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REVIEW

Neutralizing antibody: a savior in the Covid-19 disease

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Abstract

Coronavirus outbreak was declared a pandemic by World Health Organization (WHO) in March 2020. The pandemic has led to a devastating loss of life. It has shown us how infectious diseases can cause human existence at stake, and community health is important. The spike protein is the most immunogenic component of the virus. Most vaccine development strategies have focused on the receptor-binding domain (RBD) in the spike protein because it is the most specific target site that recognizes and interacts with human lung cells. Neutralizing antibodies are generated by the humoral immune system and reduce the viral load by binding to spike protein components. Neutralizing antibodies are the proteins secreted by plasma cells and serve as an important part of the defense mechanism. In the recent Covid-19 infection, neutralizing antibodies can be utilized for both diagnostic such as immune surveillance and therapeutic tools such as plasma therapy. So far, many monoclonal antibodies are in the clinical trial phase, and few of them are already in use. In this review, we have discussed details about neutralizing antibodies and their role in combating Covid-19 disease.

Keywords Receptor-binding domain (RBD) · Covid-19 · Neutralizing antibody · Angiotensin converting enzyme-2 (ACE2) receptor · Convalescent plasma transfer (CPT)

Introduction

Covid-19 is an infectious disease, which has spread globally. The disease is characterized by pneumonia and is an acute respiratory disease. It is caused by Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2). The reason it has turned into a pandemic is due to its transmission from infected individuals without symptoms. This infectious disease is the first time reported in Wuhan city, China, in December 2019, occurred due to droplet transmission of SARS-CoV-2 [1]. According to WHO, around 255 million people have been infected with this virus so far, and more than 5 million died globally, in which most of the people were either old or had other underlying complications [2]. Almost every individual has faced direct and indirect loss due to this pandemic, and it needs to be resolved.

It is the third human coronavirus which appeared in the twenty-first century, and the other two are severe acute respiratory syndrome (SARS-CoV-1) and Middle East respiratory syndrome coronaviruses (MERS-CoV), which emerged in 2002 and 2013, respectively. The mortality rate of the last two viruses was high, while they have low transmissibility and infectivity. Different molecular interactions, including receptor interaction, are involved, which determine the host range of a virus [3, 4]. SARS-CoV-2 is an enveloped virus that contains a positive-sense single-stranded RNA. The natural reservoir of this virus is a bat, and then there is a possibility of some amplifying host, which transferred this virus to humans, the final most reservoir [5]. SARS-CoV-2 is homologous to SARS-CoV-1 and uses the same mechanism to enter into human lung cells. Both belong to the beta coronavirus group. They both recognize Angiotensin converting enzyme-2 (ACE2) receptor for entering into lung cells, but SARS-CoV-2 has a higher mortality rate. It has around 96% sequence similarity at the-whole genome level with bat SARS (RaTG13) so considered as bat origin [6]. SARS-CoV-2 uses its envelope spike protein to enter the host cells by interacting with host ACE2 receptor of type II

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alveolar cell of the lung, and the DC-SIGN receptor on the respiratory dendritic cell (DC) and associated endothelial cells [7, 8]. The name coronavirus was given on the basis of the presence of around 1200 aa long S-protein, which gives a crown-like shape to the coronavirus and comes into class-1 viral fusion proteins [9–11]. The spike protein of SARS-CoV-2 is divided into N-terminal S1-ectodomain and C-terminal S2- membrane-anchored domain by cellular proteases such as cathepsin L, which cleaves the S glycoprotein at S1/S2 junction and as well as at S2 site positioned downstream of S1/S2 proteolytic cleavage [9, 12, 13]. S1 RBD of SARS-CoV-2 is mainly responsible for virus entry and has a higher affinity for ACE2 receptors than SARS CoV spike protein [14]. The S2 domain is accountable for cell membrane fusion and contains an internal fusion peptide, a putative fusion peptide region, a transmembrane domain, and two heptad-repeat domains [9, 12]. The exact molecular mechanism of SARS-CoV-2 entry into human cells is unknown, but Lu et al. observed that it might be through internal fusion protein and putative fusion protein [10]. The SARS-CoV-2 and SARS-CoV S1 domain share approximately 50 amino acids similarity. Interestingly, Gln493 and Asn501 are the key residues of the SARS-CoV-2 RBD, which are responsible for the binding to the ACE2 receptor and hence further support the idea that SARS-CoV-2 has developed capacity for a person-to-person transmission [12]. The species specificity does not only depend on receptor recognition, but it also depends on its capacity to neutralize innate immune response [15]. However, it is not very well known how SARS-CoV-2 counteracts innate immune response. There is no specific antiviral treatment for SARS-CoV-2 treatment and hence social distancing, oxygen therapy, antibiotic treatment for bacterial infection, and fluid management are suggested [16].

While the SARS-CoV-2 virus contains many structural proteins such as spike protein, nucleocapsid protein and core protein, the spike protein is the most immunogenic one and thus vaccination strategy is extensively utilizing spike protein as a candidate for administration. It depends solely on the quantity and quality of neutralization antibodies made in individuals either naturally by their immune system or by administering vaccines. Moreover, in convalescent plasma samples from those who recovered from SARS-CoV-2 infection have a large amount of RBD-specific neutralizing antibodies [17]. So far almost every country is trying to develop a vaccine for SARS-CoV-2and they are in different phases from pre-clinical to human approval stage. In USA there are so far 3 vaccines such as Pfizer BioNTech, Moderna and Johnson and Johnson are approved by Food and drug administration (FDA) for use. Success of vaccine is dependent on durability and stability of neutralization antibody titers. Besides the vaccination, FDA also approved emergency use of plasma therapy which is solely based on transfer of plasma neutralizing antibodies from convalescent patients to newly infected one. This mode of transfer is called passive immunization. The success of plasma therapy varies from person to person as it depends on time of plasma therapy, quality, and quantity of transferred antibodies. Despite the variable response, plasma therapy is still widely used across the world.

Additionally, neutralizing antibodies can also be used in diagnostic tests. As it is based on ELISA using blood so require less technical expertise and can be durable in a large population especially a preliminary test to analyze the seroconversion rate of that population. In this review we are highlighting the importance of neutralizing antibodies. We address many questions such as what are different isotypes of neutralizing antibodies and the cells which have role in production of these antibodies. How these antibodies are useful in plasma therapy and diagnostic serological test. Furthermore, we also discussed the status of neutralization potential in the era of new emerging variants.

New avenue for SARS-CoV-2 treatment using monoclonal antibody

Antibodies provide a new hope to treat Covid-19 patients because of their specific binding to viral particles and further have neutralization capacity so helping in clearance and/or reducing the viral load in body. So far, there are several monoclonal antibodies in various development stages ranging from preclinical to clinical one as shown in Table 1. Antibodies developed by natural infection in convalescent plasma have variable efficacy and data are not consistent. FDA had approved emergency use authorization (EUA) for convalescent sera in August 2020. For commercial purposes, development of monoclonal antibodies is either developed in humanized mice or purified from convalescent patients or via cell culture techniques as shown in Fig. 1. Monoclonal neutralizing antibodies which have been approved so far for therapeutic intervention include REGN-COV-2 which is IgG1 monoclonal antibody and is a combination of Casirivimab and imdevimab similarly Bamlanivimab or a combination of Bamlanivimab and etesevimab also potential neutralizing antibodies. These antibodies bind to spike RBD region and neutralize SARS-CoV-2 virus effectively [18]. There are various methods/approaches utilized to prepare neutralizing monoclonal antibodies which include: Single cell sort for memory B cells and utilization of next generation high throughput sequencing technique, flow cytometric sorting of antigen specific RBD or spike protein followed by cloning of Ig encoded genes from single B cells, phage display libraries utilized SARS-CoV-2 RBD/ SARS CoV-1 /MERS CoV S proteins, hybridoma culture technique or

Table 1 The list of monoclonal neutralizing antibodies and their detailed descriptions are enlisted [20] (source:https://www.antibodysociety.org/covid-19-biologics-tracker/)

Monoclonal antibody	Phase trial	Company name
REGN10987 and REGN10933 Humanized and human mAb cocktail [22] or Casirivimab and imdevimab combination	Clinical	Regeneron Pharmaceuticals, Inc
LY-CoV555 [23] Or combination of LY-CoV555 and LY-CoV016 or LY3819253	Clinical	Eli Lilly and company or AbCellera
VIR-7831/GSK4182136	Clinical	Vir Biotechnology, Inc. and GlaxoSmithKline plc
CT P59	Conditional approval in South Korea	Celltrion company
AZD7442 (AZD8895 + AZD1061)	Phase 3	Astra Zeneca
JS016 Human mAb [24]	Phase 2	Junshi Biosciences
TY027	Phase -3	Tychan Pte. Ltd. Singapore
BRII-96, BRII-98	Phase -3	Brii Biosciences
SCTA01	Phase 2/3	Sinocelltech Ltd
ADM03820		Ology Bioservices
BI 767551	Clinical	Boehringer Ingelheim, Cologne University Hospital (UKK), University of Marburg (UMR), and the German Center for Infection Research (DZIF
COR-101	Phase 1 /2	CORAT therapeutics
ADG20	Phase 2/3	
MW33	Pivotal Phase 2	Mabwell (Shanghai) Bioscience Co., Ltd
JS016, LY3832479, LY-CoV016	Phase 2	Junshi Biosciences / Eli Lilly and Company
DXP593	Phase 2	Beigene
COVI-AMG (STI-2020)	Phase 2	Sorrento Therapeutics, Inc
BI 767551, DZIF-10c	Phase 2/3	U. Cologne / Boehringer Ingelheim
VIR-7832	Phase 1 /2	Vir Biotechnol
HLX70	Phase 1 pending	Hengenix Biotech Inc
DXP604	Phase 1	Beigene
ADM03820	Phase 1	Ology Bioservices
HFB30132A	Phase 1	HiFiBiO Therapeutics
ABBV-47D11	Phase 1	AbbVie
C144-LS and C-135-LS	Phase 1	Bristol-Myers Squibb, Rockefeller University
LY-CovMab	Phase 1	Luye Pharma Group Ltd
JMB2002	Phase 1	Jemincare Group
LY-CoV1404, LY3853113	Phase 1	AbCellera / Eli Lilly and Company

utilization of EBV immortalized memory B cells etc. [19]. High cost involved in large scale production of monoclonal antibodies and their testing is a limiting factor for mass scale usage as a therapeutic agent.

The list of monoclonal neutralizing antibodies and their detailed descriptions are enlisted in Table 1 [20] (source:https://www.antibodysociety.org/covid-19-biologics-tracker/). Summary of monoclonal antibodies which are tested for their neutralization potential in different research labs with their potential mode of action are listed in Table 2 [21].

Different isotype of neutralizing antibodies and their role during Covid-19 infection

In the course of any infection our immune system produces two types of antibodies that is unswitched and switched isotype. In acute Covid-19 infection, neutralizing antibodies

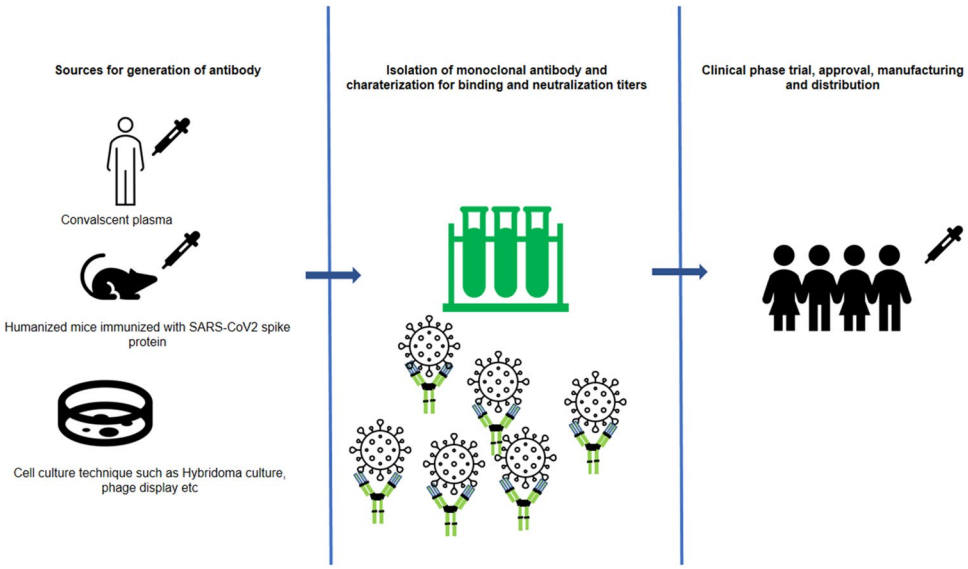

Fig. 1 A schematic of development of monoclonal antibody. Different phases of antibody generation start from (1) generation of antibody in the lab (2) Screening of antibody and (3) Commercial manufacturing, clinical trials and approval, and distribution for therapeutic use

are dominated mainly by switched type antibodies such as IgG and IgA and less by unswitched IgM type. An early report revealed the role of spike protein where they discovered that RBD specific IgG neutralizing antibodies could prevent cell-to-cell fusion between virus spike protein and ACE2 receptor and thus stop the virus entry to lung cells and further transmission of the virus as shown in Fig. 2 [25]. Additionally, when analyzing the immune repertoire from convalescent patient plasma cells by single-cell sequencing, the authors observed clonal expansion of certain IgG repertoire. Many of these expanded clones are SARS-CoV-2 antigen-specific, with high affinity for RBD specific having neutralization potential [26]. Acute Covid-19 patients have more RBD specific IgG antibodies compared to IgM and IgA. Among IgG isotype, IgG1 and IgG3 are more prevalent in acute patient plasma samples post one week of symptom development. These RBD specific IgG antibodies have strong neutralization potential during acute SARS-CoV-2 early infection, day 7 post PCR positive test. These reports show the serological importance of Covid-19 during acute infection in diagnosis [27]. Marot S et al. report came from healthcare workers analysis, which suggests that the rate of decline of RBD specific neutralizing antibodies decline rate is different among IgM, IgG, and IgA isotypes. While IgG and IgA titers are stable or reduced slightly from day 21 to 3 months during mild symptom development, IgM antibody level and its neutralization titers decline rapidly from day 21 to 2 months and continue further after 3 months. These data reflect lower persistence of IgM neutralization antibodies once developed compared to IgG and IgA [28]. Sterlin et al. have reported the importance of IgA antibodies in acute SARS-CoV-2 patients. IgA can be detected in serum, saliva as well as bronchoalveolar lavage fluid during initial time of infection and it has neutralization potential as well. IgA plasmablasts undergo peripheral expansion and homing as measured by CCR10 positivity (homing marker from lung cells to mucosal site) plasmablast and memory cells soon after the onset of symptoms and reach peak at 3 weeks towards mucosal site is seen. IgA antibody titers start declining after 3–4 weeks of symptom onset while IgG remains in plateau and detectable in serum [29]. Wajnberg A et al. demonstrated that IgG is the major antibody with higher neutralization titers and relative stability in Covid-19 recovered patients. Their data suggested that RBD IgG antibodies as a suitable candidate for plasma therapy [30]. The kinetics of neutralization antibody titers as measured by Plaque reduction neutralization titers ($PRNT_{50}$) decline are also dependent on the severity of symptoms. Severe ill patients retain antibodies longer than mild and then shorter in the asymptomatic patients as shown in Supplementary Fig. 1 [28]. Moreover, Long QX et al. demonstrated the seroconversion of different antibodies. They found that virus-specific IgG and IgM neutralizing antibodies occur simultaneously and were detected in serum starting from the first week of infection and reach a peak approximately in three weeks in all tested Covid-19 patients. Interestingly, both the antibodies are higher in the severely ill patients compared to the non-severe group. They observed simultaneous seroconversion for both IgG and IgM in almost all patients tested, and median seroconversion reached day 13 of symptom onset. This report also highlights the importance of serological test to detect IgG and IgM as a diagnostic test for Covid-19 patient who is negative for RT-PCR test at a specific window of time [31]. So, in summary these reports highlight that for passive immunization antibodies transfer IgG antibodies post day 7–21 infection are suitable to transfer into patients who have Covid-19 at early stage that is 3–7 days. Meta-analysis studies show that convalescent

Table 2 Monoclonal antibodies which are tested for their neutralization potential in different research labs with their potential mode of action

Target site for Neutralizing antibody	Examples	Mode of action
Receptor binding motif at RBD site	CC12.1, CC6.33, CA1, CB6, B38 and H4 human Mab, COV2-2196, COV2-2130 and COV2-2381 MAb	Block direct RBD-hACE2 receptor interaction, show complete competition with ACE2 for binding to RBD so prevent virus entry
N Terminal Domain or S2 domain	2–17, 5–24, 4–8 human Mab, 2 M-10B11 and 9A1 MAb, COV57 human pAbs	Prevent spike protein interaction with hACE2 and virus fusion to lung cell
RBD site but not on receptor binding motif so don't interfere hACE-2 binding directly	47D11, CR3022, S309, 2 M-10B11 human Mab, EY6A mouse MAb	Destabilize viral fusion
RBD site but not on receptor binding motif and restrict confirmational change	414–1, 553–15, CV30, Bd-368–2, BD218, BD23, H014, C121, C135, C144 and C105 human Mab,	Block RBD-hACE2 receptor interaction so prevents virus entry
Combination of two or more antibody and bind different regions	REGN 10,933 and REGN 10,933, COV2-2196, COV2-2130 and COV2-2381 Mab, B38 and H4 human Mab,	Bind to RBM and S1B core domain along with restrict confirmational change

plasma transfer (CPT) to hospitalized severely ill patients leads to less mortality rate, and it is most effective when administered within 3 days of the onset of symptoms. This treatment is safe and can be combined with other standard treatments. The limitation of these studies are absence of controls and randomized clinical trials [32, 33]. Maeda et al. highlighted about enhance neutralization potential after receiving CPT. They reported around 60% patients showed significant increased neutralization activity once treated with CPT, but half of the patient lost neutralization potential in a one-month time. They also conclude that IgG CPT neutralizing activity remain inconsistent and varies from person to person and does not positively correlated with binding antibodies specific for SARS-CoV-2 S1 [34]

B cells response during Covid-19 infection

Neutralizing antibodies are produced by B cells. B cells terminally differentiated into plasma cells and secrete antibodies into serum as shown in Supplementary Fig. 2. There are two types of B cell response: the extrafollicular B cell response, and another is the follicular response that led to the germinal center (GC) reaction. IgM neutralizing antibodies are mainly produced during extrafollicular B cell response, while switched ones such as IgG and IgA are generated during GC reaction, which are of higher affinity [35]. Successful vaccination is dependent on the long-term production of neutralizing antibodies by formation and persistence of either long-lived plasma cells or memory cells which are outcomes of GC reaction from a secondary lymphoid organ such as the spleen and lymph nodes as shown in Supplementary Fig. 2. Cross sectional study in acute SARS-CoV-2 infection patient suggested that total B cell frequency as well as plasmablast frequency increase in blood and peak at around 2 weeks post the onset of symptoms and then start to decline. While analyzing RBD specific switched memory B cells, this also showed a similar pattern to antibodies in terms of isotype. RBD specific IgG memory B cells are dominated followed by IgA and then IgM type [36].

In SARS-CoV-2 acute severe diseases, there is a reduction in GC rection thus diminished formation of long-lived plasma cells and memory B cells. Therefore, high affinity and class switched neutralizing antibody production are lowered in this situation. Moreover, this report, has shown a reduction in Bcl6 + B cells, a marker for GC B cells and T follicular helper cells in postmortem spleen and thoracic lymph node tissue. They also showed aberrantly activated B cell population in peripheral blood and TNF alpha expression. This also explains short lived neutralizing antibody production in the SARS-CoV-2 acute disease scenario and further suggests difficulties in getting herd immunity by natural infection [37, 38]. Woodruff MC et al. also confirmed

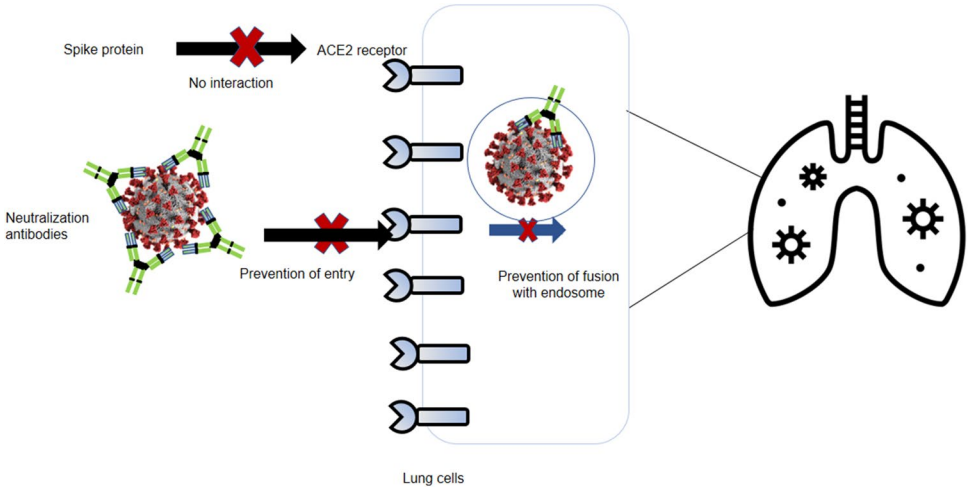

Fig. 2 Mechanism of action of neutralizing antibody: Once neutralizing antibody interacts with spike or RBD site, it prevents the entry by blocking Spike protein to ACE2 interaction. Another way is to prevent the fusion of the virus envelope to the cellular endosome

a similar finding that explained the differential outcome of antibody response for asymptomatic to severely ill patients. They showed that in the severely ill patients, extrafollicular B cell response is higher and also IgM and switched antibodies while the asymptomatic patient has higher follicular B cell response, GC reaction and memory response. Furthermore, they also revealed that severely ill patient showed high autoimmune pathology like Systemic lupus erythematosus (SLE) and had a positive correlation with autoimmunity. In severely ill patients blood sample, when they did phenotyping for the effector cells, they observed that activated naïve population expanded and differentiated into a unique double negative (DN) effector population. The extrafollicular response pathway in severely ill patients has a positive correlation with IL6, C-reactive protein, IP10 level in serum which is identified as a biomarker of Covid-19 disease. There is a robust expansion of CD138 + mature plasma cells in severely ill patients that leads to production of a large number of neutralizing antibodies compared to both asymptomatic and healthy patients, but they are the extrafollicular type [39].

For long term antibody production during reinfection, memory cells play an important role. Deng W et al. reported in rhesus macaque that suggested protection against reinfection and generation of long-term immunity. This report compared the primary challenge infection case with the challenge-rechallenge (i.e. reinfection) point in the rhesus macaque non-primate model. They found that reinfection post 28-day after recovery from primary infection showed higher IgG neutralizing antibodies against virus spike protein and have no viral load detection. This also indicates that if we give two doses in a 28-day interval of virus vaccine it offered better protection as higher production of neutralizing antibodies. Though a long-term challenge to see memory response remains unexplored to observe quantitative and qualitative long term immune response [40]. The chance of reinfection also depends on antibody level made from prior infection. Once a person recovers from acute SARS-CoV-2 infection. They are more likely to get reinfected if they remain seronegative compared to a seropositive person [41]. Among memory B cells analyzed 6 months post infection, IgG memory B cells are predominant followed by IgA memory and sustained post 6 months of infection. In contrast, IgM memory B cells are short lived one, so they start to decline gradually [42]. Antibodies generated from memory B cells after 6 months of infection showed an example of continued evolution in terms of increased neutralization potential and breadth in their repertoire, increased somatic hypermutation which indicate antigen persistence for longer duration [43].

The drawback in most of human studies so far on Covid-19 project is that they are largely based on blood sample study. So, trafficking of memory cells and long-lived plasma cells in bone marrow is still limited. Similarly for IgA study at mucosal site is still needs to be carry out in extensive manner. One recent study done in human bone marrow aspirates in mild Covid-19 infected convalescent patient shows persistence of long-lived plasma cells up to 6–8 months. They showed in longitudinal study that decay in anti-S antibody titers are rapid for 1 months then gradual decline from 1 to 4 months and very slow decrease in mean titers till 11 months. Thus, persistence of IgG antibody till 11 months post symptom onset in some convalescent patient sera. When analyzing S-binding memory B cells in blood, it is detected as soon as one month post symptom onset and remain detectable at least for 7 month [44]. This study highlighted the long-term immunity for spike protein in mild infected patients. So, in nutshell during infection, first viral loads reach at their peak level, then antibody start to develop to fight against virus and neutralize them. Later, IgG antibody predominates and provide long term immunity as shown in Supplementary Fig. 3.

Serological test

The Serological tests are meant to detect antibody levels against the SARS-CoV-2 virus. It is different from the diagnostic RT-PCR test in which there is the detection of genetic material. Serological test showed a history of whether an individual was infected with Covid-19 diseases in the past. In contrast, a diagnostic test is more suitable for an individual who is currently infected. Serological tests can be performed as early as 6 days and reach a peak on 14 to 30 days post the onset of symptom while SARS-CoV-2 RNA can be detected as early as 2–3 days post symptom onset peak at day 5 and remain detectable from 25 to 50 days. Serological tests are not recommended to the population with low disease prevalence, infectious patients at an early stage or asymptomatic or pseudo symptomatic patients, and healthcare professionals at higher risk of Covid-19 infection [45, 46]. Comparison between serological test to diagnostic test is summarized in Supplementary Table 1.

Serological tests only give the information of the quantity of spike and/or RBD specific antibody in serum and is done by ELISA as shown in Supplementary Fig. 4. But the functionality of these antibodies is determined by neutralization assay. There are mainly two type of neutralization assay, one is based on live virus neutralization titers and second on pseudo type virus neutralization in which spike is conjugated to another virus such as HIV or VSV. Live virus neutralization shows more accurate results but using pseudo type virus gives the flexibility of working in BSL2 facility instead of BSL3 for live virus culture. Neutralization titers detection technique using patient serum/plasma to detect SARS-CoV-2 antibodies are listed below in Supplementary Table-2. When $PRNT_{50}$ is quantified, the estimated time duration to drop 1:10 detection limit antibody titers in various patient, depends on disease severity. As reported, it took approximately 372, 416 and 133 days for severe, mild and asymptomatic patients respectively [47].

Mutants and neutralization potential

Few vaccines which are currently in human trial are as Pfizer BioNTech or BNT162b2, Moderna or mRNA-1273, Johnson and Johnson or Ad26.COV2.S, Covaxin or BBV152, Astrazeneca or AZD1222, Sputnik V and Gamaleya etc. Emerging new variant cause a serious concern against vaccine use for long term and its efficacy reliability. SARS-CoV-2 is a single stranded RNA virus, and the mutation rate is higher in viruses than any other pathogen such as bacteria or fungi. Mutation in spike protein causes decline in the neutralization potential of antibodies and the vaccination efficacy of an individual. The list of few variants is reported as given below in Supplementary Table 3 [52–54]:

Neutralization titers ($FRNT_{50}$) were compared for different variants of SARS-CoV-2, such as UK variant (B.1.1.7) and USA Atlanta variant (B.1 lineage), USA San Diego variant (N501Y) along with the first reported original china variant (A.1 lineage). Their neutralization potential was found to be comparable in acute and convalescent serum sample. Individual received mRNA-1273 vaccination show slight reduction in neutralization antibody titers against SARS-CoV-2 variant compared to original A.1 lineage [55]. Similarly in another report from the same group compared receptor binding and neutralization titers between USA Atlanta variant B.1 lineage and South African (B.1.351 lineage) in acute, convalescent and post second dose mRNA 1273 vaccinated serum sample. They observed a 3–fourfold reduction in both binding to receptor binding domain as well as neutralization titers against B.1.351 lineage compared to B.1 lineage. This shows one of the concerns for reducing efficacy for SARS-Cov2 vaccination as shown in Supplementary Fig. 5. It also highlighted a raising concern of every year vaccination as emerging new variants with more transmissible rate, viral fitness and the higher fatality rate can cause failure in existing vaccination as neutralization antibodies will no longer bind to spike protein and prevent entry to lung cells [56]. Additionally, Planas D. et al. have reported partial reduction in neutralization potential against SARS-CoV-2 variants developed either by natural infection or post Pfizer vaccination. In this report that reduction is more towards B.1.351 than B.1.1.7 compared to B1 prototype variant (D614G). Yadav et al. have reported that individuals administered Covaxin and convalescent sera from recovered Covid-19 patients both are able to neutralize efficiently for the variant B.1.617 (Indian variant) and B.1.17 (UK variant) when compared with B1 prototype variant (D614G) against which Covaxin is developed [54]. Invitro culture study shows moderately diminished neutralization antibody titers against SARS-CoV-2, in various tested SARS-CoV-2 variants that have variation in spike sequences generated by deletions or substitutions, when treated with monoclonal antibody or convalescent sera. This report indicated development of resistance for monoclonal antibodies [57, 58]. Results from longitudinal study have shown that after vaccination, naïve individuals showed enhanced antibody titers after second dose of mRNA vaccine while recovered patient from natural Covid-19 infection, first dose gave accelerated antibody titers against B.1.351 SARS-CoV-2 variant. This paper also showed that in naïve individuals antigen specific memory cell formation is primarily of the IgM isotype after the first dose of vaccination and IgG isotype after second dose, in contrast to that in recovered individual where it is largely IgG memory after first dose of mRNA vaccine [59]. In nutshell, as SARS-CoV-2 is now spread globally so any

new emerging variant which gave virus more fitness, transmission ability and less susceptible to existing vaccine is the biggest challenge to complete eradicate Covid-19 disease. Every country is now actively vigilant for emerging new wave of this disease due to new mutant.

Future perspective

Lack of long-term study, small cohort size and emerging variants cause a serious setback regarding the efficacy of vaccination. Long term memory B cell response and breadth of repertoire are still needed to study extensively. Production of neutralizing antibodies requires not only B cells but also T cells and innate cells. The range of symptoms and variation in antibody response has left the scientific community puzzled. How herd immunity and pre-existing immunity impact the quality and quantity of neutralization titers remain to be explored. Zoonotic origin of this disease gave a speculation of pre-existing immunity as reported by Ng et al., in which they found S2 subunit specific IgG antibodies in uninfected patients that have neutralization potential. This also reflect cross reactive nature of antibodies. There is a differential expression of these antibodies in children and adolescent one [60]. Antibody titers also showed correlation with age. While seroconversion rate is similar between pediatric vs adult patient, IgG antibody titers are negatively correlated with age in pediatric patient and moderately positive with adult patients [61]. This highlights that a differential course of vaccination might be needed in children compared to adult. Development of immune escape mutations in SARS-CoV-2 and resistant to vaccine, drug as well as neutralizing monoclonal antibodies are the biggest challenges in future eradication of the disease.

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Declarations

Conflict of interest The authors declare no potential competing interest.

Ethical approval No animal has been used in this study.

Informed consent All authors are agree to publish this manuscript in your valuable journal.

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