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Cytokine storm and the prospects for immunotherapy with COVID-19

ABSTRACT

Knowledge about the pathobiology of SARS-CoV-2 as it interacts with immune defenses is limited. SARS-CoV-2 is spread by droplets that come into contact with mucous membranes. COVID-19 is characterized by 2 or 3 stages: most patients who recover experience 2 stages of illness commencing with an asymptomatic or paucisymptomatic incubation period, followed by a nonsevere symptomatic illness lasting for several weeks, occurring in about 80% of those infected. In the remainder, a third phase marked by a severe respiratory illness, often accompanied by multisystem dysfunction, coagulopathy, and shock is observed. This phase of the illness is characterized by hypercytokinemic inflammation and is often referred to as "cytokine storm." While the immunopathogenesis remains unclear, prospects of treating this severe phase of the illness with immunotherapy are evolving, with some treatments showing promise.

AS WE LEARN ABOUT COVID-19, we recognize that there are gaping holes in our knowledge of the pathobiology of SARS-CoV-2 as it interacts with our immune defenses. Epidemiologically, we know that most people, especially young and healthy ones, do quite well at defending themselves from this infection and that even those with severe disease tend to recover without sequelae. We also know that not everyone has a relatively benign disease course and that risk factors for progression are dominated by age and comorbidities, especially cardiovascular disease, diabetes, and obesity.

While some of these clinical findings seem to have face-validity, others are not so clear. Why is age such a dominant risk factor, but on the other hand, why do some young, otherwise seemingly healthy individuals succumb to the infection? We do not yet have complete answers to these questions.

■ THREE STAGES OF DISEASE

To tackle this problem, we must first examine what is known about the interaction between the pathogen and the host immune system. SARS-CoV-2 is spread by droplets that come into contact with mucous membranes. Interestingly, not all individuals who are exposed acquire the infection. Once a person is infected, the disease progresses through 2 or 3 main stages (Figure 1).

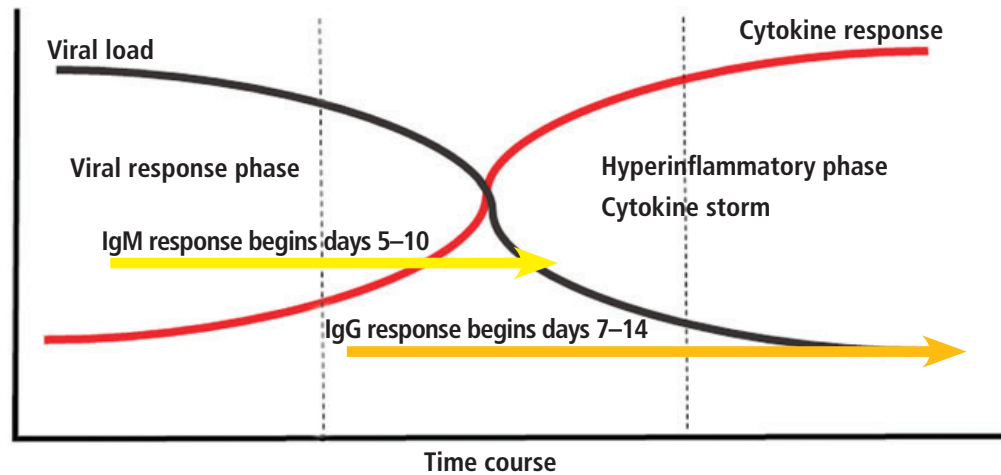
Stage 1 is an asymptomatic or paucisymptomatic incubation period in which there is a high level of viral shedding in the upper respiratory tract. Implicitly, this stage marks engagement of the innate immune system as the initial mode of host defense.

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CYTOKINE STORM

	Stage 1	Stage 2	Stage 3
	Asymptomatic	Nonsevere symptomatic	Severe respiratory-inflammatory
Immune response over time:	Innate immune activation	Adaptive immune activation	Cytokine release syndrome
Self-limiting in 80%	Viral engagement of PAMPs	Generation of specific antibodies and T-cell response	IL-1, IL-6, TNF, GM-CSF, IFN-gamma, others
Severe in 15%–20%	Low type 1 IFN	Release of DAMPs	Coagulopathy
Fatal in 1%–2%			Complement



DAMPs= damage-associated molecular patterns; GM-CSF = granulocyte macrophage colony-stimulating factor; IFN = interferon; IgM = immunoglobulin M; IL-1 = interleukin 1; IL-6 = interleukin 6; PAMPs = pathogen-associated molecular patterns; TNF = tumor necrosis factor

Figure 1. Three stages of COVID-19 disease.

Stage 2 is a period of nonsevere symptomatic illness in which viral loads peak approximately 5 days after symptom onset.¹ At this stage adaptive immunity is engaged, allowing development of specific T- and B-cell responses required to end the infectious process. The disease ends in stage 2 in approximately 80% of infected individuals.

Finally, **stage 3** is characterized by severe respiratory illness with progressive pneumonitis that may or may not lead to respiratory failure, which, in its final stages causes diffuse alveolar damage. Stage 3 is also frequently attended by progressive fever, multiorgan dysfunction, hypercoagulability, and shock.²

Immunologically, SARS-CoV-2 infects cells that express the angiotensin-converting enzyme 2 receptor, including cells of the respiratory tract, endothelial cells, and likely hematopoietic cells, including macrophages. With full engagement of the integrated immune response, the development of neutraliz-

ing antibodies is believed to be a critical event in recovery as well as the generation of virus-specific T-cell responses, ultimately leading to viral clearance.³

Attempts to correlate the stages of clinical disease described above with SARS-CoV-2 viral loads from respiratory secretions, blood, and tissues have yielded conflicting results. Some patients with advanced disease have high viral loads while others do not.⁴

■ WHY DO SOME PATIENTS GO ON TO STAGE 3?

Why host antiviral defenses fail and why some patients go on to stage 3 is not yet clear, but attempts to reconcile these findings suggest that this progression may be driven by ongoing viral infection. In support of this hypothesis is a recent study documenting that SARS-CoV-2 infection actually induces a low interferon response, an immune pathway critical for antiviral defense, while at the same time inducing

a strong inflammatory response, thus creating a perfect storm of continued viral replication and unbridled inflammation.^{5,6}

The clinical state of patients with stage 3 disease is characterized by hypercytokinemic inflammation. This syndrome has variably been referred to as “cytokine storm.” The cytokine storm of COVID-19 bears similarities to other conditions that are also referred to under this umbrella, including primary hemophagocytic lymphohistiocytosis (HLH), as well as secondary forms such as macrophage activation syndrome (MAS) and secondary HLH, which are often encountered in the setting of autoimmunity, cancer, or viral infections.^{7,8} In COVID-19, and unlike in MAS or secondary HLH, the primary target organ is the lung, leading to an acute respiratory distress syndrome. While stage 3 COVID-19 is not secondary HLH or MAS, it does share features both clinically and pathologically.⁵

Recently, a variant of this cytokine storm has been described in children with COVID-19 and has been dubbed *multisystem inflammatory syndrome in children* (MIS-C).⁹

Laboratory features are quite similar among these disorders, with marked elevations of acute-phase reactants (eg, C-reactive protein, ferritin), lymphopenia, coagulation defects, and elevated levels of numerous inflammatory cytokines; prominent among them are interleukin 6 (IL-6), IL-1, IL-2, IL-7, IL-17, granulocyte macrophage-colony stimulating factor (GM-CSF), and tumor necrosis factor (TNF).¹⁰

Why there is an increased incidence of this inflammatory late-stage complication in select young individuals and more frequently in patients who are elderly and in those with comorbidities is poorly understood. Interestingly, though, in a study attempting to further understand why otherwise-healthy individuals can die from viral illness, 30% of patients dying from H1N1 influenza were found to carry single copies of genes commonly encountered in patients with HLH,¹¹ suggesting a link between immune predisposition to HLH and outcome. We can also postulate that the chronic low-grade inflammation and an increase in self-reactivity that characterize the aging immune system also may contribute. Importantly, recent studies have shown that

TABLE 1

Immunotherapeutic strategies for COVID-19

Kinase inhibitors

Tofacitinib, baricitinib, ruxolitinib, others

Targeted therapy

Inhibitors of interleukin 1 (IL-1), IL-6, tumor necrosis factor, and granulocyte macrophage colony-stimulating factor; interferon gamma

Tolerogenic therapies

T-cell regulators, low-dose IL-2

Cellular therapies

Natural killer cell therapies, antiplasmacytoid dendritic cell, others

Passive therapy

Intravenous immune globulin, immune plasma, specific antibodies

Nonspecific therapies

Glucocorticoids, calcineurin inhibitors, mammalian target of rapamycin inhibitors

immunologic aging proceeds at different rates in different individuals; thus, mere chronological age is, not surprisingly, a relatively crude predictor of COVID-19 progression.¹²

IMPLICATIONS FOR THERAPY

From a therapeutic perspective, there is a clear need for an effective antiviral agent that can prevent viral infection in exposed individuals and limit tissue damage in those with established disease (stages 1 and 2).

In stage 3, in the absence of any effective antiviral therapy, we are relegated to supportive care. It is at this stage that the experimental use of agents designed to limit tissue damage driven by uncontrolled inflammation is being investigated. Given the similarities between stage 3 COVID-19 and other hypercytokinemic states, a variety of nonspecific immunosuppressive strategies have been proposed, such as glucocorticoids, hydroxychloroquine, colchicine, and other immunomodulators, as well as Janus kinase inhibitors and a number of targeted therapies directed at pivotal cytokines (Table 1). For now, the experience with such agents largely consists of anecdotal case reports and small clinical trials.¹³

As of this writing, more than 900 clinical trials of various therapeutics for COVID-19 are registered at clinicaltrials.gov. Agents that have been proposed or are in use include anti-

Despite gaping holes in our knowledge, treatment is evolving

IL-1, anti-IL-6, anti-GM-CSF, and anti-TNF drugs, and Janus kinase inhibitors. A conceptual framework of such therapies is displayed in Table 1.

Anti-IL-6 and anti-IL-1 agents: Center stage for COVID-19 stage 3 disease

Among these therapies, agents that target IL-6 have perhaps generated the greatest enthusiasm. Numerous IL-6–targeting agents are being tested in COVID-19 including those targeting the IL-6 receptor (tocilizumab, sarilumab) and those targeting IL-6 itself (siltuximab, clazakizumab, and sirukumab). Two of these agents—tocilizumab (NCT04320615) and sarilumab (NCT04315298)—are already in advanced stages of multicenter randomized control trials, and data should be forthcoming soon.

Interest in IL-6 is strong, as it is a pleomorphic cytokine produced by both hematopoietic and viscerosomatic cells and has far-reaching effects on immune function and diverse nonimmune physiologic processes.¹⁴ It is a key upstream driver of inflammation and has been successfully targeted therapeutically. IL-6 also has been shown to be a predictor of respiratory failure.

Of particular relevance for stage 3 COVID-19 disease, targeting IL-6 with tocilizumab is now indicated for treatment of cytokine storm accompanying chimeric antigen receptor (CAR)-T-cell therapy.¹⁵ Clinical support for advancing the study of IL-6 inhibition in COVID-19 has come from a variety of sources, including anecdotes from now-widespread off-label use of tocilizumab, case reports, and small series¹⁶ in which rapid reversal of laboratory and clinical parameters were reported. Balancing enthusiasm for such a strategy is the known pivotal role of IL-6 in host defense, particularly in defense against respiratory viruses.¹⁷

IL-1 is another inflammatory cytokine that could potentially be targeted to treat various cytokine storm syndromes. IL-1 is an upstream mediator of inflammation and is produced by

the NLRP3 inflammasome; it has been incriminated in the pathogenesis of COVID-19, having been detected in lung tissue by a variety of techniques.¹⁸

As of this writing, 3 small nonrandomized case series have demonstrated benefit of IL-1 inhibition in COVID-19.^{18,19} The largest of these series,¹⁹ while suffering from the use of a retrospectively derived comparator group, demonstrated meaningful improvement in reducing the need for mechanical ventilation with the use of anakinra, a human IL-1 receptor antagonist. Anakinra has a short half-life, a large therapeutic window, and a well-established safety profile, and can be given by subcutaneous and intravenous routes.¹⁸ Large prospective trials are now under way and results are eagerly awaited.

Safety concerns

Above all, there are serious considerations regarding untoward toxicity. Paramount among safety considerations is the potential for targeted therapies to suppress the host's immune response and further limit failing antiviral defenses. Theoretically, the short-term use of such agents is likely to be less immunosuppressive than observed in long-term clinical use, but this hypothesis remains unproven. Concerns regarding the use of Janus kinase inhibitors is of particular note since they can further serve to suppress type I and III interferons, which are critical in antiviral defense.²⁰ Also critical is the potential risk associated with the timing of therapy. Administering treatment too early may compromise antiviral immunity, while waiting too long may risk irreversible organ damage.

Other novel therapies

Other novel therapies used alone or in combination include targeting GM-CSF, granulocyte-colony stimulating factor, and Janus kinase. These have been reviewed or mentioned in several excellent narrative reviews.^{5,8,13,21} ■

The disease ends in stage 2 in about 80% of infected individuals

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Cytokines and the still-baffling clinical biology of COVID-19

Although it seems like forever, we are only months into the COVID-19 pandemic, so it should really be no surprise that there are huge gaps in our understanding of the infection and its treatment. It took decades before we developed effective therapies for HIV, hepatitis B, and non-A non-B hepatitis. But this is different. It feels different. It was on us like a tidal wave, and we are now inundated with new data 24/7. Clinical descriptions of new syndromes linked to coronavirus infection and results of small randomized and larger observational studies appear online ahead of print, and alerts are forwarded to our inboxes in a constant stream, not to mention what we hear in the nightly news. Every medical center seems to be scrambling to conduct emergent clinical trials comparing novel treatments with “usual care,” but “usual care” of COVID-19 patients is also changing at a rapid pace.

With all this information, it seems we should know more than we do about how to manage the very sick. But it even in a pandemic affecting such a large number of patients in a short window of time, with many of them experiencing measurable outcome events, well-done randomized treatment trials take time to orchestrate and complete. When morbidity and mortality of hospitalized patients is high, with no known effective therapy, the usual care of patients will likely include multiple unproven medications in an attempt to turn the tide of their infection. This can dramatically complicate the analysis of observational studies. Plus, this virus drives a complicated pathobiology.

The clinical expression and course of COVID-19 are pleomorphic and, thus far, are not easily predicted. There are asymptomatic infected individuals who are nonetheless shedding virus, and presymptomatic individuals seemingly even more infective. Most patients experience a mild to modest illness with some combination of fatigue, gastrointestinal symptoms, anosmia, and respiratory symptoms. But some, perhaps after 5 to 10 days, have a second phase of illness characterized by markedly worsened respiratory symptoms due to severe and progressive viral pneumonia. And within this latter group, some experience a dramatic clinical downturn with variable cardiopulmonary collapse, high fevers, and hypercoagulability associated with laboratory markers consistent with what has been called cytokine storm, macrophage activation syndrome (MAS), or in some other settings, hemophagocytic lymphohistocytosis (HLH). The similarity to these latter syndromes, which often respond to agents directed against the cytokines interleukin 1 (IL-1) (anakinra, canakinumab) or interleukin 6 (IL-6) (tocilizumab, sarilumab), has led to empiric use of these agents and to the initiation of multiple formal clinical trials, as discussed by Dr. Len Calabrese in this issue of the *Journal* (page 389).

But it is not as simple as patients experiencing cytokine storm just having worse disease, characterized by immune overreactivity, which needs to be quelled by blocking the culprit immune hormone. The primary culprit contributing to the progressive lung dysfunction and damage is not certain. Is it all immune damage? Or is the contin-

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ued persistence of coronavirus playing a direct or indirect role through continued activation of primordial components of the immune system? And is the hyperinflammatory response the body's last-ditch effort at controlling the virus, in which case blocking it might (in the absence of an effective antiviral therapy) be counterproductive? Hence the need for well-done, controlled, and randomized clinical trials.

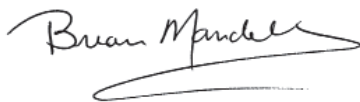
The respective significance of active viral infection and replication vs effects of ultra-high levels of inflammatory mediators (IL-1, IL-6, granulocyte-macrophage colony-stimulating factor) on lung damage and multisystem failure remains to be delineated. Elevated levels of IL-6 and downstream markers of cytokine effects (eg, ferritin) have been associated with death, but this association doesn't prove that it is not the persistence of viral replication and viral products that are in fact contributing to the ongoing elaboration of these cytokines as well as to direct damage to lung tissue.

The biology is complicated. This novel coronavirus has been shown to increase synthesis of IL-1 by stimulating its precursor pro-IL-1 as well as activating the intracellular inflammasome cascade that cleaves active IL-1 from its precursor. Additionally, there are studies that indicate the virus can antagonize the initial host antiviral mechanisms involving interferon generation and natural killer cell-mediated destruction of viral infected cells, resulting in persistence of replicating virus. This latter effect can mimic the rare genetically influenced primary HLH syndromes. Thus, blocking specific cytokines may not be sufficient therapy, unless the virus itself can also be eliminated.

Adding to this complexity, there are studies demonstrating that IL-6 can play an important antiviral role and also can be a positive influence on repair and remodeling following experimental inflammatory lung injury induced in animals by endotoxin or bleomycin.

A lack of an answer to how best to treat patients with severe COVID-19 is not the same as having a lack of information. The latter continues to grow, the former will hopefully follow. Certainly, identifying a potent antiviral agent will help enormously, and I'd expect an antiviral will work synergistically with anticytokine strategies in patients with severe disease. In the meantime, we await clinical results and biochemical analyses from the several prospective trials under way, while wading through the minefields of many well-intentioned but compromised, complicated, and hard-to-interpret observational studies.

If after reading the Calabrese article you are interested in reading more about the fascinating biology of the immune response to this virus that is so rapidly unfolding, I refer you to 2 other well-referenced reviews,^{1,2} and more articles about COVID-19 in general are available at our COVID-19 Curbside Consults section at www.ccjm.org.



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