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REVIEW

The interplay between the immune system and SARS-CoV-2 in COVID-19 patients

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Abstract

Millions of people across the globe have been affected by coronavirus disease 2019 (COVID-19), which began in Wuhan, China, and is caused by SARS-CoV-2. COVID-19 has a variety of clinical characteristics and triggers immune responses required for the elimination of the viral agent. Currently, no effective treatment options are available for targeting SARS-CoV-2 infection. Repurposing of drugs such as chloroquine, thalidomide, and leflunomide alongside convalescent plasma is being employed as a therapeutic strategy. Clinical studies have shown that both asymptomatic and symptomatic patients can have an extremely active immune response that is largely attributable to immune system modulations. This includes cytokine storm syndrome (CSS), which affects the adaptive immune system, leading to exhaustion of natural killer (NK) cells and thrombocytopenia in some cases. This review examines the interaction of SARS-CoV-2 with the host immune system and the potential for the development of appropriate immunotherapy for the treatment of COVID-19.

Introduction

In December 2019, a distinct case of pneumonia characterized by typical clinical features of viral pneumonia was reported in Hubei province of the People's Republic of China. Analysis of respiratory samples revealed that a novel coronavirus was responsible for this pneumonia, which was later referred to as "novel coronavirus pneumonia" (NCP) [1]. The disease was officially named "coronavirus disease 2019" (COVID-19) by the World Health Organization (WHO). Coronaviruses are enveloped viruses with single-stranded RNA (ssRNA) and a helical nucleocapsid, and they are responsible for infections of the respiratory and intestinal tracts. SARS-CoV was responsible for an outbreak of severe acute respiratory syndrome (SARS) in China in 2002, and an outbreak of Middle East respiratory syndrome (MERS) was caused by MERS-CoV in the Middle East [2–5]. Earlier last year, another coronavirus outbreak was reported, and the novel coronavirus was named "SARS-CoV-2" because of its similarities to SARS-CoV [6, 7]. Currently, the molecular mechanism underlying the infectious activity of SARS-CoV-2 is poorly understood, although structural analysis of the virus indicates that it may infect human cells by using angiotensin-converting enzyme 2 (ACE2) as a receptor [7]. The zoonotic spread of SARS-CoV-2 is not clear yet, but human-to-human transmission was reported at a marketplace where wild animals were sold, and studies have also indicated that the three above-mentioned viruses are likely to have originated from bats [6, 8, 9].

SARS-CoV-2 is a member of the subfamily *Coronavirinae*, which includes four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* [10]. Some members of the first two of these genera infect humans. SARS-CoV-2, like SARS-CoV and MERS-CoV, is a member of the genus *Betacoronavirus* [11]. The SARS-CoV-2 genome is similar to that of SARS-like bat coronaviruses, and SARS-CoV, it relies on ACE2 as a receptor to infect human cells [12, 13]. The S (spike) protein present on the surface of the coronavirus is responsible for recognition of the receptor. Structural modeling of the binding of SARS-CoV-2 indicates that it is predicted to attach with more than tenfold higher affinity than

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SARS-CoV [14]. However, the underlying mechanism of the pathophysiology of SARS-CoV-2 is still unknown.

Clinical illness in patients infected with SARS-CoV-2 varies from a mild respiratory condition to severe acute respiratory illness and renal failure [1]. Pneumonia is the most prevalent clinical feature of SARS-CoV-2 infection, although the length of time until the appearance of clinical symptoms after infection varies from patient to patient; the average period is 14 days [15]. Health facilities are conducting diagnosis of COVID-19, and non-invasive diagnosis involves the detection of viral RNA. Real-time PCR (qPCR) is employed for the diagnosis of COVID-19 by detecting the presence of viral RNA in sputum, the throat, and the respiratory tract. This method is highly specific, but its relatively low sensitivity can lead to false-negative results [16]. The white blood cell (WBC) count varies among patients with COVID-19, and lymphopenia is frequently observed [17]. Increased levels of lactate dehydrogenase and ferritin have also been reported. Computed tomography (CT) of the chest region may be useful in diagnosis, yet it cannot rule out the presence of COVID-19 [18]. For qPCR, the type of sample and time of collection play a key role in the diagnosis. In the early phase of infection, the virus can be detected in respiratory samples, whereas serum samples give negative results, even though studies have suggested the presence of a high viral load in the early days of infection [19]. In addition to qPCR, testing methodologies including RT-loop-mediated isothermal amplification (RT-LAMP) and RT-insulated isothermal PCR (RT-iiPCR) have also been reported. Comparative analysis focused on results of serology-based testing (e.g., ELISA) and nucleic-acid-based molecular testing (e.g., PCR) has shown that PCR has better sensitivity and specificity. A CRISPR-based testing system was developed by Zhang et al. for the detection of SARS-CoV-2 within 60 minutes using the SHERLOCK methodology [20]. This technology might pave the way for developments in point-of-care (POC) testing for COVID-19. A rapid sequencing method has also been developed for SARS-CoV-2 that is based on rapid construction of the transcriptomics sequencing library [21].

Currently, no effective treatment is available for COVID-19. The purpose of this article is to review the interactions between immune system and SARS-CoV-2 and the potential for the development of immunotherapy for the treatment of COVID-19 patients.

Immune system interference by SARS-CoV-2

Cytokine storm syndrome

SARS-CoV-2 infection leads to severe complications by targeting the adaptive immune system and causing lymphopenia [22] (Fig. 1, Table 1). It halts the production of antibodies and the T cell response, resulting in inflammation [23]. If inflammation is not controlled by the adaptive immune system within 7-10 days of infection, it distorts the adaptive immune response and results in "cytokine storm syndrome" (CSS). Previous studies have shown that CSS also occurs in patients with streptococcal toxic-shock-like syndrome (STSLS) caused by *Streptococcus suis* and in influenza patients [24, 25]. CSS is manifested by sustained fever, liver dysfunction, and coagulopathy. These are obvious clinical features of COVID-19 as well [26]. CSS results in the secretion of inflammation-specific cytokines; i.e., interleukin (IL)-1, IL-2, IL-8, IL-12, IL-18, tumor necrosis factor-alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-gamma (IFN-γ) [27]. IL-1β and IL-8 induce the expression of IL-6 and IL-17. The high IL-6 level has a suppressive effect on the immune system (Fig. 1a). By inhibiting the production of IFN-γ, it has a negative effect on the production of $CD8^+$ cytotoxic T cells [28]. As a result, COVID-19 patients are unable to produce an adaptive immune response (antigen-specific B cells and antibodies) [27]. An increased level of IL-6 leads to its movement towards the liver via the bloodstream, where it causes the induction of serum amyloid A (SAA) [29]. SAA accumulation results in chronic inflammatory diseases by producing amyloid A amyloidosis. Amyloid fibril deposits in various organs can result in their dysfunction [30]. IL-6 in bone marrow promotes megakaryocyte maturation, resulting in an elevated platelet count, thus causing blood clots. Many patients with severe COVID-19 experience clotting. This might be due to disruption of the immune system by the infection. High platelet counts are a sign of inflammation and can thus act as a biomarker of immune system dysregulation [26].

Exhaustion of NK and $CD8^+$ T cells

Natural killer (NK) cells are components of the innate immune system and exhibit lymphocytic activity against tumors and microbial infections (Table 1). Various studies have highlighted their reciprocal relationship with neighboring macrophages, endothelial cells, and T cells [37]. The antagonistic activity between NK cells and their neighboring cells helps to regulate NK cell activation and determine whether NK cells are able to perform their killing function [38]. A recent study performed in China showed that COVID-19 patients who were struggling with severe disease symptoms (SDS) had lower NK cell counts than patients with mild disease symptoms (MDS). These patients showed elevated expression of NKG2A receptors, which are inhibitory receptors present on the NK cell surface that gradually diminish its function, leading to disease progression (Fig. 1b). It has been suggested that the downregulation of

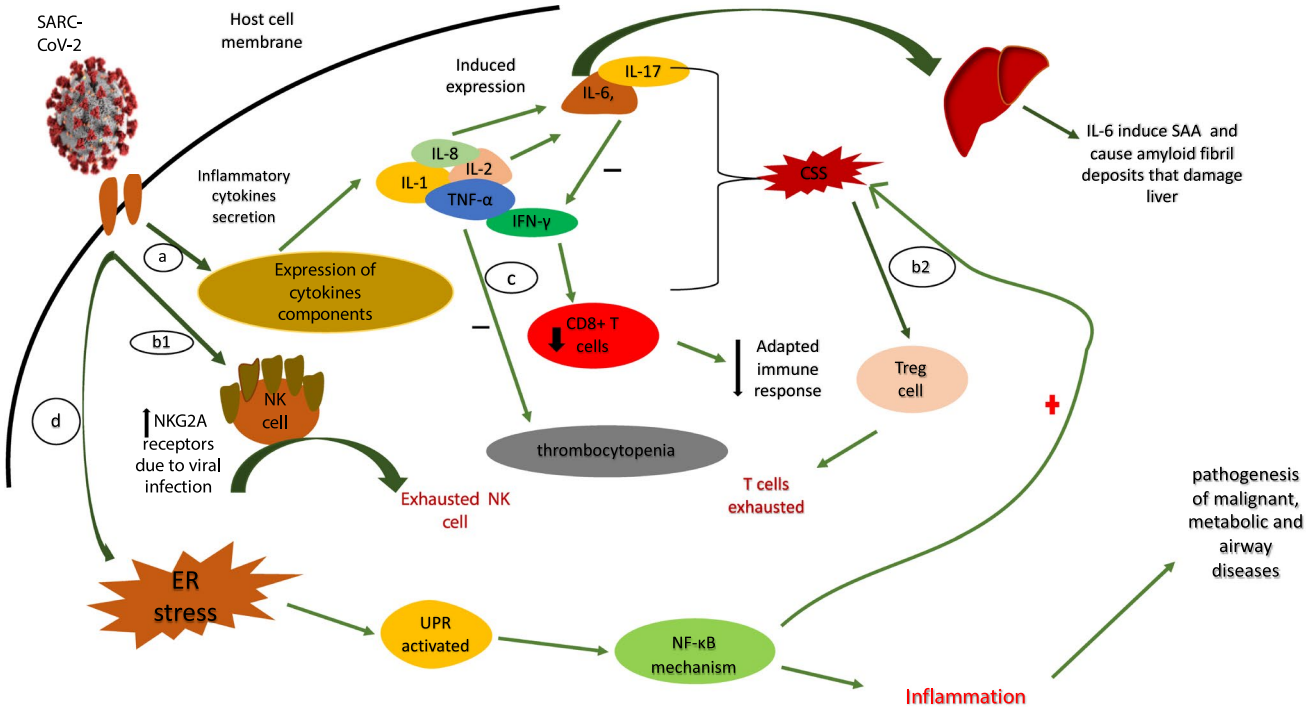

Fig. 1 Pathway illustrating four different ways by which SARS-CoV-2 interferes with the immune system. (a) SARS-CoV-2 infection results in cytokines stress syndrome by producing a cascade of inflammatory cytokines such as interleukin 1 (IL-1), IL-2, IL-8, TNF-α, tumor necrosis factor-alpha, and interferon-gamma (IFN-γ) through the activation of cytokine components. IL-2 and IL-8 further induce the expression of IL-6 and IL-17. CSS is characterized by a high level of IL-6. IL-6 causes liver damage by producing SAA serum amyloid. (b) SARS-CoV-2 infection causes the upregulation of NK cell receptors (NKG2A). High NKG2A levels halt the function of NK cells by exhausting them (b1). Simultaneously, T cell exhaustion occurs due to the activation of Treg cells because of CSS (b2). (c) Increased expression of TNF-α due to CSS causes thrombocytopenia. (d) SARS-CoV-2 infection causes ER endoplasmic reticulum stress, which activates the unfolded protein response (UPR). Prolonged stress results in activation of the NF-κB pathway, which causes inflammation and ultimately leads to the pathogenesis of malignant, metabolic, and airway diseases.

Table 1 Characteristics and clinical features of patients with immune system complications caused by SARS-CoV-2 infection

Immune-system-related complications of COVID-19	Characteristics	Clinical features	References
Cytokine storm	High level of inflammatory cytokines	IL-6: vascular leakage, coagulation TNF-α: flu-like symptoms IFN-γ: fatigue, malaise	[31]
NK cell exhaustion	NK cell function lost	chronic infection	[32]
T cell exhaustion	$CD8^+$ T cell dysfunction	Loss of effector (by producing perforins and granzymes), metabolic, memory and self-renewal function	[33]
Thrombocytopenia	Extreme reduction in platelet count	Liver damage (thrombosis), hemorrhagic complications	[34]
ER-stress-mediated inflammation	Activation of tumor-promoting cytokines ER stress caused by excessive viral protein production and modification for viral replication and infection	Inflammatory microenvironment, cancer aggressiveness, pathogenesis of metabolic and airway diseases	[35, 36]

the NKG2A receptor might play a role in controlling the disease [7].

T lymphocytes are categorized as $CD8^+$ cytotoxic lymphocytes (CTLs), CD4 helper (Th) cells, and regulatory (Treg) T cells [33]. $CD8^+$ cells eliminate the virus by producing a cascade of pathogen-killing molecules such as IFN-γ, granzymes, and perforins, while $CD4^+$ cells assist $CD8^+$ cells in clearing viral infection more effectively [39]. Treg cells suppress the activation of both $CD8^+$ and helper cells and maintain the cell count balance. A study

performed on 499 COVID-19 patients with MDS showed reduced $CD8^+$ and $CD4^+$ cell counts (70.56% of total MDS patients). About 90.5% of critical-stage patients showed an extreme reduction in their total T cell count, especially $CD8^+$ cells [40]. Another study on COVID-19 patients also showed a reduction in T cell counts and Treg cell upregulation due to a cytokine storm [33]. Treg cell activation with its surface marker forkhead box P3 (FoxP3) reduces T cell activation, and therefore, patients with COVID-19 cannot maintain long-term activation of T cells and enter a phase of "exhaustion". Exhausted T cells (T*ex*) express programmed death-1 (PD-1) and T cell immunoglobulin & mucindomain-3 (Tim-3); (CD366) markers and thus lose their effector, metabolic, memory, and self-renewal function. Targeting NKG2A receptors and Treg cell upregulation by IL-6 and IL-10 may help in preventing T cell exhaustion.

Thrombocytopenia

Thrombocytopenia occurs when the platelet count is less than 150,000/µl [41]. A decrease in platelet count is an alarming sign of severe complications of the innate immune system and in most cases results in death [42]. Thrombocytopenia is characterized by multiple organ dysfunction [43] caused by megakaryocytes present in the lungs. Morphological changes in the lung capillary bed due to mechanical ventilation or viral infection result in deranged thrombocyte defragmentation (Fig. 1c) [44]. Findings from one study indicated that IL-3, IL-6, IL-11, and dysregulated thrombopoietin (TPO) influence the production of megakaryocytes from hematopoietic cells [45]. Megakaryocyte inhibition occurs through the action of inhibitory cytokines such as interferon-α and transforming growth factor (TGF)-β [13]. Thrombocytopenia leads to hemorrhagic complications [42]. A meta-analysis of 1725 COVID-19 patients, 245 of which were critical patients, found a 57.7% reduction in platelet counts from the normal range in patients with SDS. This suggests that viral infection results in endothelial damage, which activates platelet production and aggregation, leading to lung thrombosis [34]. Therefore, in addition to cytokine storm and T cell exhaustion, thrombocytopenia can be another separate biomarker for COVID-19 infection.

ER-stress-mediated inflammation

The endoplasmic reticulum (ER) is a site where various essential protein modifications occur [46]. When the capacity of the ER to synthesize and modify proteins reaches a certain limit, it becomes overburdened and enters a stress phase. ER stress activates the unfolded protein response (UPR), which restores normal ER functioning by producing proinflammatory molecules (Fig. 1d) [47]. SARS-CoV stresses the ER in three ways: by forming double-membrane vesicles (DMV), by glycosylating its structural proteins, and by depleting the ER membrane of lipids by using them for budding and release of virions [48, 49]. Knoops et al. described the structure of SARS-CoV-affected ER using high-resolution electron tomography [50]. The study suggested that SARS-CoV utilizes the ER membrane for formation of DMVs. The SARS-CoV M protein (transmembrane protein) is glycosylated, and the glycosylation can be either O-linked or N-linked. The M protein, if not glycosylated, can induce production of interferon alpha (IFN-α) [51]. The three above-mentioned ER-stress-producing processes are required for SARS-CoV replication. Prolonged ER stress causes the UPR signaling pathway to trigger inflammation by the NF-κB pathway, leading to excessive cytokine production (Fig. 1d) [52]. UPR-mediated inflammation governs the pathogenesis of malignant, metabolic, and airway diseases [35]. SARS-CoV infection imposes stress on the ER and aggravates cancer pathogenesis in the inflammatory microenvironment. Inflammation leads to CSS, which activates tumor-promoting cytokines. Recent studies on the association of cancer aggressiveness with COVID-19 have shown that patients with an active tumor or undergoing anticancer treatment are at greater risk of death after infection with SARS-CoV-2 [36]. The specific reason for the high mortality rate in cancer patients is not yet known, but different hypothesis-based studies and reviews emphasize that ER stress could be one of the leading causes.

Immunotherapy and COVID-19

Currently, no effective and specific treatment targeting SARS-CoV-2 is available. Therapeutic strategies employed in the clinics include supportive medication (Fig. 2) [53], the majority of which do not improve the quality of life of patients. For instance, a combination of interferons along with ribavirin has shown limited efficacy against COVID-19. Glucocorticoids have also been found to be ineffective in numerous cases. Currently, clinical trials focused on the use of corticosteroids for the treatment of COVID-19 are being conducted [54], although limited data are available about their use in earlier pandemics caused by coronaviruses [55, 56]. Tocilizumab, an antagonist of interleukin-6, together with sarilumab, is being tested in clinical trials for the treatment of COVID-19 [57, 58]. Alternatively, CytoSorb, an adjunctive therapeutic molecule that absorbs a variety of cytokines, leading to a reduction in their concentration, has been shown to improve the immunopathological condition of patients [54]. A COVID-19 patient has been successfully treated with the immunomodulator thalidomide [57]. A combination of azithromycin and the antimalarial drug hydroxychloroquine has shown potential for the treatment of COVID-19 in a small non-randomized clinical trial [59]. However, limited data are available on the modulation of

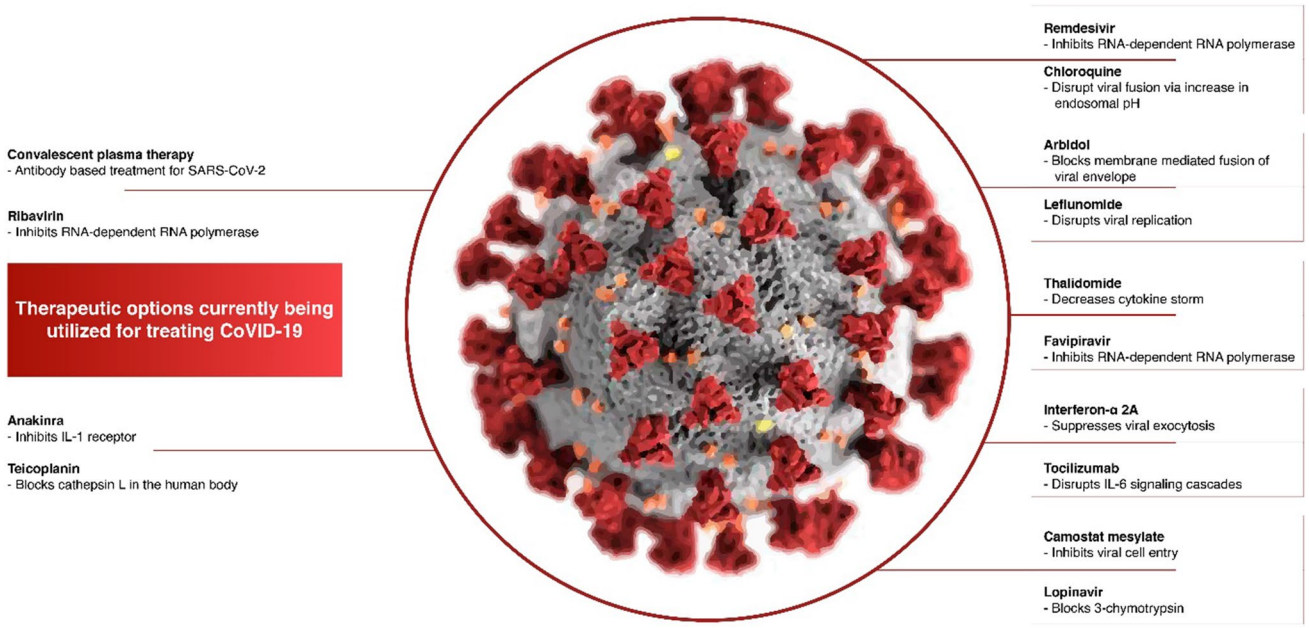

Fig. 2 Currently employed therapeutic strategies for treatment of COVID-19

the immune system via hydroxychloroquine for targeting COVID-19 [60].

SARS-CoV-2 modulates levels of both T cells and B cells, and this characteristic can be exploited to develop therapeutic strategies targeting the infectious agent. Clinical evaluation of vaccines against SARS-CoV in experimental animal models has indicated the presence of immunopathological modulation linked with Th2-cell-controlled eosinophil penetration [61, 62]. An experiment using a mouse model showed increased immunopathology instead of protection [63]. The development of vaccines based on their interaction with T cells requires a comparative evaluation of the molecular mechanisms underlying protective T cell activation and harmful T cell production [64].

A B cell response was observed in COVID-19 patients approximately 1 week after the onset of symptoms, and the nucleocapsid (N) protein is the first target of T cells [65], with the production of antibodies against S protein immediately after the onset of symptoms [66]. Antibody production can also be detected earlier in COVID-19 patients, but many patients do not develop persistent antibodies, and the possibility of reinfection in such patients remains unknown. The administration of antibodies appears to be an effective treatment for COVID-19 patients, as has been shown in cases of infection with both SARS-CoV-2 and SARS-CoV [67–71]. The molecular mechanism of protection against the virus has not been elucidated in humans. In SARS-CoV infection, neutralizing antibodies target the S protein, which binds to the ACE2 receptor on human cells [72]. Monoclonal antibodies targeting SARS-CoV can also bind to SARS-CoV-2 but with less affinity due to differences in target regions of the antibodies [73, 74]. Furthermore, cross-neutralization of SARS-CoV-2 by a mouse anti-SARS-CoV antiserum has been described [75].

Convalescent plasma has been used as a source of polyclonal antibodies against SARS-CoV-2 for the treatment of COVID19 patients [76]. Therapeutic strategies for developing monoclonal antibodies to neutralize SARS-CoV-2 have included phage library display and immunization of experimental mice [77, 78]. The absence of antibody-escape mechanisms, i. e., glycan coating of the receptor-attachment site suggests that if SARS-CoV-2 behaves in a manner similar to SARS-CoV, development of monoclonal antibodies for the treatment of infection will be successful [54, 79]. Currently, effective commercial monoclonal antibodies for the treatment of SARS-CoV-2 infection are not available, despite significant advancements in the development of therapeutic monoclonal antibodies for the treatment of COVID-19 using passive immunization approaches. Although the commercial-scale production of monoclonal antibodies for targeting SARS-CoV-2 is not cost- and time-efficient, designing an optimized and tailored antibody production platform can play a vital role in this regard. Further studies on structural and immunopathological data on coronaviruses can help in the treatment of SARS-CoV-2 using immunotherapy approaches.

Conclusion

COVID-19 has caused massive destruction worldwide, and nations are still striving to get back to normal. Scientists and physicians are working hard to find effective treatments, although robust diagnosis is still a concern because of ambiguity in the detection of viral infection. This review was an effort to highlight important possible ways by which SARS-CoV-2 interferes with the immune system and immune-based drugs used in different countries. CSS, thrombocytopenia, and T cell exhaustion provide insight into how COVID-19 interferes with the immune system, and these aspects can also help in the discovery of biomarkers for diagnosis of COVID-19. Recent studies have reported CSS to be a major factor in this disease, so targeting different aspects of CSS might help in designing drugs that mainly focus on the immune system. Many immune-system-based drugs have already been designed and a few of them are in clinical trials. These drugs have shown promising results, and immunotherapy could thus prove to be the most important future treatment of COVID-19. Thrombocytopenia is a very reliable indicator of viral infection. Also, we can look forward to integrating ER stress as a biomarker with assay-based techniques to measure ER stress and its role in disease pathogenesis.

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