IN FOCUS

Harnessing CAR T-cell Insights to Develop Treatments for Hyperinflammatory Responses in Patients with COVID-19

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Summary: Cytokine release and macrophage activation contribute to immunopathology after SARS-CoV-2 infection. We discuss approaches to decrease the morbidity and mortality in patients with COVID-19 by repurposing existing drugs previously developed for cancer therapy.

The outbreak of SARS-CoV-2 infections in December 2019 has now become a global pandemic. As of April 2020, the virus has infected almost 3 million people worldwide and continues to spread. The numbers of cases and fatalities are increasing rapidly globally. This has overburdened emergency departments and intensive care units and demands effective prophylactic and therapeutic treatments. Vaccines are important to establish herd immunity in the population, but safety and efficacy testing for them will likely require at least a year. In the meantime, there is an urgent need for effective treatment options for those who have been exposed to this life-threatening disease. Here we address measures that have been used to treat other diseases and can be repurposed to lower the morbidities and mortalities caused by COVID-19. For a review of direct antiviral measures, see ref. 1.

SARS-CoV-2 belongs to the family of betacoronaviruses and is the third of its kind to infect humans. The COVID-19 virus uses the angiotensin converting enzyme-related carboxypeptidase (ACE2) receptor to gain entry to cells, which is widely expressed in cardiopulmonary tissues on alveolar type II pneumocytes, and also in selected hematopoietic cells, particularly monocytes and macrophages. ACE2 expression is required for the immunopathology culminating in acute respiratory distress syndrome (ARDS) consequent to SARS-CoV-2 infection (2).

CYTOKINE RELEASE CONTRIBUTES TO THE MORBIDITY OF SARS-COV-2 INFECTION

Understanding the pathology of COVID-19 and the lethal immunopathologic events is central to designing effective treatment strategies. Patients with COVID-19 often exhibit high fever and elevation of proinflammatory cytokines and proteins, a disorder best termed "cytokine release syndrome" (CRS). We prefer the term CRS to "cytokine storm" for COVID-19 because the kinetics of hypercytokinemia in patients with COVID-19 are more gradual than the fulminant release observed after CAR T-cell therapy (3). Elevations of cytokines and chemokines in the blood were previously reported in patients with SARS and MERS infections (4). In a recent meta-analysis of more than 1,700 patients with COVID-19 from 10 studies, IL6 levels were consistently elevated in most patients at hospitalization, and the levels were about 3-fold higher in those requiring ICU care (5). High levels of IL6 signal transduction are central to the immunopathology of CRS that follows CAR T-cell therapies. It has been shown that treatment with antibodies to IL6R or IL6 antagonists can be extremely effective at preventing life-threatening complications. Tocilizumab, a mAb targeting IL6R, is used therapeutically for rheumatic conditions and is also colabeled by the FDA for treatment with CAR T-cell therapy (6). According to clinicaltrials.gov, there are currently at least 16 clinical trials ongoing worldwide to determine the efficacy of blocking IL6R in patients with COVID-19 exhibiting CRS.

HEMATOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AND MACROPHAGE ACTIVATION SYNDROME

Hematophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome characterized by CRS, lymphopenia, and multiorgan failure (7). Systemic elevations of cytokines, C-reactive protein (CRP), and ferritin accompanied by lymphopenia are frequent in patients with COVID-19 and are hallmarks of patients with HLH (Fig. 1). The release of CRP from the liver is primarily driven in response to systemic IL6 secretion. It is thought that activated macrophages are the source of cells releasing the cytokines and that they are the central mediators of the immunopathology in HLH. Inflammation in the liver drives the release of CRP, and in patients with COVID-19, CRP levels positively correlate with the size of the lung lesions detected using CT scans and can predict the severity of the disease (8). Consistent with HLH, accumulations of macrophages are found in the lungs of patients with COVID-19 (9), and HLH has previously been reported in patients with SARS, MERS, and other severe systemic viral infections. HLH/macrophage activation syndrome (MAS) is also seen in systemic autoimmune diseases and graft versus host disease due to allogeneic hematopoietic stem cell transplantation.

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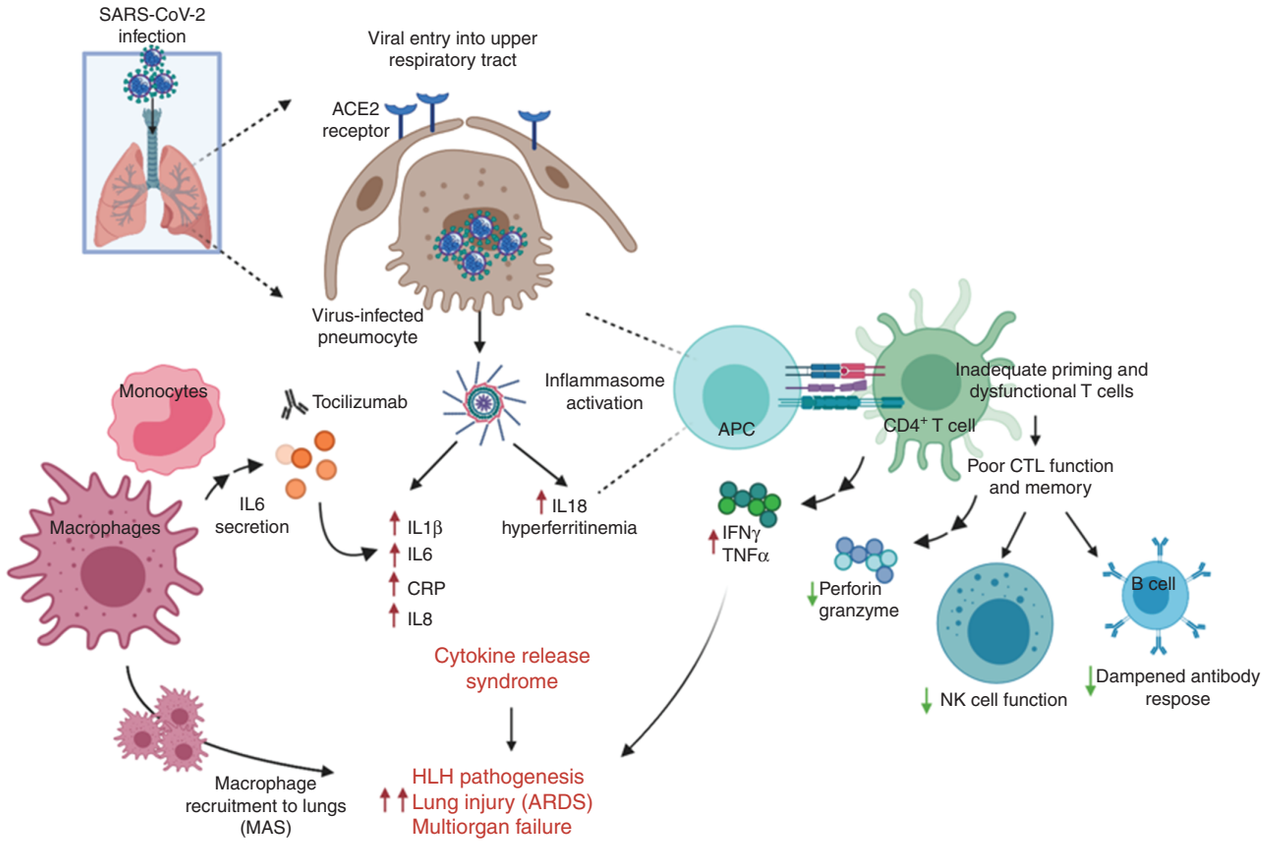

Figure 1. SARS-CoV-2 infection disables cross-talk between immune cells, causing CRS and HLH. The virus entry begins by infecting pneumocytes expressing the ACE2 receptor that recruits antigen-presenting cells (dendritic cells and macrophages) to the lungs. This activates the NLRC4 inflammasome that leads to overproduction of both IL1β and IL18, leading to IL6 and ferritin secretion by macrophages. Liver damage leads to upregulation of CRP and subsequently IL8 secretion. In addition, inadequate processing and presentation of viral proteins leads to formation of dysfunctional T-cell responses, that is, limited production of perforin and granzyme B but constant production of IFNγ and TNFα, which furthers disease progression. Upregulation of all these cytokines leads to a disorder called "cytokine release syndrome" and the recruitment of macrophages to the lungs, contributing to ARDS. CTL, cytotoxic T lymphocyte; NK cell, natural killer cell.

HLH can be secondary to systemic infections and other inflammatory conditions, as mentioned above, or familial HLH can occur due to various homozygous autosomal recessive mutations and polymorphisms. It is also possible that heterozygous genetic predispositions will be uncovered in patients with severe COVID-19. For example, whole-exome sequencing uncovered mutations in genes associated with HLH in patients with fatal H1N1 influenza infections (10). The heterozygous prevalence of HLH-associated gene mutations suggests that a genetic predisposition or epigenetic alterations could contribute to the susceptibility of SARS-CoV-2–infected patients who develop HLH. Furthermore, it is likely that genetic polymorphisms will be identified that enhance or inhibit the susceptibility to infection by SARS-CoV-2, as was recently reported for IFN-inducible lymphocyte antigen 6 complex, locus E (*LY6E*; ref. 11).

PREVENTION AND THERAPEUTIC OPTIONS FOR HLH IN SARS-COV-2 INFECTION

Drug treatments used for HLH/MAS and ARDS may also be effective in treating patients with COVID-19. Examples of agents used to treat HLH/MAS and ARDS are shown in the Supplementary Table. Etoposide and other cytotoxic agents have been used with success, particularly in familial forms of HLH. However, given that most patients with severe COVID-19 infections have other underlying illnesses, it is not likely that this approach will be tolerable without unacceptable toxicity.

Corticosteroids are known to suppress lung inflammation and the need for ventilation in patients with pneumonia but at the same time can also inhibit immune responses and pathogen clearance. However, they have not been shown to work in respiratory infections due to respiratory syncytial virus, influenza, SARS-CoV, or MERS-CoV. Evidence suggests that they should not be used to treat lung injury in COVID-19 either. Studies in patients with SARS-CoV treated with steroids showed higher levels of viremia, and patients with influenza showed higher mortality, longer hospitalization, and increased risk of secondary infections. Thus, their use should be considered only as a last treatment option, in low and regulated doses under clinical trials (12).

In mice, bacterial superantigens can cause CD28-dependent CRS, and in humans CD28 agonists can cause CRS (13). CD28 antagonists have been used to treat HLH/MAS in

patients with arthritis, and it is possible they may have a role in COVID-19.

The JAK–STAT signaling pathway forms a critical component of cytokine receptor systems, and many HLH-associated cytokines signal via this pathway. Thus, JAK inhibitors can lessen inflammation caused due to CRS. JAK inhibitors such as tofacitinib and baricitinib are safe and can establish plasma concentration of inhibition, but their efficacy in treating patients with COVID-19 remains to be determined (14). Studies have shown that in a mouse model of HLH infection with a chronic lymphocytic choriomeningitis virus (LCMV) strain, significant improvements in survival were seen after treatment with JAK inhibitors such as ruxolitinib (15). However, these inhibitors also inhibit the activity of inflammatory cytokines such as INFα, which are known to play an important role in viral clearance (14).

COVID-19 IN LOW- AND MIDDLE-INCOME COUNTRIES: CALCINEURIN INHIBITORS

On the basis of the previously mentioned study (5), a 3-fold reduction in IL6 levels might be sufficient to abrogate the need for ICU care in patients with SARS-CoV-2 infection. It is likely that cytokine antagonists to the IL6 pathway, particularly tocilizumab, will be shown to be effective at decreasing the mortality of patients with COVID-19 in the near future. Another proinflammatory cytokine working upstream of IL6 is IL1, which is also upregulated in CRS. Anakinra and other IL1R antagonists can be used to treat patients with arthritis and can be useful in CRS cases that stem from elevated IL1 levels. However, health care systems in low- and middle-income countries are already stretched thin and will be unable to afford these biologics. Thus, an urgent need emerges to uncover therapies that may be effective for patients with SARS-CoV-2 infection. For this purpose, it appears that host-directed therapies that have proved to be safe can be repurposed to treat COVID-19 infections.

One such class of immunosuppressant drugs is calcineurin inhibitors (CI), which are widely used in transplant rejection prophylaxis, arthritis, and psoriasis. Calcineurin inhibitors are a class of noncytotoxic immunosuppressants that selectively impair T-cell function by blocking NFAT signaling and downstream cytokine production. Cyclosporine (CSA), tacrolimus (FK506), and sirolimus (SRL-RAD) are different forms of CIs. CSA has a weak myelotoxicity profile and thus avoids the major side effect of neutropenia that occurs with most immunosuppressants and thus confers a risk of sepsis. In addition to immunosuppressant properties, CSA has also been shown to have direct antiviral effects by inhibiting replication of coronaviruses (16). However, LCMV-infected and FK506-treated mice have functionally impaired T cells, which leads to accumulation of macrophages in the liver and further orchestrates production of inflammatory cytokines such as TNFα and IL6 (17). Thus, if properly timed in patients after exposure to virus, CSA could serve as a broad-spectrum inhibitor to control SARS-CoV-2 infection and decrease the magnitude of cytokine release. Careful monitoring of dose and timing of immunosuppression are extremely important. This drug provides an ideal point of intervention, that is, in patients who come to the ER for admission but do not require ICU admission at a point when they have already primed the acquired immune system. This is in contrast to immunologically naïve hosts, because administration of CI immunosuppression prior to infection can be deleterious (18). Finally, in contrast to treatment of COVID-19 with biologics, CSA and tacrolimus are readily available off-the-shelf generic drugs that will be scalable in low- and middle-income countries.

IMPLICATIONS OF COVID-19 IN PATIENTS WITH CANCER AND IMMUNOTHERAPY

COVID-19 poses increased risk for patients with cancer, especially those who have recently undergone chemotherapy, radiotherapy, or immunotherapy treatment (19). Studies have shown that a significant proportion of patients with lung cancer treated with nivolumab benefit from tocilizumab in steroid-refractory immune-related adverse events. These patients also show significant increases in CRP, which decrease upon tocilizumab treatment (20). This shows not only the coincidence of treatments that modulate dysfunctional host immune responses, but also the potential complications with overlapping SARS-CoV-2 infections and cancer immunotherapies.

Patients responding to CD19 CAR T-cell therapy experience CRS, which is well managed by tocilizumab, but they also have B-cell aplasia and are regularly treated with IgG replacement therapy (3). As a result, they will be unable to mount any humoral response to viral infections. This puts CAR T-cell therapy survivors at an increased risk of complication with SARS-Cov-2 infection. There are ongoing trials to test the use of convalescent plasma as an effective treatment option for these patients. Given CRS is a common occurrence in patients with cancer following CAR T-cell treatment, ongoing clinical trials with CAR T-cell therapy are actively surveying patients for viremia and lung lesions before enrolling them on trials. This is important because comorbidities from CRS due to CAR T-cell therapy and HLH-like symptoms due to SARS-CoV 2 infection could be fatal.

CONCLUSION

As the global pandemic of COVID-19 continues, it is important that we have effective and concrete therapies that can save lives. Treatment of patients with CSA and other repurposed drugs has the potential to decrease CRS and inhibit viral replication. Such treatments have the potential to ameliorate the global burden on intensive care facilities and also potentially to reduce the R_0 to less than 1. Thus, there is an urgent need to advance experimental protocols to stop the spread of SARS-CoV-2 infections.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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