Neutrophil subsets and their differential roles in viral respiratory diseases

Yuning Zhang\textsuperscript{1} | Quanbo Wang\textsuperscript{2} | Charles R Mackay\textsuperscript{2,3} | Lai Guan Ng\textsuperscript{4,5,6,7,8} | Immanuel Kwok\textsuperscript{4}

\textsuperscript{1} Department of Research, National Skin Centre, Singapore, Singapore
\textsuperscript{2} School of Pharmaceutical Sciences, Shandong Analysis and Test Center, Qilu University of Technology (Shandong Academy of Sciences), Jinan, China
\textsuperscript{3} Department of Microbiology, Infection and Immunity Program, Biomedicine Discovery Institute, Monash University, Melbourne, Australia
\textsuperscript{4} Singapore Immunology Network (SIgN), A*STAR (Agency for Science, Technology and Research), Biopolis, Singapore
\textsuperscript{5} State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China
\textsuperscript{6} School of Biological Sciences, Nanyang Technological University, Singapore, Singapore
\textsuperscript{7} Department of Microbiology and Immunology, Immunology Translational Research Program, Yong Loo Lin School of Medicine, Immunology Program, Life Sciences Institute, National University of Singapore, Singapore, Singapore
\textsuperscript{8} National Cancer Centre Singapore, Singapore, Singapore

\textbf{Correspondence}

Lai Guan Ng, Singapore Immunology Network (SIgN), A*STAR (Agency for Science, Technology and Research), Biopolis 138648, Singapore.
Email: Ng_Lai_Guan@immunol.a-star.edu.sg

Lai Guan Ng and Immanuel Kwok are the corresponding authors.

\section{INTRODUCTION}

Neutrophils are known as early responders against bacterial or fungal infections, releasing their powerful antimicrobial arsenal to neutralize and contain the infection. Neutrophils were first reported by Elie Metchnikoff in 1883 as professional phagocytes.\textsuperscript{1} They are capable of engulfing foreign microbes and neutralizing them with various secretory granules\textsuperscript{2} and potent reactive oxygen species (ROS).\textsuperscript{3} Neutrophils also have the unique ability to release neutrophil extracellular traps (NETs) to limit the spread of infections.\textsuperscript{4} These NETs were shown to help neutralize large fungal hypha together with the secretion of cytotoxic granules.\textsuperscript{5,6} The effects of neutrophil-mediated immunity are
further amplified as neutrophils work in large numbers, forming a concerted effort to eliminate foreign threats. Although beneficial, these effects can often be detrimental to the host tissues and the immune response, including further exacerbation of organ damage, resulting in critical and life-threatening conditions. In viral diseases, however, the roles neutrophils play are not well understood. Furthermore, the recent evidence of a strong neutrophil presence in severe acute respiratory syndrome coronavirus (SARS-CoV2)-infected patients has garnered interest in the function these neutrophils elicit in the progression of COVID-19 disease.

Neutrophils are innate immune cells that are frequently overlooked in discussions of viral immunity, in part because adaptive immune cells such as T and B cells are recognized to play essential roles in initiating cytotoxic killing and antibody generation in response to viral infection, both of which are major steps toward viral clearance. While the role of neutrophils in viral infection is still unclear, growing evidence suggests that neutrophils play a role in resolving viral infection. For instance, data from preclinical mouse models of influenza A virus (IAV) infection show depletion of neutrophils by anti-Ly6G or anti-Gr1 antibody treatment led to severe disease outcomes in infected mice. Similar findings were also made in encephalitis and HSV-1 infection models, supporting the need for neutrophils to achieve optimal viral immunity. Studies have also demonstrated that mice with deficient inflammasome signaling through the NLPR3 pathway have poor neutrophil recruitment due to decreased production of neutrophil chemokines such as KC, TNF-α, and IL-1β, which results in severe pathology and mortality following an IAV infection. Neutrophils in respiratory syncytial virus (RSV) infections, on the other hand, have been proven in several studies to have no influence on viral load or pathogenicity. Although some studies suggest that neutrophils play a beneficial or redundant role in the body, clinical observation indicated that a high neutrophil count is associated with the severity of many diseases. For instance, hematologic analysis and clinical studies of COVID-19 patients’ neutrophil counts showed strong association with disease severity. It has been proposed that the neutrophil-to-lymphocyte ratio (NLR), which has been used to stratify cancer patients, may be utilized as a predictive and prognostic marker for COVID-19 patients. These results suggest that the presence of neutrophils in severe types of inflammation is linked to disease severity in COVID-19 patients. Here, we will review the current evidence of neutrophil heterogeneity and consolidate information on neutrophil function in viral respiratory infection. We expect to better understand the role of neutrophils in the immune response to viral respiratory infections and their participation in the pathology of the most severe cases.

2 | DEVELOPMENT AND FUNCTIONAL HETEROGENEITY OF NEUTROPHILS

The daily production of billions of neutrophils takes place within the bone marrow, where committed progenitors reside in specialized niches providing growth signals and developmental cues. Due to the short lifespan of neutrophils, this developmental process is critical in producing a steady supply to the circulation. Granulopoiesis is therefore well studied, and is described by the characterization of the various maturation stages through their granule content and morphologic attributes. According to their granule content and nuclear shape, neutrophils are produced in a step-wise order of early myoblasts → promyelocytes → myelocytes → metamyelocytes → band cells → segmented neutrophils. Under homeostatic conditions, only the mature segmented neutrophils exit the bone marrow, performing their various roles. However, during inflammatory conditions, hematologists typically observe immature phenotypes of neutrophils in the circulation. This is commonly known as a left shift and is used as an indicator of inflammation.

These immature phenotypes have been widely reported in various inflammatory conditions, ranging from cancer, pregnancy, stress, cardiovascular diseases, and notably viral infections. The way these immature neutrophils are characterized differs between research groups. There is no standardized approach for phenotyping neutrophil subsets, making it difficult to corroborate functional data from multiple laboratories. Some groups have proposed markers to identify these subsets, such as CD10, CD177, Olfactomedin-4 (OLFM4), and CD49d. Separating these subtypes can be difficult since they might emerge from various distinct neutrophil precursor stages with different degrees of maturity. A more profound knowledge of the ontogeny of the neutrophils will be required to overcome this issue. In recent years, several research groups have characterized neutrophil development at each step of differentiation and maturation. The advancements in single-cell technologies and sequencing techniques have led to the identification and characterization of various neutrophil progenitors and precursors in both mice and humans. This includes the early progenitors, such as the proNeus and eNePs, as well as late precursors preNeus, NePs, and NeuPs. In-depth single-cell analyses further reveal finer transcriptomic distinct developmental stages (Figure 1). These studies demonstrate that immature neutrophils can exist in the circulation during disease states, creating a heterogeneity of developmental states that can potentially elicit various unique functions in response to the inflammatory stimulus. However, it is still unclear if these immature forms go on to become functionally distinct mature neutrophils.

Mature neutrophils undergo an ageing process when released into the circulation, decreasing their expression of L selectin (CD62L) while increasing chemokine receptor CXCR4 expression. Aged neutrophils are then cleared from the circulation by specialized efferocytic macrophages present in the lung, liver, and spleen. This physiologic process regulates granulopoiesis, providing feedback signaling to the bone marrow through an IL-23 and IL-17-mediated manner. Clearance of aged neutrophils also prevents unwanted necrosis or spillage of their store of cytotoxic granules. Interfering with this clearance process results in an accumulation of circulating aged neutrophils. This build-up of exhausted phagocytes has been shown to play significant roles in disease. Aged neutrophils have a much higher phagocytic activity as compared with the non-aged neutrophils. Additionally, they respond faster toward inflammatory signals, migrating to sites of
infection to neutralize threats. A transgenic mouse model affecting the ageing process in neutrophils showed these distinctions in functionality between aged and fresh neutrophils. In this model, the group showed that mice with mostly aged neutrophils were able to survive better against fungal infections as compared with mice with mostly fresh neutrophils. However, aged neutrophils confer a worse disease pathology in a vascular inflammation model, resulting in larger infarction sizes and poorer survival. Therefore, it is important to consider the heterogeneity of neutrophil age states in addition to the developmental state of the circulating neutrophils during inflammatory states such as viral infections.

Neutrophils in the circulation also comprise a subset of myeloid-derived suppressor cells that possess immune-modulating properties observed in various conditions such as cancer, pregnancy, and sepsis. These neutrophils possess a general identity of Ly6G+CD11b+ and can have a morphologic resemblance of both immature and mature neutrophil phenotypes as described by various groups. These suppressor cells are reported to inhibit T cell proliferation and activation, dampening the immune response. They may also be akin to low-density neutrophils (LDN) found in the mononuclear fraction after density gradient separation. LDNs are also reported as a combination of immature and mature phenotypes observed first in SLE and rheumatoid arthritis. These cells have perhaps degranulated and accumulated in the bloodstream of these patients, as reported by some groups. This indicates that neutrophils can exist in various states, eliciting both beneficial and detrimental functions to the immune response.

In the lung specifically, a major subset of neutrophils exists as a marginated, intravascular pool, adhering to the endothelium of capillaries and postcapillary venules. These neutrophils have been shown to be activated once in the lung, acquiring higher expression of adhesion receptors such as CD11b but lower expression of CD62L. Notably, the authors argued that this phenomenon occurs regardless of the inflammatory condition. Recent evidence in mice further proposes that neutrophils change at the transcriptome level when they infiltrate various tissues. In the study, the group showed a lung-specific signature in the neutrophils, possessing proangiogenic genes involved in vascular growth and repair. Intravital imaging of lung neutrophils showed that they interact closely with B cells, allowing for their clearance by macrophages. A disruption of neutrophil clearance by B cell depletion

FIGURE 1 Characterization of neutrophil subsets in mice and humans. Neutrophil development is historically characterized by various morphologic structures and granules using bone marrow smears. Undifferentiated myeloblasts differentiate into promyelocytes, myelocytes, nonproliferating meta-myelocytes, band cells, and finally mature as segmented neutrophils. These stages are accompanied by stage-specific granules. The advent of high dimensional single-cell phenotyping technologies enabled both the transcriptome and protein expression characterization of the developmental continuum, giving rise to the identification of discrete subsets proposed by various groups. These enable the study of neutrophils in viral inflammatory conditions and discover subset-specific functions leading to disease pathology and resolution. Created with BioRender.com
was shown to cause pathologic consequences. This opens new questions on how tissue neutrophils, such as those in the lungs, are activated during viral respiratory diseases (VRDs) and how this might impact their function against the infection.

3 | NEUTROPHILS AND VRDs

Respiratory viruses are one of the greatest contributors to endemics and pandemics in the history of humankind, with a significant morbidity and mortality rate. The most common ones are influenza virus, RSV, parainfluenza virus, metapneumovirus, rhinovirus (RV), coronavirus, and adeno-virus. They can affect the upper respiratory tract, which presents mild to moderate symptoms such as fever, cough, sore throat, and/or running nose. This infection can progress to the lower respiratory tract, which causes damage to the lungs and results in severe symptoms such as pneumonia. In pediatric hospitalizations for lower respiratory tract illnesses (LRTIs), 40% are caused by seasonal parainfluenza virus epidemics. RV, typically associated with upper respiratory tract illnesses (URTIs) such as the common cold, has been reported to result in severe LRTIs and is the second leading cause of pneumonia and bronchiolitis in infants and young children. In IAV infections, clinical studies have reported pneumonia as the most frequent severe clinical manifestation, affecting an estimated one-third of the IAV-infected and hospitalized patients. Severe pneumonia can progressively develop into acute respiratory distress syndrome (ARDS), the most severe form of acute respiratory failure. A life-threatening respiratory condition with a pooled mortality rate of 43% across various evaluated studies, ARDS is characterized by pulmonary edema with large infiltration of neutrophils into the interstitial and bronchoalveolar space.

Infiltration of neutrophils is a common trait in VRDs, reported in IAV, RSV, metapneumovirus, RV, adeno-virus, and coronaviruses. In RSV infection, an increased number of neutrophils in the lung is a hallmark for disease severity in both humans and mice. Severe RSV-infected infants have neutrophils as the predominant cell type in the bronchoalveolar lavage (BAL). An increase in lung neutrophils, markers, and genes of neutrophil function and activation has been shown in severe IAV and SARS-CoV-2 infections. Additionally, neutrophil infiltration is also observed in URTIs. RV and adeno-virus are typical causes of URTIs, such as the common cold. In the early course of cold from symptomatic RV-infected patients, neutrophils have been shown to infiltrate the nasal mucosa and secretion. During the common cold of adeno-virus-infected children, a high level of neutrophils, HNP-1, -3, and -4 was observed in their upper respiratory tracts. This increased level of neutrophils observed across respiratory viral infections emphasizes the importance of studying the role of neutrophils in VRDs.

4 | TAKING EVIDENCE FROM IAV INFECTIONS

Before delving into the biologic significance of neutrophil subsets for viral immunity, we first integrate the current evidence of neutrophils in viral respiratory infections to ascertain their functions, both beneficial and detrimental to the host. Among the numerous research involving neutrophils and viral infections, IAV infection investigations have made significant contributions to our understanding of the several functions neutrophils can play during a VRD. IAV is the cause of the yearly seasonal flu and the global human flu pandemics. Since 1900, 5 influenza pandemics has hit the world, with the most recent 2009 IAV H1N1 causing over half a million deaths globally. Moreover, a global estimate of 5 million severely diseased and 650 thousand respiratory deaths has been associated with seasonal influenza each year. Due to its seasonal emergence and multiple subtypes, IAV is a persistent global public health concern that results in a spectrum of pathologic severity. Due to the diversity of individual patients and the difficulties of conducting mechanistic research on human individuals, IAV infection studies are widely carried out on mice, which provides clues on the pathology and responses that neutrophils participate in. It is important to consider murine models of viral infections as well as human studies as they, together, form a cohesive understanding of the mechanisms and underlying pathologies. When possible, we also incorporate relevant studies of other respiratory viruses to complement our comprehension of neutrophil function.

5 | ANTIVIRAL NEUTROPHIL FUNCTIONS IN VRDs

Neutrophils are phagocytes, and their ability to engulf viral particles suggests a possible antiviral function. Indeed, Mullarkey et al. demonstrated that neutrophils perform antibody-dependent cellular phagocytosis (ADCP) on IAV through Hemagglutinin stalk protein-specific IgG antibodies. The opsonized viral particles are phagocytosed by neutrophils resulting in the generation of ROS and perhaps the elimination of virus. In a study using a flank model of Modified Vaccinia Ankara (MVA) infection, it was demonstrated that neutrophils harboring viruses were shown to be APCs. Duffy et al. discovered the infected neutrophils that homed to the bone marrow activated residing CD8+ memory T cells specific to MVA. This effect was later seen to be abrogated by a disruption in neutrophil migration through CCR1 signaling. Additionally, Hufford et al. reported de novo synthesis of viral RNA and protein of IAV in neutrophils, suggesting how they can be infected and serve as antigen-presenter cells to CD8+ T cells for antiviral immunity function. Additionally, Hufford’s group showed an increase in IFNγ production in activated CD8+ T cells, but not CD4+ T cells, and the depletion of neutrophils through anti-Ly6G antibodies showed a significant decrease of these activated CD8+ T cells in the lungs. Notably, while being infected and act as transporters/presenters, neutrophils were incapable of supporting active IAV growth. In an elegant study by Lim et al., neutrophils were shown to secrete the chemokine CXCL12, which induced the migration of influenza-specific CD8+ T cells to the infected lung. This function of T cell recruitment was lost with either neutrophil-specific knock-out of CXCL12 or blocking its receptor, CXCRI4, through the inhibitor AMD3100.
Degranulation of antimicrobial peptides and mediators has been shown to greatly damage lung epithelial cells and tight junction integrity. These powerful proteinases and granules serve to eliminate microbial threats and contain infections. Though it seems unnecessary in viral infections, these mediators are key players in viral clearance. In VRDs like RSV, matrix metalloproteinase 9 (MMP-9) is important for viral clearance. Dabo et al. demonstrated that MMP-9 decreases RSV infectivity and modulates neutrophil recruitment and cytokine generation in the lung using an MMP-9 knock-out model of RSV infection. Another study reported similar findings, showing that secretion of MMP-9 through TLR signaling was required for neutrophil migration to the lung on IAV-infected mice. It is still unclear how MMP-9 reduces infectivity of RSV, possibly disrupting viral attachment or indirectly triggering signaling cascades of receptors MMP-9 is known to bind to. Apart from MMP-9, it was demonstrated that the neutrophil-derived cathelicidin LL-37 has direct antiviral effects, interacting with the virus and decreasing its virulence, suppressing both IAV and RSV infections. Separately, antimicrobial peptides called human neutrophil peptides can neutralize IAV through a process of viral aggregation. This process was shown to promote uptake of IAV by neutrophils, preventing further infections. Along with antimicrobial peptides, neutrophils produce considerable amounts of ROS via oxidative burst, which can be used to remove phagocytosed material or cause tissue damage. Oxidative burst has been detected in mice during IAV and RSV infection. Excessive amounts of ROS, when inflammation is prolonged, can often lead to severe disease pathology in IAV infection. Additionally, mediators like myeloperoxidase (MPO) and neutrophil elastase (NE) can either proteolyse or catalyze ROS production. MPO was shown to be important for viral clearance but also a contributor of lung tissue damage. Similarly, NE has been found in the respiratory tract and serum of severe RSV-infected infants, potentially contributing to disease pathology. Interestingly, individuals with chronic granulomatous disease exhibit ROS-deficient neutrophils but no increased vulnerability to VRDs, indicating that ROS may be primarily engaged in disease pathogenesis as the disease progresses.

ROS is also linked to another important neutrophil function: NET formation. NETosis has garnered much contention as an antiviral role in VRDs. Persistent NET formation in IAV-infected models was associated with lung damage and increased pathogenesis, as NETs are highly toxic. ARD-related histopathologic manifestations such as diffuse alveolar damage (DAD) caused by alveolar injury were entangled with NETs. NET formation and endothelial damage were detected when alveolar epithelial cells (AECs) were infected, suggesting the potential link of NET formation to alveolar damage in IAV-infected patients. Additionally, high extracellular histones levels were found in the lungs of IAV-infected mice and were shown to exacerbate lung pathology. RSV-infected epithelial cells were shown to be recognized by neutrophils, triggering NETosis, which can capture RSV particles in vitro. Additionally, histones have been shown to neutralize H3N2 and H1N1 IAV. These findings highlight NETosis as potential antiviral capabilities against VRDs. On the other hand, close examinations of patients’ sputum, lung biopsies, or mouse models with severe VRDs frequently reveal elevated NETs and neutrophil levels, suggesting neutrophils as major drivers of disease pathology and mortality in severe disease. Most of the airway obstructions during severe RSV infections were found to have NETs plugs. As a result of this, airway obstruction and poor prognosis may be linked to an increased neutrophil response by NETs. However, these observations are likely a consequence of uncontrolled disease progression, led by dysregulation of cellular signaling and unresolved inflammation.

After executing their functions, neutrophils can perform an additional function in the lung by secreting epidermal growth factor (EGF), which has been demonstrated to be required for monocyte development into competent APCs. A recent study showed that the absence of EGF significantly reduced CD8+ T cell cytokine expression and activation, and showed a marked decrease in dendritic cell maturation markers like MHCII and CD86. Collectively, the data suggest that neutrophils may carry out their antiviral activities through indirect processes.

6 | RECRUITMENT OF NEUTROPHILS TO THE INFECTED RESPIRATORY TRACT

The innate immune players in the lung include the resident lung leukocytes and the epithelial cells lining the alveolar surface and the conducting airways. The immune cells in healthy lung tissues are ~95% alveolar macrophages, 1–4% lymphocytes, and ~1% neutrophils. Pattern recognition receptors (PRRs) such as TLRs are found on alveolar walls and epithelial cells lining the respiratory tract to sense pathogen-associated molecular patterns and damage-associated molecular patterns. These epithelial cells in the airway are the first to be infected, secreting proinflammatory cytokines and chemokines to activate and recruit leukocytes into the infected respiratory tract. These mediators include MIP-1α, TNF-α, IL-1, IL-6, CXCL1, CXCL2, CXCL10, and IL-17. Enhanced levels of proinflammatory cytokines and Type I IFNs are also contributed by infected alveolar macrophages. Depending on the inflammatory stimuli, massive infiltration can occur, leading to lung tissue damage and loss of lung function.

Following IAV infection and activation of tissue-resident macrophages and epithelial cells, neutrophils are the first wave of immune cells recruited to the site of infection, which is approximately 2 days after the IAV infection. During IAV, RSV, and SARS-CoV infection, MyD88/TRIF signaling is crucial for recruitment of neutrophils to the lung. Following IAV infection, an increase in both local and systemic levels of neutrophils was commonly observed in clinical patients, mice, and ferrets. This neutrophilia correlates with the increased IL-6, CCL2, CXCL8, and CXCL10 levels and disease severity of IAV infections. The release of proinflammatory cytokines (e.g., TNF-α, IL-1, and IL-6), chemokines (CCL2, CCL7, CXCR8), complement component C5a, and leukotriene B4 attracts neutrophils to the infected respiratory airway. Similarly, these molecules were highly associated with increased disease severity.
After migration to the infected airway, neutrophils are found to confer a protective function to the host in both the early and late stages of the infection.\textsuperscript{67} Cell depletion studies in mice revealed that recuperation from severe cases of IAV depended heavily on neutrophils presence and contribution.\textsuperscript{13} Interestingly, mild IAV infection did not exhibit a similar dependency on neutrophils. Moreover, studies in mice using IAV strains with varying levels of virulence suggest that neutrophil response is dependent on the pathogenicity of the viral infection.\textsuperscript{173}

In the lungs, the release of proinflammatory cytokine IL-6 is essential to stimulate neutrophil survival and promote viral clearance, as it can reinstate antiapoptotic factors levels (Mcl-1 and Bcl-XL) suppressed by IAV.\textsuperscript{168} Low levels of IL-6, coupled with low neutrophil numbers, strongly correlated with increased disease severity. However, retention of neutrophils might not be necessarily beneficial. Retention of lung neutrophils has been associated with IAV disease severity, and observed especially in highly pathogenic IAV strains.\textsuperscript{112,155,173,174} CXCL8 (a neutrophil chemoattractant) increase in lung airspaces was correlated to elevated neutrophil numbers from recovered patients with ARDS.\textsuperscript{175,176} Notably, this increase was correlated to higher disease severity and symptoms.

As mentioned, neutrophils are needed in the early stage of IAV infection for viral clearance. They are effectors in viral clearance, but the accumulation of neutrophils are signs of dysregulated inflammatory signaling and life-threatening tissue damage. In IAV, mild lung pathology can be observed in neutrophil-depleted mice, while ARDS-like pathology and excessive neutrophil infiltration were found in macrophage-depleted mice.\textsuperscript{110} Hence, neutrophil responses need to be well balanced during IAV to have an adequate but not excessive inflammation response. It might also be more beneficial for neutrophils to have early apoptosis later into the IAV infection to prevent excessive neutrophil accumulation in the lung.

## 7 | SEX, AGE, AND THE ACCUMULATION OF NEUTROPHILS IN INFECTED LUNGS

IAV infection severity has been associated with multiple demographic factors such as age and sex.\textsuperscript{177} For both seasonal and pandemic strains of IAV, children below the age of 10 and adults above age 65 have an increased risk of disease severity. Their immunocompromised nature leaves them more vulnerable to infections. Interestingly,
young adults (15–49 years of age) have severe outcomes during IAV pandemics as compared with seasonal outbreaks, and this increased risk is seen more in females than in males. This observation was attributed to the male reproductive hormone, testosterone, that has been shown to confer protection in male mice.\textsuperscript{178} Interestingly, treatment of female mice with high doses of exogenous estradiol showed higher survival rates.\textsuperscript{179} In estradiol-treated female mice, increased neutrophil recruitment was observed, and the depletion of neutrophils reverses the protective effects of the treatment.\textsuperscript{180} This suggests that the protective effect of estradiol is influenced by its amount produced, which might account for the higher risk in females. Future studies are required to study the role of sex differences and the response toward IAV and other VRDs.

While other respiratory viruses such as the RSV, parainfluenza virus, metapneumovirus, RV, and adenovirus affect mainly children,\textsuperscript{68} the influenza virus and coronavirus have more disease complications in elderly of 65 years old and above.\textsuperscript{65,181,182} Young children can excrete the viruses earlier and over a longer period of time than adults.\textsuperscript{65} A recent study conducted by Kulkarni et al.\textsuperscript{174} between lung neutrophil infiltration and age demonstrated an increased level of neutrophils in aged compared with young mice. This observation might have been contributed by the elevated secretion of neutrophil-recruiting chemokines, CXCL1 and CXCL2, by AECs in infected, aged mice. The receptor of these neutrophil chemoattractants, CXCR2, is highly expressed on circulating neutrophils for chemotaxis to the site of inflammation.\textsuperscript{183} CXCR2 has been shown to play a major role in neutrophil migration to the lungs during influenza infection, but the neutrophils recruited were reported as dispensable for influenza viral clearance.\textsuperscript{88,184–186} This suggests that the increase in disease severity and mortality rate concerning age is related to the increased secretion of CXCL1 and CXCL2, which attract excessive numbers of neutrophils to the lungs and confer a pathogenic effect.\textsuperscript{174} This corresponds to ageing studies in humans, where individuals aged above 60 have deficiencies in circulating neutrophils and reductions in neutrophil effector functions such as phagocytosis of bacteria, production of NETs, ROS, and migration.\textsuperscript{187}

However, a study conducted by Lu et al.\textsuperscript{188} demonstrated increased resistance to IAV infection in aged as compared with young mice. While younger mice showed a faster viral clearance, they also had a higher mortality rate and tissue damage. Due to their weakened immune system, aged mice cleared viruses more slowly without causing tissue damage. Studies by Kulkarni et al.\textsuperscript{174} and Lu et al.\textsuperscript{188} employed the same viral strain (H1N1; PR8) and mouse strain (female C57BL/6), however their findings were inconsistent. Notably, in the second study by Lu et al.,\textsuperscript{188} the age range of the mice is significantly older. Thus, this age difference in mice may account for the observed disparity in experimental results. Hence, this suggests that stronger immune responses might not result in better survival rates, and therapies targeted at reducing excessive neutrophil levels may have to take into consideration the age group and sex of the patient.

With age, neutrophil functions such as phagocytosis and ROS production decline.\textsuperscript{189,190} Numerous retrospective studies have demonstrated, severe COVID-19 disease is frequently observed in elderly patients.\textsuperscript{22,191–194} Transcriptomic and cytokine analyses of aged COVID-19 patients revealed higher degradation signatures and IFN-γ signaling. This was also similarly seen using a non-human primate model of SARS-CoV2 infection, including a higher level of VEGF in the lungs of old macaques.\textsuperscript{195} Interestingly, the airway epithelial cells of children with SARS-CoV2 possessed augmented antiviral sensing and immune cell activation.\textsuperscript{196} This, along with the higher viral sensing and IFN production in myeloid cells, prevents children from acquiring severe disease pathology.

## 8. CORONAVIRUS (SARS, MERS, SARS-COV2): HIGH NEUTROPHIL COUNTS OBSERVED IN CORONAVIRUS-INFECTED PATIENTS

Coronaviruses are enveloped, positive-sense single-stranded RNA viruses with spike-like structures on their viral surfaces when observed under the electron microscope.\textsuperscript{197,198} Similar to IAV, they can cause mild to severe respiratory infections in humans that progress toward fatal outcomes.\textsuperscript{197} Since 2000, the emergence of highly pathogenic coronavirus as pandemics has been an unsolved global public health concern. SARS-CoV in 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 have caused a high mortality rate of 9.6% and 35.5%, respectively.\textsuperscript{199}

Only a few investigations have been conducted to determine the involvement of neutrophils in SARS-CoV and MERS-CoV infection. Animal models that are ideal for studying these coronaviruses are currently being investigated.\textsuperscript{200–203} In the acute phase of SARS-CoV infection, a high neutrophil count and a low number of CD4+ and CD8+ T cells were observed in patients’ blood, which was correlated with unfavorable consequences.\textsuperscript{204–206} The excessive neutrophils may have contributed to lesion formation in SARS-CoV-infected patients via secretion of granules and ROS that give rise to necrosis in neighboring cells and recruitment of other inflammatory cells. For MERS-CoV infection, high levels of IL-8 (CXCL8), IL-1α, and IL-1β were expressed in the lower respiratory tract of patients.\textsuperscript{207} These cytokines positively correlated with case fatality rates that were reported. Notably, the high expression level of IL-8 and IL-1β in MER-CoV patients can be drawn parallel to the studies conducted with IAV.\textsuperscript{175,176} This suggests a similar inflammatory response observed in both VRDs, allowing us to understand deeper the mechanisms that underlie coronavirus infections through IAV infection studies.

Since December 2019, the world was hit by SARS-CoV-2. It is a novel, highly contagious and pathogenic coronavirus that has resulted in more than a million deaths worldwide. Transmitted between humans via respiratory droplets and close contact, SARS-CoV-2 causes COVID-19, which primarily presents as respiratory symptoms.\textsuperscript{208–210} To this date, SARS-CoV-2 has overtaken both SARS-CoV and MERS in the total number of infected individuals and death toll.\textsuperscript{210–212} Viral pneumonia caused by SARS-CoV-2 can be grouped into 2 phenotypes: a milder pneumonia phenotype and an ARDS-like phenotype. COVID-19 patients can recover from mild pneumonia or progressively develop to the more ARDS-like severe symptoms.\textsuperscript{213} During this
progression, conditions such as atelectasis (blockage of the airway) and lung derecruitment (collapse of the lungs) are developed. Progression into ARDS occurs 20–30% of the time. Primary histologic manifestation of severe COVID-19 includes bilateral DAD, pulmonary edema, and hyaline membranes.\textsuperscript{214} DAD is also associated with high mortality in patients. Postmortem histopathologic analysis of COVID-19 patients reveals endothelialitis, which is the inflammation of the endothelium and alveolar wall injury with the presence of neutrophils and lymphocytes.\textsuperscript{215,216} This suggests that neutrophils and lymphocytes contribute to the lung tissue damage and pathogenesis of COVID-19.

Hence, the association between the NLR and COVID-19 disease severity has been studied and identified as an early indicator for severe COVID-19 disease.\textsuperscript{10,11,217–221} A retrospective cohort study in Wuhan consisting of 210 COVID-19 patients (87 of them are severe cases) showed a higher NLR of 6-fold difference in severe cases compared with mild cases, with an increased level of neutrophils and a significant reduction in T cell level.\textsuperscript{218} Neutrophil markers including resistin, lipocalin-2, hepatocyte growth factor, IL-8, G-CSF, and dual endothelin-1 and VEGF signal peptide-activated Receptor (DEspR) have also been identified as predictors of severe COVID-19 disease.\textsuperscript{97,222} In critical cases, high NLR and neutrophilia have been associated with excessive ROS, which may contribute to COVID-19 disease severity through induction of disease manifestation such as tissue damage, atherosclerosis, thrombosis, and endothelialitis.\textsuperscript{223} Additionally, excessive ROS may cause an imbalance of iron homeostasis and contribute to inefficient oxygen transport.\textsuperscript{224} Furthermore, the elevation of circulating NETs was observed.\textsuperscript{225} Studies in patients revealed a pathogenic role of NETs in disease progression,\textsuperscript{93,226–239} contributing to immunothrombosis. In vitro experiments found SARS-CoV-2 capable of activating healthy neutrophils and inducing the release of NETs, which promotes lung epithelial apoptosis.\textsuperscript{226} These suggest that both excessive ROS and NET formation from dysregulated levels of neutrophils in the lung contribute to disease pathology. Hence, NETosis is a potential therapeutic target against severe COVID-19 disease progression.\textsuperscript{240} R406, an ATP-competitive SYK inhibitor and active metabolite of fostamatinib, inhibited NETosis of healthy donor neutrophils in COVID-19 patient plasma, demonstrating its potential to inhibit NETosis in COVID-19 patients.\textsuperscript{241} Interestingly, these neutrophil-related pathologies closely resemble IAV infections, reinforcing how neutrophil functions can be closely studied using IAV as a model.

To better study COVID-19, animal models are actively being developed.\textsuperscript{242,243} While angiotensin-converting enzyme 2 (ACE2) is the functional receptor in humans for SARS-CoV-2, ACE2 in mice does not bind to the virus effectively.\textsuperscript{244} Strategies to overcome this problem are being explored. Currently, no mouse model replicates all aspects of COVID-19 displayed in humans.\textsuperscript{245} However, continued refinement may result in models even for these aspects of the human disease. Recent studies using a transgenic mouse model with human ACE2 demonstrated that the neutrophil mediator S100A8/9 is highly expressed in SARS-CoV2 infection and inhibition through the drug paquinimod reduced pathogenesis.\textsuperscript{246} These models will pave the way for new insights into the mechanisms that neutrophils partake in during SARS-CoV2 and other VRDs to elucidate possible treatment regimens that can target neutrophil subsets at various stages of the disease.

9 | NEUTROPHIL HETEROGENEITY IN VRDS

The current literature on neutrophils in VRDs, covered in this review, provides the foundation for understanding the role of neutrophil subsets in VRDs. There are many layers of heterogeneity to consider, resulting from environmental, developmental, and activation states.\textsuperscript{275,247,248} The first layer of heterogeneity is the neutrophil’s infection status. IAV-infected neutrophils were shown to produce less ROS, cathelicidin LL-37, and lipocalin.\textsuperscript{249–251} This implicates their functional capacity for viral clearance and may explain higher viral loads that are observed with high infiltrating neutrophil numbers. As discussed, these infected neutrophils can also act as antigen-presenters for CD8+ T cell immunity.\textsuperscript{106}

The second layer of heterogeneity is the neutrophil’s developmental status. During inflammation, the appearance of immature band cells has been observed, marked as a “left shift” of precursor neutrophils.\textsuperscript{33} Retrospective studies of infants with various VRDs noted high frequencies of immature neutrophils, and this observation was not influenced by bacterial coinfections.\textsuperscript{252} Cortjens et al.\textsuperscript{253} further identified 3 blood neutrophil subsets during viral infections in infants, namely immature CD16loCD62Lhi neutrophils, mature CD16hiCD62Llo neutrophils, and a suppressive CD16hiCD62Llo subset. The group previously identified the suppressive CD16hiCD62Llo subset in LPS-treated individuals.\textsuperscript{254} It is unclear if this LPS-induced CD16hiCD62Llo subset is analogous to the VRD-induced subset identified. Recent evidence with single-cell sequencing technologies has shown that neutrophils in SARS-CoV2-infected patients are frequently developmentally immature, with lower expression levels of S100a8, S100a9, CD10, and CD101.\textsuperscript{255} These neutrophils were also reported to consist of proneutrophils, preneutrophils, and immature neutrophils that associate highly with severe COVID-19 disease.\textsuperscript{256} Immature neutrophils, in particular, showed a strong correlation with disease severity and is suggested to perform better than the NLR.\textsuperscript{257} Martinelli et al.\textsuperscript{258} used microarray profiling and compared immature bone marrow neutrophils with mature blood neutrophils, showing that immature neutrophils lacked type-1 IFN signaling receptors and associated genes. They further showed the lack of STAT-1 phosphorylation during IFNz or IFNγ stimulation in immature neutrophils.\textsuperscript{258} These results suggest a differential ability to respond toward VRDs, leading to a lower propensity for NET formation and cytokine release. Single-cell analysis of SARS-CoV2-infected patient leukocytes confirms this, showing proNeus and preNeus in the blood have much lower expression of IFN signaling genes.\textsuperscript{259} Moreover, both proNeus and preNeus have low or no expression of CD16 required for ADCP as mentioned earlier. This further differentiates the antiviral potential between mature and immature neutrophil subsets.

The role of immature neutrophils during VRDs is still unclear. It is proposed that their appearance andaccumulation is a consequence
of the high inflammation present in the patients that stimulates the premature mobilization of immature neutrophils from the bone marrow to the circulation and sites of inflammation. These banded nuclei cells are perhaps less efficient in providing viral clearance and forming NETs and may trigger the recruitment of more immature granulocytes to compensate for the loss of efficiency in viral clearance. Studies on immature neutrophils during inflammatory conditions suggest a high ROS function and migration capacity, but low NETs and phagocytosis function. This was also shown with in vitro human studies, demonstrating an increased immature neutrophil migration through CXCL8 signaling, but a higher propensity for NET formation correlating with severe COVID-19 disease.

The third layer of heterogeneity is the neutrophil’s activation status. Reports of LDNs in VRDs have suggested a degranulated form of neutrophils capable of immunosuppression. This subset of neutrophils consisted of a mixture of immature and mature phenotypes, unlike what is commonly thought. However, recent evidence of LDNs questions this difference with normal density neutrophils (NDNs). Identified a specific CD16 subset, LDN subset that was shown to possess enhanced cytokine production upon stimulation. RNA sequencing analysis further suggests increased phagocytosis and degranulation function when compared with CD16 subset. However, recent evidence of LDNs questions this difference with normal density neutrophils (NDNs). Were able to generate LDNs from activating NDNs with TNF-α, fMLP, or LPS. These LDNs were shown to have no significant difference in ROS production or surface marker expression. Moreover, no differential effect on T cell proliferation or IFNγ production was observed. One key difference was the lowered ability for NET formation, possibly linked to the decrease of granules such as NE and MPO required for NETosis. Similarly, LDNs of patients with severe fever with thrombocytopenia syndrome (SFTS) was shown to be derived from NDNs after culture with SFTS media. However, Li’s group showed that LDNs could secrete higher amounts of IL-8, IL-6 and IL-17, suggesting a contributor of higher cytotoxicity to endothelial cells. In their study, Li showed that LDNs had higher viral loads, signifying possible differences in antigen-presentation potential.

10 | FUTURE OPPORTUNITIES AND CHALLENGES

Neutrophils can play various roles during VRDs. However, some of these activities that aid in viral clearance can become harmful to the host when dysregulated. Complicated by a spectrum of heterogeneity in neutrophil subtypes and states, identifying pathologic neutrophils remains a significant hurdle in devising biomarkers and novel treatment strategies. Current evidence of neutrophils in VRDs lack a consensus of identifying neutrophil subsets. Lung neutrophils are, at physiologic conditions, different from blood neutrophils phenotypically. Surface markers such as CD62L and CD11b, which are used to identify suppressive and activated neutrophils, were shown to be lower in expression in lung neutrophils. Moreover, many surface markers, used in differentiated neutrophil subsets might alter drastically depending on severity and disease progression. It is obvious that emergency granulopoiesis occurs during VRDs, mobilizing immature precursors into the periphery. Therefore, an inflammation-stable maturation marker, such as CD10 (human) and CD101 (mouse), could be incorporated into future studies to dissect development-specific changes in neutrophil activity during VRDs. Future work also should focus on lung neutrophils from BAL samples as the role of the local environment is increasingly appreciated. We anticipate that these future findings will lead to novel interventions and therapies capable of modulating neutrophil activity to improve clinical outcomes.

ACKNOWLEDGMENTS
We thank all members of L.G.N. laboratory for their kind support. L.G.N. and I.K. are supported by Singapore Immunology Network (SlgN) core funding, A*STAR, Singapore. C. R. M. is funded under the University and Institute Innovation Team Project of Jinan (Grant No. 2020GXRC036) and the Innovation Pilot Project of Integration of Science, Education and Industry of Shandong Province, China (Grant No. 2020KC-ZD011). We apologize to all colleagues whose work was omitted from this article. All figures were created with Biorender.com.

AUTHORSHIP
Y.Z., I.K., C.R.M, Q.W. and L.G.N. wrote and edited the manuscript. L.G.N reviewed the manuscript. Y.Z. and I.K. designed the graphics. I.K. and L.G.N. supervised the work and conceptualised the review focus.

DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Segal AW. How neutrophils kill microbes. Annu Rev Immunol. 2005;23:197-223.
2. Borregaard N. Neutrophils, from marrow to microbes. Immunity. 2010;33:657-670.
3. Winterbourn CC, Kettle AJ, Hampton MB. Reactive oxygen species and neutrophil function. Annu Rev Biochem. 2016;85:765-792.
4. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. 2018;18:134-147.
5. Brinkmann V. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532-1535.
6. Branzk N, Lubojemska A, Hardson SE, et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nat Immunol. 2014;15:1017-1025.
7. Kruger P, Safrarzadeh M, Weber ANR, et al. Neutrophils: between host defence, immune modulation, and tissue injury. PLoS Pathog. 2015;11:e1004651.
8. Peiseler M, Kubes P. More friend than foe: the emerging role of neutrophils in tissue repair. J Clin Invest. 2019;129:2629-2639.
9. Mortaz E, Alipoor SD, Adcock IM, et al. Update on neutrophil function in severe inflammation. Front Immunol. 2018;9. 10.3389/fimmu.2018.02171. Epub ahead of print.
10. Liu Y, Du X, Chen J, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect. 2020;90:1344532020302085.
11. Yang A-P, Liu J, Tao W, et al. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol. 2020;10.1016/j.immuni.2020.106504. Epub ahead of print April 13.
12. Egan CE, Sukhumavasi W, Bierly AL, et al. Understanding the multiple functions of Gr-1(+) cell subpopulations during microbial infection. Immuno Res. 2008;40:35-48.
33. Honda T, Uehara T, Matsumoto G, et al. Neutrophil left shift and white blood cell count as markers of bacterial infection. Clin Chim Acta. 2016;457:46-53.

34. Lipiński M, Rydzewska G. Immature granulocytes predict severe acute pancreatitis independently of systemic inflammatory response syndrome. Przegląd Gastroenterol. 2017;12:140-144.

35. Roehr MHA, Wang JY. Immature granulocytes in pregnancy: a story of Virchow, anxious fathers, and expectant mothers. Am J Hematol. 2011;86:307-308.

36. Reader BF, Nicole PD, Andrew TJ, et al. Social stress enhances immature neutrophil release from bone marrow in murine Aspergillus fumigatus-induced allergic airway inflammation. Brain Behav Immun. 2014;40:e21.

37. Katahira Y, Higuchi H, Matsushita H, et al. Increased granulopoiesis in the bone marrow following Epstein-Barr virus infection. Sci Rep. 2019;9:13445.

38. Monteiro Júnior JD de M, de Oliveira Cipriano Torres D, Filho DCS. Hematological parameters as prognostic biomarkers in patients with cardiovascular diseases. Curr Cardiol Rev. 2019;15:274-282.

39. Marini O, Costa S, Bevilacqua D, et al. Mature CD10+ and immature CD10− neutrophils present in G-CSF−treated donors display opposite effects on T cells. Blood. 2017;129:1343-1356.

40. Zhou G, Yu L, Fang L, et al. CD177+ neutrophils as functionally activated neutrophils negatively regulate IBD. Gut. 2018;67:1052-1063.

41. Alder MN, Mallela J, Opoka AM, et al. Olfactomedin 4 marks a subset of neutrophils in mice. Innate Immun. 2019;25:22-33.

42. Plivev BK, Shmidt EI, Ivanova AV, et al. Circulating CD35(-)/CD49d(+) neutrophils in influenza virus infection patients. Hum Immunol. 2012;73:1087-1090.

43. Xie X, Shi Q, Wu P, et al. Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. Nat Immunol. 2020;21:1119-1133.

44. Zhu YP, Padgett L, Dinh HQ, et al. Identification of an early unipotent neutrophil progenitor with pro-tumoral activity in mouse and human bone marrow. Cell Rep. 2018;24:2329-2341.e8.

45. Dinh HQ, Eggert T, Meyer MA, et al. Coexpression of CD71 and CD117 identifies an early unipotent neutrophil progenitor population in human bone marrow. Immunity. 2020;53:319-334.e6.

46. Kwok I, Becht E, Xia Y, et al. Combinatorial single-cell analyses of granulocyte-monocyte progenitor heterogeneity reveals an early uni-potent neutrophil progenitor. Immunity. 2020;53:303-318.e5.

47. Kim M-H, Yang D, Kim M, et al. A late-lineage murine neutrophil precursor population exhibits dynamic changes during demand-adapted granulopoiesis. Sci Rep. 2017;7:39804.

48. Muench DE, Olsson A, Ferchen K, et al. Mouse models of neutropenia reveal progenitor-stage-specific defects. Nature. 2020;582:109-114.

49. Adrvoer JM, Nicolás-Ávila JA, Hidalgo A. Aging: a temporal dimension for neutrophils. Trends Immunol. 2016;37:334-345.

50. Kolarzewska E. The older the faster: aged neutrophils in inflammation. Blood. 2016;128:2280-2282.

51. Stark MA, Huo Y, Burcin TL, et al. Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. Immunity. 2005;22:285-294.

52. Nathan C. Points of control in inflammation. Nature. 2002;420:846-852.

53. Uhl B, Vladiu Y, Zuchtriegel G, et al. Aged neutrophils contribute to the first line of defense in the acute inflammatory response. Blood. 2016;128:2327-2337.

54. Adrvoer JM, del Fresco C, Crainicicuc G, et al. A neutrophil timer coordinates immune defense and vascular protection. Immunity. 2019;50:390-402.e10.

55. Gabrilovich DI. Myeloid-derived suppressor cells. Cancer Immunol Res. 2017;5:3-8.
56. Schrijver IT, Théroude C, Roger T. Myeloid-derived suppressor cells in sepsis. Front Immunol. 2019;10.1038/s41590-019-1263-1. Epub ahead of print February 27.

57. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. Nat Immunol. 2018;19:108-119.

58. Hacbarth E, Kajdacsy-Balla A. Low density neutrophils in patients with systemic lupus erythematosus, rheumatoid arthritis, and acute rheumatic fever. Arthritis Rheum. 1986;29:1334-1342.

59. Grieshaber-Bouyer R, Nigrovic PA. Neutrophil heterogeneity as therapeutic opportunity in immune-mediated disease. Front Immunol. 2019;10.1038/s41590-019-0346-x. Epub ahead of print.

60. Sagiv JY, Michaeli J, Assi S, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Rep. 2015;10:562-573.

61. Hassan M, Hellebrekers P, Chen N, et al. On the origin of low-density neutrophils. J Leukoc Biol. 2020;107:809-818.

62. Fortunati E, Kuzmier KM, Grutters JC, et al. Human neutrophils switch to an activated phenotype after homing to the lung irrespective of inflammatory disease. Clin Exp Immunol. 2009;155:559-566.

63. Ballestero I, Rubio-Ponce A, Genua M, et al. Co-option of neutrophil fates by tissue environments. Cell. 2020. 10.1016/j.cell.2020.10.003. Epub ahead of print October 23.

64. Kim JH, Podstawksa J, Lou Y, et al. Aged polymorphonuclear leukocytes cause fibrinous interstitial lung disease in the absence of regulation by B cells. Nat Immunol. 2018;19:192-201.

65. Seitz R, Heiden M, Offergeld R, et al. Influenza virus. Transfus Med Hemotherapy. 2009;36:32-39.

66. Nichols WG, Peck Campbell AJ, Boeckh M. Respiratory viruses other than influenza virus: impact and therapeutic advances. Clin Microbiol Rev. 2008;21:274-290. Table of contents.

67. Tate MD, Brooks AG, Reading PC. The role of neutrophils in the upper and lower respiratory tract during influenza virus infection of mice. Respir Res. 2008;9:57.

68. Boncristiani HF, Criado MF, Arruda E. Respiratory viruses. Encyclopedia of Microbiology. Elsevier:500-518.

69. Schuster JE, Williams JV. Human Metapneumovirus. Pediatr Rev. 2013;34:558-565.

70. Cheemarla NR, Guerrero-Plata A. How does the human metapneumovirus regulate neutrophil infiltration into the airways?: Future Virol. 2018;13:233-235.

71. Williams JV, Harris PA, Tolleson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N Engl J Med. 2004;350:443-450.

72. Branche A, Falsey A. Parainfluenza virus infection. Semin Respir Crit Care Med. 2016;37:538-545.

73. Scott MK, Chomnanard C, Lu X, et al. Human adenovirus associated with severe respiratory infection, Oregon, USA, 2013–2014. Emerg Infect Dis. 2016;22:1044-1051.

74. Hayden FG. Rhinovirus and the lower respiratory tract. Rev Med Virol. 2004;14:17-31.

75. Ljubin-Sternak S, Meštrović T, Ivković-Jureković I, et al. The emerging pathogen in upper and lower respiratory tract infections in children – clinical and molecular epidemiological study from croatia, 2017–2019. Front Microbiol. 2019;10:2737.

76. Aponte FE, Taboada B, Espinoza MA, et al. Rhinovirus is an important pathogen in upper and lower respiratory tract infections in Mexican children. Virol J. 2015;12:31.

77. Louie JK, Roy-Burman A, Guardia-LaBar L, et al. Rhinovirus associated with severe lower respiratory tract infections in children. Pediatr Infect Dis J. 2009;28:337-339.

78. Liu P, Xu M, Cao L, et al. Impact of COVID-19 pandemic on the prevalence of respiratory viruses in children with lower respiratory tract infections in China. Virol J. 2021;18:159.

79. Garg S, Jain S, Dawood FS, et al. Pneumonia among adults hospitalized with laboratory-confirmed seasonal influenza virus infection—United States, 2005–2008. BMC Infect Dis. 2015;15:369.

80. Maruyama T, Fujisawa T, Suga S, et al. Outcomes and prognostic features of patients with influenza requiring hospitalization and receiving early antiviral therapy. Chest. 2016;149:526-534.

81. Casalino E, Antoniol S, Fidouh N, et al. Influenza virus infections among patients attending emergency department according to main reason to presenting to ED: a 3-year prospective observational study during seasonal epidemic periods. PLoS One. 2017;12:e0182191.

82. Lee K-Y. Pneumonia, acute respiratory distress syndrome, and early immune-modulator therapy. Int J Mol Sci. 2017;15.

83. Williams AE, José RJ, Mercer PF, et al. Evidence for chemokine synergy during neutrophil migration in ARDS. Thorax. 2017;72:66-73.

84. Kirsebom FCM, Kaurar F, Nurliev R, et al. Neutrophil recruitment and activation are differentially dependent on MyD88/TRIF and MAVS signaling during RSV infection. Mucosal Immunol. 2019;12:1244-1255.

85. Totura AL, Whitmore A, Agnihotthram S, et al. Toll-Like Receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. mBio. 2015;6:e00638-15.

86. Wareing MD, Shea AL, Inglis CA, et al. CXCR2 Is required for neutrophil recruitment to the lung during influenza virus infection, but is not essential for viral clearance. Viral Immunol. 2007;20:369-378.

87. McCarthy MK, Zhu L, Procario MC, et al. IL-17 contributes to neutrophil recruitment but not to control of viral replication during acute mouse adenovirus type 1 respiratory infection. Virology. 2014;456-457:259-267.

88. Camp JV, Jonsson CB. A role for neutrophils in viral respiratory disease. Front Immunol. 2017;8:550.

89. McNamara PS. Bronchoalveolar lavage cellularity in infants with severe respiratory syncytial virus bronchiolitis. Arch Dis Child. 2003;88:922-926.

90. Goritcka M, Makris S, Kaurar F, et al. Alveolar macrophage-derived type I interferons orchestrate innate immunity to RSV through recruitment of antiviral monocytes. J Exp Med. 2015;212:699-714.

91. Cavallaro EC, Liang K-K, Lawrence MD, et al. Neutrophil infiltration and activation in bronchiolitis airways are independent of viral etiology: neutrophil activity in viral bronchiolitis. Pediatr Pulmonol. 2017;52:238-246.

92. Tang BM, Shojaei M, Teoh S, et al. Neutrophils-related host factors associated with severe disease and fatality in patients with influenza infection. Nat Commun. 2019;10:3422.

93. Zuo Y, Yalavarthi S, Shi H, et al. Neutrophil extracellular traps in COVID-19. JCI Insight. 2020. 10.1172/jci.insight.138999. Epub ahead of print April 24.

94. Xiong Y, Liu Y, Cao L, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Inf. 2020;9:761-770.

95. Lucas C, Wong P, Klein J, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature. 2020;584:463-469.

96. Investigators MOSAIC, Dunning J, Blankley S, et al. Progression of whole-blood transcriptional signatures from interferon-induced to neutrophil-associated patterns in severe influenza. Nat Immunol. 2018;19:625-635.

97. Meizlish ML, Pine AB, Bishai JD, et al. A neutrophil activation signature predicts critical illness and mortality in COVID-19. Blood Adv. 2021;5:1164-1177.

98. Turner RB. The role of neutrophils in the pathogenesis of rhinovirus infections. Pediatr Infect Dis J. 1990;9:832-835.

99. Priyadharshini VS, Ramirez-Jimenez F, Molina-Macip M, et al. Human neutrophil defensin-1, -3, and -4 are elevated in nasal aspirates from...
children with naturally occurring adenovirus infection. Can Respir. J. 2018;2018:1-6.

100. Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature. 2009;459:931-939.

101. Cavallazzi R, Ramirez JA. Influenza and Viral Pneumonia. Clin Chest Med. 2018;39:703-721.

102. WHO. Influenza (Seasonal). World Health Organisation Available from: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal). 2018.

103. Thangavel RR, Bouvier NM. Animal models for influenza virus pathogenesis, transmission, and immunology. J Immunol Methods. 2014;410:60-79.

104. Mullarkey CE, Bailey MJ, Golubeva DA, et al. Broadly neutralizing hemagglutinin stalk-specific antibodies induce potent phagocytosis of immune complexes by neutrophils in an Fc-dependent manner. mBio. 7:e01624-16.

105. Duffy D, Perrin H, Abadie V, et al. Neutrophils transport antigen from the dermis to the bone marrow, initiating a source of memory CD8+ T Cells. Immunity. 2012;37:917-929.

106. Hufford MM, Richardson G, Zhou H, et al. Innate defense against neutrophils within the infected lungs act as antigen presenting cells for anti-viral CD8+ T cells. PloS One. 2012;7:e46581.

107. Ivan FX, Tan KS, Phoon MC, et al. Neutrophils infected with highly virulent influenza H3N2 virus exhibit augmented early cell death and rapid induction of type I interferon signaling pathways. Genomics. 2013;10:101-112.

108. Lim K, Hyn Y-M, Lambert-Emo K, et al. Neutrophil trails guide influenza-specific CD8+ T cells in the airways. Science. 2015;349:aa4352-aa4352.

109. Moraes TJ, Zawarzska JH, Downey GP. Neutrophil granule contents in the pathogenesis of lung injury. Curr Opin Hematol. 2006;13:21-27.

110. Narasaraju T, Yang E, Samy RP, et al. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonia. Am J Pathol. 2011;179:199-210.

111. Dabo AJ, Cummins N, Eden E, et al. Matrix metalloproteinase 9 exerts antiviral activity against respiratory syncytial virus. PloS One. 2015;10:e0135970.

112. Bradley LM, Douglass MF, Chatterjee D, et al. Matrix metalloprotease 9 mediates neutrophil migration into the airways in response to influenza virus-induced toll-like receptor signaling. PLoS Pathog. 2012;8:e1002641.

113. Fridman R, Toth M, Chwyrcova I, et al. Cell surface association of matrix metalloproteinase-9 (gelatinase B). Cancer Metastasis Rev. 2003;22:153-166.

114. Ellerbroek SM, Halbleib JM, Benavidez M, et al. Phosphatidylinositol 3-kinase activity in epidermal growth factor-stimulated matrix metalloproteinase-9 production and cell surface association. Cancer Res. 2001;61:1855-1861.

115. Hartshorn KL, White MR, Tecle T, et al. Innate defense against influenza A virus: activity of human neutrophil defensins and interactions of defensins with surfactant protein D. J Immunol Baltim Md. 1950;2006;176:6962-6972.

116. Doss M, White MR, Tecle T, et al. Interactions of alpha-, beta-, and theta-defensins with influenza A virus and surfactant protein D. J Immunol Baltim Md. 2009;182:7878-7887.

117. Akaike T, Noguchi Y, Ijiri S, et al. Pathogenesis of influenza virus-induced pneumonia: involvement of both nitric oxide and oxygen radicals. Proc Natl Acad Sci USA. 1996;93:2448-2453.

118. Bataki EL, Evans GS, Everard ML. Respiratory syncytial virus and neutrophil activation. Clin Exp Immunol. 2005;140:470-477.

119. Johansson C, Kirsebom FCM. Neutrophils in respiratory viral infections. Mucosal Immunol. 2021;14:815-827.

120. Vlahos R, Stambas J, Bozinovski S, et al. Inhibition of Nox2 oxidative activity ameliorates influenza virus-induced lung inflammation. PLoS Pathog. 2011;7:e1001271.

121. Borregaard N. Development of neutrophil granule diversity. Ann N Y Acad Sci. 1997;832:62-68.

122. Sugamata R, Dobashi H, Nagao T, et al. Contribution of neutrophil-derived myeloperoxidase in the early phase of fulminant acute respiratory distress syndrome induced by influenza virus infection: r. Sugamata et al. Microbial Immunol. 2012;56:171-182.

123. Emboriadou M, Hatzistilianou M, Magnisali C, et al. Human neutrophil elastase in RSV bronchiolitis. Ann Clin Lab Sci. 2007;37:79-84.

124. Abu-Harb M, Bell F, Finn A, et al. IL-8 and neutrophil elastase levels in the respiratory tract of infants with RSV bronchiolitis. Eur Respir J. 1999;14:139.

125. Anjani G, Vignesh P, Joshi V, et al. Recent advances in chronic granulomatous disease. Genes Dis. 2020;7:84-92.

126. N T. Neutrophils as possible therapeutic targets in severe influenza pneumonia. J Infect Pulm Dis. 2016;2. 10.16966/2470-3176.115. Epub ahead of print.

127. Zhu L, Liu L, Zhang Y, et al. High level of neutrophil extracellular traps correlates with poor prognosis of severe influenza a infection. J Infect Dis. 2018;217:428-437.

128. Ashar HK, Mueller NC, Rudd JM, et al. The role of extracellular histones in influenza virus pathogenesis. Am J Pathol. 2018;188:135-148.

129. Funchal GA, Jaeger N, Czepielewski RS, et al. Respiratory syncytial virus fusion protein promotes TLR-4–dependent neutrophil extracellular trap formation by human neutrophils. PloS One. 2015;10:e0124082.

130. Geerdink RJ, Hennis MP, Westerlaken GHA, et al. LAIR-1 limits neutrophil extracellular trap formation in viral bronchiolitis. J Allergy Clin Immunol. 2018;141:811-814.

131. Muraro SP, De Souza GF, Gallo SW, et al. Respiratory syncytial virus induces the classical ROS-dependent NETosis through PAD-4 and necroptosis pathways activation. Sci Rep. 2018;8:14166.

132. Hägglund S, Blodörn K, Näslund K, et al. Proteome analysis of bronchoalveolar lavage from calves infected with bovine respiratory syncytial virus—Insights in pathogenesis and perspectives for new treatments. PloS One. 2017;12:e0186594.

133. Cortjens B, de Boer OJ, de Jong R, et al. Neutrophil extracellular traps cause airway obstruction during respiratory syncytial virus disease: nETs in RSV disease. J Pathol. 2016;238:401-411.

134. Hoeksema M, Tripathi S, White M, et al. Arginine-rich histones have strong antiviral activity for influenza A viruses. Innate Immun. 2015;21:736-745.

135. Radermecker C, Detrembleur N, Guiot J, et al. Neutrophil extracellular traps infiltrate the lung airway, interstitial, and vascular compartments in severe COVID-19. J Exp Med. 2020;217. 10.1084/jem. 20201012. Epub ahead of print September 14.

136. Bendib I, de Chaisemartin L, Granger V, et al. Neutrophil extracellular traps are elevated in patients with pneumonia-related acute respiratory distress syndrome. Anesthesiology. 2019;130:581-591.

137. Heltzer ML, Coffin SE, Maurer K, et al. Immune dysregulation in severe influenza. J Leukoc Biol. 2009;85:1036-1043.

138. Tahaghoghi-Hajighorbani S, Zafari P, Masoumi E, et al. The role of dysregulated immune responses in COVID-19 pathogenesis. Virus Res. 2020;290:198197.

139. Lim K, Kim T, Trzeciak A, et al. In situ neutrophil efferocytosis shapes T cell immunity to influenza virus. Nat Immunol. 2020;21:1046-1057.

140. Martin TR. Innate immunity in the lungs. Proc Am Thorac Soc. 2005;2:403-411.

141. Janeway CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197-216.

142. van de Sandt CE, Kreijtz JHCM, Rimmelzwaan GF. Evasion of immune complexes by neutrophils in an Fc-dependent manner. mBio. 7:e01624-16.
143. Williams MR, Aucita V, Newton G, et al. Emerging mechanisms of neutrophil recruitment across endothelium. Trends Immunol. 2011;32:461-469.

144. Pittman K, Kubes P. Damage-associated molecular patterns control neutrophil recruitment. J Innate Immun. 2013;5:315-323.

145. Chen X, Liu S, Goraya MU, et al. Host immune response to influenza a virus infection. Front Immunol. 2018;9:320.

146. Waugh T, Ching JCH, Zhou Y, et al. Influenza A virus (H1N1) increases airway epithelial cell secretion by up-regulation of potassium channel KCNN4. Biochem Biophys Res Commun. 2013;438:581-587.

147. Tavares LP, Teixeira MM, Garcia CC. The inflammatory response triggered by Influenza virus: a two edged sword. Inflamm Res. 2017;66:283-302.

148. Schmitz N, Kurrer M, Bachmann MF, et al. Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. J Virol. 2005;79:6441-6448.

149. Short KR, Kroeeze EJBV, Fouchier RAM, et al. Pathogenesis of influenza-induced acute respiratory distress syndrome. Lancet Infect Dis. 2014;14:57-69.

150. Ma W-T, Yao X-T, Peng Q, et al. The protective and pathogenic roles of IL-17 in viral infections: friend or foe?. Open Biol. 2019;9:190109.

151. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. Mol Med. 2011;17:293-307.

152. Höagner K, Wolff T, Pleschka S, et al. Macrophage-expressed IFN-β contributes to apoptotic alveolar epithelial cell injury in severe influenza virus pneumonia. PLoS Pathog. 2013;9:e1003188.

153. Kobasa D, Jones SM, Shinya K, et al. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature. 2007;445:319-323.

154. Lee SMY, Gardy JI, Cheung CY, et al. Systems-level comparison of host-responses elicited by avian H5N1 and seasonal H1N1 influenza viruses in primary human macrophages. PLoS One. 2009;4:e8072.

155. Perrone LA, Plowden JK, García-Sastre A, et al. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. PLoS Pathog. 2008;4:e1000115.

156. Dienz O, Rud JG, Eaton DG, et al. Essential role of IL-6 in protection against H1N1 influenza virus by recruiting neutrophil survival in the lung. Mucosal Immunol. 2012;5:258-266.

157. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. JG enV i r o l. 2017;98:12451-12456.

158. Stifter SA, Bhattacharyya N, Pillay R, et al. Functional interplay between Type I and II interferons is essential to limit influenza virus replication and mortality in mice. PLoS Pathog. 2013;9:1002.

159. Tumpey TM, García-Sastre A, Taubenberger JK, et al. Pathogenicity of influenza A viruses with genes from the 1918 pandemic virus: functional roles of alveolarnmacrophages and neutrophils in limiting virus replication and mortality. J Virol. 2005;79:14933-14944.

160. Prince LR, Whyte MK, Sabroe I, et al. The role of TLRs in neutrophil activation. Curr Opin Pharmacol. 2011;11:397-403.

161. Prince LR, Whyte MK, Sabroe I, et al. The role of TLRs in neutrophil activation. Curr Opin Pharmacol. 2011;11:397-403.

162. Cline TD, Beck D, Bianchini E. Influenza virus replication in alveolar macrophages: balancing protection and pathogenesis. JG enV i r o l. 2013;75:3077-3086.

163. Bjornson AB, Mellencamp MA, Schiff GM, et al. Complement is activated in the upper respiratory tract during influenza virus infection. Am Rev Respir Dis. 1993;148:545-554.

164. Groeneveld J, Raijmakers PGHM, Hack EC, et al. Interleukin-8-related neutrophil elastase and the severity of the adult respiratory distress syndrome. Cytokine. 1995;7:746-752.

165. Miller EJ, Cohen AB, Nagao S, et al. Elevated levels of NAP-1/Interleukin-8 are present in the airspaces of patients with the adult respiratory distress syndrome and are associated with increased mortality. Am Rev Respir Dis. 1992;146:427-432.

166. Belperio JA, Keane MP, Burdick MD, et al. Critical role for CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. J Clin Invest. 2010;120:2423-2431.

167. Belperio JA, Keane MP, Burdick MD, et al. Critical role for CXCR2 and CXCR4 ligands during the pathogenesis of ventilator-induced lung injury. J Clin Invest. 2002;110:1703-1716.

168. Sawant KV, Xu R, Cox R, et al. Chemokine CXCL1-mediated neutrophil trafficking in the lung: role of CXCR2 activation. J Innate Immun. 2015;7:647-658.
46. Loske J, Röhmel J, Lukassen S, et al. Pre-activated antiviral innate immunity in the upper airways controls early SARS-CoV-2 infection. J Infect. 2021;148:e139.

47. Kong M, Zhang H, Cao X, et al. Higher level of neutrophil-to-lymphocyte ratio in COVID-19. J Infect. 2020;81:e18-e25.

48. Darbousset R, Thomas GM, Mezouar S, et al. Tissue factor–positive neutrophil extracellular traps in coronary thrombus of a case series. J Am Coll Cardiol. 2020;76:1213-1217.

49. Laforge M, Elbim C, Frère C, et al. Tissue damage from neutrophil extracellular traps: a review. Blood. 2020;136:2699-2708.

50. Narasaraju T, Tang BM, Herrmann M, et al. Neutrophil-derived NETs and COVID-19. J Exp Med. 2020;217:e20201129.

51. Blasco A, Coronado M-J, Hernández-Terciado F, et al. Assessment of neutrophil extracellular traps in coronary thrombus of a case series of patients with COVID-19 and myocardial infarction. JAMA Cardiol. 2021;6:469.

52. Zhang Y, Han K, Du C, et al. Carboxypeptidase B blocks ex vivo activation of the anaphylatoxin-neutrophil extracellular trap axis in neutrophils from COVID-19 patients. Crit Care. 2021;25:51.
Zhangle et al.

231. Ng H, Havervall S, Rosell A, et al. Circulating markers of neutrophil extracellular traps are of prognostic value in patients with COVID-19. Arterioscler Thromb Vasc Biol. 2021;41:988-994.

232. Thierry AR, Roch B. Neutrophil extracellular traps and by-products play a key role in COVID-19: pathogenesis, risk factors, and therapy. J Clin Med. 2020;9:2942.

233. Ondracek AS, Lang IM. Neutrophil extracellular traps as prognostic markers in COVID-19: a welcome piece to the puzzle. Arterioscler Thromb Vasc Biol. 2021;41:995-998.

234. Middleton EA, He X-Y, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood. 2020;136:1169-1179.

235. Obermayer A, Jakob L-M, Haslbauer JD, et al. Neutrophil extracellular traps in severe COVID-19-associated lung injury. Dis Markers. 2021;2021:1-10.

236. Radermecker C, Detrembleur N, Guiot J, et al. Neutrophil extracellular traps infiltrate the lung airway, interstitial, and vascular compartments in severe COVID-19. J Exp Med. 2020;217:e20201012.

237. Ackermann M, Anders H-J, Bily R, et al. Patients with COVID-19: in the dark-NETs of neutrophils. Cell Death Differ. 2021;28:3125-3139.

238. Leppkes M, Knopf J, Naschberger E, et al. Vascular occlusion by neutrophil extracellular traps in COVID-19. EBioMedicine. 2020;58:102925.

239. Arcanjo A, Logullo J, Menezes CCB, et al. The emerging role of neutrophil extracellular traps in severe acute respiratory syndrome coronavirus 2 (COVID-19). Sci Rep. 2020;10:19630.

240. Barnes BJ, Adrover JM, Baxter-Stoltzus A, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. J Exp Med. 2020;217:e20200652.

241. Strich JR, Ramos-Benitez MJ, Randazzo D, et al. Fostamatinib inhibits neutrophil extracellular traps induced by COVID-19 patient plasma: a potential therapeutic. J Infect Dis. 2021;223:981-984.

242. Dinnon KH, Leist SR, Schäfer A, et al. A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. Nature. 2020;586:560-566.

243. Johansen MD, Irving A, Montagutelli X, et al. Animal and translational models of SARS-CoV-2 infection and COVID-19. Mucosal Immunol. 2020;13:877-891.

244. Wan Y, Shang J, Graham R, et al. Receptor recognition by the novel coronavirus from Wuhan: a temporary but not final analysis. Cell. 2020;186:269-284.e16.

245. Muñoz-Fontela C, Dowling WE, Funnell SGP, et al. Animal models for SARS-CoV-2 infection and COVID-19. Cell Mol Life Sci. 2020;77:3371-3392.

246. Martinelli S, Urosevic M, Daryadel A, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. Cell. 2020;0. 10.1016/j.cell.2020.08.002. Epub ahead of print August 5.

247. Deniset JF, Kubes P. Neutrophil heterogeneity: bonafide subsets or by-products of inflammation? Curr Opin Immunol. 2020;0. 10.1016/j.coi.2020.06.004. Epub ahead of print August 5.

248. Wang L, Ai Z, Khyrattty A, et al. ROS-producing immature neutrophils in giant cell arteritis are linked to vascular pathologies. JCI Insight. 2020;5. 10.1172/jci.insight.139163. Epub ahead of print October 15.

249. Navarini AA, Recher M, Lang KS, et al. Immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. Crit Care Med. 2013;41:820-832.

250. Tripathi S, Verma A, Kim E-J, et al. LL-37 modulates human neutrophil polarization states. J Leukoc Biol. 2020;107:931-938.

251. Robinson KM, McHugh KJ, Mandalapu S, et al. Influenza A virus exacerbates Staphylococcus aureus pneumonia in mice by attenuating antimicrobial peptide production. J Infect Dis. 2014;209:865-875.

252. Noyola E, Noor A, Sweeney N, et al. Prevalence of bandemia in respiratory viral infections: a pediatric emergency room experience. Front Pediatr. 2020;8:845.

253. Cortjens B, Ingelse SA, Calis JC, et al. Neutrophil subset responses in infants with severe viral respiratory infection. Clin Immunol. 2017;176:100-106.

254. Pillay J, Tak T, Kamp VM, et al. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. Cell Mol Life Sci. 2013;70:3813-3827.

255. Silvin A, Chapuis N, Dunsmore G, et al. Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19. Cell. 2020;0. 10.1016/j.cell.2020.08.002. Epub ahead of print August 5.

256. Schultz-Schrepping J, Reusch N, Pacilik D, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. Cell. 2020;0. 10.1016/j.cell.2020.08.001. Epub ahead of print August 5.

257. Arcanjo A, Logullo J, Menezes CCB, et al. The emerging role of neutrophil extracellular traps in severe acute respiratory syndrome coronavirus 2 (COVID-19). Sci Rep. 2020;10:19630.

258. Carissimo G, Xu W, Kwok I, et al. Whole blood immunophenotyping uncovers immature neutrophil-to-VD2 T-cell ratio as an early marker for severe COVID-19. Nat Commun. 2020;11:5243.

259. Belchamber K, Thein O & Hazeldine J et al. Altered neutrophil phenotype and function in non-ICU hospitalised COVID-19 patients correlated with disease severity. Preprint, Intensive Care and Critical Care Medicine. Epub ahead of print June 8, 2021. DOI: 10.1101/2021.06.08.21258535.

260. Morrissey SM, Geller AE, Hu X, et al. A specific low-density neutrophil population correlates with hypercoagulation and disease severity in hospitalized COVID-19 patients. JCI Insight. 2021;6. 10.1172/jci.insight.148435. Epub ahead of print May 10.

261. Cabrera LE, Pekkarinen PT, Alander M, et al. Characterization of low-density granulocytes in COVID-19. PLoS Pathog. 2021;17:e1009721.

262. Hay S, Selaru T, Fairhurst A-M. Low-density neutrophils in systemic lupus erythematosus. Arthritis Rheumatol. 2020;72:1587-1595.

263. Hardisty GR, Llanwarne F, Minns D, et al. High purity isolation of low density neutrophils casts doubt on their exceptionality in health and disease. Front Immunol. 2021;12:2057.

264. Li Y, Li H, Wang H, et al. The proportion, origin and pro-inflammation roles of low density neutrophils in SFTS disease. BMC Infect Dis. 2019;19:109.

265. Tak T, Rygiel TP, Karnam G, et al. Neutrophil-mediated suppression of influenza-induced pathology requires CD11b/CD18 (MAC-1). Am J Respir Cell Mol Biol. 2018;58:492-499.

266. Carissimo G, Xu W, Kwok I, et al. Whole blood immunophenotyping uncovers immature neutrophil-to-VD2 T-cell ratio as an early marker for severe COVID-19. Nat Commun. 2020;11:5243.

267. Martinelli S, Urosevic M, Daryadel A, et al. Induction of genes mediating interferon-dependent extracellular trap formation during neutrophil differentiation. J Biol Chem. 2004;279:41423-41432.

268. Manz MG, Boettcher S. Emergency granulopoiesis. J Exp Med. 2014;209:865-875.

269. Tariq T, Rygiel TP, Karnam G, et al. Neutrophil-mediated suppression of influenza-induced pathology requires CD11b/CD18 (MAC-1). Am J Respir Cell Mol Biol. 2018;58:492-499.

270. Manz MG, Boettcher S. Emergency granulopoiesis. Nat Rev Immunol. 2014;14:302-314.

271. How to cite this article: Zhang Y, Wang Q, Mackay CR, Ng LG, Kwok I. Neutrophil subsets and their differential roles in viral respiratory diseases. J Leukoc Biol. 2022;111:1159–1173. https://doi.org/10.1002/JLB.1MR1221-345R