydroxy succinimide ester (PBASE) to immobilize the specific antibody and can detect viral antigens with a sensitivity of 1 fg/ml. Furthermore, Cady et al. [32] introduced a multiplexed grating-coupled fluorescent plasmonics biosensor platform for the measurement of antibodies against SARS-CoV-2 in human blood serum and dried blood spot samples.

Immunotherapy

Immunotherapy has been shown to reliably enhance immune protection against cancer and viral infections, by modifying the patient's own immune system or using antibodies provided from external sources. As the aggravation of physical damage and the increase in mortality of COVID-19 patients are all related to insufficient production of anti-SARS-CoV-2 antibodies, the use of antibody immunotherapy in improving the course of the disease and reducing mortality is of great research value. To that end, this section collects and describes the effects of several existing immunotherapies that use antibodies, with the hope of pointing out the future direction of immunotherapy.

Specific nAb-based therapies are being used to treat people who have been infected with the virus, and these therapies show great promise in reducing hospitalizations and deaths. Table 2 shows the summary of nAb-based therapy studies. A summarized diagram showing the current evidence for immunoassay and immunotherapy as well as the targeting sites is presented in Fig. 1.

Convalescent Plasma Therapy

Convalescent plasma (CP) has been reported to be used in treating COVID-19 patients in the early stages of the SARS-CoV-2 pandemic, in the absence of approved specific antiviral agents. In a recent study [33], CP was used to treat 10 patients with severe COVID-19. Three days after the infusion of 200 ml CP, their clinical symptoms and blood oxygen saturation improved significantly, the level of viral loads and C-reactive protein decreased, and blood lymphocytes increased. After 7 days of treatment, CT scans revealed that all patients' lung lesions had been absorbed. Similarly, Zeng et al. [34] reported that CP therapy improved clinical symptoms rapidly and was well tolerated in patients with severe COVID-19. Their research also demonstrated that CP could increase and maintain high nAb levels, as well as cause virus RNA to vanish within 7 days. The US FDA has approved CP to treat patients with severe COVID-19, but it is conditional on doctor approval [35]. It is worth noting that all patients received standard care, including antiviral and hormonal therapy, in addition to CP transfusion. Although CP can be obtained easily by removing blood cells of the donated blood, leaving behind plasma and antibodies, the large-scale application of CP treatment still faces significant challenges. These difficulties include a large number of CP deficiencies and donor-dependent variations in antibody specificities and titers [36]. Before CP therapy can be used widely, it must first be thoroughly evaluated in large-scale randomized controlled clinical trials. In addition, for CP therapy, a minimum effective nAb titer and optimal treatment time point must be determined.

RBD-Targeting nAb

Among the four structural proteins in SARS-COv-2, the S protein plays a key role in viral infection and pathogenesis [37]. It comprises subunits S1 and S2: S1 harbors the N-terminal domain (NTD) and the receptor-binding domain (RBD), whereas S2 harbors heptad repeat 1 (HR1) and HR2 [38]. The blood serum of COVID-19 patients contains a variety of SARS-CoV-2 antibodies, and RBD-binding antibodies have a higher neutralizing activity than non-RBD–binding antibodies. Rogers et al. [39] found that although viral infection induces a strong immune response against the non-RBD regions of the S protein, only a small part of this response is neutralizing. Brower et al. [40] isolated 19 nAbs, which target multiple antigenic sites on the S protein, and discovered that 74% of them targeted the RBD. Among them, COVA1-18 and COVID2-I5 were found to have the highest neutralizing activity against authentic SARS-CoV-2 virus, with half-maximum inhibitory concentrations (IC50) of 0.007 and 0.009 ug/ml, respectively. Ju et al. [41] isolated and characterized 206 monoclonal antibodies (mAbs) specific for the RBD from single B cells from eight individuals infected with SARS-CoV-2. P2C-1F11 and P2B-2F6 were two kinds of nAbs with IC50 values of 0.03 and 0.05 ug/ml, respectively.

Shi et al. [42] isolated two specific human mAbs (designated CA1 and CB6) targeting the RBD site from a patient recovering from COVID-19 infection. CB6 exhibited potent

Table 2 Summary of studies on nAb-based therapies

Publication	Ab type	Ab ID	Target	IC50 against pseudotyped SARS-CoV-2 (ug/ml)	IC50 against authentic SARS-CoV-2 (ug/ml)	Affinity (nmol/l)	Source	Registration number	Type of trial
Wu et al. [105]	mAb mAb	B38 H4	RBD RBD	NR NR	0.177 0.896	70.1 4.48	COVID-19 convalescent	NCT04375046 NR	Clinical trial; phase 1 Initial isolation of nAbs
Cao et al. [106]	mAb	BD-368-2	RBD	0.0012 (HIV)	0.015	0.82	COVID-19 convalescent	NR	Animal model study
Shi et al. [42]	mAb	CB6	RBD	0.23~0.41 (HIV)	0.036	2.49	COVID-19 convalescent	NCT04780321	Clinical trial; phase 2
Ju et al. [41]	mAb	P2C-1F11	RBD	0.03 (HIV)	0.03	2.21	COVID-19 convalescent	NR	Initial isolation of nAbs
Hansen et al. [107]	Cocktail Cocktail	REGN10987 REGN10933	RBD RBD	0.008 (HIV) 0.008 (HIV)	0.007 0.009	NR NR	Genetically humanized VI mice as well as COVID-19 convalescent	NCT04426695 NCT04425629	Clinical trial; phase 2 Clinical trial; phase 3
Rogers et al. [39]	mAb	CC6.29	RBD	0.002 (MLV)	0.007	1.2	COVID-19 convalescent	NR	Animal model study
Brouwer et al. [40]	mAb mAb	COVA1-18 COVA2-25	RBD RBD	0.008 (HIV) 0.008 (HIV)	0.007 0.009	NR NR	COVID-19 convalescent	NR NR	Animal model study
Seydoux et al. [108]	mAb	CV30	RBD	0.03 (HIV)	NR	3.6	COVID-19 convalescent	NR	Initial isolation of nAbs
Liu et al. [43]	mAb mAb mAb mAb	2-15 2-17 5-24 4-8	RBD NTD NTD NTD	0.005 (VSV) 0.168 (VSV) 0.013 (VSV) 0.032 (VSV)	0.0007 0.007 0.008 0.009	NR NR NR NR	COVID-19 convalescent	NR NR NR NR	Initial isolation of nAbs
Wu et al. [109]	mAb mAb	n3088 n3130	RBD RBD	3.3 (HIV) 3.7 (HIV)	2.6 4	3.7 (S1) 55.39 (S1)	Phage-displayed single-domain antibody	NR NR	Initial isolation of nAbs
Sun et al. [110]	mAb	ab6	RBD	NR	0.35	11	Phage-displayed single-domain antibody	NR	Initial isolation of nAbs
Chi et al. [50]	mAb mAb	4A8 0304-3H3	Non-RBD (S1, S-ECD) Non-RBD (S2, S-ECD)	49 (HIV) NR	0.61 0.04	92.7 (S1) 4.52 (S2)	COVID-19 convalescent	NR NR	Initial isolation of nAbs
Jiang et al. [111]	Cross-neutralizing nAb	47D11	RBD	0.061 (VSV)	0.57	9.6 (RBD)	hACE2 transgenic mouse	NCT04644120	Clinical trial; phase 1
Sun et al. [112]	Cross-neutralizing nAb	S309	RBD	0.24 (MLV)	0.079	<0.001 (RBD)	SARS convalescent	NR	Initial isolation of nAbs
Sia et al. [113]	Cross-neutralizing nAb	ADI-55688,55,689,55,690,55,951,55,993,56,000,56,010,56,032,56,046	RBD	0.05~1.40 (MLV)	0.05~1.40	<10	SARS convalescent	NR	Animal model study
Chi et al. [50]	Cross-neutralizing nAb	COVA1-16 COVA2-02	RBD RBD	0.131 (HIV) NR	0.745 <10	NR NR	COVID-19 convalescent	NR NR	Initial isolation of nAbs
Tai et al. [70]	Cross-neutralizing nAb	18F3,7B11	18F3: SARS-CoV and SARS-CoV-2 RBDs; 7B11: SARS-CoV RBD and not fully conserved in SARS-CoV-2 RBD	<10 (HIV)	NR	NR	SARS-CoV RBD-immunized mouse	NR	Initial isolation of nAbs
Baum et al. [60]	mAb	REGEN10933,REGN10987	RBD	NR	NR	NR	Genetically humanized mice as well as COVID-19 convalescent	NCT04425629, NCT04426695, NCT04452318	Clinical trial; phase 3 Clinical trial; phase 2 Clinical trial; phase 3

Table 2 (continued)

Publication	Ab type	Ab ID	Target	IC50 against pseudotyped SARS-CoV-2 (ug/ml)	IC50 against authentic SARS-CoV-2 (ug/ml)	Affinity (nmol/l)	Source	Registration number	Type of trial
Lv et al. [114]	mAb	H014v	RBD	3 nM	38 nM	27.8 nM	The phage antibody library	NR	Initial isolation of nAbs
Tychan [115]	mAb	TY027	RBD	NR	NR	NR	Phage display	NCT04429529	Clinical trial; phase 1
Chen et al. [116]	mAb	LY3819253/Ly-CoV555	RBD	NR	NR	NR	COVID-19 convalescent	NCT04441918, NCT04427501	Clinical trial; phase 1 Clinical trial; phase 1
Ju et al. [41]	mAb	JS106 (CB6)	RBD	NR	NR	NR	COVID-19 convalescent	NCT04441918	Clinical trial; phase 1
SAb [117]	pAb	SAB-185	RBD	NR	NR	NR	Transgenic cattle	NCT04468958, NCT04469179	Clinical trial; phase 1 Clinical trial; phase 1
Sorrento Therapeutics Inc. [118]	mAb	STI-1499 (COVI-GUARD)	RBD	NR	NR	NR	COVID-19 convalescent	NCT04454398	Clinical trial; phase 1
Haschke et al. [119]	hrsACE2	APN01	RBD	NR	NR	NR	Recombinant human ACE2 protein	NCT00886353, NCT04335136	Clinical trial; phase 1 Clinical trial; phase 2
Gaborit et al. [120]	Cocktail	XAV-19	RBD	NR	2.5	NR	Humanized animals	NCT04453384	Clinical trial; phase 2
Brii Biosciences Ltd [121]	mAb	BRII-196, BRII-198	RBD	NR	NR	NR	COVID-19 convalescent	NCT04479631, NCT04479644, NCT04787211	Clinical trial; phase 1 Clinical trial; phase 1 Clinical trial; phase 2
Sinocelltech Ltd. [122]	mAb	SCTA01	RBD	NR	NR	NR	Mice	NCT04483375	Clinical trial; phase 1

IC50 half-maximum inhibitory concentrations, *Ab* antibody, *mAb* monoclonal antibodies, *pAb* polyclonal antibody, *nAb* neutralizing antibody, *hrsACE2* human recombinant soluble ACE2, *NTD* N-terminal domain, *RBD* receptor-binding domain, *HIV* human immunodeficiency virus, *VSV* vesicular stomatitis virus, *MLV* Moloney murine leukemia virus, *NR* not reported
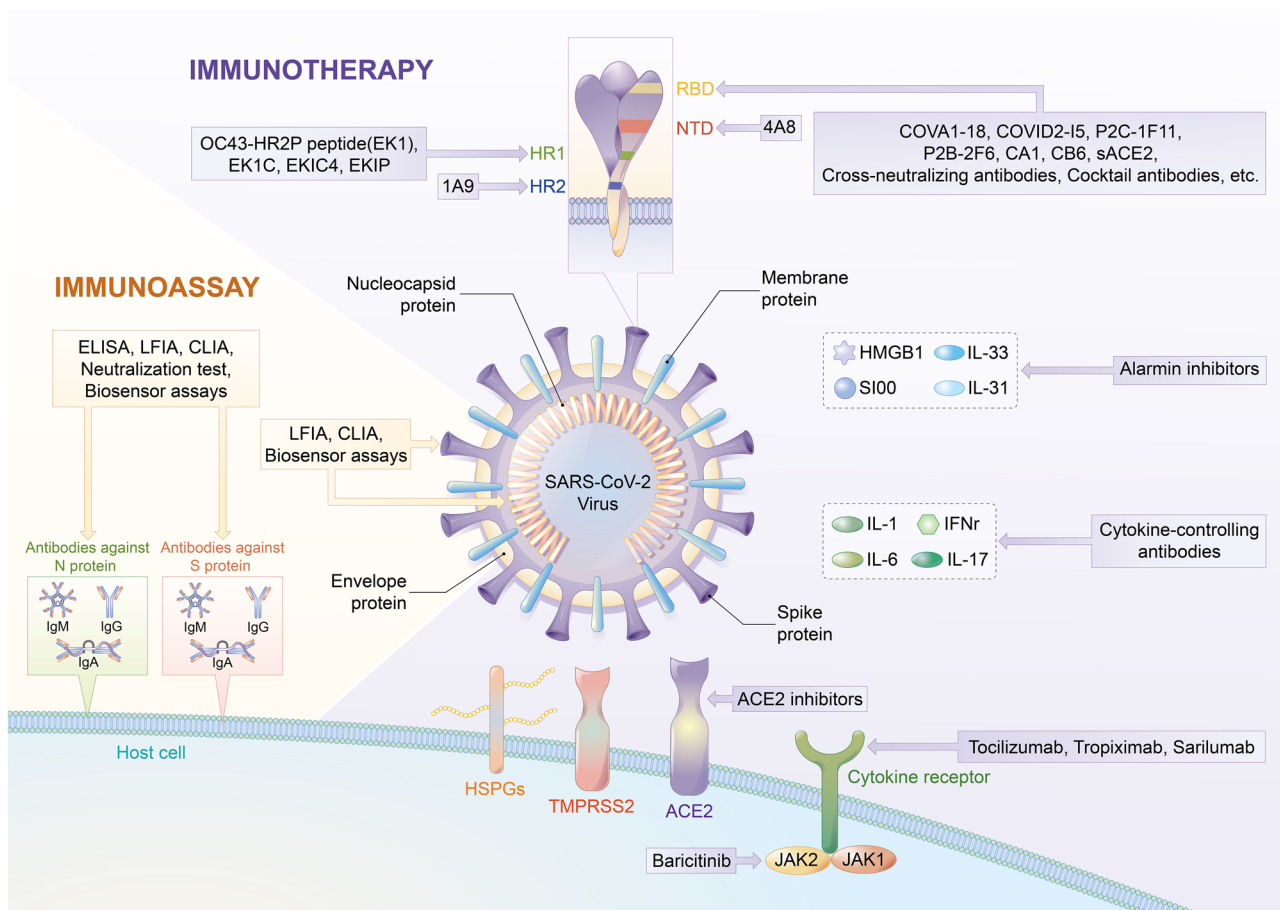
Fig. 1 Schematic summary of immunoassay and immunotherapy for COVID-19. Among the four structural proteins of SARS-Cov-2, S and N proteins are commonly used as biomarkers for immunoassay. Two common technologies used in rapid antigen detection are LFIA and CLIA. As for antibody detection, ELISA, LFIA, CLIA, and neutralization tests are common assays. In addition, biosensor devices are developed for antigen and antibody detection. Immunotherapy includes neutralizing-antibody–based treatments targeting different regions of SARS-CoV-2, which include RBD and NTD on the S1 protein, as well as HR1 and HR2 on the S2 protein. Immunotherapy targeted on host cell ACE2 receptor, HSPGs, and IL-6 receptor can effectively inhibit the host cell receptor and cytokine storm. Antibodies that control the production of cytokines and alarmin inhibitors also play a role in inhibiting cytokine storms. *S protein* spike protein, *N protein* nucleocapsid protein, *LFIA* lateral flow immunoassay, *CLIA* chemiluminescence immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RBD* receptor-binding domain, *NTD* N-terminal domain, *HR* heptad repeat, *sACE2* soluble ACE2, *HSPGs* heparan sulfate proteoglycans

neutralization activity against SARS-CoV-2 in vitro, with an IC50 of 0.23–0.41 ug/ml for pseudovirus and 0.036 ug/ml for euvirus. In prophylactic settings, CB6 inhibited SARS-CoV-2 infection in rhesus monkeys, with a peak viral load of less than 103 RNA copies/ml. In treatment settings, CB6 inhibited the pathological lung damage and reduced the viral load by 3 lg. The RBD-binding antibodies that compete directly with ACE2 are the preferred prophylactic and therapeutic applications, and as reagents for defining antibody epitopes for vaccine design. Liu et al. [43] described the isolation of 61 SARS-CoV-2-neutralizing mAbs from five COVID-19 patients. Cryo-electron microscopy reconstructions revealed that the antibodies recognize the spike in its closed, 'all RBD-down' state. A 1.5-mg/kg mAb2-15 reduced both the infectious virus titers and RNA copy numbers in lung tissue by 4 logs in a golden hamster prevention experiment.

However, the problem of the continuous variation of RBD exists. Mutations in the mutant strain's spike gene cause adaptations to the nAbs, making the vaccine less effective. Existing vaccines and therapeutic antibodies have been reported to be less effective in treating the newly discovered SA variant (B.1.351), cocktail therapy, or convalescent plasma as well. Targeting a relatively stable conserved peptide could be a response strategy to the high RBD variation of SARS-CoV-2. According to research, the antibody induced by the cross-reaction of SARS-CoV and MERS-CoV is targeted at the conserved peptide of coronavirus [44]. 3Clpro is related to virus replication, and

the corresponding gene may be the coronavirus conserved sequence [45]. On the other hand, approaches to develop modified molecules with higher RBD-competing activity might be an option. Higuchi et al. [46] discovered that soluble ACE2 (sACE2), an engineered highly compatible extracellular domain of ACE2, effectively neutralizes the N501Y mutant strain and the escaped virus from convalescent plasma in a hamster model, and is currently undergoing phase II clinical trials in Europe. Lei et al. [47] created an ACE2-human IgG1 fragment crystallizable (Fc) fusion by connecting the extracellular domain of human ACE2 to the Fc region of the human immunoglobulin IgG1. This recombinant protein can neutralize pseudoviruses expressing S proteins of SARS-CoV-2 or SARS-CoV. It has also been demonstrated that the soluble recombinant AociCE2 receptor has a high affinity for the SARS S protein, with an affinity of 1.70 nM, which is comparable to mAb affinities. One potential limitation of the recombinant ACE2 strategy is that the increase in extracellular ACE2 levels may have unanticipated effects on the body, disrupting the normal combination of ACE2 in tissues [48].

Non-RBD Regions Targeting nAb

nAbs directed against non-RBD regions were detected in the plasma of COVID-19 convalescent patients [49], including nAbs against the N-terminal domain (NTD) and the S2 protein. S2 and NTD are generally not competitive inhibitors of RBD binding to ACE2 due to their distance from the binding site. Antibodies against S2 and NTD may inhibit virus binding to ACE2 or fusion with the cell membrane in an indirect manner by preventing the conformational change of S protein [50]. Chi et al. [50] isolated an antibody (4A8) from convalescent patients with COVID-19, which proved that the binding on the NTD of the SARS-CoV-2 S protein had a high neutralizing effect. Liu et al. [43] isolated three NTD-directed antibodies from five SARS-CoV-2-infected patients. In this study, all three antibodies showed high potency, with an IC50 value of 0.007 to 0.109 μg/ml against authentic SARS-CoV-2.

Previous studies have isolated several neutralizing mAbs to the S2 subunit, especially heptad repeat (HR) loops including HR1 and HR2 domains [51–53]. The HR region in the S2 subunit is conserved among various coronaviruses that can infect humans (HCoVs), and plays a central role in HCoV infections by forming the six-helix bundle (6-HB) core structure that mediates viral fusion [54]. 1A9 antibody is the only monoclonal antibody known to bind to the HR2 domain of the SARS-CoV-2 coronavirus S2 subunit and could neutralize in vitro infection of severe acute respiratory syndrome coronavirus [51]. Xia et al. [55] reported a modified OC43-HR2P peptide (EK1) that can bind HR1s of multiple HCoVs and form stable complexes, thereby inhibiting S protein-mediated fusion. In vivo studies demonstrated that intranasal

administration of EK1 revealed highly protective effects and safety profiles, underscoring its clinical potential. Structural studies of EK1 highlighted its broad compatibility in accommodating HR1s from different HCoVs, thus consolidating its broad-spectrum inhibitory effect against pan-CoVs. As the potent stability of the SARS-CoV-2 6-HB structure might reduce the antiviral efficacy of EK1, Xia et al. [56] improved the inhibitory activity of EK1 by constructing lipopeptides EK1C and EK1P. Among a series of cholesteryl EK1 with multiple linkers, lipopeptide EK1C4 exhibited the most potent inhibitory activity against entry of pseudotyped coronaviruses and against in vitro infection by live coronaviruses. Intranasal application of EK1C4 showed strong protection of mice against HCoV-OC43 infection, suggesting that EK1C4 could be used to prevent and treat current and future SARS-related coronavirus infections. Moreover, Hoffmann et al. [57] found that the entry inhibitors EK1 and EK1C4 would still be active against the recently emerged SARS-CoV-2 variants B.1.1.7, B.1.351, and P.1 that harbor mutations in the S protein, indicating their strong inhibitory effects. In addition to the well-known protein targets, glycan targets are also worth studying because the S protein of SARS-CoV-2 is highly glycosylated. The isolated s309 nAb recognized glycan epitope on RBD indicated that the glycosylation of S protein might affect the development of nAbs against SARS-CoV-2 [58].

Cocktail Antibodies

The effect of convalescent plasma has been demonstrated in many trials and early clinical applications, but its application has been limited. The S protein of SARS-CoV-2 has undergone mutations that may make it resistant to single-targeting nAbs [40]. Mutations in the S protein's E484, F490, Q493, and S494 have been shown to cause SARS-CoV-2 complete or partial resistance to some potential therapeutic antibodies [59]. To address this issue, preclinical studies show that combining two or more nAbs (cocktail antibodies) targeting distinct epitopes is a more effective and superior antibody therapy. The REGN10933 + REN10987 antibody cocktail, which binds to two nonoverlapping RBD epitopes, was able to neutralize all identified mutants [60–62].

Weinreich et al. [63] found that an antibody cocktail against SARS-CoV-2 can improve the prognosis by increasing the virus clearance rate, especially for patients with a slow start of virus immune response, and become an effective antiviral therapy. They showed that the REGN-COV2 antibody cocktail reduced the viral load and had a greater impact on patients who had not yet started the immune response or had a higher viral load at baseline. The safety results of the combined dose group of REGN-COV2 and placebo group were similar. Currently, the antibody cocktail is primarily used to treat confirmed COVID-19 patients who do not need supplemental

oxygen and who are at high risk of progressing to severe COVID-19 [64]. It has been approved for use in the US and Brazil and is undergoing clinical trials in the UK.

Cocktail therapy has been used in other fields to varying degrees. Cocktail antibody therapy outperforms single antibody therapy in the treatment of SARS-CoV-2, particularly in terms of producing resistance to mutated viruses. It is necessary to develop better cocktail antibody combinations for different virus variants so that the treatment can be more targeted.

Cross-Neutralizing Antibodies

The primary amino acid sequences of S proteins in SARS-CoV and SARS-CoV-2 share 77.2% amino acid sequence identity, with 79.59% similarity and 74% identity in RBD domains [65, 66]. The high homology of two viral proteins allows for the isolation of SARS-CoV and SARS-CoV-2 cross-reactive antibodies. However, it is reported that SARS-CoV and SARS-CoV-2 cross-reactive antibodies can only neutralize one of the two viruses, but not the other, such as CR3022 [67]. Another antibody 515–5 isolated from patients with COVID-19 can effectively neutralize the SARS-CoV-2 virus and has only a weak but detectable neutralization effect on SARS-CoV [68]. Wan et al. [66] showed that the humanized antibody H014 could effectively neutralize SARS-CoV and SARS-CoV-2 by binding to a novel RBD conformational epitope. The cross-neutralizing antibody s309 isolated by Pinto et al. [69] from the memory B cells of SARS convalescent patients was shown to have high neutralizing activity against SARS-CoV and SARS-CoV-2 pseudovirus. Brouwer et al. [40] identified COVA1-16 and COVA2-02 from three COVID-19 patients, with IC50s of 2.5 and 0.61 μg/ml against pseudotyped SARS-CoV-2, respectively. Tai et al. [70] identified six SARS-CoV RBD-specific neutralizing mAbs that cross-reacted with SARS-CoV-2 RBD. Two of them, 18F3 and 7B11, can neutralize SARS-CoV-2 infection. It is worth noting that SARS-CoV and SARS-CoV-2 cross-neutralizing mAbs have recently been proved not to compete with hACE2 (e.g., nAbs ADI-55689/ADI-56046, 47D11, and S309), which indicates that there may be conserved epitopes between SARS-CoV-2 and SARS-CoV in addition to hACE2 binding sites [41, 66, 71, 72].

Immunotherapy Based on Inhibiting Host Cell–Virus Binding

Creating an antibody-like molecule that binds to host cells is another potentially promising strategy. The viral entry protein ACE2 and heparan sulfate proteoglycans (HSPGs) are all potential targets. Kruse et al. [48] proposed an antibody or single-chain antibody fragment (scFv) that could be engineered to deliver the SARS-CoV-2 S protein's RBD by binding ACE2 and saturating available sites in host cells. ACE2 inhibitors are now commonly used as therapeutic drugs in addition to therapeutic antibodies, but studies have shown that inhibitor-blocked ACE2 can still bind to SARS-CoV-2 RBD in the closed conformation [46], implying that ACE2 inhibitors are ineffective as antiviral infection drugs.

Viral entry requires not only binding to the ACE2 receptor, but also priming of the virus's spike (S) protein by the transmembrane protease serine 2 (TMPRSS2) by cleavage of the S proteins. The TMPRSS2 and ACE2 dual inhibitors in COVID-19 would be a novel antiviral class of drugs called "entry inhibitors." For this purpose, Baby et al. [73] analyzed approximately 2800 US FDA-approved drugs by a virtual docking tool and found that lopinavir and valrubicin have the potential of dual-target inhibition whereby preventing SARS-CoV-2 entry to the host. In addition, due to the essential metabolic roles of ACE2, Baughn et al. [74] believed that TMPRSS2 may be a better candidate for targeted therapies. Leng et al. [75] have reported that intravenous transplantation of ACE2- and TMPRSS2- mesenchymal stem cells (MSCs) that are free from COVID-19 infection, improved the outcome of 7 enrolled patients with COVID-19 pneumonia 2–4 days after MSC transplantation. HSPGs are the primary sites for direct contact between the virus and cell surface [76]. Clausen et al. [77] demonstrated that the SARS-CoV-2 S protein interacts with heparan sulfate and ACE2 on the cell surface via RBD. Independently, ACE2 or heparan sulfate can bind to RBD of S protein, and the binding sites are adjacent. Heparan sulfate functions as a scaffold in the formation of a ternary complex with S protein and ACE2. Heparan sulfate increases the openness of RBD and promotes its binding to ACE2. Similarly, Clausen et al. [77] demonstrated that heparan sulfate promoted the entry of spike-dependent viral. Altogether, these findings point to HSPGs as a co-receptor for SARS-CoV-2 entry. The development of antibodies that target HSPGs could be an effective therapeutic strategy.

Immunotherapy Based on Inhibiting Cytokine Storm

The pro-inflammatory cytokine IL-6 may initiate an inflammatory cascade known as cytokine storm during the COVID-19 immune responses, resulting in increased alveolar–capillary blood–gas exchange dysfunction [66, 78]. Cytokine release syndrome is a significant contributor to acute respiratory distress syndrome and multiple organ failure. Many studies have found that cytokine storm is one of the important causes of the death of MERS, influenza, and SARS [79, 80]. IL-6 can be used as a target cytokine in the treatment of COVID-19-related acute respiratory distress syndrome. Clinical data show that serum IL-6 levels are significantly higher in ICU patients [81]. Michot et al. [82] reported a patient with respiratory failure associated with COVID-19. He had a rapid favorable outcome after two infusions of the anti-IL-6 receptor inhibitor tocilizumab, which indicated that anti-IL-6 receptor inhibitor therapy could reduce

the risk of progression to SARS by mitigating the cytokine storm in the lungs with COVID-19. REMAP-CAP researchers [83] have shown that treatment with interleukin-6 receptor antagonists tropiximab and sarilumab improves the prognosis of critically ill patients, including survival, who receive organ support in the intensive care unit.

There are also some other cytokine antagonists, such as Baricitinib and fluvoxamine, that have been used in clinical trials with promising results. Baricitinib is a highly selective, effective, and safe JAK1 and JAK2 inhibitor that can inhibit IL-2, IL-6, IL-10, IFN-γ, and others. Baricitinib also inhibits endocytosis and virus aggregation through AP2-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK) [84].

Several studies have begun to investigate the feasibility of other cytokine therapies, in addition to treating patients with cytokine inhibitors. Tjan et al. recommended neutralizing antibody therapy in the early stages of infection and steroid therapy in severe cases after virus clearance was confirmed [85]. Other research has suggested that there may be existing monoclonal antibodies or specific antagonists that control the production of cytokines like IL-6, IL-1, IL-17, and IFN-γ [86]. The use of such cytokine-controlling antibodies can significantly improve the efficacy of neutralizing antibody therapy. Alarmins, such as HMGB1, IL-31, IL-33, and S100, have been shown in some studies on the mechanism of cytokines to aggravate cytokine storm and disease [87]. Alfarouk et al. described the specific mechanism of cytokine storms from the perspective of mitochondria: mitochondria are the focal point of cytokine storms. In the elderly population, the rational use of alarmins inhibitors or pharmacological or non-pharmacological modulators of mitochondrial function in neutralizing antibody therapy may improve efficacy and patient life quality [87, 88].

Autoantibodies Produced by Cross-Reaction Between Viral Epitopes and Autoepitopes

Autoantibodies are antibodies that mistakenly target and react with a person's own tissues, organs, cells, and cell components. Usually, the immune system is able to discriminate between foreign substances ("non-self") and the body's own cells ("self"). When the immune system fails to distinguish between "self" and "non-self", a large number of autoantibodies are produced, resulting in the development of autoimmune diseases. Autoimmune diseases activate complement; damage tissue; promote systemic inflammation [89]; cause damage to the skin, mucous membranes, and organs; as well as vascular inflammation such as thrombotic microangiopathy [90].

A variety of autoantibodies may be produced by COVID-19 patients, causing a prolonged period of immunodeficiency (T-cell and B-cell dysfunction [91], increased risk of new events of respiratory, diabetes, and cardiovascular diseases [92]), and a significant decline in the body function in some

patients after recovery, a condition known as "post COVID-19 syndrome" [93].

Anti-NETs (neutrophil extracellular traps) antibodies have been discovered in COVID-19 patients' serum [94]. NETs are bactericidal networks that form after the death of neutrophils and play a significant role in the pathogenesis of thrombovasculitis. However, the precise mechanism by which these antibodies inhibit NET clearance in the serum remains unknown [95]. Another research explored the development of autoantibodies in COVID-19 patients who had no prior autoimmune disease, and found that COVID-19 is linked to human autoantibody production [96].

On the other hand, autoantibodies may be closely related to cytokine storms, exacerbating the body's inflammatory response and leading to acute respiratory distress (ARD) or acute renal failure (ARF). In other words, COVID-19 patients with positive autoantibodies (antinuclear and anti-neutrophilic cytoplasmic antibodies (ANCA)) had the worst clinical outcomes [96]. A study of the clinical and laboratory characteristics of 21 severe COVID-19 cases in China found that the prevalence of anti-52 kDa SSA/Ro antibody, anti-60 kDa SSA/Ro antibody, and antinuclear antibodies were 20%, 25%, and 50%, respectively [97].

The potential mechanism of autoantibody production has not been fully explained. One of the current hypotheses is the molecular simulation of infectious pathogens. Autoantigen is an endogenous antigen that is recognized as non-self by the immune system. Some SARS-CoV-2 epitopes showed cross-reactivity with autoantigen. By exposing the epitope to produce cross-reacting antibodies, the virus induces an autoimmune response [98]. Vojdani and Kharrazian [99] discovered that 21 out of 50 human tissue antigens had moderate to high strength responses to SARS-CoV-2 antibody, suggesting an antigenic cross-reaction between the SARS-CoV-2 epitope and various human tissue antigens. This cross-reaction can result in high titers of autoantibodies, inducing Guillain–Barre syndrome [100], lupus, antiphospholipid syndrome, and ANCA-associated vasculitis [98]. Franke et al. [101] found that high levels of antibodies were detected in patients' cerebrospinal fluid. Indirect immunofluorescence of unfixed mouse brain sections showed the strongest IgG binding in most cases, implying that some human SARS-CoV-2 monoclonal antibodies may bind to brain tissue and induce autoimmune responses.

Therefore, when developing a neutralizing antibody against SARS-CoV-2 for clinical use, its cross-reaction should be taken into account to avoid autoimmune damage. Because autoantibodies are common in severe COVID-19 cases, and various cross-reaction mechanisms are still unknown, it is difficult to consider the overall effect of neutralizing antibody therapy on the body in a comprehensive manner. Therefore, it is critical to choose appropriate neutralizing antibodies against virus epitopes.

Conclusions

In recent years, understanding the immune response, especially the antibody immunity response, has led to the development of various clinical strategies for the detection and treatment of diseases such as COVID-19. Immunology against SARS-CoV-2 still represents several clinical and translational challenges, and more research is required to tailor immunoassays and immunotherapies to further improve detection and treatment efficacy.

Authors' Opinion

The emergence of the COVID-19 pandemic resulting from the spread of the SARS-CoV-2 has inspired intensive efforts to develop immunoassays and immunotherapy to support clinical diagnosis and treatment. Lateral flow immunoassay (LFIA) that directly detect viral antigens or antibodies in clinical samples without any sample preparation step is necessary for the fast diagnosis of COVID-19. Highly sensitive immunological methods such as CLIA and ELISA can be used for high-throughput and accurate diagnosis. Virus-neutralizing tests, on the other hand, are crucial as they determine whether antibodies have the ability to neutralize the virus. A variety of biosensing strategies have also been developed.

The use of specific antibody immunotherapy is now one of the most effective therapeutic methods for COVID-19. In the early stages of the SARS-CoV-2 pandemic, convalescent plasma (CP) has been used in treating COVID-19 patients. Subsequently, monoclonal neutralizing antibodies (nAb) that interfere with different steps of viral infection were developed, with some showing considerable improvements in clinical results. The immunology against SARS-CoV-2 viruses still represents several clinical and translational challenges, and more research is required to tailor immunoassays and immunotherapies to further improve detection and treatment efficacy.

Declarations

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