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Review Article

IMMUNOPATHOLOGICAL ROLES OF NEUTROPHILS IN VIRUS INFECTION AND COVID-19

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ABSTRACT—Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been spread around the world and is currently affecting global public health. Clinical evidence indicates that the elevated number of peripheral neutrophils and higher ratio of neutrophils-to-lymphocytes are correlated with severe outcomes in COVID-19 patients, suggesting the possible immunopathological role of neutrophils during SARS-CoV-2 infection. As an abundant innate immune cell type, neutrophils are well known for their contributions to antimicrobial defense. However, their dysfunction is also associated with different inflammatory signatures during the pathogenesis of infection. Herein, in this mini-review, we summarize the recent progress on the potential role of neutrophils during COVID-19-associated inflammatory responses. In particular, we highlight the interactions between neutrophils and viruses as well as the relationship of neutrophils with cytokine storm and thrombosis in COVID-19 patients. Lastly, we discuss the importance of neutrophils as potential therapeutic targets for COVID-19.

KEYWORDS—COVID-19, cytokine storm, inflammation, neutrophils, SARS-CoV-2, thrombosis

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel mutational RNA virus, causing the coronavirus disease 2019 (COVID-19) outbreak that is initially reported in December 2019 in Wuhan, China (1, 2). As it spreads throughout the world, the COVID-19 is now defined as a pandemic and becomes a great threat to global public health. Patients with SARS-CoV-2 infection typically show symptoms of fever, dry cough, shortness of breath, and myalgia (1–4). Although most of the infected patients are asymptomatic or manifest only mild pneumonia, nearly 10% to 20% of severe cases develop acute respiratory distress syndrome (ARDS) characterized by life-threatening respiratory failure (3, 5). This severe subgroup has robust pulmonary inflammation and disproportionate immune response that may lead to high levels of circulating cytokines, excessive lung damage, thrombotic tendency, and even multiple-organ dysfunction (4, 6). Therefore, viral load may not be the sole driving factor that dominates the progression of severe COVID-19 (7, 8). The diversified immune responses observed in different COVID-19 patients represent crucial components in determining the outcome of disease. However, it remains largely unknown how SARS-CoV-2 infection could trigger severely dysregulated immune responses (9). Hence, better understanding the roles of various immune cells in different stages of COVID-19 will be urgently needed for developing precision-based therapies.

To date, clinical observations have shown alterations in hematology and immunity in patients with COVID-19 (5, 9–12). In particular, the increased number of neutrophils and higher ratio of neutrophil-to-lymphocyte have been reported in most COVID-19 patients (9, 10). Importantly, these two clinical characterizations are independent risk factors associated with fatal outcomes (5, 10, 12). Furthermore, the autopsy samples showed neutrophilic mucositis and robust neutrophil infiltration in pulmonary capillaries and alveolar spaces (13, 14). Together, these results implicate that neutrophils may play critical roles in the pathogenesis of COVID-19 infection. Given that neutrophils are the most abundant type of leukocytes in human circulation and key effector cells of innate-immune system, they could initiate the first wave of host defense against most infections including viruses (15). In addition to their recognition and elimination of invading microorganisms, neutrophils are also involved in a variety of pathogenic conditions (such as proinflammation, immuno-thrombosis, and tissue damage), which are affected by disease progression, aging, and microbiome fluctuations (16). The divergent involvement of neutrophils in different disease pathophysiology makes them attractive targets for pharmaceutical drugs.

In this mini-review, we aimed to summarize the emerging pathological roles of neutrophils in COVID-19. First, we outlined the interactions between neutrophils and viruses, followed by highlighting the relationship of neutrophils with

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cytokine storm and thrombosis in COVID-19. We next discussed the important roles of neutrophils in COVID-19-associated inflammatory responses. Lastly, we proposed prospective therapeutic strategies on precision-modulating of immune cells.

THE ROLES OF NEUTROPHILS IN VIRUS INFECTION

Despite the critical roles of neutrophils in the prevention of bacterial infection, a growing body of studies have revealed their contributions to recognition and elimination of viruses (17, 18). Pattern recognition receptors (PRRs) are presented in myeloid cells (i.e., neutrophils) to recognize invading pathogen by detecting pathogen-associated molecular patterns (PAMPs). With respect to viruses, their PAMPs are nucleic acids that are generally recognized by a subset of PRRs, toll-like receptors (TLRs). For example, TLR3, TLR7, and TLR8 recognize RNA viruses, while TLR9 senses DNA viruses (18–20). When influenza viruses bind to the endosomal TLR7 in neutrophils, their enhanced phagocytosis provides an antiviral activity (21). Although influenza viruses are not associated with LPS, TLR4 still participates in the host defense against influenza infection (18, 22). For example, lack of TLR4 decreases the degree of phagocytosis of apoptotic cells by neutrophils and increases mortality among influenza virus-infected mice (22). Furthermore, regarding SARS-CoV infections, a mouse genetic study by Totura et al. (23) highlighted the important roles of TLR adaptor signaling in protecting innate immune response. Interestingly, TLR3-, TLR4-, TRAM-, and TLR3/TLR4 adaptor TRIF-knockout mice are more susceptible to SARS-CoV than control wild-type mice, evidenced by weight loss, increased lung injury, and higher mortality (23). In addition to TLRs, retinoic acid-inducible gene (RIG)-like receptors are also involved in the interactions with intracellular viruses (24). For instance, after stimulating human neutrophils with viral double-stranded RNA, the expression levels of RIG-I, melanoma differentiation-associated protein 5 (MDA5), and other antiviral genes (i.e., type I interferon (IFN) and IFN-responsive gene) are significantly increased (25). However, the neutrophils isolated from MDA5-knockout mice partially lose the capacity of promoting IFN-β production (25). Similarly, during the early stage of respiratory syncytial virus (RSV) infection, the RVS nucleic acid binds to RIG-I and MDA5, which subsequently activates nuclear factor kappa-B and interferon regulatory factor (IRF) three translocation into the nucleus, where these transcription factors promote type I IFN production (26, 27) (Fig. 1). Collectively, these studies indicate that recognition of PAMPs by neutrophils is the first-line approach to eliminate invading viruses, thus limiting the excessive inflammatory response caused by viruses. Hence, promoting the neutrophil-mediated virus clearance is a potential therapeutic strategy for viral infections.

Besides the antiviral roles, neutrophils are noteworthy for their pro-inflammatory function. During virus infection, an appropriate host-immune response is essential to maintain and restore tissue homeostasis. An unbalanced neutrophilic inflammation could result in the sustained immune activation and tissue injury, which contribute to the pathogenesis of inflammatory diseases (11, 28). In response to severe respiratory tract viral infection, a large number of activated neutrophils are recruited to the airway, causing alveolar-capillary injury by releasing a variety of proteolytic enzymes and chemokines/cytokines, and eventually leading to pulmonary diseases such as ARDS (17). For example, during the pathogenesis of RSV lower respiratory tract infection (LRTI), the viruses initially bind to the intercellular cell adhesion molecule-1 (ICAM-1) localized on the surface of epithelial cells, which then increase the production of chemoattractant IL-8 that drives the release of neutrophils from bone marrow (29, 30). Meanwhile, both RVS infection and activated epithelial cells trigger the activation of endothelial cells, which drives the release of soluble ICAM-1 (31). As an endothelial adhesion molecule, ICAM-1 is responsible for neutrophil adhesion and promotes neutrophil migration across the endothelium into tissues (29). Once neutrophils accumulate in the inflamed tissue, they could cause sustained damage to lung tissues through releasing several toxic factors including reactive oxygen species (ROS), myeloperoxidase (MPO), neutrophil extracellular traps (NETs) (32) (Fig. 1). Moreover, the degree of neutrophil infiltration is closely related to clinical severity and viral load in the cases of RSV-LRTI (33). As for the influenza infection, excessive recruitment and activation of neutrophils are major contributing factors for lung injury. Recent evidence further reveals the presence of extensive NETs in lung tissues of mice infected with lethal dose of influenza virus (34). This suggests the detrimental role of neutrophil-released NETs in alveolar–capillary damage. Indeed, *in vitro* studies have confirmed that NETs could exacerbate endothelial cell damage during myalgia (34). Recently, Zhu et al. (35) reported that the transcriptional regulator BCL6 in neutrophils could suppress the expression of apoptotic genes including Pmaip1, Casp3, and Pdcd4, leading to the prolonged neutrophil survival in the site of inflammation. To the contrary, mice with BCL6-deficient neutrophils demonstrate enhanced resistance to influenza infection and decreased inflammatory responses (35) (Fig. 1). Therefore, while neutrophils are beneficial to confine viral infection, it is equally critical to avoid excessive neutrophil activation and thereby, prevent inflammatory damage. Future studies investigating neutrophil infiltration and phenotypic activation should be warranted.

SARS-CoV-2 is an enveloped virus with four structural proteins, similar to other two highly pathogenic coronaviruses (CoVs) including SARS-CoV and the Middle East Respiratory Syndrome Corona Virus (MERS-CoV) (36). Different viruses interact with various cellular receptors with distinct ligands. For example, the membrane receptor dipeptidyl peptidase 4 is sensitive to recognize the spike protein of MERS-CoV, whereas the angiotensin-converting enzyme 2 (ACE2) receptor is essential for the cellular entry of SARS-CoV and SARS-CoV-2 (37). Recent studies have shown that ACE2 is ubiquitously expressed on the surface of endothelial cells and alveolar epithelial type II cells, but is rarely expressed in immune cells (38). However, immune cells such as T cells can be potentially infected by CoVs, which indicates additional mechanisms of infection, such as activation of CD147 by CoVs (38–40). Nonetheless, it remains unclear whether SARS-CoV-2 can directly infect neutrophils, and if it does, one question will be asked which

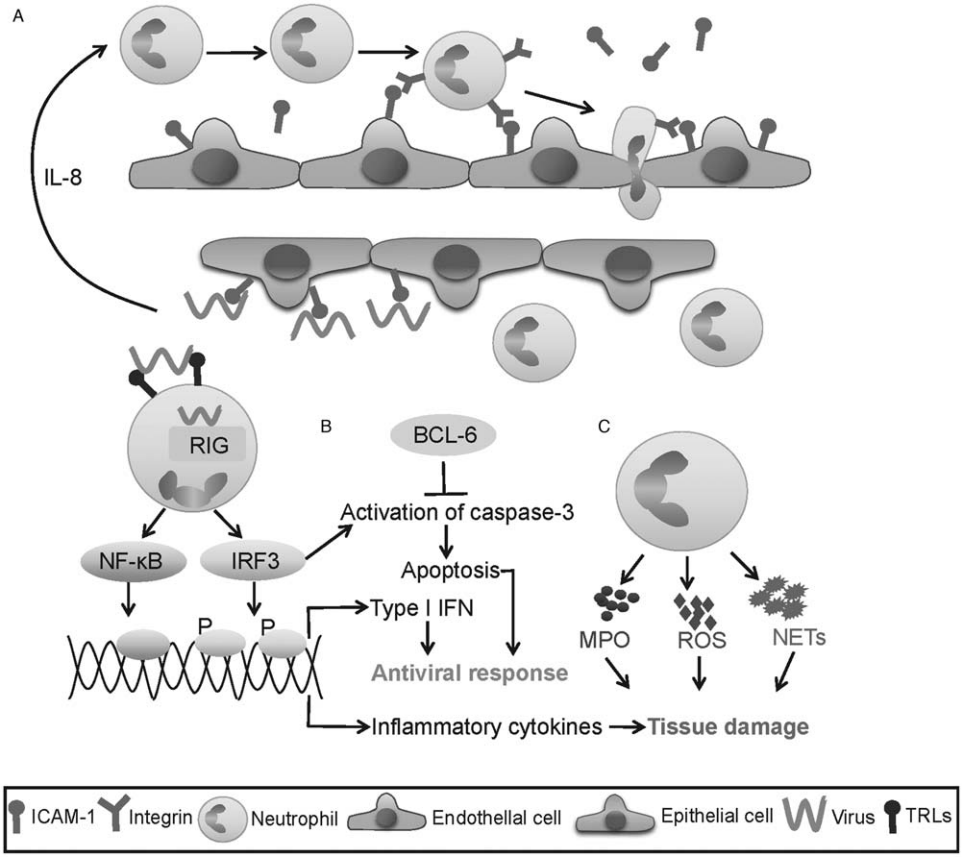

FIG. 1. **Overview of neutrophil biology during virus infection in the lung.** As the first-line defense against microbial infection, neutrophils play indispensible roles in inflammatory stimuli by recognizing pathogens and maintaining tissue homeostasis. A, The interactions between invading viruses and ICAM-1 on the epithelial cell surface promote the production of chemoattractant IL-8 and soluble ICAM-1. IL-8 is responsible for the release of neutrophils from bone marrow. And ICAM-1 on the endothelial cell surface binding with neutrophil integrin is beneficial for neutrophils migrating across the endothelium into tissues. B, Recruited neutrophils recognize PAMPs on invading pathogens through their pattern-recognition receptors. The nucleic acids from viruses can be recognized by TLRs and RIG-like receptors followed by activating/transferring NF-κB and IRF3 into the nucleus. These transcription factors promote the production of type I IFN, which participates in the antiviral process. Moreover, IRF3 is a positive regulator of apoptosis, which is instrumental in controlling excessive inflammation. C, Activated neutrophils release various inflammatory cytokines and toxic factors such as MPO, ROS, and NETs, leading to the excessive inflammatory response. BCL-6 indicates B-cell lymphoma-6; ICAM-1, intercellular cell adhesion molecule-1; IFN, interferon; IL-8, interleukin-8; IRF3, interferon regulatory factor 3; MPO, myeloperoxidase; NETs, neutrophil extracellular traps; NF-κB, nuclear factor kappa-B; PAMPs, pathogen-associated molecular patterns; RIG, retinoic acid-inducible gene; ROS, reactive oxygen species; TLRs, toll-like receptors.

molecular signal affects the uptake of SARS-CoV-2 by neutrophils. Thus, it is necessary to explore the possible receptors associated with interactions between neutrophils and SARS-CoV-2 under the umbrella of antiviral treatment. In addition, the pathological roles of neutrophils in COVID-19 (Table 1) require further investigation. Lastly, published studies have demonstrated that activated neutrophils not only promote cytokine storm, but also play vital roles in initiating thrombosis (41). This may at least partially explain why severe COVID-19 is correlated with cytokine storm and thrombosis. Of interest, the cytokine storm can reversely regulate neutrophils activation and recruitment (41, 42). The underlying mechanisms are summarized below.

NEUTROPHILS AND CYTOKINE STORM IN COVID-19

It has been recognized that severe COVID-19 patients have higher concentrations of cytokines [i.e., IL-1β, IL-6, IL-17, TNF-ααα] AND INFLAMMATORY CHEMOKINES INCLUDING CXC-CHEMOKINE LIGAND (CXCL) 1, CXCL2, CXCL8, CXCL10, CC-CHEMOKINE LIGAND (CCL) 2, CCL7, AS WELL AS GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) (43, 44). THESE MEDIATORS HAVE BEEN WIDELY REPORTED TO AFFECT NEUTROPHIL MATURATION, ACTIVATION, AND RECRUITMENT (FIG. 2). FOR EXAMPLE, STUDIES HAVE REVEALED THAT G-CSF BINDS TO THE RECEPTOR ON NEUTROPHIL PRECURSOR CELLS FOLLOWED BY PROLIFERATION, LEADING TO THE RELEASE OF NEUTROPHILS FROM BONE MARROW (45). THE SYNTHESIS OF G-CSF IS MODULATED BY IL-17 PRODUCED BY NEUTROPHIL-REGULATORY T CELLS, WHILE THE LEVELS OF IL-17 ARE AFFECTED BY IL-23 PRODUCED BY MACROPHAGES AND DC CELLS (46). REMARKABLY, THE PRODUCTION OF IL-23 MAY BE INHIBITED IN MACROPHAGES AND DC CELLS DURING THEIR PHAGOCYTOSIS OF APOPTOTIC NEUTROPHILS (47). TOGETHER, NEUTROPHIL PRODUCTION IS REGULATED BY THE COMPLEX IMMUNE SYSTEM OF IL-23/IL-17/G-CSF AXIS. IN ADDITION TO COLONY-STIMULATING FACTORS, THE PRODUCTION OF CHEMOKINE CXCL8 IS ALSO REGULATED BY IL-17 IN IL-6-DEPENDENT TH17 CELLS, WHOSE NUMBER IS INCREASED SUBSTANTIALLY DURING SARS-COV-2 INFECTIONS (48). NOTABLY, IT HAS BEEN CONFIRMED THAT CXCL8 IS A NEGATIVE REGULATOR OF NEUTROPHIL

Table 1. **The roles of neutrophil-released effectors in COVID-19**

Effector molecules released by activated neutrophils	Function of effector molecules	Potential role in COVID-19	References
Neutrophil elastase (NE)	Degradation of extracellular matrix and epithelial junction structure	Alveolar–capillary damage	52
Myeloperoxidase (MPO)	Destroy the structures of cadherins and cytoskeletal proteins	Epithelial cell apoptosis and necrosis	52
	Formation of NETs	Cytokine storm	41, 53
		Thrombus formation	41, 61, 63, 64
Reactive oxygen species (ROS)	TGF-β production	Lymphopenia	51

MPO indicates myeloperoxidase; NE, neutrophil elastase; NETs, neutrophil extracellular traps; ROS, reactive oxygen species; TFPI, tissue factor pathway inhibitor; TGF-β, transforming growth factor-β.

cell apoptosis and prolongs their survival (48). As for other chemokines, previous studies indicated that CXCL1 and CXCL2 could bind to CXCR2 on neutrophils, which results in the conformational transformation of integrins (45). These high-affinity conformational integrins then engage their ligands such as ICAM localized on the endothelial cell surface for tight adhesion of neutrophils to endothelial cells, allowing for neutrophils migration into tissues (49). In addition, the massive ROS released by neutrophils is responsible for the tissue damage during infection (32). Of interest, in the case of COVID-19, ROS is also associated with the development of lymphopenia through stimulating TGF-b signaling in Treg cells (50, 51). Recently, Schönrich et al. (51) reported that the TNF-α release during cytokine storm exacerbated ROS production through a positive feedback loop by activating NADPH oxidases. This TNF-α-mediated ROS production may be an important contributing factor for the damage observed in distal tissues such as the brain in COVID-19 patients (51).

SARS-CoV-2 initially enters alveolar epithelial cells *via* engaging ACE2 receptor, triggering activation of macrophages, DC cells and T lymphocytes in severe COVIC-19 patients, but activated neutrophils play essential roles in regulating cytokine storm (41, 48). For example, the SARS-CoV-2-induced cytotoxic proteins released by neutrophils such as neutrophil elastase (NE) and MPO could affect the

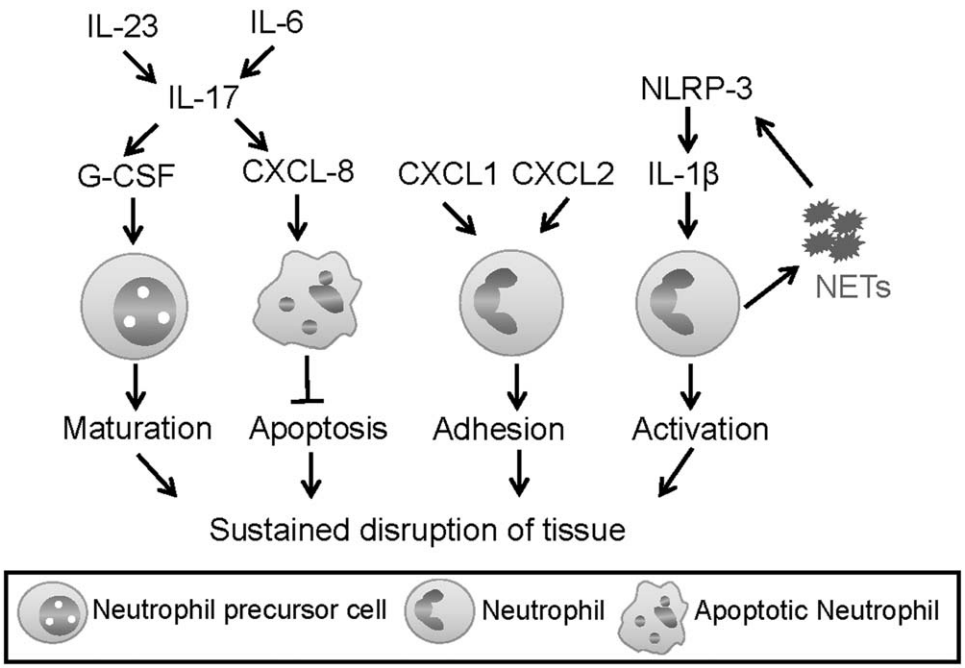

Fig. 2. **Interaction between cytokines and recruited neutrophils during SARS-CoV-2 infection.** Among patients with COVID-19, high concentrations of various cytokines can be observed. Despite that these cytokines are mainly released by macrophages and T cells, they are involved in the regulation of neutrophil function. The axis of IL-23/IL-17/G-CSF promotes neutrophil maturation and proliferation in bone marrow. And the axis of IL-6/IL-17/CXCL8 shows negative regulation of neutrophil apoptosis and prolongs their survival. Furthermore, CXCL1 and CXCL2 are responsible for the adhesion of neutrophils to endothelial cells by affecting the conformational transformation of integrin. In addition to the regulation of neutrophil function, cytokines are affected by the activated neutrophils. IL-1β generated by NLRP3 can promote neutrophils to participate NET formation and is responsible for neutrophil activation and recruitment. In turn, the NETs can further activate NLRP3 and thereby, inducing more production of IL-1β. Persistent activation of neutrophils results in the sustained disruption of lung tissues. CXCL1 indicates CXC-chemokine ligand 1; CXCL2, CXC-chemokine ligand 2; CXCL8, CXC-chemokine ligand 8; G-CSF, granulocyte colony-stimulating factor; IL-6, interleukin-6; IL-17, interleukin-17; IL-23, interleukin-23; IL-1β, interleukin-1β; NETs, neutrophil extracellular traps; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain protein 3.

structures of cadherins and cytoskeletal proteins, leading to the epithelial cell apoptosis and necrosis (52). Furthermore, the disruption of epithelial barrier impels an increased secretion of proinflammatory cytokines from neutrophils. Recently, Yaqinuddin and Kashir suggested that IL-1β and neutrophil-derived NETs may form a NLRP3-mediated feed-forward loop resulting in severe COVID-19 (53). Notably, IL-1β generated by activated NLRP3 inflammasome in resident macrophages can activate and recruit excessive neutrophils into alveolar spaces and enhance NET formation. In turn, the NETs can further activate NLRP3 inflammasome that induces more production of IL-1β (53). Together, the positive IL-1β/NETs feedback loop is closely related to the excessive inflammation observed in COVID-19 patients. Moreover, IL-1β can stimulate the production of IL-6, which is a major mediator within the cytokine storm (54). Consequently, targeting the IL-1β/NETs loop may represent a new strategy for the treatment of severe COVID-19.

NEUTROPHILS AND THROMBOSIS IN COVID-19

D-dimer, as a fibrin degradation product, is widely used for the prediction of venous thromboembolism (i.e., deep venous thrombosis) and pulmonary embolism, because it is presented in the blood after a blood clot is degraded by fibrinolysis (55, 56). Kollias et al. (55) recently showed that increased concentrations of D-dimer in the blood were closely associated with poor outcome in severe COVID-19 patients. Autopsy further demonstrated deep venous thrombosis in 58% of patients with COVID-19 (7 of 12), and pulmonary embolism was the direct cause of death in 33% of patients with COVID-19 (4 of 12) (56). Meanwhile, the microscopic features of lung autopsy samples revealed a significant fibrin deposition and endothelial cell necrosis within septal capillary injury (57). Hence, such strong correlation between thromboembolic events and the severity of COVID-19 implicates the key roles of coagulation cascade during SRAS-CoV-2 infection. It is widely accepted that activation of coagulation system involves in innate immune response, characterized by the immunothrombotic process. Despite that macrophages/monocytes participate in activation of coagulation, neutrophils are also associated with thrombosis (58). Previous studies suggested that accumulation of neutrophils in the site of endothelial injury is prior to platelet activation; however, the activated platelets could promote neutrophil adhesion and migration by enhancing expression of adhesion molecules including ICAM-1 and VCAM-1 on endothelial cells (59, 60). Furthermore, neutrophils can be directly activated through the interaction with platelets. This process is modulated by multiple platelet molecules such as P-selectin, β-integrin, TREM-1 ligand (61). Additionally, the neutrophil-derived serine proteases such as cathepsin G and NE are involved in the coagulation cascade through the introduction of chemical endothelial injury, activation of coagulation factor X, and the cleavage of tissue factor pathway inhibitor (60, 62).

Furthermore, recent clinical study has revealed that sera from COVID-19 patients had higher levels of cell-free DNA, (MPO-DNA, and citrullinated histone H3; the latter two are specific markers of NETs (63). These three mediators are correlated with neutrophil count, D-dimer, lactate dehydrogenase, and platelet levels, suggesting the significant roles of NETs in coagulation cascade during SARS-CoV-2 infection (41, 63, 64). Importantly, prior studies have shown that NETs can promote thrombus formation even without the involvement of activated platelets (61). This process requires the participation of coagulation factors IX and XII (65). In addition, NETs are responsible for the accumulation of coagulation components including platelets, leukocytes, and clotting factors, which contributes to the intravascular NET-induced hemodynamic disorder and eventually leads to thrombin generation (58). Recently, the analysis of pulmonary autopsy samples from COVID-19 patients further revealed higher concentrations of complement components such as C5b-9, C4d, and C3d, suggesting the important roles of complement system in the pathogenesis of severe COVID-19 (57). Indeed, knockout of either C3- or C5 in mice could impair the recruitment of immune cells and platelets to the site of injury, leading to weaker thrombus (66). One recent study by Ward and Fattahi (67) has demonstrated that C5a is involved in neutrophil activation and NET formation. Likewise, C5b-9 plays a critical role in the activation of NLRP3 inflammasome followed by indirect modulation of NET formation (67). Remarkably, the extracellular histones released by NETs can promote platelet activation *via* interacting with TLR2 and TLR4 (68). Therefore, the role of neutrophil-released NETs in complement-associated thrombosis should be further investigated in COVID-19 patients.

POTENTIAL THERAPEUTIC STRATEGIES TARGETING NEUTROPHILS IN COVID-19

At present, it is well accepted that SARS-CoV-2-related ARDS is one of the major causes of death in severe COVID-19 patients. In a retrospective study including 52 critically ill patients with SARS-CoV-2 infection in China, 32 (61.5%) patients died at 28 days, of whom 26 (81%) patients had ARDS (69). Thus, aggressive treatment of ARDS may dramatically improve survival rate of severe COVID-19 patients. Along this line, NE is a proteolytic enzyme secreted by activated neutrophils, and it has been shown to regulate neutrophil migration and disruption of epithelial barrier (70). Moreover, NE can escape from the regulation of endogenous protease inhibitors at the site of inflammation, which keeps it continuously activated and leads to ARDS (71). By contrast, Sivelestat sodium, a selective neutrophil elastase inhibitor, can inhibit the NE-related degradation of extracellular matrix and epithelial junction structure, further implicating its potential protective role in alleviating ARDS. However, clinical efficacy of sivelestat sodium remains controversial after preclinical and clinical studies of ARDS (71–73). Recently, Samurahara Central Hospital from Japan reported that two patients with COVID-19 at risk of ARDS were successfully weaned from ventilator 14 days after sivelestat sodium treatment (74). In China, sivelestat sodium is also approved to enter COVID-19 clinical trial by NMPA (Approval NO. 2020S00126). However, future studies are required to evaluate its reliability in inhibiting NE activity and the specific efficacy in the treatment of SARS-CoV-2-related ARDS.

CONCLUSION AND FUTURE DIRECTION

Although treatments targeting neutrophils may not kill SRAS-CoV-2 virus directly, they are beneficial to control unbalanced immune response and hyper-inflammation. It is worth noting that the appropriate therapeutic strategies should be carefully selected according to the progress of COVID-19 infection. For example, antiviral and strong immune stimulatory therapies should be administrated in the initial stage of viral infection, whereas immunosuppressive therapy should be performed once the cytokine storm is evident (42). In addition, better understanding neutrophil responses and molecular mechanisms of cellular activation during SARS-CoV-2 infection is needed, as filling this knowledge gap will provide in-depth guidance in characterizing patients based on neutrophil profiles and offers precision-based therapies (i.e., focusing on controlling viral infection by activating neutrophils vs. inhibiting neutrophil function to prevent excessive inflammatory damage). Because of the complex immunopathological changes in COVID-19, the exploration of adjunct therapies targeted at immune cells (such as regulation of neutrophil migration, accumulation and NETs formation) in combination with antiviral drugs may be of value for reducing mortality rate in patients with COVID-19.

REFERENCES

1. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, et al.: Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395(10223):507–513, 2020.
2. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, et al.: Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* 180(7):934–943, 2020.
3. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al.: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395(10223):497–506, 2020.
4. Zhao Z, Xie J, Yin M, Yang Y, He H, Jin T, Li W, Zhu X, Xu J, Zhao C, et al.: Clinical and laboratory profiles of 75 hospitalized patients with novel coronavirus disease 2019 in Hefei, China. *medRxiv* 2020. 2020.03.01.20029785.
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, et al.: clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 323(11): 1061–1069, 2020.
6. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, et al.: Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 8(4):420–422, 2020.
7. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, Péré H, Charbit B, Bondet V, Chenevier-Gobeaux C, et al.: Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* 369(6504):718–724, 2020.
8. Merad M, Martin JC: Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol* 20(6):355–362, 2020.
9. Zhou Z, Ren L, Zhang L, Zhong J, Xiao Y, Jia Z, Guo L, Yang J, Wang C, Jiang S, et al.: Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell Host Microbe* 27(6):883.e2–890.e2, 2020.
10. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, Luo M, Chen L, Zhao Y: Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *J Infect* 81(1):e6–e12, 2020.
11. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, et al.: Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395(10229): 1054–1062, 2020.
12. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, Zhang M, Tan J, Xu Y, Song R, et al.: Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. *J Transl Med* 18(1):206, 2020.
13. Middleton EA, He XY, Denorme F, Campbell RA, Ng D, Salvatore SP, Mostyka M, Baxter-Stoltzfus A, Borczuk AC, Loda M, et al.: Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood* 136(10):1169–1179, 2020.
14. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS: Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med* 8(7):681–686, 2020.
15. Kobayashi SD, Malachowa N, DeLeo FR: Influence of microbes on neutrophil life and death. *Front Cell Infect Microbiol* 7:159, 2017.
16. Németh T, Sperandio M, Mócsai A: Neutrophils as emerging therapeutic targets. *Nat Rev Drug Discov* 19(4):253–275, 2020.
17. Giacalone VD, Margaroli C, Mall MA, Tirouvanziam R: Neutrophil adaptations upon recruitment to the lung: new concepts and implications for homeostasis and disease. *Int J Mol Sci* 21(3):851, 2020.
18. Stegelmeier AA, van Vloten JP, Mould RC, Klafuric EM, Minott JA, Wootton SK, Bridle BW, Karimi K: Myeloid cells during viral infections and inflammation. *Viruses* 11(2):168, 2019.
19. Carignan D, Herblot S, Laliberté-Gagné M, Bolduc M, Duval M, Savard P, Leclerc D: Activation of innate immunity in primary human cells using a plant virus derived nanoparticle TLR7/8 agonist. *Nanomedicine* 14(7):2317–2327, 2018.
20. Saitoh S, Miyake K: Regulatory molecules required for nucleotide-sensing Toll-like receptors. *Immunol Rev* 227(1):32–43, 2009.
21. Wang JP, Bowen GN, Padden C, Cerny A, Finberg RW, Newburger PE, Kurt-Jones EA: Toll-like receptor-mediated activation of neutrophils by influenza A virus. *Blood* 112(5):2028–2034, 2008.
22. Hashimoto Y, Moki T, Takizawa T, Shiratsuchi A, Nakanishi Y: Evidence for phagocytosis of influenza virus-infected, apoptotic cells by neutrophils and macrophages in mice. *J Immunol* 178(4):2448–2457, 2007.
23. Totura AL, Whitmore A, Agnihothram S, Schäfer A, Katze MG, Heise MT, Baric RS: Toll-like receptor 3 Signaling via trif contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *mBio* 6(3):e00638–e00715, 2015.
24. Futosi K, Fodor S, Mócsai A: Neutrophil cell surface receptors and their intracellular signal transduction pathways. *Int Immunopharmacol* 17(3):638–650, 2013.
25. Tamassia N, Le Moigne V, Rossato M, Donini M, McCartney S, Calzetti F, Colonna M, Bazzoni F, Cassatella MA: Activation of an immunoregulatory and antiviral gene expression program in poly(I:C)-transfected human neutrophils. *J Immunol* 181(9):6563–6573, 2008.
26. Lifland AW, Jung J, Alonas E, Zurla C, Crowe JE Jr, Santangelo PJ: Human respiratory syncytial virus nucleoprotein and inclusion bodies antagonize the innate immune response mediated by MDA5 and MAVS. *J Virol* 86(15):8245–8258, 2012.
27. Yoboua F, Martel A, Duval A, Mukawera E, Grandvaux N: Respiratory syncytial virus-mediated NF-kappa B p65 phosphorylation at serine 536 is dependent on RIG-I, TRAF6, and IKK beta. *J Virol* 84(14):7267–7277, 2010.
28. Schett G, Neurath MF: Resolution of chronic inflammatory disease: universal and tissue-specific concepts. *Nat Commun* 9(1):3261, 2018.
29. Juliana A, Zonneveld R, Plötz FB, van Meurs M, Wilschut J: Neutrophil-endothelial interactions in respiratory syncytial virus bronchiolitis: an understudied aspect with a potential for prediction of severity of disease. *J Clin Virol* 123:104258, 2020.
30. Villenave R, Shields MD, Power UF: Respiratory syncytial virus interaction with human airway epithelium. *Trends Microbiol* 21(5):238–244, 2013.
31. Chang CH, Huang Y, Anderson R: Activation of vascular endothelial cells by IL-1alpha released by epithelial cells infected with respiratory syncytial virus. *Cell Immunol* 221(1):37–41, 2003.
32. Funchal GA, Jaeger N, Czepielewski RS, Machado MS, Muraro SP, Stein RT, Bonorino CB, Porto BN: Respiratory syncytial virus fusion protein promotes TLR-4-dependent neutrophil extracellular trap formation by human neutrophils. *PLoS One* 10(4):e0124082, 2015.
33. Russell CD, Unger SA, Walton M, Schwarze J: The human immune response to respiratory syncytial virus infection. *Clin Microbiol Rev* 30(2):481–502, 2017.
34. Narasaraju T, Yang E, Samy RP, Ng HH, Poh WP, Liew AA, Phoon MC, van Rooijen N, Chow VT: Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am J Pathol* 179(1):199–210, 2011.
35. Zhu B, Zhang R, Li C, Jiang L, Xiang M, Ye Z, Kita H, Melnick AM, Dent AL, Sun J: BCL6 modulates tissue neutrophil survival and exacerbates pulmonary inflammation following influenza virus infection. *Proc Natl Acad Sci U S A* 116(24):11888–11893, 2019.
36. Paules CI, Marston HD, Fauci AS: Coronavirus infections-more than just the common cold. *JAMA* 323(8):707–708, 2020.
37. Sallenave JM, Guillot L: Innate immune signaling and proteolytic pathways in the resolution or exacerbation of SARS-CoV-2 in Covid-19: key therapeutic targets? *Front Immunol*; 2020;(11):1229, 2020.

38. Radzikowska U, Ding M, Tan G, Zhakparov D, Peng Y, Wawrzyniak P, Wang M, Li S, Morita H, Altunbulakli C, et al.: Distribution of ACE2, CD147, CD26, and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. *Allergy* 75:2829–2845, 2020.
39. Chu H, Zhou J, Wong BH, Li C, Chan JF, Cheng ZS, Yang D, Wang D, Lee AC, Li C, et al.: Middle east respiratory syndrome coronavirus efficiently infects human primary T lymphocytes and activates the extrinsic and intrinsic apoptosis pathways. *J Infect Dis* 213(6):904–914, 2016.
40. Wang K, Chen W, Zhou Y-S, Lian J-Q, Zhang Z, Du P, Gong L, Zhang Y, Cui H-Y, Geng J-J, et al.: SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. *bioRxi*; 2020. 2020.03.14.988345.
41. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, Daßler-Plenker J, Guerci P, Huynh C, Knight JS, et al.: Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med* 217(6):e20200652, 2020.
42. Jamilloux Y, Henry T, Belot A, Viel S, Fauter M, El Jammal T, Walzer T, François B, Sève P: Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. *Autoimmun Rev* 19(7):102567, 2020.
43. Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S: Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin Rheumatol* 39(7):2085–2094, 2020.
44. Sokolowska M, Lukasik Z, Agache I, Akdis CA, Akdis D, Akdis M, Barcik W, Brough H, Eiwegger T, Eliaszewicz A, et al.: Immunology of COVID-19: mechanisms, clinical outcome, diagnostics and perspectives-a report of the European Academy of Allergy and Clinical Immunology (EAACI). *Allergy* 75:2445–2476, 2020.
45. Liew PX, Kubes P: The neutrophil's role during health and disease. *Physiol Rev* 99(2):1223–1248, 2019.
46. Ley K, Smith E, Stark MA: IL-17A-producing neutrophil-regulatory Tn lymphocytes. *Immunol Res* 34(3):229–242, 2006.
47. Stark MA, Huo Y, Burcin TL, Morris MA, Olson TS, Ley K: Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity* 22(3):285–294, 2005.
48. Pelaia C, Tinello C, Vatrella A, De Sarro G, Pelaia G: Lung under attack by COVID-19-induced cytokine storm: pathogenic mechanisms and therapeutic implications. *Ther Adv Respir Dis* 14:1753466620933508, 2020.
49. Phillipson M, Kubes P: The neutrophil in vascular inflammation. *Nat Med* 17(11):1381–1390, 2011.
50. Sandoval R, Lazcano P, Ferrari F, Pinto-Pardo N, Billault CG, Utreras E: TNF-α increases production of reactive oxygen species through Cdk5 activation in nociceptive neurons. *Front Physiol* 9:65, 2018.
51. Schönrich G, Raftery MJ, Samstag Y: Devilishly radical NETwork in COVID-19: oxidative stress, neutrophil extracellular traps (NETs), and T cell suppression. *Adv Biol Regul* 77:100741, 2020.
52. Akgun E, Tuzuner MB, Sahin B, Kilercik M, Kulah C, Cakiroglu HN, Serteser M, Unsal I, Baykal AT: Proteins associated with neutrophil degranulation are upregulated in nasopharyngeal swabs from SARS-CoV-2 patients. *PLoS One* 15(10):e0240012, 2020.
53. Yaqinuddin A, Kashir J: Novel therapeutic targets for SARS-CoV-2-induced acute lung injury: targeting a potential IL-1β/neutrophil extracellular traps feedback loop. *Med Hypotheses* 143:109906, 2020.
54. Eda H, Burnette BL, Shimada H, Hope HR, Monahan JB: Interleukin-1β-induced interleukin-6 production in A549 cells is mediated by both phosphatidylinositol 3-kinase and interleukin-1 receptor-associated kinase-4. *Cell Biol Int* 35(4):355–358, 2011.
55. Kollias A, Kyriakoulis KG, Dimakakos E, Poulakou G, Stergiou GS, Syrigos K: Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action. *Br J Haematol* 189(5):846–847, 2020.
56. Wichmann D, Sperhake JP, Lütgehetmann M, Steurer S, Edler C, Heinemann A, Heinrich F, Mushumba H, Kniep I, Schröder AS, et al.: Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med* 173(4):268–277, 2020.
57. Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, Baxter-Stoltzfus A, Laurence J: Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl Res* 220:1–13, 2020.
58. Noubouossie DF, Reeves BN, Strahl BD, Key NS: Neutrophils: back in the thrombosis spotlight. *Blood* 133(20):2186–2197, 2019.
59. Frantzeskaki F, Armaganidis A, Orfanos SE: Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation. *Respiration* 93(3):212–225, 2017.
60. Mócsai A: Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J Exp Med* 210(7):1283–1299, 2013.
61. Seitz R, Gürtler L, Schramm W: Thromboinflammation in COVID-19: Can α_2-macroglobulin help to control the fire? *J Thromb Haemost* 19:10, 2020.
62. Massberg S, Grahl L, von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, Brinkmann V, Lorenz M, Bidzhekov K, Khandagale AB, et al.: Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med* 16(8):887–896, 2010.
63. Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, Blair C, Weber A, Barnes BJ, Egeblad M, et al.: Neutrophil extracellular traps in COVID-19. *JCI Insight* 5(11):e138999, 2020.
64. Price LC, McCabe C, Garfield B, Wort SJ: Thrombosis and COVID-19 pneumonia: the clot thickens! *Eur Respir J* 56(1):2001608, 2020.
65. Gould TJ, Vu TT, Swystun LL, Dwivedi DJ, Mai SH, Weitz JI, Liaw PC: Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms. *Arterioscler Thromb Vasc Biol* 34(9):1977–1984, 2014.
66. de Bont CM, Boelens WC, Pruijn GJM: NETosis, complement, and coagulation: a triangular relationship. *Cell Mol Immunol* 16(1):19–27, 2019.
67. Ward PA, Fattahi F: New strategies for treatment of infectious sepsis. *J Leukoc Biol* 106(1):187–192, 2019.
68. Semeraro F, Ammollo CT, Morrissey JH, Dale GL, Friese P, Esmon NL, Esmon CT: Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood* 118(7): 1952–1961, 2011.
69. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, et al.: Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 8(5):475–481, 2020.
70. Kaynar AM, Houghton AM, Lum EH, Pitt BR, Shapiro SD: Neutrophil elastase is needed for neutrophil emigration into lungs in ventilator-induced lung injury. *Am J Respir Cell Mol Biol* 39(1):53–60, 2008.
71. Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A: The role of neutrophil elastase inhibitors in lung diseases. *Chest* 152(2):249–262, 2017.
72. Lee JM, Yeo CD, Lee HY, Rhee CK, Kim IK, Lee DG, Lee SH, Kim JW: Inhibition of neutrophil elastase contributes to attenuation of lipopolysaccharide-induced acute lung injury during neutropenia recovery in mice. *J Anesth* 31(3):397–404, 2017.
73. Tagami T, Tosa R, Omura M, Fukushima H, Kaneko T, Endo T, Rinka H, Murai A, Yamaguchi J, Yoshikawa K, et al.: Effect of a selective neutrophil elastase inhibitor on mortality and ventilator-free days in patients with increased extravascular lung water: a post hoc analysis of the PiCCO Pulmonary Edema Study. *J Intensive Care* 2(1):67, 2014.
74. Available at: https://www.botanical.jp/library_view.php?library_num=679. Accessed May 22, 2020.