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Review Article

Understanding the role of neutrophils in acute respiratory distress syndrome

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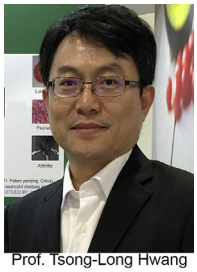
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ABSTRACT

Acute respiratory distress syndrome (ARDS) is difficult to treat and is associated with a high mortality rate. The most severe form of coronavirus disease 2019 (COVID-19) also leads to life-threatening ARDS. Neutrophil counts are positively correlated with disease severity in ARDS. Neutrophil activation not only plays a significant role in immune defense against infections, but also causes tissue damage and leads to inflammatory diseases. Activated neutrophils rapidly migrate to inflamed lung tissue, releasing toxic granular contents and generating neutrophil extracellular traps. In the last few decades, it has become apparent that neutrophils occupy a central role in ARDS pathology. In this review, we summarize the neutrophil inflammatory responses and their relationships to ARDS. According to the current literature, understanding the function of neutrophils may be helpful in the treatment of ARDS.

Acute respiratory distress syndrome (ARDS) is a life-threatening respiratory condition with an increasing incidence rate, ranging from 20 to 40%, and a mortality rate of approximately 40% [1,2]. On account of the outbreak of coronavirus disease 2019 (COVID-19), 29–42% COVID-19 patients developed ARDS and 15–52% of COVID-19 ARDS cases resulted in fatality [3,4]. Initially, the American-European Consensus Conference for ARDS defined acute lung injury

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(ALI) and ARDS as separate conditions. ALI was regarded as a less severe presentation of ARDS [5]. The Berlin definition of ARDS was updated, classified into mild, moderate, and severe grades based on the pulmonary oxygenation level. Due to its similarity to mild ARDS, ALI was removed from the Berlin definition [6]. The clinical symptoms and signs of ARDS are characterized by hypoxemia, marked diffuse bilateral pulmonary infiltrates, and extensive pulmonary edema. Studies of histological events of ARDS demonstrated that pulmonary edema is induced by increased vascular permeability, followed by protein-rich fluid in the alveolar space and accumulation of activated immune cells. The diffuse alveolar damage and accumulated immune cells lead to compromised gas exchange and respiratory failure [7,8]. Neutrophil infiltration in inflamed lung is a hallmark of ARDS [9]. Activated neutrophils trigger oxidative stress, release proteases, and form neutrophil extracellular traps (NETs), resulting in lung damage. However, neutrophils also play a role in the repair of inflamed lung tissues. Neutrophils are involved in the proliferation of type II pneumocytes during the repair phase after inflammatory lung injury [10]. It has been reported that neutropenia does not result in improved recovery in ARDS patients [11,12]. Although numerous studies have explored the pathogenesis of ARDS, there is still a lack of consensus regarding ARDS progression and effective pharmacotherapeutic treatment. Understanding the functional implications of neutrophils will allow exploration of applicable therapeutic strategies to reduce neutrophil-induced inflammatory lung injury. This review will focus on the roles of neutrophils and related immune products associated with ARDS.

Overview of lung inflammatory injury

The pathogenesis of ARDS is complex and classified as direct or indirect. Most commonly, direct injury results from infectious damage to lung tissue. The causes of lung injury by infectious stimuli are not fully known. Pneumococcus and influenza A virus originally infect bronchial epithelium, induce upper airway and alveolar damage, recruit neutrophils and macrophages, and amplify cytokine and chemokine production [13,14]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects pulmonary tissues, resulting in accumulation of massive amounts of immune cells and leads to an inflammatory cytokine storm. In COVID-19 ARDS patients, cytokine storm is a common characteristic. Patients with COVID-19 ARDS have elevated plasma levels of inflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, granulocyte colony-stimulating factor, interferon gamma, and tumor necrosis factor alpha (TNF-α). An excess of inflammatory cytokines further causes immune cell infiltration into inflamed lungs to induce alveolar damage and diminish lung function [3,15,16]. Another cause of direct lung injury is mechanical tissue damage, such as pulmonary contusion and inhalation of injurious materials. Pulmonary contusions primarily contribute to ARDS in traumatic patients by priming the innate immune response and enhancing the activity of toll-like receptor 4 resulting in excess production of pro-inflammatory mediators [17,18]. Additionally, indirect pulmonary injuries can be explained by the "2-hit hypothesis" which states that an inflammatory response related to extra-pulmonary area stimulus (first hit) is followed by systemic inflammatory response (second hit) that can induce lung injury. Common triggers of indirect lung injury induced by systemic inflammatory response include sepsis, shock, acute pancreatitis, bone fracture, and massive blood transfusion [19,20]. It has been shown that severe systemic inflammatory response resulting from sepsis produces numerous circulating inflammatory mediators. These mediators reach the bronchial and alveolar tissues and activate resident macrophages that attract inflammatory cells. Excess recruitment of immune cells not only occludes lung microcirculation but also releases cytotoxic products to damage surrounding tissues [21,22]. Moreover, the pathogenesis of transfusion-related acute lung injury (TRALI) occurs through antibody-dependent mechanisms. The first hit contributes to the underlying inflammatory condition of the patient. The second hit is related to antibodies in the transfused blood product [23]. Neutrophils are thought to mediate the development of TRALI. The antibodies from transfused blood components bind to the pulmonary endothelium followed by accumulation and activation of neutrophils. Activated neutrophils undergo respiratory burst and release ROS, release proteolytic enzymes by degranulation, and form NETs, which further contribute to lung inflammation [24].

Role of neutrophils in ARDS

Our research, along with other studies, has provided insights into the pathogenic role of neutrophils in various inflammatory states, including sepsis and ARDS [25,26]. Neutrophils are the first immune cells recruited to the site of inflammation following stimulation by chemotactic factors released from damaged pulmonary tissues. Both exogenous and endogenous inflammatory stimuli can be recognized by specific receptors of human neutrophils. This further promotes the recruitment and activation of circulating neutrophils. These activated neutrophils produce several cytotoxic products, including ROS, granular enzymes, NETs, and various pro-inflammatory cytokines [27]. Moreover, these immune products trigger several chemotactic signals to induce positive feedback, further enhancing inflammation. The overwhelming activation of neutrophils contributes to surrounding tissue damage and even lung dysfunction [Fig. 1] [28]. In COVID-19 ARDS patients, higher counts of neutrophil are observed and represent a predictor of poor outcome [29]. Therefore, strategies to diminish neutrophils in lung tissue, including decreasing neutrophil recruitment and impairing neutrophil immune functions, have been predicted to attenuate lung injury.

Neutrophil recruitment in ARDS

Neutrophil migration from the circulatory system into inflamed lung tissue can be initiated by both infectious and sterile inflammatory stimuli. Neutrophils attach to the vascular endothelium, and then migrate following a

chemokine gradient [30]. These chemoattractive signals are recognized by local immune cells, which produce inflammatory mediators that further boost neutrophil recruitment. In ARDS, endothelial cells activate and capture circulating neutrophils [31,32]. Neutrophils are sequestered through selectin-mediated binding, triggering "inside-out" activation of integrins, such as CD11a/CD18, which binds to intercellular adhesion molecules (ICAMs) from the endothelium [33].

Neutrophil rolling is a form of migration along the endothelial wall and is facilitated by specialized structure like membrane extensions and uropods. The rolling neutrophils become flattened and polarized to generate the forward-moving force, and then slowly crawl along the endothelial cells [34]. This slow rolling, or crawling, delays the neutrophils' passage through the inflammatory tissue. This phenomenon augments neutrophil recruitment through chemokine signaling. Crawling neutrophils can either pass between the endothelial cells or through them to exit the vessel lumen. Finally, the migrating neutrophils detach from the vessel wall and move into the inflamed respiratory tissues [35–37].

Once activated, pulmonary neutrophils exhibit various immune responses, including increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and release of proteolytic enzymes [38,39]. Overwhelming NADPH oxidase activity and proteolytic enzyme release are associated with surrounding respiratory tissue damage. Therefore, strategies to reduce neutrophil migration may be useful in the alleviation of pulmonary tissue damage [40,41]. Consistently, several chemical compounds were shown to diminish neutrophil migration in animal models. Decreasing neutrophil recruitment decreases production of cytotoxic mediators [42,43], whereas increased neutrophils in pulmonary tissue demonstrably contributes to the pathogenesis of ARDS. Notably, studies of human and animals with neutropenia and ARDS showed poor recovery [11,12]. Evidences that neutrophils promoted both pulmonary inflammation and repair explain these findings. Neutrophils participate in the remodeling of damaged lung tissue through release of MMP-9 and the Wnt/β-catenin pathway [44,45]. The role of neutrophil recruitment in ARDS is complex and still needs to be explored.

ARDS and neutrophil oxidative stress

In response to infectious and sterile stimuli, neutrophils contribute to the progression of ARDS through the assembly and activation of the ROS-producing NADPH oxidase complex (NOX2) [46,47]. NOX2, located on the membrane, reduces oxygen into superoxide anion and releases it outside the cell. Superoxide anion is highly reactive and spontaneously dismutates into a more stable hydrogen peroxide (H_2O_2). H_2O_2 can pass through cell membrane and distribute to either extracellular or intracellular areas. The enzyme myeloperoxidase uses H_2O_2 to produce a series of more reactive products, including hydroxyl radicals and hypochlorous acid [48,49]. ROS are harmful to pulmonary tissues, indicating that reducing ROS production is beneficial to mitigate lung inflammatory injury [50,51]. SARS-CoV-2 infections cause redox imbalance and induce ROS accumulation. Oxidative stress produced from downregulation of superoxide dismutase 3 expression in lung tissues is observed in elderly patients with

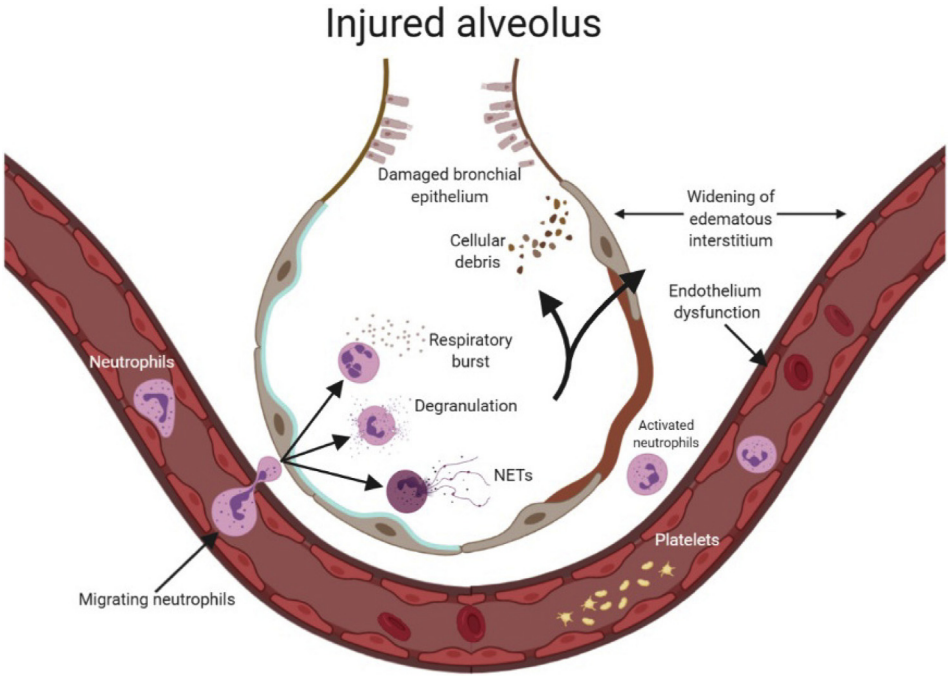

Fig. 1 Schematic diagram of neutrophil-mediated lung injury. Neutrophils migrate to inflamed lung tissue. This is followed by respiratory burst, degranulation, and NET formation in an injured alveolus. These immune responses lead to bronchial epithelial damage, cell debris production from dying cells attracting more immune cells, and endothelium dysfunction causing widening of edematous interstitium, further impairing oxygenation.

COVID-19, and the oxidative damage is closely related to poor outcome in elderly COVID-19 patients [52,53].

Several mechanisms of ROS are known. First, ROS directly damages the DNA of target cells. Previous research has shown that ROS produced from activated neutrophils induces oxidative DNA damage in respiratory epithelial cells [54,55]. Moreover, hyperoxia with 95% oxygen induces either neutrophil influx or oxidative DNA damage in neonate lung epithelial cells [56]. Second, ROS triggers peroxidation of lipid membranes, thus contributing to target cell lysis. The products of lipid peroxidation are also used as a measure of the level of tissue injury. In animal lung injury models, the level of lipid peroxidation was related to the severity of pulmonary tissue injury [57,58]. Third, ROS induces intracellular protein phosphorylation and transcription factor activation to promote the release of pro-inflammatory mediators. For instance, mitogen-activated protein (MAP) kinases can be activated by ROS to mediate cell death in response to stress, suggesting that the phosphorylation of MAP kinases have a major role in promotion of lung inflammatory injury [59,60]. Moreover, ROS triggers the activation of NF-κB, an important transcription factor that controls pro-inflammatory mediator release, to augment lung inflammatory injuries [61,62]. Research on the anti-oxidant α-tocopherol showed that ROS production and related expression of NF-κB in activated neutrophils was decreased, and lung inflammatory injury was ameliorated in the presence of α-tocopherol [63]. Similarity, another study showed that paraquat, an oxidant inducer, triggered ROS generation and release of inflammatory mediators, such as TNF-α, and IL-1β. The mechanisms of paraquat to heighten lung inflammation included activation of Jun N-terminal kinase (JNK) MAP kinase and NF-κB [64]. ROS damages the pulmonary endothelium, which is the barrier between the blood and vessels. Intracellular calcium signals are activated by ROS to increase endothelium permeability during pulmonary inflammatory process [65,66]. Cooperatively, neutrophil oxidative stress induces pulmonary endothelial and epithelial barrier dysfunctions. Barrier dysfunction increases neutrophil infiltration into lung tissues, and then these accumulated pulmonary neutrophils secrete several cytotoxic agents followed by worsening of pulmonary tissue damage, further enhancing the progression of ARDS [67].

ARDS and neutrophil degranulation

Neutrophil granules contain both pro- and anti-inflammatory substances that can be released to kill pathogens or for remodeling tissues. This process is called degranulation [68,69]. Neutrophils release membrane-bound vesicles, which contain various types of cytoplasmic granules associated with the progression of ARDS. The primary granules (also known as azurophilic granules) contain myeloperoxidase and many proteolytic proteins, including neutrophil elastase and cathepsin G. These granular proteins play major roles in microbicidal responses [70]. The secondary granules contain proteins that degrade the extracellular matrix. The tertiary granules contain few antimicrobials but store several metalloproteases [71,72]. During the inflammatory process, signals from receptors on the plasma membrane are transduced to the cytoplasm, which trigger the delivery of granule-associated proteins to the cell surface and secretion of their contents out of the cells. The regulation mechanisms are numerous, including calcium signaling, src family kinases, MAP kinases, and GTPase-related signaling [73,74].

Neutrophilic infiltration into respiratory inflammatory tissues is a means of defending the host against pathogens, however, overwhelming extracellular release of neutrophil granular enzymes have been implicated in triggering collateral pulmonary tissue damage in ARDS [75,76]. Neutrophil elastase is a primary granular proteolytic enzyme and impacts lung inflammatory injury. Elastase can degrade pulmonary extracellular matrix proteins and cleave epithelial-cadherin to break respiratory cell-cell adhesion. Moreover, elastase has been shown to damage pulmonary vascular endothelial glycocalyx during the pulmonary inflammatory process [77–79]. Myeloperoxidase, the other primary granular enzyme, catalyzes H_2O_2 to produce toxic ROS, which has been demonstrated to be a local mediator of alveolar damage [80]. The family of matrix metalloproteinases (MMPs) are released from the secondary and tertiary granules. Previous research showed that the expression of MMPs was correlated with the progression of ARDS. The enhanced expression of MMPs was related to inflammation-induced endothelial injury and impaired oxygenation in ARDS [81,82]. Another study revealed that MMP inhibitors suppressed MMP expression in plasma and lung tissue, decreased inflammatory mediator release, and attenuated lung inflammatory injury [83,84].

ARDS and NET formation

NETs are extracellular fibrous net-like structures, which have neutrophil granular proteins, such as myeloperoxidase or neutrophil elastase, coated on the backbone structures of DNA [85,86]. The mechanisms that induce NET formation have been explored, but signaling pathways involved are not completely understood. Raf/MEK/Erk signaling is activated to induce ROS production in the process of NET formation. Upon activation, the granular enzymes, myeloperoxidase and elastase, are released into the nucleus to induce nuclear disintegration. These granular enzymes coupled with PAD4 trigger chromatin decondensation and release. This mixture of granular enzymes and chromatin is expulsed, followed by cell membrane rupture [87–89].

NETs are thought to be part of an innate immune mechanism for pathogen clearance; however, there are increasing concerns about the potential of NETs to initiate and propagate inflammatory damages in host tissues. Interestingly, both sterile and infective inflammations promote NET formation. During sterile inflammation, the components of NETs were found in lung and plasma of TRALI patients and mice. Treatment with DNase 1 decreased NET formation and lung injury [90,91]. Moreover, NET formation was observed in ventilator-induced lung injury. Previous human and animal studies showed that NET formation in the alveolar space was higher in bronchoalveolar lavage fluid from subjects with ventilator-induced ARDS. However, decreasing NET formation was not related to the shortening of mechanical ventilation [92–94]. During infective inflammation, NETs captured respiratory syncytial virus or influenza virus particles in patients and mice with respiratory infection. Nevertheless, cumulative

NET formation not only obstructed small airways, but also damaged alveolar–capillary to induce lung inflammatory injury [95–97]. The characteristics of lung tissue in ARDS demonstrated intensive neutrophil infiltration of lung tissue and NETs entwined with alveoli were exhibited in infected mice. The progression of inflammation further induced alveolar damage and pulmonary edema. Treatment with DNase I degraded NETs and reduced inflammatory cytokines, indicating that decreasing NET formation ameliorated lung injury and improved survival [98–100].

The severity and mortality of COVID-19 ARDS are correlated to higher neutrophil counts and NET level [29,101]. NETs play a significant role in producing a cytokine storm [102,103]. Furthermore, NETs have the ability to trigger immunothrombosis. The pulmonary capillary from patients with COVID-19 ARDS is filled with microthrombus and NETs. Microthrombi and fibrin deposition in lungs results in reduced oxygenation and further induces ARDS in COVID-19 patients [104–106].

Conclusion

Although neutrophil immune responses are involved in ARDS, many questions remain unanswered. Neutrophils produce pro-inflammatory mediators and also augment inflammatory damage, implying that targeting neutrophils is a potential therapeutic for ARDS and COVID-19. However, on the basis of evidence supporting the fact that neutropenia does not attenuate ARDS, perhaps neutrophils play different roles in various phases of acute inflammation. The eventual goal is to determine when and how neutrophil activation is beneficial so as to tilt the balance toward benefit rather than harm.

Conflicts of interest

The authors declare no competing financial interests.

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REFERENCES

[1] Pham T, Rubenfeld GD. Fifty years of research in ARDS. The epidemiology of acute respiratory distress syndrome. A 50th birthday review. Am J Respir Crit Care Med 2017;195:860–70.
[2] Sigurdsson MI, Sigvaldason K, Gunnarsson TS, Moller A, Sigurdsson GH. Acute respiratory distress syndrome: nationwide changes in incidence, treatment and mortality over 23 years. Acta Anaesthesiol Scand 2013;57:37–45.
[3] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
[4] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, 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 2020;180:1–11.
[5] Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med 1994;149:818–24.
[6] Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. J Am Med Assoc 2012;307:2526–33.
[7] Papazian L, Calfee CS, Chiumello D, Luyt CE, Meyer NJ, Sekiguchi H, et al. Diagnostic workup for ARDS patients. Intensive Care Med 2016;42:674–85.
[8] Bourenne J, Carvelli J, Papazian L. Evolving definition of acute respiratory distress syndrome. J Thorac Dis 2019;11:S390–3.
[9] Zemans RL, Matthay MA. What drives neutrophils to the alveoli in ARDS? Thorax 2017;72:1–3.
[10] Paris AJ, Liu Y, Mei J, Dai N, Guo L, Spruce LA, et al. Neutrophils promote alveolar epithelial regeneration by enhancing type II pneumocyte proliferation in a model of acid-induced acute lung injury. Am J Physiol Lung Cell Mol Physiol 2016;311:L1062–75.
[11] Yacoub AT, Mojica L, Jones L, Knab A, Alrabaa S, Greene J. The role of corticosteroids in adult respiratory distress syndrome caused by viridans group streptococci bacteremia in neutropenic patients. Mediterr J Hematol Infect Dis 2014;6:e2014055.
[12] Mokart D, van Craenenbroeck T, Lambert J, Textoris J, Brun JP, Sannini A, et al. Prognosis of acute respiratory distress syndrome in neutropenic cancer patients. Eur Respir J 2012;40:169–76.
[13] Gotts JE, Bernard O, Chun L, Croze RH, Ross JT, Nesseler N, et al. Clinically relevant model of pneumococcal pneumonia, ARDS, and nonpulmonary organ dysfunction in mice. Am J Physiol Lung Cell Mol Physiol 2019;317:L717–36.
[14] Shah RD, Wunderink RG. Viral pneumonia and acute respiratory distress syndrome. Clin Chest Med 2017;38:113–25.
[15] Yeleswaram S, Smith P, Burn T, Covington M, Juvekar A, Li Y, et al. Inhibition of cytokine signaling by ruxolitinib and implications for COVID-19 treatment. Clin Immunol 2020;218:108517.
[16] McElvaney OJ, McEvoy N, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, et al. Characterization of the inflammatory response to severe COVID-19 illness. Am J Respir Crit Care Med 2020;202:812–21.
[17] Hoth JJ, Martin RS, Yoza BK, Wells JD, Meredith JW, McCall CE. Pulmonary contusion primes systemic innate immunity responses. J Trauma 2009;67:14–21.
[18] Daurat A, Millet I, Roustan JP, Maury C, Taourel P, Jaber S, et al. Thoracic Trauma Severity score on admission allows to determine the risk of delayed ARDS in trauma patients with pulmonary contusion. Injury 2016;47:147–53.
[19] Eworuke E, Major JM, Gilbert McClain LI. National incidence rates for acute respiratory distress syndrome (ARDS) and ARDS cause-specific factors in the United States (2006-2014). J Crit Care 2018;47:192–7.
[20] Hoegl S, Burns N, Angulo M, Francis D, Osborne CM, Mills TW, et al. Capturing the multifactorial nature of ARDS - "Two-hit" approach to model murine acute lung injury. Phys Rep 2018;6:e13648.

[21] Deitch EA. Gut-origin sepsis: evolution of a concept. Surgeon 2012;10:350–6.
[22] Whitney JE, Zhang B, Koterba N, Chen F, Bush J, Graham K, et al. Systemic endothelial activation is associated with early acute respiratory distress syndrome in children with extrapulmonary sepsis. Crit Care Med 2020;48:344–52.
[23] Roubinian N. TACO and TRALI: biology, risk factors, and prevention strategies. Hematology Am Soc Hematol Educ Program 2018;2018:585–94.
[24] Jongerius I, Porcelijn L, van Beek AE, Semple JW, van der Schoot CE, Vlaar APJ, et al. The role of complement in transfusion-related acute lung injury. Transfus Med Rev 2019;33:236–42.
[25] Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A. The role of neutrophil elastase inhibitors in lung diseases. Chest 2017;152:249–62.
[26] Yang SC, Chang SH, Hsieh PW, Huang YT, Ho CM, Tsai YF, et al. Dipeptide HCH6-1 inhibits neutrophil activation and protects against acute lung injury by blocking FPR1. Free Radic Biol Med 2017;106:254–69.
[27] Yang SC, Chen PJ, Chang SH, Weng YT, Chang FR, Chang KY, et al. Luteolin attenuates neutrophilic oxidative stress and inflammatory arthritis by inhibiting Raf1 activity. Biochem Pharmacol 2018;154:384–96.
[28] Aulakh GK. Neutrophils in the lung: "the first responders". Cell Tissue Res 2018;371:577–88.
[29] Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect 2020;81:e6–12.
[30] Williams AE, Jose RJ, Mercer PF, Brealey D, Parekh D, Thickett DR, et al. Evidence for chemokine synergy during neutrophil migration in ARDS. Thorax 2017;72:66–73.
[31] Zindel J, Kubes P. DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. Annu Rev Pathol 2020;15:493–518.
[32] Vassallo A, Wood AJ, Subburayalu J, Summers C, Chilvers ER. The counter-intuitive role of the neutrophil in the acute respiratory distress syndrome. Br Med Bull 2019;131:43–55.
[33] Lam FW, Da Q, Guillory B, Cruz MA. Recombinant human vimentin binds to P-selectin and blocks neutrophil capture and rolling on platelets and endothelium. J Immunol 2018;200:1718–26.
[34] Hind LE, Vincent WJ, Huttenlocher A. Leading from the back: the role of the uropod in neutrophil polarization and migration. Dev Cell 2016;38:161–9.
[35] Proebstl D, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, Jones GE, et al. Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. J Exp Med 2012;209:1219–34.
[36] Matsumoto M, Hirata T. Moesin regulates neutrophil rolling velocity in vivo. Cell Immunol 2016;304–305:59–62.
[37] Maas SL, Soehnlein O, Viola JR. Organ-specific mechanisms of transendothelial neutrophil migration in the lung, liver, kidney, and aorta. Front Immunol 2018;9:2739.
[38] Zhao C, Itagaki K, Gupta A, Odom S, Sandler N, Hauser CJ. Mitochondrial damage-associated molecular patterns released by abdominal trauma suppress pulmonary immune responses. J Trauma Acute Care Surg 2014;76:1222–7.
[39] Endo D, Fujimoto K, Hirose R, Yamanaka H, Homme M, Ishibashi KI, et al. Genetic phagocyte NADPH oxidase deficiency enhances nonviable Candida albicans-induced inflammation in mouse lungs. Inflammation 2017;40:123–35.
[40] Qin X, Zhu G, Huang L, Zhang W, Huang Y, Xi X. LL-37 and its analog FF/CAP18 attenuate neutrophil migration in sepsis-induced acute lung injury. J Cell Biochem 2019;120:4863–71.
[41] Faller S, Hausler F, Goeft A, von Itter MA, Gyllenram V, Hoetzel A, et al. Hydrogen sulfide limits neutrophil transmigration, inflammation, and oxidative burst in lipopolysaccharide-induced acute lung injury. Sci Rep 2018;8:14676.
[42] Chen CY, Tsai YF, Huang WJ, Chang SH, Hwang TL. Propofol inhibits endogenous formyl peptide-induced neutrophil activation and alleviates lung injury. Free Radic Biol Med 2018;129:372–82.
[43] Baudiss K, de Paula Vieira R, Cicko S, Ayata K, Hossfeld M, Ehrat N, et al. C1P attenuates lipopolysaccharide-induced acute lung injury by preventing NF-kappaB activation in neutrophils. J Immunol 2016;196:2319–26.
[44] Blazquez-Prieto J, Lopez-Alonso I, Amado-Rodriguez L, Huidobro C, Gonzalez-Lopez A, Kuebler WM, et al. Impaired lung repair during neutropenia can be reverted by matrix metalloproteinase-9. Thorax 2018;73:321–30.
[45] Zemans RL, Briones N, Campbell M, McClendon J, Young SK, Suzuki T, et al. Neutrophil transmigration triggers repair of the lung epithelium via beta-catenin signaling. Proc Natl Acad Sci USA 2011;108:15990–5.
[46] Nguyen GT, Green ER, Mecsas J. Neutrophils to the ROScue: mechanisms of NADPH oxidase activation and bacterial resistance. Front Cell Infect Microbiol 2017;7:373.
[47] Teixeira G, Szyndralewiez C, Molango S, Carnesecchi S, Heitz F, Wiesel P, et al. Therapeutic potential of NADPH oxidase 1/4 inhibitors. Br J Pharmacol 2017;174:1647–69.
[48] Winterbourn CC, Kettle AJ, Hampton MB. Reactive oxygen species and neutrophil function. Annu Rev Biochem 2016;85:765–92.
[49] Pak O, Sydykov A, Kosanovic D, Schermuly RT, Dietrich A, Schroder K, et al. Lung ischaemia-reperfusion injury: the role of reactive oxygen species. Adv Exp Med Biol 2017;967:195–225.
[50] Wagner J, Strosing KM, Spassov SG, Lin Z, Engelstaedter H, Tacke S, et al. Sevoflurane posttreatment prevents oxidative and inflammatory injury in ventilator-induced lung injury. PloS One 2018;13:e0192896.
[51] Nadeem A, Siddiqui N, Al-Harbi NO, Attia SM, AlSharari SD, Ahmad SF. Acute lung injury leads to depression-like symptoms through upregulation of neutrophilic and neuronal NADPH oxidase signaling in a murine model. Int Immunopharm 2017;47:218–26.
[52] Abouhashem AS, Singh K, Azzazy HME, Sen CK. Is low alveolar type II cell SOD3 in the lungs of elderly linked to the observed severity of COVID-19? Antioxid Redox Signal 2020;33:59–65.
[53] Silvagno F, Vernone A, Pescarmona GP. The role of glutathione in protecting against the severe inflammatory response triggered by COVID-19. Antioxidants 2020;9:624.
[54] Gould NS, White CW, Day BJ. A role for mitochondrial oxidative stress in sulfur mustard analog 2-chloroethyl ethyl sulfide-induced lung cell injury and antioxidant protection. J Pharmacol Exp Ther 2009;328:732–9.
[55] Park JS, Park YJ, Kim HR, Chung KH. Polyhexamethylene guanidine phosphate-induced ROS-mediated DNA damage caused cell cycle arrest and apoptosis in lung epithelial cells. J Toxicol Sci 2019;44:415–24.
[56] Auten RL, Whorton MH, Nicholas Mason S. Blocking neutrophil influx reduces DNA damage in hyperoxia-exposed newborn rat lung. Am J Respir Cell Mol Biol 2002;26:391–7.
[57] Binder CJ, Papac-Milicevic N, Witztum JL. Innate sensing of oxidation-specific epitopes in health and disease. Nat Rev Immunol 2016;16:485–97.
[58] Fukuhara K, Nakashima T, Abe M, Masuda T, Hamada H, Iwamoto H, et al. Suplatast tosilate protects the lung against

hyperoxic lung injury by scavenging hydroxyl radicals. Free Radic Biol Med 2017;106:1–9.
[59] Usatyuk PV, Vepa S, Watkins T, He D, Parinandi NL, Natarajan V. Redox regulation of reactive oxygen species-induced p38 MAP kinase activation and barrier dysfunction in lung microvascular endothelial cells. Antioxid Redox Signal 2003;5:723–30.
[60] Dias-Freitas F, Metelo-Coimbra C, Roncon-Albuquerque Jr R. Molecular mechanisms underlying hyperoxia acute lung injury. Respir Med 2016;119:23–8.
[61] Jiang K, Guo S, Yang C, Yang J, Chen Y, Shaukat A, et al. Barbaloin protects against lipopolysaccharide (LPS)-induced acute lung injury by inhibiting the ROS-mediated PI3K/AKT/NF-kappaB pathway. Int Immunopharm 2018;64:140–50.
[62] Wang J, Huang J, Wang L, Chen C, Yang D, Jin M, et al. Urban particulate matter triggers lung inflammation via the ROS-MAPK-NF-kappaB signaling pathway. J Thorac Dis 2017;9:4398–412.
[63] Rocksen D, Ekstrand-Hammarstrom B, Johansson L, Bucht A. Vitamin E reduces transendothelial migration of neutrophils and prevents lung injury in endotoxin-induced airway inflammation. Am J Respir Cell Mol Biol 2003;28:199–207.
[64] Shen H, Wu N, Wang Y, Han X, Zheng Q, Cai X, et al. JNK inhibitor SP600125 attenuates paraquat-induced acute lung injury: an in vivo and in vitro study. Inflammation 2017;40:1319–30.
[65] Di A, Mehta D, Malik AB. ROS-activated calcium signaling mechanisms regulating endothelial barrier function. Cell Calcium 2016;60:163–71.
[66] Liu XY, Xu HX, Li JK, Zhang D, Ma XH, Huang LN, et al. Neferine protects endothelial glycocalyx via mitochondrial ROS in lipopolysaccharide-induced acute respiratory distress syndrome. Front Physiol 2018;9:102.
[67] Kellner M, Noonepalle S, Lu Q, Srivastava A, Zemskov E, Black SM. ROS signaling in the pathogenesis of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Adv Exp Med Biol 2017;967:105–37.
[68] Lacy P. Mechanisms of degranulation in neutrophils. Allergy Asthma Clin Immunol 2006;2:98–108.
[69] Mollinedo F. Neutrophil degranulation, plasticity, and cancer metastasis. Trends Immunol 2019;40:228–42.
[70] Bedouhene S, Dang PM, Hurtado-Nedelec M, El-Benna J. Neutrophil degranulation of azurophil and specific granules. Methods Mol Biol 2020;2087:215–22.
[71] Soehnlein O. Multiple roles for neutrophils in atherosclerosis. Circ Res 2012;110:875–88.
[72] Pham CT. Neutrophil serine proteases: specific regulators of inflammation. Nat Rev Immunol 2006;6:541–50.
[73] Miranda-Ribera A, Lecchi C, Bronzo V, Scaccabarozzi L, Sartorelli P, Franciosi F, et al. Down-regulatory effect of alpha 1-acid glycoprotein on bovine neutrophil degranulation. Comp Immunol Microbiol Infect Dis 2010;33:291–306.
[74] Armstrong CL, Miralda I, Neff AC, Tian S, Vashishta A, Perez L, et al. Filifactor alocis promotes neutrophil degranulation and chemotactic activity. Infect Immun 2016;84:3423–33.
[75] Rebetz J, Semple JW, Kapur R. The pathogenic involvement of neutrophils in acute respiratory distress syndrome and transfusion-related acute lung injury. Transfus Med Hemother 2018;45:290–8.
[76] Moraes TJ, Zurawska JH, Downey GP. Neutrophil granule contents in the pathogenesis of lung injury. Curr Opin Hematol 2006;13:21–7.
[77] Mebratu YA, Tesfaigzi Y. IL-17 plays a role in respiratory syncytial virus-induced lung inflammation and emphysema in elastase and LPS-injured mice. Am J Respir Cell Mol Biol 2018;58:717–26.
[78] Suzuki K, Okada H, Takemura G, Takada C, Kuroda A, Yano H, et al. Neutrophil elastase damages the pulmonary endothelial glycocalyx in lipopolysaccharide-induced experimental endotoxemia. Am J Pathol 2019;189:1526–35.
[79] Boxio R, Wartelle J, Nawrocki-Raby B, Lagrange B, Malleret L, Hirche T, et al. Neutrophil elastase cleaves epithelial cadherin in acutely injured lung epithelium. Respir Res 2016;17:129.
[80] Aratani Y. Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys 2018;640:47–52.
[81] Fligiel SE, Standiford T, Fligiel HM, Tashkin D, Strieter RM, Warner RL, et al. Matrix metalloproteinases and matrix metalloproteinase inhibitors in acute lung injury. Hum Pathol 2006;37:422–30.
[82] Zinter MS, Delucchi KL, Kong MY, Orwoll BE, Spicer AS, Lim MJ, et al. Early plasma matrix metalloproteinase profiles. A novel pathway in pediatric acute respiratory distress syndrome. Am J Respir Crit Care Med 2019;199:181–9.
[83] Wang CT, Zhang L, Wu HW, Wei L, Xu B, Li DM. Doxycycline attenuates acute lung injury following cardiopulmonary bypass: involvement of matrix metalloproteinases. Int J Clin Exp Pathol 2014;7:7460–8.
[84] Albaiceta GM, Gutierrez-Fernandez A, Garcia-Prieto E, Puente XS, Parra D, Astudillo A, et al. Absence or inhibition of matrix metalloproteinase-8 decreases ventilator-induced lung injury. Am J Respir Cell Mol Biol 2010;43:555–63.
[85] Van Avondt K, Hartl D. Mechanisms and disease relevance of neutrophil extracellular trap formation. Eur J Clin Invest 2018;48:e12919.
[86] Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303:1532–5.
[87] Hakkim A, Fuchs TA, Martinez NE, Hess S, Prinz H, Zychlinsky A, et al. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. Nat Chem Biol 2011;7:75–7.
[88] Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 2018;18:134–47.
[89] Burgener SS, Schroder K. Neutrophil extracellular traps in host defense. Cold Spring Harb Perspect Biol 2020;12:a037028.
[90] Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J Clin Invest 2012;122:2661–71.
[91] Thomas GM, Carbo C, Curtis BR, Martinod K, Mazo IB, Schatzberg D, et al. Extracellular DNA traps are associated with the pathogenesis of TRALI in humans and mice. Blood 2012;119:6335–43.
[92] Bendib I, de Chaisemartin L, Granger V, Schlemmer F, Maitre B, Hue S, et al. Neutrophil extracellular traps are elevated in patients with pneumonia-related acute respiratory distress syndrome. Anesthesiology 2019;130:581–91.
[93] Yildiz C, Palaniyar N, Otulakowski G, Khan MA, Post M, Kuebler WM, et al. Mechanical ventilation induces neutrophil extracellular trap formation. Anesthesiology 2015;122:864–75.
[94] Mikacenic C, Moore R, Dmyterko V, West TE, Altemeier WA, Liles WC, et al. Neutrophil extracellular traps (NETs) are increased in the alveolar spaces of patients with ventilator-associated pneumonia. Crit Care 2018;22:358.
[95] Cortjens B, de Boer OJ, de Jong R, Antonis AF, Sabogal Pineros YS, Lutter R, et al. Neutrophil extracellular traps cause airway obstruction during respiratory syncytial virus disease. J Pathol 2016;238:401–11.

[96] Juliana A, Zonneveld R, Plotz 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 2020;123:104258.
[97] Ashar HK, Mueller NC, Rudd JM, Snider TA, Achanta M, Prasanthi M, et al. The role of extracellular histones in influenza virus pathogenesis. Am J Pathol 2018;188:135–48.
[98] Liu S, Su X, Pan P, Zhang L, Hu Y, Tan H, et al. Neutrophil extracellular traps are indirectly triggered by lipopolysaccharide and contribute to acute lung injury. Sci Rep 2016;6:37252.
[99] Gan TT, Yang YL, Hu F, Chen XC, Zhou JW, Li Y, et al. TLR3 regulated poly I:C-induced neutrophil extracellular traps and acute lung injury partly through p38 MAP kinase. Front Microbiol 2018;9:3174.
[100] Lefrancais E, Mallavia B, Zhuo HJ, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. JCI Insight 2018;3:e98178.
[101] Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. Am J Hematol 2020;95:834–47.
[102] Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. J Exp Med 2020;217:e20200652.
[103] Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, et al. Neutrophil extracellular traps in COVID-19. JCI Insight 2020;5:e138999.
[104] Whyte CS, Morrow GB, Mitchell JL, Chowdary P, Mutch NJ. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. J Thromb Haemost 2020;18:1548–55.
[105] Zhang H, Zhou P, Wei Y, Yue H, Wang Y, Hu M, et al. Histopathologic changes and SARS-CoV-2 immunostaining in the lung of a patient with COVID-19. Ann Intern Med 2020;172:629–32.
[106] Middleton EA, He XY, Denorme F, Campbell RA, Ng D, Salvatore SP, et al. Neutrophil extracellular traps (NETs) contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 2020;136:1169–79.