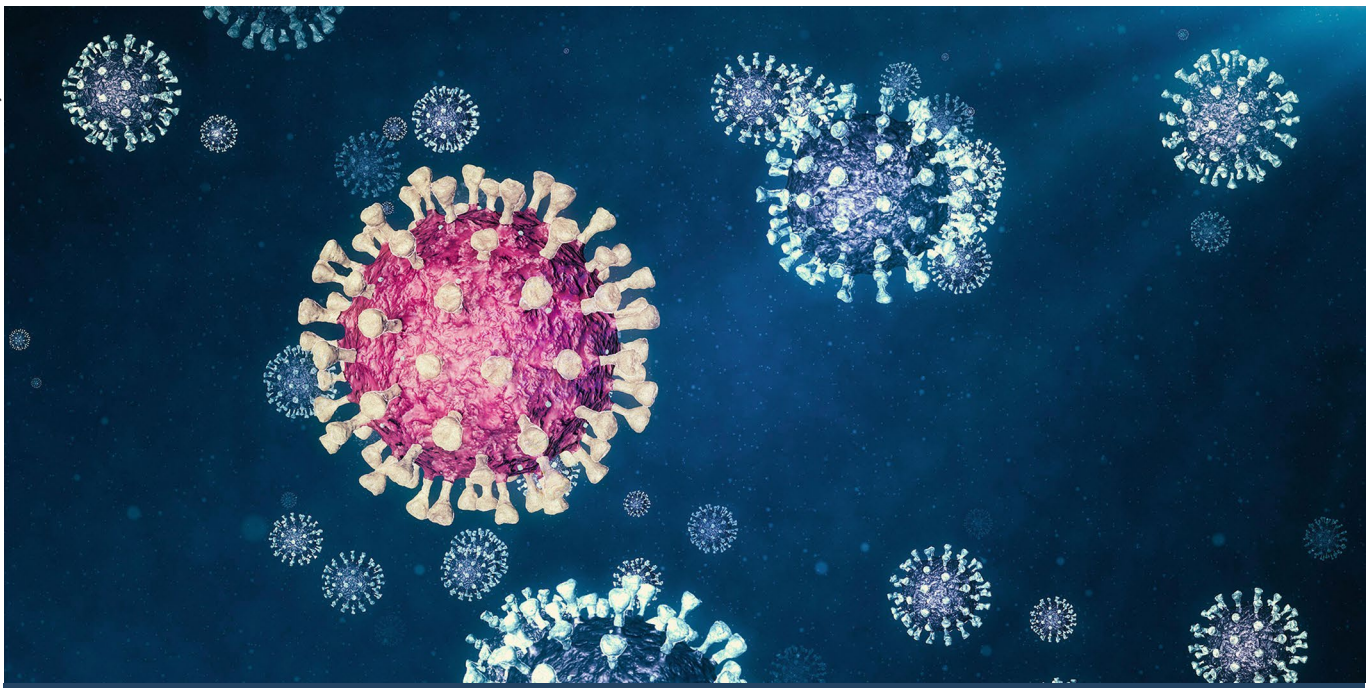

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# Immune status could determine efficacy of COVID-19 therapies

Emerging evidence suggests that SARS-CoV-2 can drive a diverse array of immune processes, raising the risk that immunosuppressant agents that are in clinical trials might be effective for some patients but detrimental for others.

*Megan Cully*

Nearly as soon as the SARS-CoV-2 virus was identified, clinicians started working with repurposed antivirals such as Gilead's remdesivir and the HIV drug combo lopinavir–ritonavir to establish whether these agents had activity against the coronavirus. As ICUs filled up with infected patients who had acute respiratory distress syndrome (ARDS), seemingly caused by runaway immune responses and the resulting cytokine storms, clinicians set about assessing whether approved anti-inflammatory agents could be used to keep immune responses in check and avoid collateral damage. Several months on, the COVID-19 pipeline — spanning more than 1,100 interventional trials on ClinicalTrials.gov, as of mid-June — remains dominated by these two strategies.

But a growing number of front-line intensivists are advocating a more nuanced approach to immune modulation in COVID-19. Dampening the immune system when patients are fighting off infections can be a dangerous approach, says Richard Hotchkiss, an intensivist at Washington University in St Louis. Instead, patients might need immune-boosting agents to help fend off infection. "I'm personally tired of reading about the cytokine storm," says Hotchkiss. "That's the predominant theory, but I believe that's not correct."

Oncologists Santosh Vardhana and Jedd Wolchok, both at the Memorial Sloan Kettering Cancer Center, recently outlined their case for immunotherapies for the treatment of COVID-19 in the *Journal of Experimental Medicine*, based on their experience with cancer patients who became infected with SARS-CoV-2. The immune system takes on different forms with COVID-19, they wrote. While many patients have hallmarks of inflammation early on in the infection, the virus can outlive the initial burst of immune activity. As T cells battle the infection they become metabolically exhausted and dysfunctional, and these exhausted cells need to be replaced with new ones. In COVID-19 patients, those reinforcements never arrive.

In Vardhana's view, the underlying pathology in patients who don't respond to immune-cooling therapies might not be persistent inflammation. "I would suggest that the problem is viral breakthrough," he says. Viral replication spirals out of control as T cell function declines.

If this is the case, the therapeutic goal for host-directed COVID-19 drugs should instead be to nudge patients towards a 'Goldilocks' level of immune activity. Not so hot as to cause organ failure, and not so cold that the virus can run amok. To get to this point, researchers need a better understanding of the pathophysiology of COVID-19, the immune responses it drives, and the ability to incorporate this into clinical trial design.

### Anti-inflammatories first

Corticosteroids — low-grade, orally available and generic anti-inflammatory drugs — are a first-choice weapon for physicians treating unwanted inflammation in everything from skin allergies to autoimmune disorders. During the SARS and MERS outbreaks in 2003 and 2012, respectively, severely ill patients were treated with corticosteroids, although with hindsight these may not have been beneficial. And physicians have been giving these drugs to patients with COVID-19 from the get-go.

But in one of the first reports on COVID-19, corticosteroid administration did not alter mortality, and instead reduced viral clearance. Although interim guidance from the WHO currently advises against corticosteroid use for SARS-CoV-2 infection, many hospitals still use these to treat the signs of hyperinflammation. Numerous ongoing clinical trials of this drug class should provide a more concrete answer on how effective they are.

While corticosteroids are a blunt tool, researchers are hopeful that more precise scalpels that inhibit specific pro-inflammatory pathways will be more effective. And IL-6 is a leading contender.

> You cannot cripple or seriously impair the host immune response to the virus and expect the patient to do well

In one of the earliest clinical reports on COVID-19 in 69 patients from China, high levels of IL-6 were associated with low oxygen saturation. In a German study of 40 patients, high IL-6 levels predicted the patients that would go on to respiratory failure. The damaging effects of IL-6 in COVID-19 likely arise from both its capacity to stimulate the immune system and its direct effects on other organs.

The IL-6 receptor (IL-6R)-blocking antibody tocilizumab provides a means of testing the hypothesis that directed anti-inflammatories will provide therapeutic benefit. And the agent is already approved not only for autoimmune disorders, but also for cytokine release syndrome (CRS), in which the immune system becomes hyperactivated in response to anti-cancer CAR-T cell therapies.

Some researchers see parallels between CRS and COVID-19 immune responses as a rationale for trials of IL-6 and IL-6R inhibitors in COVID-19, because both are hyperinflamed states with elevated levels of IL-6. In Vardhana's experience, COVID-19 looks vaguely like CRS, but "with the volume turned way down." For example, seizures and shock feature in CRS, but not in COVID-19.

Preliminary results from clinical trials of antibody therapies that target IL-6 or IL-6R have been mixed. In three small trials of tocilizumab in 20 or fewer patients — two in China and one France — treatment reduced mortality and/or ICU admission. Reassuringly, in a 154-patient trial in the USA, tocilizumab reduced mortality in mechanically ventilated patients. But Sanofi and Regeneron's IL-6-targeting sarilumab failed to show efficacy in a larger 270-patient trial. Multiple larger trials remain ongoing.

Many researchers are optimistic. "If tocilizumab and other interventional studies that target those cytokines are shown to be helpful, then that's a pretty strong indicator that [hyper-inflammation] is part of what's at play," says Bruce Walker, an infectious diseases specialist at the Ragon Institute in Boston.

Miriam Merad, an immunologist at the Icahn School of Medicine at Mount Sinai, is also bullish on the potential with IL-6 blockade. Despite concerns from others that IL-6-blockade could be problematic because it partially suppresses T cell activation, she points out that many other cytokines can functionally replace IL-6 activity without the harmful effects on other organs. "I wouldn't be too afraid of IL-6 blockade," she says. In CAR-T cell recipients who are treated with IL-6 blockers for CRS, for example, the transplanted T cells still function, she points out.

At the same time, other cytokines could also be causing widespread damage, she speculates, and inhibitors of these may also be therapeutically useful. Trials of other interleukin-blocking therapies — including those targeting IL-1, IL-8 or IL-33 — are ongoing.

Broader immune-suppressing strategies are also being investigated. More than a dozen trials of JAK inhibitors, which are used to treat rheumatoid arthritis and other autoimmune conditions, are registered or underway in COVID-19. JAKs are key signalling molecules that lie downstream of numerous cytokines, including IL-6.

However, JAK inhibitors make both Hotchkiss and Merad nervous. Not only might these broadly anti-inflammatory agents reduce the capacity of the host to clear SARS-CoV-2, they might also leave patients even more susceptible to opportunistic infections like bacterial pneumonia. In autopsy studies, up to 50% of COVID-19 patients who died of their disease had co-infections. The contribution of these secondary infections to mortality is not clear, but could be substantial.

Vardhana thinks that the way forward requires a more careful consideration of disease pathophysiology. There may be a window soon after hospitalization during which immunosuppressive therapies, including steroids and IL-6 blockers, are effective. "Anecdotally, if we give steroids to some of those very early patients who are ramping up, it pulls them back," says Vardhana. He calls these patients "early toxic progressors" — recently infected individuals with high cytokine levels who could benefit from immune-cooling drugs.

But, patients who have had the infection for a week or more — Vardhana's "late dwindlers" — likely have a different immunological profile, in which the major problem could be the lack of effective T cells. "In patients who have been in the hospital for more than 3 or 4 days … steroids don't do anything," he says. They can provide a temporary respite from the organ-attacking cytokines, but they also dampen the virus-attacking immune response, which Vardhana believes is the real culprit.

Hotchkiss agrees. "Maybe a subset of patients have this cytokine storm," he says, "however, it's clear that a number of patients progress to a more immune-suppressed state."

Hotchkiss is also concerned that some of the antibody-based therapies, such as the IL-6 or IL-6R-targeted ones, may outstay their usefulness in patients' blood. In sepsis, which is characterized by immune hyperactivation and ongoing infection, the early hyper-inflammatory response lasts just 24–48 hours, he explains. But some of these anti-inflammatory drugs have half-lives of 3–5 days. So these therapies might still be inhibiting the immune system as it is winding down. "You cannot cripple or seriously impair the host immune response to the virus and expect the patient to do well," he says.

### Bolstering the immune system

Interest in immune-boosting agents, consequently, is on the rise — with a focus on the time course of the disease.

The immune response changes during the course of many infections, and some of those changes depend on how long the infection lasts. Viruses such as HIV, HBV and HCV can take months to produce symptoms,

by which point the infections are considered chronic. Acute infections like influenza show symptoms within 2 days of exposure, and most people clear the virus within 7 days. COVID-19 shows up 5–7 days after exposure, and for some people, the disease can persist for weeks or even months.

"It's not really a truly chronic infection in the way that hepatitis B virus (HBV) or HIV are, but it's also not really an acute infection the way H1N1 was," says Vardhana. "It kind of falls in the middle, and I think that's a big part of the challenge here." The chronicity of the infection could explain why both immune-suppressing and immune-boosting approaches may be viable ways forward at different times.

Already, the uncertainty over whether to suppress or enhance the immune system has led to trials investigating diametrically opposing treatment strategies. Recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF, another pro-inflammatory cytokine) is approved for the reconstitution of myeloid cells after bone marrow transplantation, and blocking antibodies have been investigated for treating leukaemia. Clinical trials investigating recombinant GM-CSF or GM-CSF-blocking antibodies are both currently recruiting cohorts of 80–200 patients.

The sepsis community has seen this story before. After decades of clinical trial failures with anti-inflammatories to suppress cytokine storms and prevent organ damage, sepsis researchers have turned to immune-activating strategies. These to have yet to deliver conclusive evidence of their usefulness, but IL-7 and anti-PD1/PDL1 therapies have shown preliminary signs of efficacy in early clinical trials.

Hotchkiss argues that similar strategies could be effective in COVID-19. In particular, he has his eye on IL-7, which prevents T cell death, promotes T cell proliferation and reverses lymphopenia in patients with sepsis. T cells from COVID-19 patients proliferate in response to IL-7, he notes. A clinical trial of IL-7 in 48 patients in the UK is already underway, and another is registered in France. Vardhana agrees that using IL-7 "is a really good idea".

### Intervening with interferons

In other infections, particularly those that can become chronic, doctors often combine virus-targeted therapies with immune boosters. Immune-boosting interferon α (IFNα) plus the antiviral drug ribavirin was the mainstay of HCV therapy until 2014, for example, when more potent antivirals became available.

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Transcriptomic analyses suggest that something may be amiss in the interferon response in patients with COVID-19. In cells and animal models, SARS-CoV-2 induced abnormally low levels of transcripts downstream of type I and type III interferons, juxtaposed with high levels of cytokines such as IL-6, Benjamin tenOever, of the Icahn School of Medicine at Mount Sinai, and his colleagues recently reported. Patients with COVID-19 had the same pattern, they found. Given that interferons are the body's first-line defenders against infection, therapeutically boosting the early type I or type III interferon responses could help patients fight off infections, enabling them to clear the virus before hyperinflammation becomes a problem.

Clinical evidence also supports the role for interferons. In a 127-person trial, IFNβ, in combination with the repurposed antiviral drugs lopinavir–ritonavir and ribavirin, decreased the length of SARS-CoV-2 infection. IFNβ, a type I interferon, is the only immune-boosting strategy being investigated in the WHO's SOLIDARITY trial, again in combination with lopinavir–ritonavir.

Now, Merad and her collaborators are planning to test IFNλ, a type III interferon, in patients with mild or moderate disease. The IFNλ receptor is only expressed on epithelial cells and other non-immune cells, so this molecule shouldn't immediately lead to organ-damaging cytokine production. A similar trial of 140 patients is underway in Canada.

As seen in tenOever's transcriptomic analysis, declining immune function and hyperinflammation are not necessarily mutually exclusive processes, she adds. "You can imagine reducing the cytokines that we know are the most damaging — so here I'm thinking TNF, IL-6, IL-8 — while potentially boosting a T cell and B cell response," she says, "but it is not straightforward."

So the Goldilocks strategy might actually look more like a Scandinavian sauna routine. Boost the interferon response in patients with mild or early disease, cool the immune system in patients with high IL-6 levels, and heat it back up when the T cells become exhausted.

### Restoring exhausted T cells

A few different immune-boosting strategies are on the table to address T cell exhaustion.

T cells that cannot keep up with energy demands during prolonged battles reduce their function through a series of steps, first described in 1998 in mice infected with lymphocytic choriomeningitis virus (LCMV). Subsequent work has shown that humans with chronic HIV, HCV or HBV infections have exhausted T cells that, although they keep the infection to a smouldering level, are unable to extinguish it.

A key marker of T cell exhaustion is PD1, a receptor that is upregulated on these cells and reduces T cell function. Antibodies targeting PD1, its ligand (PDL1) or CTLA4, another inhibitory surface receptor, have revolutionized cancer therapy by reactivating the anticancer immune response.

But PD1-targeting antibodies first showed efficacy in the LCMV model. And the first demonstration that PD1 inhibition was relevant to human disease was from Walker's lab, in which PD1-blocking antibodies increased T cell numbers and function in HIV-infected people.

Trials investigating anti-PD1 therapies in COVID-19 are registered in Hong Kong and underway in France.

Researchers are divided on the wisdom of these trials.

"They're definitely worth trying," says Hotchkiss.

But Walker isn't so sure. "I just haven't seen enough data at this point to feel comfortable about that," he says. "PD1 blockade in cancer patients can cause death from activation of immune function," he highlights. "It's not a silver bullet: it takes the brakes off the whole immune system and that worries me."

"I almost wonder if CTLA4 blockade would be better in this setting," muses Vardhana. "The challenge with PD1 blockade is that it also depletes your memory and/or naive T cell pool by pushing your remaining cells towards an activated state," he says. He favours therapies that maintain or bolster activity in the existing pool of active cells; "I worry that without that, you're just going to further terminal T cell exhaustion if you're not clearing the antigen," he says.

Another strategy focuses on giving T cells an energy boost to get back into the fight.

Exhausted T cells are metabolically distinct from active ones. Chronic viral infections or tumours induce redox stress in T cells, which leads to cell death and lymphodepletion, explains Vardhana.

Box 1 | **Clinical trial challenges in the ICU**

Interpreting data from clinical trials for patients with severe COVID-19 isn't straightforward, partly because of the ICU environment in which these trials are conducted.

By the time patients with COVID-19 are admitted to the ICU, they have often already received multiple different treatments, in various orders and combinations, including off-label therapies. So to recruit enough patients into a study, the patient population is often not uniform. And co-morbidities skew survival outcomes, particularly in small trials. "I think that's basically why most therapeutics in critical care settings have failed," says Santosh Vardhana, at the Memorial Sloan Kettering Cancer Center.

Even determining the correct outcome measure is difficult. Acute respiratory distress syndrome (ARDS) caused by COVID-19 is a syndromic condition, with multifactorial underlying biological causes. "Personally, I would never use mortality as an outcome metric in ARDS because that is affected so significantly by the underlying cause, and it's not always necessarily dictated by whether you have ARDS or not," notes Vardhana. Therapies that improve lung function won't alter mortality if the underlying cause of death is COVID-19-associated kidney failure, for example. He favours extubation due to clinical improvement as an end point for ARDS.

End point selections also depend on what parameters can be measured. Quantitative PCR for SARS-CoV-2 isn't universally available, for example, so viral load can't be used as an outcome. And sample collection is challenging. Hospitals that have been overwhelmed during this pandemic have limited personal protective equipment, notes Bruce Walker, an infectious diseases specialist at the Ragon Institute in Boston, and so sample collection is done by overworked doctors and nurses rather than by research staff. Broncioalvaeolar lavage — a technique to collect samples from the lungs — produces aerosols, so many institutes prohibit this procedure in patients with COVID-19. And when samples can be collected, they might be infectious, requiring additional biocontainment measures. All this adds logistical complexity to trials in the ICU.

"It's quite a tribute to the medical community that they're able to actually conduct these trials when there's so much chaos," says Walker.

And various lines of evidence suggest that antioxidants like *N*-acetylcysteine can restore the balance. Vardhana is leading a phase II trial of *N*-acetylcysteine in 86 patients with COVID-19, and hopes this compound can restore T cell function.

All of these clinical trials will determine whether immune-boosting strategies are safe, or whether they push patients into a hyperinflamed state.

### Tracking immune status

If patients do need to be treated on the basis of their immune status, more work is needed to define what this would look like. Under the current WHO guidelines — which are often used to set up trial inclusion and exclusion criteria — patients transition from 'mild' to 'severe' disease when they need a ventilator. But this reflects the level of care they need, rather than the underlying biological process. So, what about other biomarkers that can be used to identify those patients who are most likely to respond to a given immunomodulating agent?

All the single-protein biomarkers that have been developed to stratify sepsis patients have been problematic, cautions Hotchkiss. He fears that the same might be true for COVID-19 biomarkers. "You really need a functional assay" to identify how many active T cells are present in patient blood samples, he adds.

Integrating multiple biomarkers into a signature could be another useful approach. A group led by Tiannan Guo at Westlake University analysed blood from 46 COVID-19 patients and 53 uninfected individuals using mass spectrometry. The resulting proteomic and metabolomic fingerprint, validated in other cohorts, includes 22 proteins and 7 metabolites that might be used to evaluate severity of disease.

Markus Ralser, a biochemist at Charité Universitätsmedizin Berlin, is working on a similar broad-based approach. He's been developing a mass spectrometry-based proteomic analysis tool for years, with speed, cost and precision in mind, for applications in clinical oncology settings. COVID-19 has shifted his focus. "The pandemic makes it obvious that we have to find ways to do things on a much faster timescale," Ralser says.

In an unbiased screening of blood from 48 patients with COVID-19, Ralser identified a set of 27 proteins, including transcripts downstream of IL-6, that could potentially be used to classify patients by disease severity. The biggest shift in the proteomes occurred not at ventilation, but when patients first needed oxygen. Subsequent studies could help to define the patient populations that will benefit from immune-suppressive or immune-activating therapies, he hopes.

For Walker, the big question is what's going on in the tissues, where the vast majority of the lymphocytes are located. Getting tissue samples is challenging, though (BOX 1).

To add yet another layer of complexity, a small subset of individuals are presenting with COVID-19-related complications. Case reports are emerging of children with a Kawasaki disease-like condition now known as paediatric inflammatory multisystem syndrome (PIMS), and of adults with Guillain-Barré syndrome accompanying SARS-CoV-2 infection. "We ought to be looking at all the parameters related to autoimmunity," notes Walker.

Understanding the immune response to COVID-19 — this not-quite-acute, not-quite-chronic infection — could be important for potential future pandemic viruses with similar profiles as well.

It could also be critical to the development of COVID-19 vaccines. There's been a relative paucity of data on T cell responses and little effort on generating T cell vaccines, says Walker. "If we end up with an antibody-based vaccine that's partially effective, then we need to know what the immune responses are that account for some people recovering and other people not," Walker says, as this will guide both therapies and vaccine development.

And despite huge advances in the understanding of COVID-19 since SARS-CoV-2 first emerged, the field is still in its infancy. Although the unique ways in which this virus interacts with the immune system are still shrouded in mystery, these could determine the best ways to coax patients' immune systems into the sweet spot.

"It's possible that we're going to revise everything we know in the weeks to come, and that's ok," says Merad, "as long as we remain open to the data."