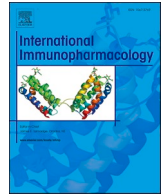ELSEVIER

Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Immune response variables and viral mutations impact on COVID-19 reinfection and relapse

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ABSTRACT

The possibility of human reinfection with SARS-CoV-2, the coronavirus responsible for COVID-19, has not previously been thoroughly investigated. Although it is generally believed that virus-specific antibodies protect against COVID-19 pathogenesis, their duration of function and temporal activity remain unknown. Contrary to media reports that people retain protective antibody responses for a few months, science does not exclude reinfection and disease relapse shortly after initiating all immune responses during the primary onset of COVID-19. Despite production of antiviral antibodies, activated CD4+/CD8+ lymphocytes, and long-lived memory B cells, susceptibility to reinfection in humans for extended periods cannot be precluded due to repeated exposures to coronavirus or potential reactivation of the virus due to incomplete virus clearance.

However, the mechanism of reinfection remains unknown. The biological characteristics of SARS-CoV-2, such as emergence of multiple mutations in the virus RNA molecules, transmissibility, rates of infection, reactivation and reinfection, can all affect the trajectory of the virus spread. Innate and adaptive immune response variables, differences in underlying diseases, and comorbidities, particularly in high risk individuals, can influence the dynamics of the virus infection. In this article, immune parameters and viral mutations pertaining to reinfection and disease relapse are reviewed and scientific gaps are discussed.

1. Introduction

More than a century after the 1918 influenza pandemic, the world is facing another pandemic. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused coronavirus disease of 2019 (COVID-19), with over 181,521,067 confirmed cases and more than 3,900,000 cases of death since the pandemic began [1]. SARS-CoV-2 infection occurs in three stages: Stage I is an asymptomatic incubation period during which the virus may be detectable. During this period, infected individuals act as hidden carriers and may unknowingly spread the virus. Stage II is characterized by mild-to-moderate symptoms and the virus is detectable in this stage. Stage III is characterized by severe acute respiratory symptoms caused by a sharp increase in the viral load [2].

Approximately 15% of the confirmed COVID-19 cases experience severe symptoms [3] with the likelihood being higher for vulnerable patients and older adults [4]. There are reported cases of children being affected by the rare pediatric inflammatory multisystem syndrome (PIMS) [5,6] leading to severe and life-threatening infection in previously healthy children and adolescents [7]. Confirmation of SARS-CoV-2 infection through antibody and nucleic acid testing in the majority of these patients, as well as hyperinflammatory manifestations similar to those seen in adults affected with COVID-19, provide compelling evidence for a link between SARS-CoV-2 and PIMS [8]. At least eighty distinct SARS-CoV-2 genotypic variants have been identified [9]. With such a diverse heterogeneity, developing a highly efficacious vaccine may be difficult, raising concerns about the possibility of reinfection. Most of previously

hospitalized patients who re-tested positive for SARS-CoV-2 RNA were young and had mild symptoms with no disease progression when they were re-admitted. Interestingly, the same virus-positive patients tested negative for virus RNA within 2–3 weeks of leaving the hospital [10]. It is probable that individuals may have been infected with two genetically distinct SARS-CoV-2 strains [11]. Thus, the risk of reinfection in COVID-19 patients is a serious concern. In relation to infectious diseases, the first question is always whether the immune system is capable of detecting and neutralizing the causative agents. Even though an infection may elicit immune responses, several aspects of infection should be clarified promptly. In this review, the association between reinfection and immunity is examined in an attempt to find answers to the following question: do re-infections and relapses occur because of immune insufficiency, immune responses variables, or the emergence of virus mutants following the initial infection?

2. Cytokine storm and lung damage

SARS-CoV-2 requires the angiotensin-converting enzyme II (ACE2) receptor-binding domain (RBD) to enter and infect host cells. The SARS-CoV-2 spike (S) protein contains two S1 and S2 subunits that bind with host cell receptors via the RBD S1 subunit, thereby simplifying the fusion process with the host cell (S2 subunit) [12]. ACE2 is associated with endosomal cysteine proteases, cathepsin B and L (CatB/L), and transmembrane serine protease 2 (TMPRSS2), which is required for the fusion of the viral and cellular membrane S2, and internalization in the pulmonary epithelium [13,14]. Target cells internalize ACE2 together with SARS-CoV-2 by endocytosis and as a result, the expression of ACE2 in surface of infected cells decreases, while the serum angiotensin II levels

increase, acting as a pro-inflammatory cytokine. In this regard, it is assumed that a renin-angiotensin system (RAS) is engaged in ARDS development following SARS-CoV-2 infection [15,16]. Similarly to how ACE2 receptor inhibitors reduced SARS-CoV-1-induced acute respiratory distress syndrome (ARDS) [17], it seems that efforts to develop ACE2 receptor inhibitors (also known as ACE2 blockers such as enalapril, lisinopril, captopril, perindopril [18,19]) for SARS-CoV-2 may be beneficial (Fig. 1A).

While most COVID-19 patients are asymptomatic or have mild symptoms or no clinical signs, some develop serious symptoms, including severe respiratory conditions, developed ARDS, and lung damage requiring intensive care [20,21]. Serum levels of IL2 and IL12 were significantly higher in patients with asymptomatic or mild COVID-19 infection than those with severe or moderate disease [22]. In severe COVID-19, increased serum cytokines level is associated with the pathogenesis of the disease [23]. Cytokine storms, as defined by hypercytokinemia of proinflammatory cytokines (e.g., IL-1β, IL-2, IL-6, IL-8, IL-17, CCL3, G-CSF, GMCSF, IP-10, MCP-1, TNF-α) are positively correlated with disease severity and multiorgan damage in infected patients [23-26], and result in disseminated vascular coagulation, leakage, and vascular permeability in COVID-19 patients and are associated with disease severity and lung damage [27,28]. Respiratory symptoms are common in patients with cytokine release syndrome (CRS), which can manifest as hypoxia, dyspnea, and bilateral lung involvement in ARDS [29]. CRS symptoms range from flu-like symptoms to severe life-threatening inflammation. Elevated cytokine and chemokine levels in CRS, as well as an increase in neutrophil–lymphocyte ratio (NLR) in severe cases of COVID-19 [27] are associated with increased inflammation and lymphocytopenia [25,28,30]. (Fig. 1B).
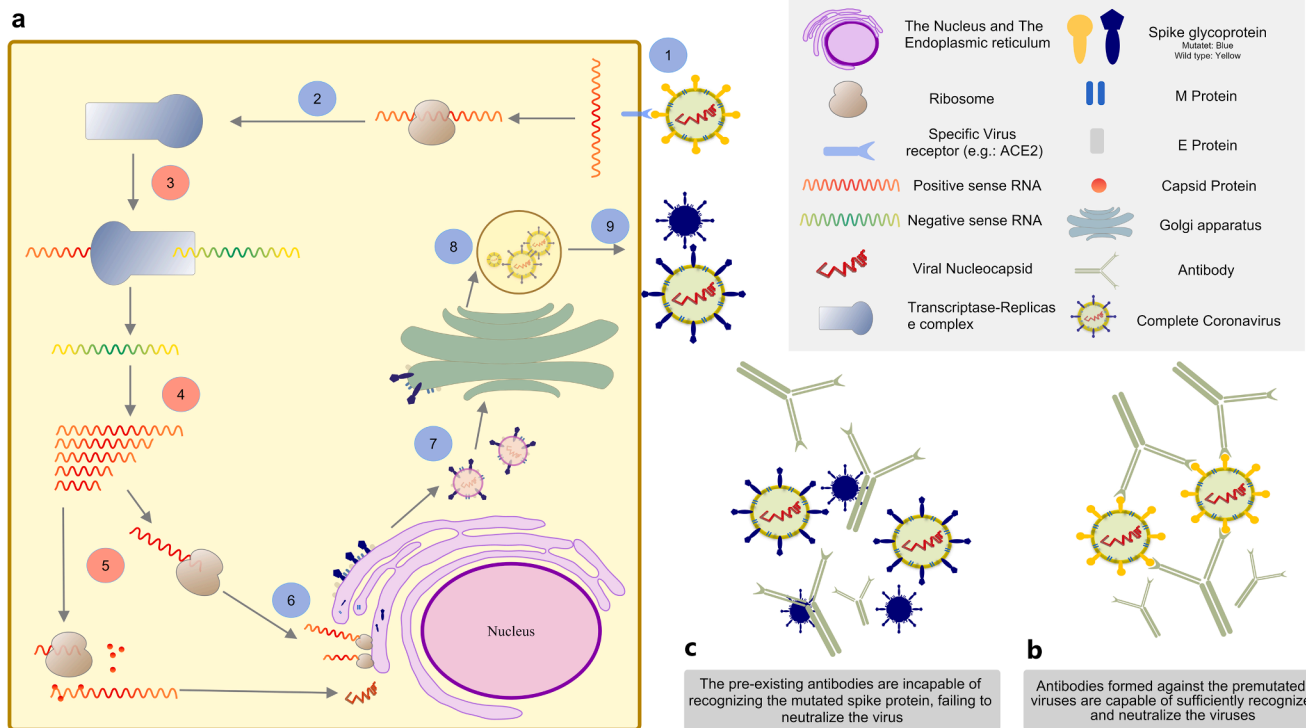

Fig. 1. A simple structure of a coronavirus and key steps involved in one round of the virus's replication. The viral replication cycle involves 9 key steps: *1.* the virus enters the cell via receptor-mediated endocytosis, and releases its genome into the cytoplasm. *2.* Cell's ribosomes translate the RNA, producing the Transcriptase-Replicase complex. *3.* The Transcriptase-Replicase uses the positive sense RNA and produces a negative sense RNA. *4.* Multiple variable sized mRNAs are formed with the template of the negative sense RNA. *5.* The mRNAs are then translated by host cell's ribosomes into structural and nonstructural proteins. *6.* Envelope proteins are placed on endoplasmic reticulum's (ER) surface. Then the nucleocapsid which is formed in the cytosol is drawn on ER's surface. *7.* Vesicles containing viral proteins and nucleocapsid, enter the Golgi apparatus. *8.* Complete virions are formed and then transported by vesicles to cell's surface. *9.* Complete viruses are released by exocytosis. Steps 3 to 5 (red circles) are highly prone to mutations and errors, which can affect biosynthesis and changes in the viral proteins, including envelop receptors/markers on the surface of the virus, making the preexisting antibodies unable to recognize the surface proteins.— the changes that can contribute to reinfection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

According to recent reports, lymphopenia is associated with attenuated CD4 and CD8 T cells in mild and acute COVID-19 infections [20,31-33]. Lymphocytopenia and variation in the total neutrophil count may be directly associated with disease and death [34,35]. Increased serum levels of high-sensitivity C-reactive protein (Hs-CRP) and procalcitonin, two master inflammatory factors are associated with an increased risk of organ damage and death [36].

People with secondary immunodeficiencies are more likely to contract SARS-CoV-2 than people with primary immunodeficiency, but they are no more likely to develop severe COVID-19 than the general population. Also, respiratory symptoms are one of the significant causes of morbidity and even mortality among patients suffering from different forms of primary immunodeficiencies and they are observed both in children and adults [37]. But, patients with secondary immunodeficiencies have worse outcomes than those with primary immunodeficiencies [38]. Evidence has shown that cellular senescence is associated with aging, contributes to the development of lung damage, and facilitates respiratory infections in elderly [39]. Thus, old age is an influencing factor in the mortality rate of COVID-19 particularly among those over 60. However, the World Health Organization (WHO) has emphasized that young people are not immune to the virus and must adhere to health attendants' instructions to prevent the spread of the virus [40].

3. Immune response variables

During viral infection, lung epithelial cells and macrophages/monocytes are activated by viral pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) which are localized at the cell surface and play a critical role in host defense against microbial pathogens by macrophages demonstrating the influential role of innate immune responses to clear pathogens [41] which leads to enhanced interferon (IFN) production [42]. Innate immunity and its associated chemokines activate the antiviral signaling network in target cells, thereby initiating the adaptive immune response. However, an excessive activation of TLR signaling pathways and high levels of proinflammatory cytokines attract immune cells, particularly monocytes and T cells, resulting in an inflammatory disorder [20,43]. Generally, IFN-I signaling demonstrated a protective effect on mammalian cells from virus infections during SARS-CoV-1 and MERS-CoV infection [44,45]. The study on MERS-CoV-infected mouse model demonstrated that, unlike SARS-CoV, IFN-I signaling is required for MERS-CoV clearance [45]. Thus, it appears that its role in SARS-CoV-2 will be considerable. SARS-CoV-2 is more susceptible to IFN-I/III treatment than SARS-CoV-1, according to early experimental findings [46,47]. (Table 1).

According to a comparison study between survivors and deceased COVID-19 patients, decreased percentages and counts in CD3, CD4, and CD8 lymphocyte subsets were observed in deceased COVID-19 patients, implying that these are useful prognostic markers for organ injury, severe pneumonia and fatal cases [36]. Additionally, the numbers of both helper- and suppressor-T cells were significantly reduced (P-values of less than 0. 001), whereas attenuated levels of regulatory T cells were more pronounced in severe cases. In fact, a balance of naïve and memory T cells appears critical for maintaining an effective immune response. While naive T cells protect against previously and new unknown infections by secreting an abundance and modulated amount of cytokines, memory T cells are involved in antigen-specific immune response. Impairment of the balance mentioned above is associated with hyperinflammation, whereas COVID-19 relapse is associated with reducing the number of memory T cells [48,49].

After effector T cells undergo apoptosis in response to viral/antigen clearance, a pool of memory T cells is generated that are programmed to prevent re-infection. CD4+ memory T cells, upon re-stimulation, trigger B cells and other immune cells via cytokine production. On the other hand, cytotoxic memory T cells aid in the destruction of infected cells during subsequent infection [50,51]. Case studies in recovered SARS-CoV-1 patients demonstrated that both CD4+ and CD8+ memory T

Table 1
Immune response associated with SARS-CoV-2 reinfection and disease relapse.

CK	WBC	Neutralizing Antibody
• CRS contains from flu-like symptoms to severe life-threatening inflammation to ARDS. • Increases serum levels of cytokines and chemokines (IL-1β, IL-2, IL-6, IL-8, IL-17, CCL3, G-CSF, GMCSF, IP-10, MCP-1, TNF-α) in severe COVID-19	• Increase in NLR leads to acute lung injury and is often associated with total lymphocytopenia. • Decreases regulatory CD4 cells, CD8 cells, B cells, NK cells, monocytes, eosinophils, basophils • Increases naive Th cells in severe cases. • Decrease number of T-mem cells. • B-mem cells may be involved in long-term protection from reinfection.	• B cell response, as evaluated virus-specific IgM, IgG, IgA and nAbs, generally occur around day 14 after infection initiated. • 96.8% of patients exhibit IgG and IgM seroconversion around 20 days after symptoms initiate • Virus-specific IgG develops in 100% of patients in 17–19 days, while IgM in 94.1% in 20–22 days after symptom initiation. • The antibodies against SARS-CoV-2 may be protective and even curative. But, this has not been observed for a number of patients that tested positive after complete remission.

Abbreviations: CK = cytokine CRS = cytokine release syndrome, NLR = neutrophil–lymphocyte ratio, IL = Interleukins (IL-1β, IL-2, IL-6, IL-8, IL-17), Cytokines (IFN-γ, MCP-1, TNF-α, IP-10), Chemokines (CCL2, CCL3, CCL5, CXCL10).

cells could elicit immune response from 3 months to 6 years in the absence of antigens [52]. In COVID-19 patients, decreased regulatory T cell populations and memory T cells may be associated with an inflammatory response that results in cytokine storm and organ failure [27].

Evidence of cross-reactive immune responses is not the same as evidence of protection. Laboratory studies on four components of the immunity (antibodies, B-mem, T-helper, and NK cells) indicate that virus-specific IgG to Spike proteins remained stable for 6+ months, whereas memory CD4 and CD8 lymphocytes declined with a half-life of 3–5 months [53,54]. This could imply that survivors of COVID-19 are protected and capable of cross-reacting for months, if not years when pre-existing immunity is recalled to fight reinfection. While similar responses are expected for various pathogens, absolute protection hardly upholds for certain RNA viruses, particularly SARS-like viruses that are capable of mutating their receptor binding proteins and changing their cell tropisms, resulting in reinfections in exposed individuals.

All vaccines, including two mRNA vaccines (BNT162b2 and mRNA-1273) and a DNA vaccine (AZD1222), encode genetic information, activating the immune system to produce the spike (S) viral antigen. All three vaccines were shown to induce neutralizing antibodies against wild-type spike protein (D614) or D614G mutants, as well as cellular immune responses during phase I and II clinical trials [55,56]. Additionally, studies on mRNA-1273 have evaluated its ability to improve the CD8+ T cell response [57]. Two significant consequences of the humoral immune response to infection or vaccination are rapid serological immunity as a result of antibodies produced by antibody-secretion cells (ASCs) and memory B cells generation [58,59], which can produce new antibodies via the creation of new ASCs [60]. The BNT162b2 mRNA SARS-CoV-2 vaccine induces (S) protein-specific neutralizing antibodies with protective immunity [61]. mRNA vaccines demonstrate the ability to elicit not only humoral but also cell-mediated adaptive responses, to activate T-cell helper (Th) and cytolytic T lymphocytes [62] and also to induce the release of IFN I [63]. The

delivered genetic cargo based on adenovirus vectors shows that immunization with AZD1222 induces rapid activation of both humoral and cellular responses, with higher response rates at the second dose [56].

The vaccine, known as replication-incompetent adenovirus type 26 (Ad26).COV2.S, is a vector vaccine encoding a stabilized variant of the SARS-CoV-2 spike (S) protein. Ad26.COV2.S induces humoral immunity, T helper cell (Th cell) 1 and cytotoxic T cell responses being stable for at least 14 week [64]. Moreover, Ad26.COV2 and AZD1222 both can emulate viral infection and induce expression of IFN along with memory T and B cells [56,65].

4. Neutralizing antibody seroconversion

Evaluation of the antibody seroconversion is beneficial for the clinical assessment of infections. Endogenous neutralizing antibodies (eNAbs) induced by viruses or vaccines may provide a source of antibodies to monitor and also predict risk of SARS-CoV-2 reinfections and disease relapse. Achieving accurate estimates of eNAbs against a given strain of SARS-CoV-2 ought to be considered in relation to the mutability and genetic polymorphism of coronaviruses. Notably, while donor plasma or plasma exchange therapy is promising [66], its true success may largely depend on a timely access to diagnostic test results to ensure the presences of efficacious eNAbs. The reactions of the antibody response to SARS-CoV-2 have recently been well realized [67]. SARS-CoV-2 evokes a B cell response, as evidenced by virus-specific IgM, IgG and IgA, as well as neutralizing IgG antibodies (nAbs) during the infection period, which is typically between days 4 and 14 [68]. Notably, 96.8% of evaluated cases exhibit IgG and IgM seroconversion around 20 days after symptoms begin; virus-specific IgG develops in 100% of patients in 17–19 days, while virus-specific IgM in 94.1% of cases within 20–22 days after symptom initiation [69]. Moreover, sera of COVID-19 patients exhibit cross-reactivity to nucleocapsid antigens of SARS-CoV-1 [70]. Despite broad and prevalent immune responses, escaping from disease severity is challenging in high risk individuals.

Binding of antibodies to the SARS-CoV-2 antigens indicates that the S1 domain has more immunogenicity than the RBD domain resulting in higher titer of IgG and IgA antibodies and also more efficient virus neutralization antibodies [71]. nAbs are able to neutralize and suppress virus interaction with the host entry receptor, ACE2. Even though antibodies against SARS-CoV-2 and SARS-CoV-1 related S/N proteins cross-react with S protein of MERS-CoV, no cross-reaction was observed against the RBD of SARS-CoV-1 and MERS-CoV [72]. Furthermore, COVID-19 plasma fails to neutralize either SARS-CoV-1 or MERS-CoV. It was concluded that the neutralization is virus specific and is most likely mediated by epitopes in the RBD [73,74].

IgG specific SARS-CoV-2 spike protein was acquirable up to 60 days after the onset of symptoms, while IgG titers decreased up to eight weeks after symptom initiation. Reactive memory B cells (B-mem) may play a role in long-term protection against reinfection [74]. Conservation specific antibodies, such as IgG and neutralizing Abs (NAbs) are generated by B cells in response to viral infection. These antibodies are able to suppress the virus from entering the infected cells and protects against viral reinfection [66]. Studies on both SARS-CoV-1 and MERS-CoV have demonstrated that virus-specific antibody responses diminish over time. Therefore, SARS-CoV-2 related immunity is likely to decline following primary infection; additional evaluation will be required to determine the degree of long-term protection [74].

The antibodies against SARS-CoV-2 may be protective and even curative, as demonstrated in the cross-sectional serotherapy experiments using a passive immunization approach to reduce virus burden during infections [75]. However, this was not observed in a number of patients who tested positive following complete remission [76-78]. Consistent with this, a faster recovery rate has not been observed in SARS-CoV-2 patients with high titers of neutralizing antibodies than patients with lower titer. eNAbs appear insufficient to provide a robust protection against the virus. Furthermore, patients who have recently been infected but have only mild symptoms and have fully recovered may be susceptible to reinfection and disease relapse [79]. Given the critical role of both humoral and cellular immunity in eliciting protective immunity against SARS-CoV-2 reinfection, adequate and sustained immune memory responses, such as neutralizing antibodies and memory T cells, would be necessary.

5. SARS-CoV-2 mutations

Coronaviruses are enveloped, single-stranded, positive sensed RNA viruses that are typically used to generate transcriptase-replicas complexes synthesizing a complementary negative sensed RNA that serves as a template for the subsequent synthesis of mRNAs. Coronavirus utilizes open reading frames (ORFs) to generate mRNAs processed into nonstructural proteins, and then new structural proteins are formed by subsequent processing of the mRNAs [80]. As described previously, progeny viruses can harbor a quite significant amount of mutations and alterations during each replication cycle. This process can repetitively occur and continue as new virus strains are transmitted to other cells. The mutated genes translate to changes in viral proteins on the surface of the virus. Thus, as an RNA virus, coronaviruses would be susceptible to mutations [81]. Nonetheless, it is believed that SARS-CoV-2 has a low overall mutation rate; this could be due to the genetic proofreading mechanism found in CoVs [82,83]. However, because SARS CoV-2 has the largest genome of all RNA-containing viruses, such genomic changes can accumulate rapidly during each viral replication cycle [81,84].

SARS-CoV-2 mutates approximately two nucleotides per month. The N501Y mutation is associated with the RBD and results in an increased binding affinity for ACE2 compared to the wild type in human and murine cells [85]. Another mutation, D614G, is highly prevalent in the global COVID-19 pandemic [86]. Fortunately, while this mutation may be associated with higher viral RNA levels in patients than the variant without the mutation and is required for SARS-CoV-2 infectivity [87,88], there is no convincing evidence that SARS-CoV-2 infection with the G614 mutant is associated with disease severity [86,89].

Additionally, the ORF8 protein of SARS-CoV-2 exhibits structural changes associated with the virus ability to spread. It has a low homology with SARS-CoV-1 among all viral proteins, which mediates the escape from the immune system by interacting with major histocompatibility complex molecules class I (MCH-I) and down-regulating the surface expression of MHC-I by lysosomal degradation via an autophagy-dependent mechanism on various cells. Overexpression of ORF8 has also been suggested to disturb antiviral activity of IFN-1 signaling [90]. The spike deletion (at position 69–70) is thought to allow the virus to evade human immune system, and it has also been linked to some RBD changes, including N501Y, N439K and Y453F [91,92]. Thus far, the most notable mutation appears to be D614G, which was initiated in Europe and had a beneficial effect on the severity of COVID-19 infection. Others assert that G614 is highly contagious and has the potential to spread rapidly [86]. In another study conducted in the United States, a second infection occurred approximately 140 days after the initial infection. The patient presented with symptoms in both incidents but with fewer symptoms during the second infection (Table 2). The genomic analysis revealed that the two isolates, associated with the two separate incidents, were most likely from distinct strains [93]. Whether the G614 mutation results from the virus's natural evolution and poses a significant risk of expanding the COVID-19 pandemic is unknown.

The B.1.1.7, B.1.351, and P.1 variants circulating in the United States and all around the world are classified as variants which have caused concern [94]. The RBD mutation which increases affinity to ACE2 is present in B.1.1.7 and B.1.351 [95]. Also, P.1 contains mutations at the same RBD residues [96].

SARS-CoV-2 variants were first recognized in the British (B.1.1.7), Brasil (P.1) and South African (B.1.351also termed 501Y.V2) and have recently been found in the US and in other world regions [97-99].

Table 2

Potentially new strains of SARS-CoV-2.

Mutations		Prevalence	Outcomes	Ref.
RBD	**Spike protein**			
N501Y		Relatively high frequency in the global genome data (~3000 sequences)	Enhanced binding affinity to ACE2 receptor	[85]
	Amino acid 69–70 deletion	England	Apparently evades human immune system	[91,134]
	D614G	High frequency in the global genome	G614 might spread rapidly, more contagious?	[86]
	ORF8	Frequent in USA	ORF8 protein downregulating the MHC-I on different cell, which might favor the virus to evade immunity	[135]
B.1.1.7		British	increased transmissibility with increased the risk of hospitalization the BNT162b2 and AZD1222 are effective	[99,100,107]
P.1		Brasil	higher transmission rate than wild type vaccines showed low efficacy	[97,102,105]
B.1.351		South African	resistant to NTD, RBD, convalescent plasma more evidences needed but BNT162b2 is effective	[98,108]

Abbreviations: RBD = receptor binding domain, ORF = open reading frame, MHC-I = histocompatibility complex molecules class I.

Graham and his collogue in their study indicated that the B.1.1.7 mutation has increased transmissibility, which has contributed in the sharp rise in cases in the UK [100]. It has been reported that B.1.1.7 has high transmission rate with 43%–82% [101] and a study in Denmark found that B.1.1.7 increased the risk of hospitalization [99]. Moreover P.1 has shown 2.6 times higher transmission rate than previous circulating SARS-CoV-2 variants [102]. Recently reported that B.1.1.7 is resistant to neutralization by most monoclonal antibodies(MAbs) which attaches to the N-terminal domain (NTD) of spike protein and relatively refractory to some of the MAbs targeted to the RBD but has no more resistant to convalescent plasma or vaccination.

However, Findings on B.1.351 showed that this variant has the same behavior on neutralization by most NTD mAbs but it is refractory by multiple mAbs to the RBD and also is resistant to neutralization by convalescent plasma and vaccination. The resistance is largely due to E484K, a mutation shared by B.1.351 and P.1 [96,103,104].

Most of vaccines showed also very low efficacy for P.1 mutation [105,106]. AZD1222 was noted 70% efficacy against B.1.1.7 [107] but only 10% efficacy for B.1.351 [108]. Moreover, BNT162b2 and NVX-CoV2373 had similar efficacy from 81.5% to 95% for B.1.1.7 mutation. Better efficacy for B.1.351 with vaccination was elicited by BNT162b2 with 100% efficacy and then Ad26.COV2.S 72% efficacy [105,109,110].

6. COVID-19 Re-infection

Numerous respiratory viruses, including human coronaviruses, can induce reinfections. Reinfections with respiratory viruses can occur as a result of a weakened immune response (e.g., respiratory syncytial virus), reinfection with a different genotype/species of virus (e.g., rhinoviruses), or due to the high mutability of the viruses (e.g., influenza virus) [111].

The first confirmed case of SARS-CoV-2 reinfection that occurred in the USA was a 25-year-old man with no known immune disorders [11]. The second infection exhibited more severe symptoms than the first one and the patient tested positive for IgG and IgM antibodies. The genetic heterogeneity of the specimens was discovered to be greater than what could be explained by short-term in vivo evolution [11].

Another case report came from Belgium, involving a 51-old-woman with mild symptoms and a seronegative antibody response; she still had mild symptoms in the second infection [112]. Others described a 46-year-old man with mild symptoms who tested positive for the polymerase chain reaction (PCR) and antibody (IgM+/IgG−) tests. The patient was reinfected two days after a close contact with a COVID-19 patient and developed disease despite seropositivity for both IgM and IgG antibodies; the virus might belong to two distinct strains [113] (Table 1).

Notably, 40% of asymptomatic patients are antibody negative while 12.9% of symptomatic individuals are antibody positive approximately eight weeks after infection [114]. In general, most patients exhibit anti-virus antibodies between days 10–14 following the onset of symptoms. Antibody levels may be low or below the detection limit in patients with mild symptoms [115].

Another case involved a 70-year-old patient who tested positive for SARS-CoV-2 PCR but was IgG antibody negative at the time of infection. After complete recovery and PCR negativity, the patient reinfected with SARS-CoV-2, resulting in positive molecular and serological tests [116].

Moreover, a report from Hong Kong indicated a 33-year-old healthy man with mild symptoms testing negative for anti-virus antibodies during the initial infection. He was discharged following a period of treatment and two negative SARS-CoV-2 RT-PCR tests. The patient was reinfected and while he remained asymptomatic, he became IgG positive, and RNA sequencing confirmed virus infection; the results revealed considerably two distinct variants [117]. Anti-SARS-CoV-2 antibody was initially detected in another case, during the second reinfection; it was believed that the virus was likely regulated in part by low-titer antibody [118]. However, the positive retest in a 21-year-old patient on her third admission might be due to the additional time required for clearance of viral gene segments from the initial infection [119].

In The Lancet, Christian Hansen and colleagues reported their study investigated the risk of becoming positive for SARS-CoV-2 by PCR for the second time, assumed to indicate re-infection [120]. The study determined that generally past infection confers 80.5% protection against re-infection, which declines to 47.1% in those aged 65 years or older.

Another real case of re-infection was defined by Wang To et al. The study confirmed that patient had recurrent clinical signs by a different strain of SARS-CoV-2. This case was investigated by epidemiological, clinical, serological (IgG seroconversion), and genomic analyses [121].

SIREN study which looks for answers to the questions about SARS-CoV-2 reinfection adds to other studies that the infection does protect against re-infection and possibly in an antibody-dependent manner [122]. Additionally, the findings indicate that infection and antibody response protects on par with or better than currently available SARS-CoV-2 vaccines [123-125].

7. Discussion

Now there is the concern about the advent of several variants over the globe and the efficacy of the vaccines against these variants [126]. However, all changes in the RNA sequence cannot render SARS-CoV-2 as

a super pathogenic or a mutating into a new variant resistance to drugs or vaccine.

Each year, a new influenza vaccine is given, in part due to the ever-evolving nature of coronaviruses – which infect, propagate, and transmit from cell to cell in order to complete their replication cycle during an infection, and which can then be sequestered in cells and evade the immune system. Specific mutations may be advantageous to the host and deter the virulence, hence reduce the viral spread and disease prevalence. While most reinfection studies are conducted on a single patient, a new strain of the virus may cause a second infection. It's tempting to speculate that the initial infection with SARS-CoV-2 may not elicit robust and stable immune responses in all individuals. Immune suppression is a remote possibility as a primary reason for reinfection, even though recovery from COVID-19 is attributed to anti-SARS-CoV-2 antibodies [89] and lymphocytic cell counts return to normal in most cases [127].

It is unknown whether all suspected COVID-19 relapses were reinfected. Due to the variety of diagnostic and serological testing platforms used and confounding variables, determining the statistical significance of these observations is difficult. One possible mechanism for reinfection in COVID-19 is the cellular transport pathway with the release of SARS-CoV-2 exosomes as a reason for reappearance of viral RNA in the recovered COVID-19 patients during the silence time period [128]. This Trojan horse-like behavior may aid the virus in combating the host's defenses and propagating the infection.

Neutralizing antibodies against SARS-CoV-2 confer immunity against reinfection, but their turnover appears to be rapid, as their concentration persists for nearly two months and continues to wane [129]. This depreciation might particularly be more pronounced on the mucosal surfaces of virus entry points than in blood circulation, which remains unknown.

As the number of infections increases, the probability of a functional change in the structure of the virus increases. New field isolates of the SARS-CoV-2 can serve as interesting models for testing this hypothesis. Reinfection may not necessarily culminate into the emergence of a new disease, although it is plausible. While this hypothesis may appear implausible, its neglection has the potential for re-emergence of diseases.

Among the theories regarding reinfection, there is also debate about whether or not the reported cases were completely cured and the shedding of the virus stopped. It is assumed that with the remission of the clinical disease, shedding continues for a median of 20 days and possibly for more extended periods, as has been observed in some cases [130]. Such cases may represent false positives and a red flag for reinfection. On the other hand, reinfection can occur in individuals with underlying immunological disorders due to impaired regulation of the immune responses or production of the antibodies.

The presence of SARS-CoV-specific CD4 T-cells and memory CD8 T-cells are essential factors for providing long-term immunity against COVID-19 [131]. A positive serology test after a few months may be insufficient to induce immunity in recently recovered COVID-19 patient [132]. It is unknown whether changes or deficiencies in the serum levels and activity of anti-SARS-CoV-2 antibodies or SARS-CoV-2-specific cytokines (such as IFITM) make patients susceptible to reinfection and disease relapse (Table 1).

There are many reports regarding confirmatory virus RNA testing in IgG-positive patients who have recovered [84]. Discordant diagnostic test results may be interpreted differently due to the low accuracy of commercial IgG kits, different types of sampling, such as the presence of viral RNA in fecal swabs without evidence of replication-competent virus isolation, and hidden carriers. Clinically symptomatic patients who recovered from COVID-19 but subsequently developed reinfection or severe disease relapse could result from a variety of immune response variables, reactivation in hidden carriers, or reinfection with a different strain. Mutations that result in the rapid spread of a new strain and the emergence of slightly different disease are relatively rare. Currently,

there are several variants of the virus under investigation [133]. A mutated copy of SARS-CoV-2 emerged in England, U.K. in December 2020, and was immediately picked up by news media attention for its apparent rapid spread. There is no evidence that it is an escape mutant with markedly altered virulence that causes an unusual or slightly different disease. Likewise, it is unknown whether it would be protected with vaccine.

The limitations of these studies are lack of large study population. Different molecular diagnostic tests could exhibit incomparable positive predictive values (PPV) and negative predictive values (NPV), even though certain biopsies for the confirmatory tests may have been always obtained. However, prior exposure shortly before reinfection may not guarantee a complete immunity.

Considering the rapid onset of acute pulmonary symptoms, and the time constraints involved in identifying the right collection of donors' plasma pool, the clinical utility of the plasma exchange therapy that depends on the presence of high titer neutralizing antibodies is challenging. The risk of reinfection and transmissibility of the virus largely depends on response variables, durability of the SARS-CoV-2 antibody-mediated immunity and memory T cells recall responses. These variables, combined with the quantitative assessments of new strains transmissions can better explain the individual susceptibility, and provide useful information regarding the accuracy of the incidents (low, moderate, high) and rates of reinfection and disease relapse rates.

8. Conclusion

The potential of SARS-CoV-2 reinfections ought to be prospectively monitored in healthcare practices and clinical trial investigations. Pathogens that are highly infectious and rapidly changing, such as rampant influenza viruses and SARS-CoV-2, can cause pandemics at any time and in any location. Being symptomatic in patients who have recovered from COVID-19 can be due to either reinfection by the virus or reactivation of the virus in hidden carriers. On the other hand, the issues of low level of antibody in patients with anergic or compromised immune response, as well as mild or moderate disease and their susceptibility to reinfection cannot be precluded. Moreover, physicians should consider the likelihood of genetic mutations observed during the recurrence course rather than reinfection with the same strain. Taken together, the diversity of the virus's genetic variants, the short half-life of neutralizing antibodies, the possibility of reinfection leaves strategies like herd immunity and efficacy of vaccines questionable and highlights the importance of preventive strategies in mitigating the spread of the disease.

In conclusion, the findings suggest that the possibility of presumptive recurrence. However, further studies are necessary to definitively understand the perception of COVID-19 recurrence and its underlying mechanisms. These findings should be regarded as preliminary until quantitative pharmacovigilance data are available to resolve the evolving issues surrounding SARS-CoV-2 reinfection and COVID-19 relapse

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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