Review

COVID-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome?

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Competing interests: none declared.

ABSTRACT

A severe outbreak of coronavirus disease 2019 (COVID-19) emerged in China in December 2019, and spread so rapidly that more than 200,000 cases have so far been reported worldwide; on January 30, 2020, the WHO declared it the sixth public health emergency of international concern. The two previously reported coronavirus epidemics (severe acute respiratory syndrome [SARS] and Middle East respiratory syndrome [MERS]) share similar pathogenetic, epidemiological and clinical features to COVID-19. As little is currently known about SARS-CoV-2, it is likely that lessons learned from these major epidemics can be applied to the new pandemic, including the use of novel immunosuppressive drugs.

Introduction

Coronavirus disease 2019 (COVID-19) is a clinical syndrome caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in China in December 2019 and then spread rapidly worldwide. It was declared a pandemic by the World Health Organisation (WHO) on March 11, 2020.

SARS-CoV-2 is a beta-coronavirus like the two other viruses that have caused fatal infections over the last 20 years: the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV). It is an enveloped, positive-sense, single stranded RNA virus with a nucleocapsid, and full-genome sequencing has shown that it is closely related to SARS-CoV, with which it shares about 79% of its genome (1, 2). In particular, molecular modelling has shown similarities between the receptor-binding domains of SARS-CoV and SARS-CoV-2 (also called spike proteins), which are the most immunogenic part of the virus and probably bind the same angiotensin converting enzyme 2 (ACE2) receptors in order to gain cell entry (1-3), thus suggesting that a similar pathogenic mechanism is involved in both viral infections (Fig. 1). Interestingly, ACE2 receptors are not only expressed on alveolar epithelial type II cells (4), which represent 83% of all ACE2-expressing cells, but also on heart, kidney, endothelium, and gut cells (5).

Up to March 17, 2020 there were more than 194,000 confirmed cases of COVID-19 worldwide and more than 7,800 infection-related deaths (source: WHO). Although SARS-CoV-2 is less lethal than MERS-CoV insofar as most patients remain asymptomatic or develop mild symptoms, up to 10-20% (especially older people and those with underlying medical co-morbidities) develop a severe disease characterised by interstitial pneumonia and the rapid development of acute respiratory distress syndrome (ARDS) or septic shock with high levels of acute-phase reactants and features of the macrophage activation syndrome (MAS) such as hyperferritinaemia, hepatic dysfunction and diffuse intravascular coagulation (6). Understanding the underlying mechanism(s) leading from mild to severe disease as a result of immune dysfunction and cytokine dysregulation is a key requirement for identifying an effective treatment for critical patients.

Cytokine dysregulation
Cytokine dysregulation in other viral respiratory diseases

In order to investigate the crucial role of the immune system during the course of COVID-19, it may be use-
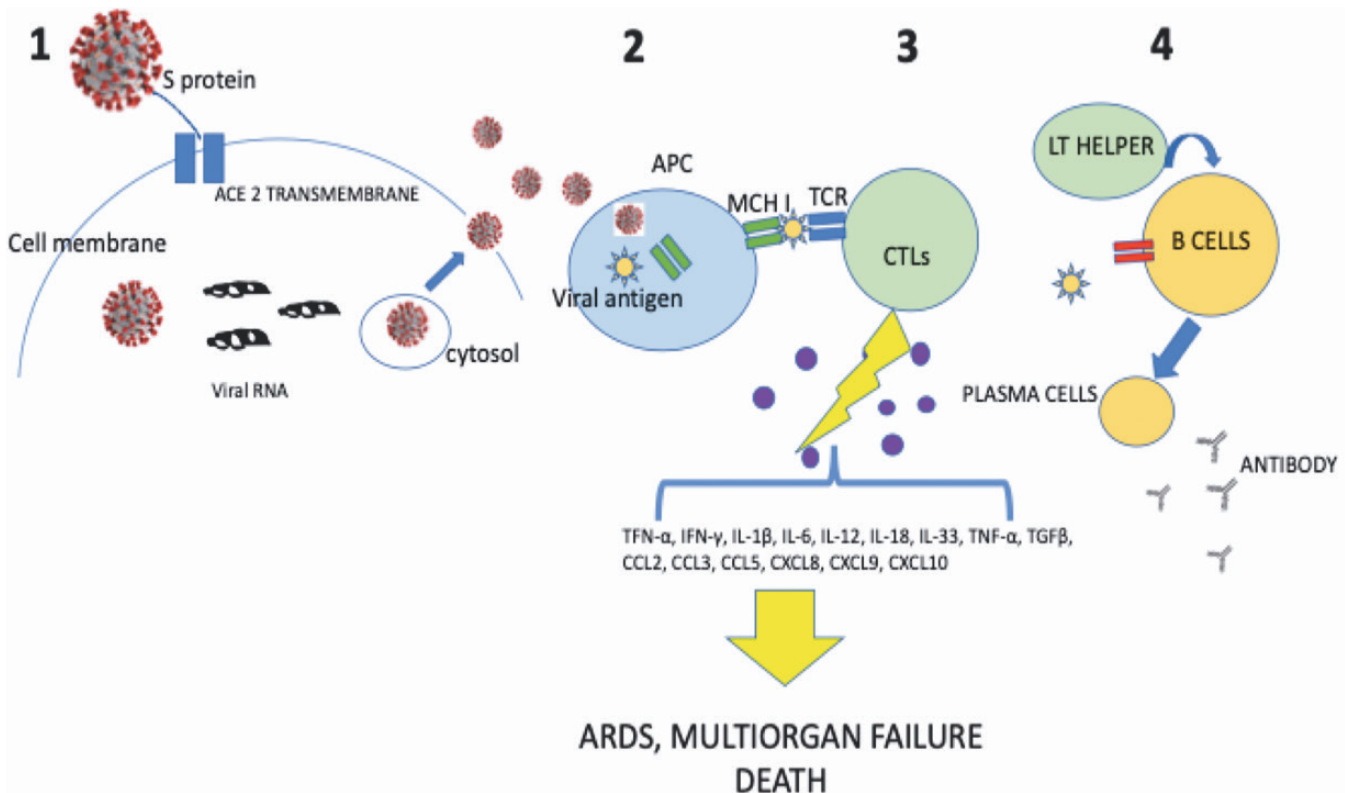
Fig. 1. Pathogenesis of COVID-19.
1. SARS-COV2 entry, replication and release. The virus binds to its ACE2 cell receptor by means of its spike glycoprotein (S protein) and then enters the cell cytoplasm where it releases its RNA genome, begins to replicate, and forms and releases new viral particles.
2. Antigen presentation. The viral antigen is presented to antigen-presenting cells (APCs) that present the antigenic peptides by means of the major histocompatibility complex (MHC). Antigen presentation stimulates both (3) cellular and (4) humoral immunity.
3. Immune effector cells release large amounts of cytokines and chemokines (a cytokine storm) that may rapidly provoke acute respiratory distress syndrome (ARDS), single or multiple organ failure, and eventually death.

ful to compare it with similar viral illnesses. SARS and MERS are acute respiratory diseases caused by similar coronaviruses and associated with high morbidity and mortality rates.

The three diseases are not only similar in pathological terms (7), but also strikingly similar in terms of their clinical presentation and epidemiology. This is especially true for SARS, the outbreak of which dates back to the beginning of this century and, like COVID-19, started in China. It caused a respiratory disease that was more severe in men, less severe in children, and led to a mortality rate of nearly 10%: up to one-third of the patients required ventilatory support and even intensive care (8).

The early hypothesis that SARS was due to cytokine dysregulation (9) was subsequently confirmed by various findings. First of all, it induced abnormally low levels of antiviral cytokines, particularly type I interferons (IFNs), which form part of the very early im-

mune response to viral infections as they are secreted upon stimulation by pathogen-derived nucleic acids (10). Both *in vitro* and *in vivo* studies showed the substantially low secretion of type I IFNs (11-13), which that may suppress Th1 and favour Th2 responses, whereas the results of studies of IFN-gamma were conflicting (13, 14).

Subjects with SARS have high levels of pro-inflammatory cytokines and chemokines that are associated with T cell depletion, pulmonary inflammation, and extensive lung damage (15). Chemokines, such as IP-10 and MCP-1, are highly expressed during the course of the disease (8, 15), and may play a key role in the development of lung disease by leading to the accumulation of immune cells in the lungs (13). They have also been putatively implicated in the development of immune-driven diseases (16). Increased concentrations of interleukin (IL)-6 are associated with severe disease (14), and it is worth not-

ing that higher levels of IL-6 correlating with symptomatology are also found in patients with respiratory syncytial virus (RSV) infection, although the level of suppressor of cytokine signalling 3 (SOCS3), which regulates the negative feedback mechanism of IL-6, is much higher than in patients with SARS-CoV infection and suggests intensively enhanced IL-6 activation (13). Plasma TNF-α seems to be moderately up-regulated in SARS patients (12, 14, 17), although *in vitro* studies (18) suggest a mechanism of TNF-alpha induction mediated by the shedding of ACE2, the portal allowing the cell entry of SARS-CoV. This may indicate differences in cytokine/chemokine up-regulation in lung and plasma.

Lymphopenia is notable during the course of SARS, and its causes are a subject of debate. It may be due to the virus directly, or to the white blood cell redistribution *via* chemotaxis or apoptosis (17, 19) as the massive infiltra-

tion of CD8^{+} T cells in the pulmonary interstitium plays a vital role in clearing SARS-CoV by inducing immune injury (20). It is important to remember that these T cells may be dysfunctional: an *in vitro* study of innate antiviral immunity within the lungs has shown that the epithelial cytokines produced upon SARS-CoV infection (particularly IL-6 and IL-8) can impair the ability of T cells to prime dendritic cells, and compromise the ability of macrophages and dendritic cells to clear the invading pathogen, thus leading to a failure to promote an effective adaptive immune response (17). It is worth noting that the people who died during the 1997 H5N1 influenza outbreak showed lymphoid depletion associated with a high titre of circulating cytokines, including IL-6 (21). MERS involves a similar mechanism of cytokine up-regulation and antiviral cytokine down-regulation (22), and the maintenance of type I IFN responses is a key element in viral clearance (23).

All these data suggest that the widespread lung damage associated with SARS may be caused more by an exaggerated immune response than the virus itself. However, at the time of the SARS outbreak, it was still the pre-biological agent era and there were very few immunosuppressive drugs capable of specifically targeting the up-regulated parts of the immune response. Corticosteroids were tried but were not really beneficial in treating SARS (24) or MERS (25) and, although IFN preparations were used to prevent SARS, their effectiveness was not confirmed by properly conducted trials. The success of the fight against SARS was therefore mainly due to efficacious epidemiological control measures.

Cytokine dysregulation in COVID-19
Cytokine dysregulation is of particular interest in patients with COVID-19, who have higher levels of inflammatory cytokines; however, what is more interesting is that, as was observed during the SARS outbreak, some of the cytokines seem to be up-regulated, especially in patients with more severe disease. Huang *et al.* found that IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A and TNF-α levels correlated with disease severity (*i.e.* higher levels in intensive care unit (ICU) patients) (6), and Diao *et al.* found that disease severity correlated with TNF-α, IL-6 and IL-10 levels (26), thus documenting TNF-α hyperproduction in the serum of COVID-19 patients (something that was not observed in patients with SARS). Interestingly, another study (27) found above-normal IL-6 levels in only one-third of its patients with mild COVID-19 but in 76% of those with severe disease. As mentioned above in relation to SARS, IL-6 can suppress normal T cell activation, which may explain the presence of lymphopenia (28), and Diao *et al.* not only found that their ICU patients had lower CD4^{+} and CD8^{+} T cell counts (in particular, *all* of the ICU patients had low CD8^{+} counts), but also that TNF-α and IL-6 concentrations negatively correlated with total T cell, CD4^{+} and CD8^{+} counts (26). Highly cytotoxic CD8^{+} T cells may explain immune-mediated tissue damage (7), and functionally exhausted T cells highly expressing inhibitory factors such as PD-1 (26) may reflect the immune dysfunction observed in COVID-19 patients as the immune response is abnormally skewed towards immunosuppressive Th-2 (6).

Given its weight during the course of COVID-19, it is clear that immune dysregulation is a highly important therapeutic target, but what is not yet clear is the cause of such a large-scale release of inflammatory cytokines. This requires further research in order to be able tackle the problem at its roots. One hypothesis suggests that a crucial role is played by cell pyroptosis, a pro-inflammatory form of cell apoptosis (*i.e.* the rapid viral replication causing cell apoptosis may lead to the massive release of inflammatory mediators) (29), and others have underlined the importance of antibodies against spike protein (anti-S-IgG) as promoters of pro-inflammatory monocyte/macrophage accumulation in the lungs (30). It is also necessary to take into account gender differences in the severity of SARS and COVID-19 as significantly more ICU patients are males (31, 32). Females show an enhanced immune response that increases their susceptibility to the development of autoimmune diseases (33) but, as this is not in line with the development of a cytokine storm in the case of COVID-19 infection, other factors may play a role.

Anti-rheumatic therapies in the potential treatment of COVID-19
The main challenge is to identify an effective treatment against SARS-CoV-2 and, although no specific drug or vaccine has yet been registered, some molecules have been proposed on the basis of their pharmacological properties. These include a number of drugs normally used to treat rheumatological diseases (34), although their possible adverse effects always need to be borne in mind (35).

Chloroquine
Chloroquine is an old drug that is used to treat and prevent malaria, and as an immunomodulator in rheumatological clinical practice. Some studies have shown that it has broad-spectrum antiviral activity, particularly against SARS and avian influenza A H5N1 (36, 37), and it has recently been included in the Chinese and Italian guidelines for the treatment of COVID-19 (38, 39). Chloroquine acts by increasing the endosomal pH necessary for viral/host cell fusion and, as demonstrated by studies of SARS-CoV, it can also interfere with the glycosylation of ACE2 receptors, which may inhibit viral entry into target cells (40). Multicentre clinical trials conducted in China have shown that chloroquine treatment leads to clinical improvement, improves patient outcomes, and reduces hospitalisation without increasing adverse events (41), and it is now being evaluated in an open-label trial (ChiCTR2000029606).

Baricitinib
Artificial intelligence predicts that janus kinase (JAK) inhibitors (particularly baricitinib) may block viral entry into pneumocytes because they target members of the numb-associated kinase (NAK) family, including adaptor-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK), which are both involved in viral endo-

cytosis. Baricitinib is currently used to treat rheumatoid arthritis as it can also limit systemic inflammatory responses and cytokine production by inhibiting the JAK-STAT pathway (42). It may therefore be useful in the treatment and prevention of the cytokine dysregulation associated with COVID-19 (43, 44) as it could affect the host inflammatory response and viral entry into cells. However, curiously enough, tofacitinib (another JAK inhibitor) shows no measurable inhibition of AAK1 (44).

Tocilizumab
As mentioned above, coronaviruses such as SARS-CoV and MERS-CoV can induce the uncontrolled cytokine and chemokine response known as a "cytokine storm" (45), and SARS-CoV-2 seems to induce the same mechanism. The cytokine storm leads to the over-activation of effector T cells and the bulk production of pro-inflammatory cytokines, which in turn lead to plasma leakage, vascular permeability, and disseminated intravascular coagulation. The levels of these cytokines negatively correlate with absolute lymphocyte counts, thus inducing T cell exhaustion and apoptosis and causing acute lung injury (ALI), and ARDS (46). A similar phenomenon is observed in the case of the cytokine release syndrome (CRS) associated with chimeric antigen receptor T cell (CAR-T) therapy (47). One of the key mediators of autoimmunity, inflammation (48), viral cytokine storms, and CRS-induced damage is IL-6 (49). Patients with SARS-CoV-2 infection have high plasma IL-6 levels, especially those with a more severe presentation and high C-reactive protein (CRP) levels (50). Some studies have shown that the use of tocilizumab, a humanised monoclonal antibody against IL-6 receptors, can reverse the cytokine storm (47), and this has led to it being used to treat COVID-19. A recent Chinese retrospective study of 21 patients with critically severe COVID-19 found that tocilizumab seemed to improve hypoxemia, fever, CRP levels, and CT scan abnormalities in most of the patients without leading to any significant adverse reactions (51). It is also interesting to note that an experimen-

tal model of mice pulmonary fibrosis shows an increase in IL-6 levels (52), which suggests that the pharmacologic inhibition of IL-6 may attenuate pulmonary inflammation and fibrosis (53). The off-label use of tocilizumab in the interstitial lung disease (ILD) caused by rheumatological diseases has been widely studied and is approved in many countries (54-58). A multicentre, randomised controlled trial of tocilizumab in patients with COVID-19 pneumonia and high IL-6 levels has been approved in China (ChiCTR2000029765), and the Chinese and Italian guidelines have recently included tocilizumab in their recommendations for the treatment of COVID-19 (38, 39). According to the Italian guidelines (39), tocilizumab can only be used at the end of the initial high viral load phase of COVID-19 (*e.g.* in patients who have been apyretic for more than 72 hours or seven days after the onset of symptoms) in patients with interstitial pneumonia and severe respiratory insufficiency, rapidly worsening respiratory exchanges, and high levels of IL-6 or D-dimer/CRP/ferritin/ fibrinogen.

Anakinra
Another cytokine that plays a central role in cytokine storm is IL-1, and some studies have found that SARS-CoV-2 causes pyroptosis with the release of IL-1β (59). Anakinra is a recombinant human IL-1 receptor antagonist, and a re-analysis of data from a phase III randomised trial of anakinra in severe sepsis indicated a significant improvement in survival of septic patients with features of macrophage activation syndrome (MAS) in the absence of any severe adverse reactions (60).

Anti-TNF-α agents
TNF-α is one of the major mediators of acute and chronic systemic inflammatory responses, promotes the production of other cytokines and chemokines, and seems to play an important role in an animal model of endotoxin-induced septic shock (61). It also plays a central role in autoimmune diseases, and anti-TNF-α drugs are widely used in rheumatological clinical practice. TNF-α levels are only moderately high

in patients with SARS, but higher levels have been observed in patients with COVID-19 and these correlate with disease severity (62). It has been suggested that anti-TNF-α treatment of COVID-19 may be a potential option (63), and a randomised, controlled trial of adalimumab has been registered (ChiCTR2000030089).

Corticosteroids
Systemic corticosteroids have been widely used in managing patients with severe viral ARDS even though their use in the treatment of severe ARDS is controversial, and they should not be routinely used in patients with COVD-19 (39). Studies have been demonstrated that early use of hydrocortisone as those during the outbreak of SARS-CoV infection was associated with a higher plasma SARS-CoV viral load with delayed viral clearance (64). Yet treatment with systemic corticosteroid in patients with severe influenza A(H1N1) was associated with an increase in mortality. They can suppress the exuberant systemic inflammatory response associated with ARDS (66), but there is no evidence that they lead to a clinical improvement in patients with viral interstitial pneumonia, particularly SARS, MERS and H1N1 (25, 64, 65-68), and they may even exacerbate lung injury (69). All these data support the hypothesis that a systemic corticosteroid use would likely exceed any benefit for treatment of viral infections (64). On the other hand, corticosteroids may modulate cytokine release so can suppress the exuberant systemic inflammatory response that occurs in ARDS (66). Data from multicentre randomised clinical trials emphasise the possibility that corticosteroids could reduce treatment failure rate, the number of hospitalising days and time to clinical stability (65). Methylprednisolone can be used in patients with late-stage ARDS and rapid disease progression as it seems to improve symptoms and lung lesions, but it does not increase overall survival (69, 70).

Conclusions
COVID-19 is a viral-induced illness whose outcome seems to be determined by the extent of the host immune sys-

tem imbalance. The primary immune response is a positive response that leads to viral clearance in the majority of cases. However, for reasons that are still unclear, the secondary immune response may be exaggerated and challenge tissue integrity, in some cases leading to multiple organ failure, ARDS and death (6). This exaggerated response is known as a cytokine storm for which there is still no specific or effective treatment. The most recently approved anti-rheumatic drugs may prove to be strong allies in the fight against COVID-19 as they can precisely target the key steps of the immune response that became dysregulated during the course of the disease (71). This could not be done at the time of the SARS epidemic, but these new targeted agents may become essential elements of the new concept of precision medicine: for example, all patients with severe COVID-19 could be screened for excessive inflammation by measuring cytokine levels in order to identify the patients who are eligible for a specific immunosuppressive treatment. However, for now, the only effective way of containing this viral outbreak and avoiding hundreds of needless deaths is to implement all of the measures aimed at reducing transmission, including inhibiting people's movement and social activities (72).

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