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Review Article

Making Sense of the Cytokine Storm in COVID-19

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Abstract

The term "cytokine storm" was coined, describing the immune response to organ transplantation. This term was more recently applied to describe extreme cases of bird flu infections. Hyperactivity of the immune system is the hallmark of a cytokine storm. Cytokines are essential messenger molecules that trigger, mediate, and promote an inflammatory response. However, when a cytokine storm is triggered, an overactive immune system attacks not only the infection sites but also otherwise healthy tissues and organs within the body. A cytokine storm is a frequently documented occurrence in SARS-CoV-2.

During a cytokine storm, CCL2, CXCL12, and TNFα are profoundly upregulated. IL-1B, IFNγ, and cytokines are most actively upregulated in SARS-CoV-2—even when a cytokine storm is absent. Subsequently, inflammation of the lung tissue occurs, damaging or destroying the alveoli, possibly resulting in acute respiratory distress syndrome (ARDS). Moreover, respiratory inflammation and ARDS in SARS-CoV-2 appear to induce blood clotting and thrombosis. The application of anticoagulants does not always prevent the formation of thrombotic plaques and blood clots.

Local coagulation events, such as deep vein thrombosis (DVT), are posited to result from microvesicle shedding of endothelial cells. If blood vessels become damaged, megakaryocytes can become pronounced in the lungs, heart, and renal glomeruli microvasculature. Results of autopsies suggest that COVID-19 leads to platelet formation and coagulation in patients with comorbidities of hypertension, hypercholesterolemia, diabetes, obesity, or a combination thereof.

The pathophysiology of COVID-19 is multifactorial. Ongoing research has confirmed specific factors as being directly responsible for the signs, symptoms, and sequelae of COVID-19, while other factors are still being questioned and new postulates are being put forth. The more data uncovered regarding the immune response to COVID-19, the better the chances for developing more specific and effective treatments and prevention against the source of the deadly pandemics, COVID-19.

Keywords: *Alveoli; Chemokines; Convalescent Plasma; Immune Markers; Immune Response; Inflammation; Interferon; Interleukin; Thrombosis; Viscosity*

Abbreviations

ARDS: Acute Respiratory Distress Syndrome; CSF: Colony-Stimulating Factor; DAD: Diffuse Alveolar Damage; DVT: Deep Vein Thrombosis; IFNa: Interferon Alpha; IFNg: Interferon Gamma; IL-1B: Interleukin-1B; IL-2: Interleukin-2; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-12: Interleukin-12; IL-18: Interleukin-18; IL-33: Interleukin-33; SOFA: Sequential Organic Failure Assessment; Th1: T Helper Cell 1; Th2: T Helper Cell 2; TF: Tissue Factor; TNFa: Tumor Necrosis Factor Alpha

Introduction

Immune response in COVID-19

One of the most frequently documented connections between SARS-CoV-2 and the immune system comes from the cytokine storm. Cytokines are essential messenger molecules that trigger, mediate, and promote an inflammatory response [1]. If a cytokine storm is triggered, an overactive immune system attacks the infection sites, other tissues, and organs in the body, endangering overall physiological processes and organ function. Also, an overly strong inflammation response—even within the lungs as an organ primarily affected by COVID-19—may have significant and life-threatening consequences. The sequelae of the cytokine storm scars and destroys respiratory tissues.

The lungs depend on the presence of fine-structured alveoli to increase the total surface area on which oxygen and carbon dioxide are exchanged. If a substantial amount of the surface area is disrupted or destroyed during the inflammatory response, lung function will be significantly and adversely affected.

The expression cytokine storm was first introduced to describe the immune response to organ transplantation, which underscores the massive immune response [1]. This term was more recently used to describe extreme cases of bird flu (H5N1) infections [2]. Cytokine storms signify hyperactivity of the immune system; thus, much can be learned about the general immune response of the body, as the same molecules are involved during normal function and hyperactivity.

Discussion

Inflammation in the lung tissue can lead to acute respiratory distress syndrome (ARDS), wherein inflammation locally destroys the alveolar tissue. This destruction not only impedes gas exchange between air and blood by reducing the available surface area of the alveoli but also increases the permeability of the alveolar surface, allowing plasma to leak into the alveoli. This pathologic permeability of the alveoli results in the lungs filling with fluid (causing edema) gradually. Thus, impeding the afflicted patients capacity to breathe appropriately and adequately [3].

Similar to MERS-CoV and SARS-CoV-1, the immune response in SARS-CoV-2 patients is characterized by an upregulation of interferon-gamma (IFN-γ), chemokines (such as CCL2 and CXCL10), interleukins (the most prominent of which are IL-1B, IL-6, and IL-12), and TNFα—to name the most prominent ones.

Chemokines are chemical messenger molecules that attract cells (specific white blood cells) to their target destination. Interleukins are secreted molecules that promote the proliferation or differentiation of immune cells, enabling the body to mount a local immune response without keeping inflammation mediators lastingly upregulated. Other classes of molecules, such as IFN-γ and TNF-α, exert multiple functions in regulating the immune response [1–3].

During a cytokine storm, CCL2, CXCL12, and TNFα are significantly upregulated. In general, IL-1B, IFNγ, and cytokines are most strongly upregulated in SARS-CoV-2—even without a cytokine storm emerging. These factors have been implicated in T helper cell 1 (Th1) activation in SARS-CoV-1 and MERS-CoV. SARS-CoV-2 also features the involvement of T helper cells 2 (Th2) via IL-4 and IL-10. These interleukins are involved in suppressing immune reactions, suggesting that the immune response to SARS-CoV-2 contains some aspects of a balance in inflammation-mediating molecules [1,2].

Table 1 illustrates an overview of various inflammatory mediators and cytokine markers released in response to a SARS-CoV-2 infection.

Inflammatory marker	Function	Reference
Interferon-alpha (IFNa)	Promotion of anti-viral gene expression.	(Coperchini., *et al*. 2020) [1]
Interferon gamma (IFNg)	Promotion of anti-viral gene expression.	(Coperchini., *et al*. 2020) [1]
Interleukin-1B (IL-1B)	Co-stimulation of T helper cells; proliferation and maturation of B cells; activation of natural killer (NK) cells; acute-phase reaction during inflammation, targeting endothelium and macrophages.	(Coperchini., *et al*. 2020) [1]
Interleukin-2 (IL-2)	Differentiation and growth of T cells.	(Coperchini., *et al*. 2020) [1]
Interleukin-6 (IL-6)	Differentiation of activated B cells into plasma cells; antibody secretion by plasma cells; differentiation of hematopoietic stem cells (HSCs); inflammation response, utilizing T cells.	(Zhang., *et al*. 2020) [2]
Interleukin-10 (IL-10)	Promotion of cytokine production in macrophages; B cell activation; inhibition of cytokine production in Th1 cells; stimulation of Th2 cells.	(Coperchini., *et al*. 2020) [1]
Interleukin-12 (IL-12)	Differentiation of activated T cells into cytotoxic T cells; mediation of IFN-g and TNF-a production in NK cells.	(Coperchini., *et al*. 2020) [1]
Interleukin-18 (IL-18)	Induction of IFN-g production in Th1 and NK cells; promotion of NK cells activity.	(Coperchini., *et al*. 2020) [1]
Interleukin-33 (IL-33)	Induction of cytokine production in Th2 cells.	(Coperchini., *et al*. 2020) [1]
Tumor necrosis factor-alpha (TNFa)	Mediation of inflammation response.	(Coperchini., *et al*. 2020) [1]
Colony-stimulating factor (CSF)	Increases cytokine production by macrophages at inflammation sites.	(Coperchini., *et al*. 2020) [1]
CCL2	Attracts immune cells to the sites of inflammation.	(Coperchini., *et al*. 2020) [1]
CCL3	Attracts immune cells to the sites of inflammation.	(Coperchini., *et al*. 2020) [1]
CCL5	Attracts immune cells to the sites of inflammation.	(Coperchini., *et al*. 2020) [1]
CXCL8	Attracts immune cells to the sites of inflammation.	(Coperchini., *et al*. 2020) [1]
CXCL9	Attracts immune cells to the sites of inflammation.	(Coperchini., *et al*. 2020) [1]
CXCL10	Attracts immune cells to the sites of inflammation.	(Coperchini., *et al*. 2020) [1]

Table 1*: Interferons, Interleukins, and other immune response markers in MERS-CoV, SARS-CoV-1, and SARS-CoV-2.*

Besides respiratory inflammation and ARDS, SARS-CoV-2 seems to induce blood clotting and thrombosis. Anticoagulants are not always able to entirely prevent the formation of thrombotic plaques and blood clots [4]. One possible rationale for these sequelae is increased blood viscosity in individuals severely affected by a SARS-CoV-2 infection. In Maier CL., et al. (2020), infected patients demonstrated blood viscosity values more than 95% of the normal range. The increased blood viscosity was highly correlated with an elevated sequential organic failure assessment (SOFA) score with $R^2 = 0.7072$ and $p < 0.001$ [4].

According to the finding of this research, blood plasma in affected patients had significantly elevated fibrinogen levels, a known cause of elevated plasma viscosity [4]. It is unclear what causes fibrinogen concentrations to rise in CoV-2 patients suffering respiratory inflammation.

In a recent case study, Rapkiewicz., *et al.* (2020) found that COVID-19 patients often show signs of multiple thromboses. The researchers observed that all seven autopsied patients died of complications related to SARS-CoV-2 infections. Also, they found platelet-rich thrombi in the pulmonary, hepatic, renal, and cardiac microvasculature" [5].

Although it is unclear if all patients died of thromboses, the results point to irregular coagulation as one possible lethal complication of COVID-19. Notably, a control group with nine patients that had died while exhibiting non-COVID-19 ARDS symptoms, showed significantly reduced levels of thrombi, in comparison. This finding suggests that coagulation is not an indirect outcome from respiratory conditions or cytokine storms, but is specific to SARS-CoV-2 infection [5].

The researchers also discovered elevated counts of megakaryocytes in the heart and lungs—which are an essential part of platelet production—suggesting a procoagulatory state in individuals eliciting severe symptoms from SARS-CoV-2 infections. Thrombi could be detected even when patients were given anticoagulants, suggesting a transition into a prothrombotic state—a feature that begins very early in the progression of a SARS-CoV-2 infection [5].

These findings were corroborated by observations of thrombi in multiple and diverse locations. However, there remains the possibility that the thrombi were formed commonly—for example, through deep vein thrombosis (DVT)—and distributed throughout the body. The researchers searched for such DVTs [5].

The thrombi found in alveolar capillaries could be one explanation for the low level of blood oxygen that individuals with severe SARS-CoV-2 symptoms often experience. In that context, all seven autopsies showed evidence of diffuse alveolar damage (DAD), which may have been brought about by thrombosis formation or a singular cause of multiple local coagulation [5].

The presence of megakaryocytes was especially pronounced in the lungs, heart, and renal glomeruli microvasculature. Often, these cells were not only associated with platelets but also virions [5]. This finding suggested that the interactions of viral particles with the immune system might somehow produce platelets and enhance the local coagulation process.

Moreover, the preceding adds an alternative explanation for multiple organ failure to the cytokine storm hypothesis. If circulation through microvessels is disrupted, the corresponding organs do not receive sufficient oxygen and cannot benefit from the typical exchange of gases and nutrients between tissues and blood vessels, leading to the shut-down of the affected organ.

It has been hypothesized that local coagulation events, such as DVT, result from microvesicle shedding of endothelial cells, sometimes leading to the formation of embolism during chemotherapy if blood vessels become damaged [6]**.** However, Rapkiewicz., *et al.* (2020) did not find any evidence of disruptions in the microcapillary structures [5]. Nonetheless, other studies found disrupted ultrastructure of blood vessels in fatal COVID-19 cases [7], including destroyed endothelial cell membranes and the infiltration of alveolar tissues by T-cells. Furthermore, a different set of genes was upregulated in those tissues compared to autopsies from individuals dying of non-COVID-19 related influenza [8], including cytoskeletal genes and genes involved in ephrin and Wnt signaling [9].

Consistent with the upregulation of pro-angiogenesis genes, the researchers observed increased angiogenesis around the affected regions of vascular damage [10]. The disruption of the microvasculature may be due to the expression of tissue factor (TF) or damage to the glycocalyx [5]**.**

In a comparable study, Fox., *et al.* (2020) found—in a series of ten autopsies of patients that had died of COVID-19—that the lungs revealed the presence of multiple thromboses, microangiopathies, and hemorrhages. The patients were of African-American origin, concomitant with the overwhelming race of COVID-19 cases within the study area in New Orleans, Louisiana, USA [11].

Albeit the number of n = 10 cases is small, it is in line with the n = 7 severe cases found by Rapkiewicz., *et al.* (2020) and Ackermann., *et al.* (2020). Blood coagulation and thrombosis are potentially life-threatening. Thus, they can be viewed as contributing to the low oxygen levels in blood frequently seen in severe COVID-19 cases. However, it is unclear if coagulation is one of the causes of respiratory complications in SARS-CoV-2 infections or merely a consequence of inflammation, which can weaken the involved tissues, predisposing toward coagulation and thrombosis [5,10].

Fox., *et al.* (2020) observed damage in the endothelial cells' hyaline membranes in all patients, even those that had not been connected to a ventilator, mitigating the possibility that tissue damage resulted from external forces [11].

Within the patients examined in the study that died of complications of COVID-19, DAD, widespread thrombosed capillaries, and microangiopathy (from thrombosis and hemorrhage in the lungs could be considered significant contributors to mortality) [11]. However, in contrast to Ackermann., *et al.* (2020) and Spehlmann, et al. (2020), no significant thromboses in tissues other than the lungs were observed [6,10,11].

Compared to other SARS-CoV infections, the observed aggregation of platelets in the lungs and the presence of thrombi in the pulmonary tissue is a feature specific to SARS-CoV-2. This observation presents an adjunct to information about the pathophysiology and mechanism of the disease. Both cytokine storm and platelet aggregation may combine to mediate fatal lung pathogenesis in those suffering severe symptoms from COVID-19.

The autopsies analyzed in Spehlmann., *et al.* (2020) showed that most patients suffered from hypertension, high cholesterol, diabetes, obesity, or a combination thereof [6]. Thus, COVID-19 may lead to platelet formation and coagulation in patients afflicted by those conditions. It is reasonable to ascertain whether obesity, diabetes, hypertension, and related conditions are associated with pulmonary thromboses and platelet formation [12].

Overall, the findings and hypotheses described in this review point to the usefulness of convalescent plasma. Exchanging such plasma with the current plasma that the patient carries may lead to a reduction in viscosity, being of significant benefit for recovery [4].

Conclusion

Much is occurring in the immune response to COVID-19. The aftermath of the immune response and the cytokine storm seems to be a result of a multitude of factors. Some factors have been confirmed as directly participatory, while other factors are still being questioned and investigated. Born of dire necessity, current research is gaining momentum, and new answers are being determined constantly; however, new questions are being spawned. The more data uncovered regarding the immune response to SARS-CoV-2, the better the chances of developing more specific and effective treatments, including prevention. For now, the immune response and cytokine storm remain highly complicit in this deadly pandemic.

Conflict of Interest Statement

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Supplementary Note

This paper is based on prior doctoral research: Chen M.H. (2019). "SARS-CoV-2: Dynamic Stimulation and Control of the Immune System by Integrated Therapies" (unpublished doctoral dissertation).

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