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Neutrophils as defense cells? Immunobiology and pathophysiology in human respiratory infectious diseases

¿Los neutrófilos como células de defensa? Inmunobiología y fisiopatología en las enfermedades infecciosas respiratorias humanas

Edwin U Rojas-Valles,[*,‡,¶] Carlos Alberto Magaña-González,[§,¶] María Teresa Herrera-Barrios[‡]

*Medical College, Universidad Nacional Autónoma de México, Mexico City; ‡Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City; § Universidad Autónoma del Estado de Hidalgo, Mexico.
¶Both authors contributed equally to the writing of the article.

ABSTRACT. The immune system protects us from infections and the entry of any pathogen activates innate immunity. Neutrophils are part of this type of response and are the most abundant in the blood with a short half-life that increases when they are activated. They are generated in the bone marrow during granulopoiesis and their release into the blood depends on the binding of CXCR4-CXCL12. They are the first cells to reach the site of infection or inflammation, and their bactericidal mechanisms are phagocytosis, degranulation, production of ROS, NET, cytokines, and chemokines. During infections, they carry out phagocytosis characterized by direct phagosome-granule fusion, and the pathogens are killed by the action of toxic granule proteins and oxidant molecules (ROS and hypochlorous acid). Pathogens or cytokines promote degranulation which, together with the production of ROS and hypochlorous acid, act on proteins, DNA, and bacterial membranes favoring their elimination. Neutrophils produce NET to trap pathogens and prevent their spread, and they are also a source of cytokines and chemokines, which is why they participate in the regulation of the immune response. In human infectious diseases, their participation can help, or contribute to a poor prognosis, causing tissue damage. This review aims to know the generalities of neutrophils and their participation in human respiratory diseases such as COVID-19 and influenza, tuberculosis, and histoplasmosis.

Keywords: neutrophils, COVID-19, influenza, tuberculosis, histoplasmosis.

RESUMEN. El sistema inmunológico nos protege de las infecciones y la entrada de cualquier patógeno activa la inmunidad innata. Los neutrófilos son parte de este tipo de respuesta y son los más abundantes en la sangre con una vida media corta que se incrementa cuando están activados. Se generan en la médula ósea durante la granulopoyesis y su liberación a la sangre depende de la unión de CXCR4-CXCL12. Son las primeras células en llegar al sitio de infección o inflamación, y sus mecanismos bactericidas son la fagocitosis, la desgranulación, la producción de especies reactivas de oxígeno, trampas extracelulares de neutrófilos, citocinas y quimiocinas. Durante las infecciones, llevan a cabo la fagocitosis caracterizada por la fusión directa fagosoma-gránulo, y los patógenos mueren por la acción de proteínas granulares tóxicas y moléculas oxidantes (especies reactivas de oxígeno y ácido hipocloroso). Los patógenos o citocinas favorecen la desgranulación que, junto con la producción de especies reactivas de oxígeno y ácido hipocloroso, actúan sobre las proteínas, ADN y las membranas bacterianas favoreciendo su eliminación. Los neutrófilos producen trampas extracelulares de neutrófilos para atrapar los patógenos y evitar su propagación y, además, son fuente de citocinas y quimiocinas, por lo que participan en la regulación de la respuesta inmune. En las enfermedades infecciosas humanas su participación puede ayudar, o contribuir a un mal pronóstico, provocando daño tisular. Esta revisión tiene como objetivo conocer las generalidades de los neutrófilos y su participación en enfermedades respiratorias humanas como COVID-19, influenza, tuberculosis e histoplasmosis.

Palabras clave: neutrófilos, COVID-19, influenza, tuberculosis, histoplasmosis.

Correspondence:
Dra. María Teresa Herrera Barrios,
Departamento de Investigación en Microbiología, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Ciudad de México.
E-mail: teresa_herrera@iner.gob.mx

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Abbreviations:
cit-H3 = citrullinated histone 3.
G-CSF = granulocyte-colony stimulated factor.
HMGB1 = High mobility group box 1.
$HOCl^-$ = hypochlorous acid.
MCP-3 = monocyte chemotactic protein-3.
MPO = myeloperoxidase.
NAR = neutrophil-albumin ratio.
NE = neutrophil elastase.
NET = neutrophil extracellular traps.
NLR = neutrophil-lymphocyte ratio.
PKC = protein kinase C.
ROS = reactive oxygen species.
PTB = pulmonary tuberculosis.
IAV = influenza type A virus.

INTRODUCTION

The immune system includes cells involved in innate immunity or acquired immunity to maintain body homeostasis. The entry of any pathogen triggers an innate response that is quick to eliminate the pathogen and prevent disease. This is mediated by the recognition of pathogen-associated molecular patterns and cellular damage-associated molecular patterns without generating immunological memory. Neutrophils are part of this response and their bactericidal mechanisms are phagocytosis, degranulation, production of reactive oxygen species (ROS), neutrophil extracellular traps (NET), cytokines and chemokines.

1. Neutrophils

1.1 Origin and characteristics: they are generated in the bone marrow by granulopoiesis from a myeloid precursor and it has been estimated that a healthy adult produces 1-2 $\times 10^{11}$. Hematopoietic stem cells are located in the spaces created by osteoblasts and endothelial cells characterized by low blood flow and lower oxygen tension, while the more mature and cell-dividing cells are near the abluminal side of the sinusoids, a special vascular structure of the bone marrow.[1] After maturation, neutrophils are released into the blood and this process depends on the interaction of their chemokine receptor CXCR4 and the chemokine CXCL12 produced by stromal cells in the bone marrow.[2]

Their homeostasis is regulated by phagocytosis of apoptotic neutrophils by macrophages and dendritic cells in tissues, reducing their proliferation in an IL-23/IL-17A/G-CSF axis-dependent manner.[3] Phagocytosis decreases the production of interleukin 23 (IL-23), causing decreased production of IL-17A by neutrophil regulatory T lymphocytes or Th17 (Tn/Th17), which are located in mesenteric lymphoid nodules.[4] Consequently, low levels of IL-17A decrease the production of granulocyte-colony stimulated factor (G-CSF) by fibroblasts and endothelial cells reducing the production of mature neutrophils. On the other hand, inflammation or infection causes an increase in G-CSF favoring granulopoiesis, production and recruitment of neutrophils.[2,5] In addition, neutrophil homestoasis involves their cell death by necrosis, necroptosis, NET and pyroptosis *(Figure 1)*.[6,7]

They constitute 50-70% of circulating leukocytes, with a diameter of 7-10 μm, a segmented nucleus, high granule content and the presence of secretory vesicles in their cytoplasm.[2,5] Its half-life is eight to 20 hours without stimulus, although after its migration to tissues it lasts 1-4 days.[6]

They recognize pathogens through their membrane receptors such as: scavenger receptors, mannose receptors, Dectin 1, CD14, FcγR, CiqR, CR1, CR3, colectins, Toll-like receptors, NOD-like receptors.[7]

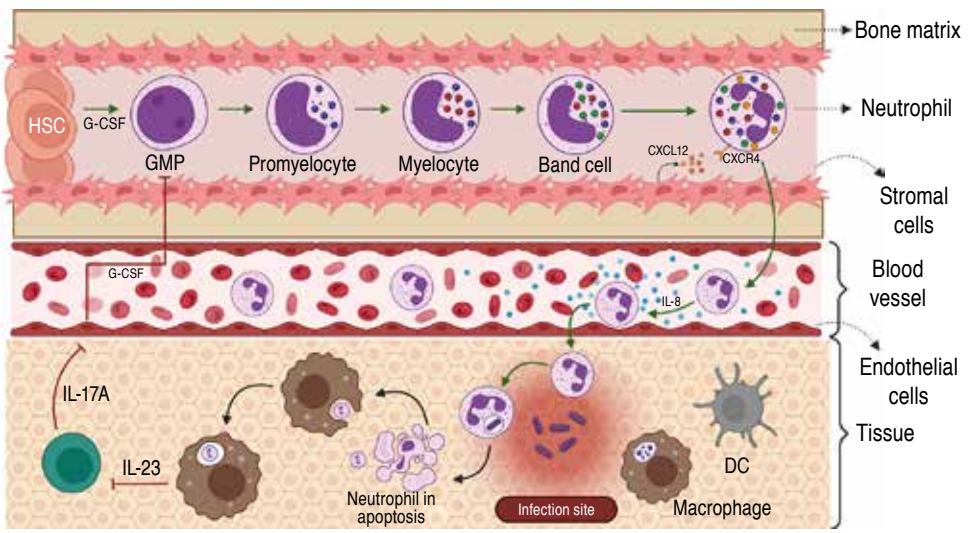

Figure 1:

The origin, maturation and homeostasis of human neutrophils. Neutrophils are generated in the bone marrow and during their maturation acquire cytoplasmic granules and are released into the bloodstream in an IL-23/IL-17A/G-CSF axis-dependent mechanism. These are directed to the site of infection in response to the chemotactic chemokine IL-8. (CXCL8) (Created by BioRender.com). DC = dendritic cell. G-CSF = granulocyte-colony stimulating factor. GMP = granulocyte/monocyte progenitor. HSC = hematopoietic stem cells.

Their cytoplasmic granules are classified into: Primary/azurophil granules, containing myeloperoxidase (MPO), serine proteases, neutrophil elastase (NE), proteinase 3, cathepsin G, azurocidin, α-defensins (HNP-1, HNP-2, and HNP-3), serprocidins, and Bactericidal-permeability-increasing protein (BPi). Secondary/specific granules, containing matrix metalloproteinase 8 (MMP8), lactoferrin, LL-37, lipocalin 2, haptoglobin, Pentraxin 3 and olfactomedin 4. Tertiary granules/gelatinase contain gelatinase B, MMP8, MMP9, Arginase-1, LL-37 and lysozyme, and secretory vesicles containing albumin, cytokines, membrane receptors (CR1, CR3, C1qR, FcγR, CD14, FPR1), cell adhesion molecules (CD11b/CD18, CD67) and part of the nicotinamide adenine dinucleotide phosphate oxidase (NADPH) complex. The granule proteins are acquired during granulopoiesis.[2,5,8,9]

2. Bactericidal mechanisms

2.1 Phagocytosis: is a process of ingestion and elimination of particles or pathogens that enter the organism larger than 0.5 μm, including apoptotic bodies.[10,11] Neutrophils, macrophages, monocytes and dendritic cells are classified as professional phagocytes, as they perform this task with great efficiency,[10] while fibroblasts, epithelial and endothelial cells are considered non-professional phagocytes in charge of eliminating dead cells to maintain homeostasis.[10,11]

It starts with ligand binding to phagocytic receptors, which are divided into opsonic receptors such as: IgG crystallizable fragment receptors (FcγR) and complement receptors (CiqR, CR1, CR3); and non-opsonic receptors such as: mannose receptors, Dectin 1, CD14, collectins, TLRs, C-type lectin and scavenger receptors.[10,12] Compared to macrophages, phagocytosis by neutrophils is rapid as the phagosome directly fuses with the cytoplasmic granules in less than 60 seconds, allowing rapid clearance of pathogens *(Figure 2A)*.[13,14]

Binding of the pathogen or particle causes a rapid oxidative and non-oxidative response by the assembly of the NADPH oxidase complex on the phagosome membrane.[9]

2.2 Degranulation: these cells also combat extracellular pathogens by releasing their cytoplasmic granules. Two types of signals are required: a) β2 integrin-dependent; and b) activation of receptors such as Mac1/CR3, FcγR and G Protein-Coupled Receptors (GPCR). This process involves the Rab and Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins involved in the control of vesicular trafficking *(Figure 2B)*.

Degranulation of secretory vesicles and tertiary granules is rapid; however, primary granules require neutrophils preactivated with proinflammatory cytokines, chemokines or microbial components. Neutrophils prevent excessive degranulation of tertiary granules by increasing ROS production, as the dysregulated process can cause tissue damage.[2]

Phagocytosis and degranulation cause the assembly of the NADPH oxidase complex at the membrane, causing the production of ROS.[9,15]

2.3 Production of reactive oxygen species (ROS): the interaction of neutrophils with the pathogen triggers the oxidative burst involving the NADPH oxidase-NOX2 enzyme system, which assembles on the cell membrane and generates two $\bullet O_2^-$ molecules and the enzyme superoxide dismutase generates H_2O_2 that acts as an antimicrobial by reacting with the thiol groups of enzymes, proteins, DNA and bacterial membranes.[11,16-18]

In addition, MPO utilizes H_2O_2 and catalyzes the reaction with chloride ions, forming a hypochlorous acid (HOCl-) that is highly reactive with thiol groups and methionine residues *(Figure 2C)*.[11,19]

It is important to mention that some pathogens have generated a defense against ROS, but neutrophils possess other alternative bactericidal mechanisms.[20]

2.4 Production of neutrophil extracellular traps (NETs): neutrophils are killed by NETosis where NETs are generated, which are extracellular fibers composed of DNA, cytosolic proteins and antimicrobial granules that trap, neutralize and eliminate pathogens. This process begins with the loss of the lobular shape of the nucleus and disassembly of the nuclear membrane, loss of the permeability of the granular membranes, inactivation of histones by the action of NE that degrades the central histone H1 causing the decondensation of chromatin, causing the mixing of chromatin in the cytosol with the cytosolic and granular components, as well as the loss of the permeability of the cell membrane that allows the release of NETs into the extracellular space *(Figure 2D)*.[15,21,22]

It has been reported that the proteins identified in NETs may vary depending on the stimulus, since with the stimulus Phytohemagglutinin (PMA, Phorbol-Myristate-Acetate) 24 proteins were identified, and with *Pseudomonas aeruginosa* 80 proteins were identified;[15,21] although histones, NE, MPO, calprotectin, cathelicidins, α-defensins and actin are always found.[21]

NETs are formed by two pathways: a) NADPH oxidase-dependent (lytic, most studied) which is activated by antibodies, microorganisms, cholesterol and mitogens (PMA, concanavalin A).[23,24] The stimuli activate protein kinase C (PKC), which activates the assembly of NADPH oxidase to the membrane, initiating the production of ROS, which disintegrate the membranes of the nucleus and granules, allowing NE and MPO to interact with histones to facilitate chromatin decondensation.[23,24]

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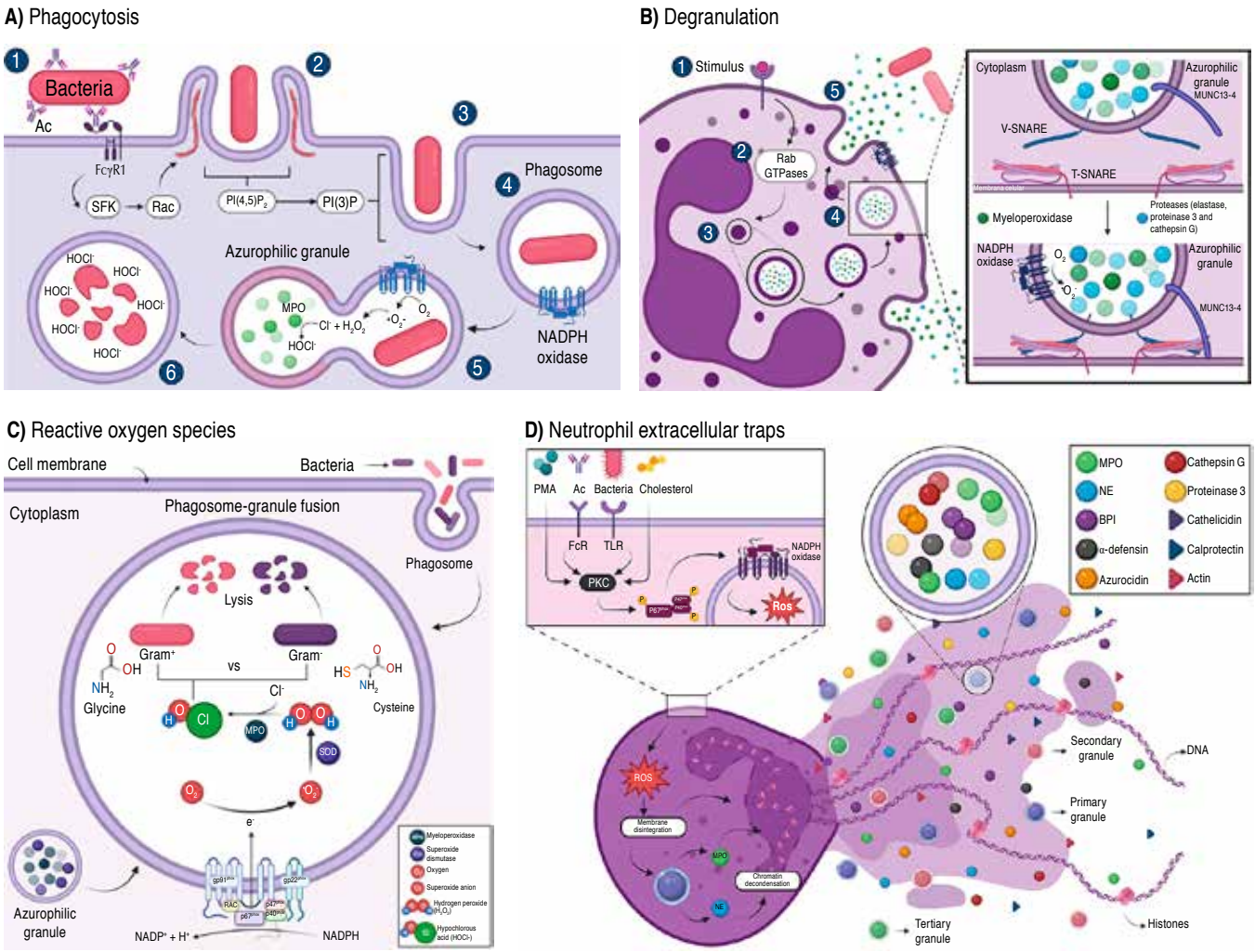

Figure 2: Bactericidal mechanisms of neutrophils. **A)** Phagocytosis. 1) Bacteria opsonized with IgG antibodies bind to FcγR1 receptors on the neutrophil membrane activating signaling (SFK-Rac), uncoupling the activation of Rab GTPases. 2) Actin fibers reorganize and form pseudopodia, associated with changes in membrane phospholipids (PI(4,5)P_2 to PI(3)P) to envelop the bacterium. 3) The membrane is enveloped with the bacterium. 4) The phagosome is released into the cytoplasm and the NADPH oxidase complex is assembled to the membrane. 5) The phagosome fuses with the azurophilic granules, where NADPH oxidase produces ROS and MPO in the granules acts on H_2O_2 to produce hypochlorous acid (HOCl-). 6) Finally, HOCl-, ROS and proteases act to eliminate pathogens. **B)** Degranulation. Neutrophils stimulated with pathogens, microbial components cytokines or chemokines and respond by secreting their granules containing bactericidal molecules. 1) Stimulation of the neutrophil is accomplished by the interaction of the ligand with the FcγR receptor on the membrane. 2) Rab GTPases-mediated signaling is triggered. 3) The granules move through the actin fibers by the Rab. 4) Rab triggers the response of Munc13-4 molecules that interact with SNARE proteins, facilitating the binding of V-SNAREs (from granules) to T-SNAREs (from the membrane). In addition, NADPH oxidase is coupled to produce ROS. 5) The fusion of the membranes (cell and granule) allows the azurophilic granules to discharge their contents and ROS to the outside of the cell to act against extracellular pathogens. **C)** Reactive oxygen species (ROS). After phagocytosis of the pathogen the phagosome fuses with the azurophil granule and NADPH oxidase assembles to the membrane. NADPH oxidase catalyzes the reaction that generates one molecule of $NADP^+$ and one H^+, allowing two electrons to be released into the phagosome-granule that reduce oxygen (O_2) to superoxide anion($•O_2^-$), and superoxide dismutase catalyzes the dismutation of $•O_2^-$ to hydrogen peroxide (H_2O_2). From H_2O_2, MPO catalyzes the reaction that produces hypochlorous acid ($HOCl^-$), which has antimicrobial properties and acts on the cysteine or glycine of Gram-negative and Gram-positive bacteria, respectively. **D)** Neutrophil extracellular traps (NET). Some pathogens have the ability to escape phagocytosis or neutrophil degranulation; however, one of the alternatives for neutrophils to die is programmed death called NETosis, where NETs are produced. The NADPH oxidase-dependent or lytic pathway is triggered by stimulations (pathogens, antibodies, cholesterol and mitogens) that cause PKC activation and promote the assembly of the NADPH oxidase complex to the membrane and the production of ROS. The nuclear and granule membranes are disintegrated by ROS and chromatin is decondensed by the action of NE and MPO. DNA fibers are launched outward to trap pathogens and carry with them granules and components with bactericidal activity.

[Created with BioRender.com].

MPO = myeloperoxidase. NADPH = nicotinamide adenine dinucleotide phosphate oxidase. NE = neutrophil elastase. NET = neutrophil extracellular traps. PKC = protein kinase C. ROS = reactive oxygen species. SFK = Src family kinases. SNARE = Soluble N-ethylmaleimide sensitive factor -NSF– **A**ttachment protein **R**eceptor.

On the other hand, b) in NADPH oxidase-independent (non-lytic, less studied) the nucleus condenses and the nuclear membranes separate, forming vesicles with DNA that are expelled into the extracellular medium, releasing chromatin.[25] This mechanism prevents the spread of pathogens, although it also has direct bactericidal properties. For example, NE acts on outer membrane proteins and virulence factors of enterobacteria.[15] The presence of NET is implicated in inflammatory and autoimmune disorders, such as acute respiratory distress syndrome, thrombosis in COVID-19, and in rheumatoid arthritis.[22]

2.5 Cytokine and chemokine production: neutrophils are a source of cytokines and chemokines to interact with T lymphocytes, B lymphocytes, macrophages and dendritic cells; participating in the regulation of innate and acquired immunity.[26] They produce pro- and anti-inflammatory, immunoregulatory cytokines, G-CSF and important CXC-type chemokines in cell migration into the tissue and vice versa *(Table 1)*.[27-34]

3. Neutrophils in infectious diseases

3.1 COVID-19: in December 2019, a new form of coronavirus called SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), a single-stranded, positive-sense enveloped RNA virus belonging to the β-coronaviruses, spread in Wuhan, China. The World Health Organization (WHO) declared the pandemic on March 11, 2020.[35] Until November 16, 2023, there were 772,011,164 confirmed cases and 6,979,786 deaths worldwide.[36] In patients with COVID-19, the presence of NE, MPO, citrullinated histone 3 (cit-H3), NET and platelets have been shown to be associated with vascular occlusion, necroinflammation and oxidative stress.[37]

In the severe form of COVID-19, inflammation and «cytokine storm» (IL-1β, IL-2, IL-6, IL-7, IL-8, IL-17, TNF, IFN-γ, IP-10, GM-CSF, MCP-1 and IL-10) have been described leading to the development of acute respiratory distress syndrome.[38,39] Neutrophil recruitments to the site of infection and the formation of NETs have been described as contributing to thrombus formation and respiratory distress *(Figure 3)*.[39]

The neutrophil-lymphocyte ratio (NLR) based on the number of neutrophils and lymphocytes in the blood together with the neutrophil-albumin ratio (NAR) are considered as biomarkers of infection and systemic inflammation. NLR values are useful for prognosis, as values less than 3 indicate mild systemic inflammation, 3 to 5 moderate inflammation, and greater than 5 are indicative of severe inflammation,[40] acute respiratory failure syndrome is the primary cause of death in COVID-19 patients. Together, neutrophilia, NLR and NAR in the early stages of infection correlate with the severity of infection.[41]

In the severe form of COVID-19 there is neutrophilia in blood and lung tissue with increased IL-1β, IL-6 and D-dimer; while NETs have the potential to propagate inflammation, microvascular thrombosis and cytokine storm in the lungs.[42,43]

In vascular occlusion NLR, MCP-3 (monocyte chemotactic protein-3) and IL-8 promote neutrophilia in patients with mild and severe COVID-19, forming aggregates of neutrophils and thrombocytes that target mainly the pulmonary vessels,[44] platelet-fibrin complexes target small pulmonary arteries and thrombi target pulmonary capillaries.[42] Neutrophils and NETs favor necroinflammation,[45] by infiltration of NET aggregates that form thrombi in pulmonary vessels, inducing vasculitis and finally necrosis that favors cytokine storm, causing further inflammation.[46]

Among the critical complications of COVID-19 is thrombosis which has been associated with elevated levels of free DNA, cit-H3, MPO-DNA complex and NET identified in arteriolar microthrombi. Released DNA, NET, MPO and cathepsin G have cytotoxic effects on pulmonary epithelium and endothelial cells *(Table 2)*.

Potential markers of NET associated with symptoms have been described, for example: elevated levels of DNA, citH3, NE and the MPO-DNA complex are associated with admission to intensive care, mechanical ventilation and short-term mortality. The MPO-DNA complex has been associated with sequential organ failure, NE and Histone-DNA associated with lung damage, renal failure, body temperature and MPO associated with days with severe hypoxia.[41] The release of NETs and ROS causes imbalance between ROS production and antioxidant mechanisms, increasing tissue injury.[47]

3.2 Influenza: caused by viruses of the genus Influenzavirus, belonging to the *Orthomyxoviridae* family, a negative-sense monocaterial RNA virus, which are transmitted by aerosols affecting cells of the respiratory tract and type II pneumocytes.[48,49] Annual epidemics result in 3 to 5 million severe cases and 290,000 to 650,000 deaths.[50]

Influenza A, B, and C viruses affect humans, but only A and B are of medical importance.[49,51] influenza type A virus (IAV) is the most common virus and has several subtypes, which are classified according to their antigenic variation in their surface proteins: hemagglutinin and neuraminidase.[49]

It causes seasonal epidemics and manifests as an acute illness with mild to severe symptoms; however, it can become complicated leading to hospitalization or death.[48,49] Complications affect at-risk groups (children or older adults) and those with comorbidities (chronic heart or lung disease, diabetes mellitus and immunosuppression).[48]

Table 1: Cytokines and chemokines secreted by neutrophils.

	Function	Target cell	References
Cytokines			
IL-1α	- Proinflammatory effect - Promotes proliferation and differentiation - Endogenous pyrogen	T and B lymphocytes, MN, eosinophils, DC and fibroblasts	27,30
IL-1β	- Increases differentiation and IL-9, RORγt and IRF4 expression	Subpopulations of T lymphocyte subpopulations: T_H9 and T_H17	27,30
IL-6	- Promotes inflammation - Hematopoiesis - Differentiation	T and B lymphocytes	27,30
IL-17	- Proinflammatory effect - Increases production of IL-1, IL-6, TNF-α, G-CSF, GM-CSF and chemokines that attract MN and neutrophils	Endothelial cells, epithelial cells and fibroblasts	27,30
IL-18	- Promotes T_H1 lymphocyte differentiation - Induces IFN-γ production by T lymphocytes - Increases cytotoxic activity of NK lymphocytes	T lymphocytes and NK cells	30
TNF-α	- Regulates the growth and differentiation of various cell types - Promotes angiogenesis, bone resorption and thrombotic processes - Suppresses lipogenic metabolism	Neutrophils, macrophages, fibroblasts and T and B lymphocytes	27,30
MIF	- Promotes activation - Inhibits macrophage migration	Macrophages	30
IL-1 Ra	- Anti-inflammatory activity - IL-1 antagonist, blocking its binding to the receptor, preventing a proinflammatory response	MN, lymphocytes, fibroblasts and endothelial cells	31
TGF-β	- Anti-inflammatory effect - Inhibits growth, differentiation and functions of various cell types - Promotes angiogenesis and tissue repair - Stimulates the production of IgA antibodies	T and B lymphocytes, MN, macrophages and fibroblasts	27,30
IL-22	- Proinflammatory and anti-inflammatory effect - Stimulates transcription of protein genes with microbicidal activity	Keratinocytes	27,30
IL-23	- Promotes differentiation - Induces IL-17A and IL-17B synthesis	T_H17 lymphocytes	27,30
G-CSF	- Growth and differentiation of neutrophil precursors	Neutrophils	27,30
Chemokines			
CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8 (IL-8)	- Proinflammatory activity - Neutrophil recruitment	Neutrophils	18,32
CXCL4	- Proinflammatory activity - Platelet aggregation	Platelets	27
CXCL9, CXCL10, CXCL11	- Proinflammatory activity - Recruitment of effector T-lymphocytes	Effector T lymphocytes	23,27
CCL2	- Proinflammatory activity - Leukocyte recruitment	MN and basophils	32
CCL3, CCL4	- Proinflammatory activity - Leukocyte recruitment - T-lymphocyte-DC interaction	Macrophages, NK lymphocytes, T-lymphocytes and DCs	27,33

Continue Table 1: Cytokines and chemokines secreted by neutrophils.

	Function	Target cell	References
Chemokines			
CCL17, CCL22	- Migration and activation	T_H2 lymphocytes, regulatory T lymphocytes and basophils.	27,33
CCL18	- Activation	Lymphocytes T_H2	33
CCL19	- Migration to lymph nodes	DC and T lymphocytes	22,32
CCL20	- Migration to intestinal lymphoid tissue	T_H17 lymphocytes, B lymphocytes and DCs	33
CCL23	- Chemoattractive activity	T lymphocytes, MN and neutrophils	34

DC = dendritic cell. G-CSF = granulocyte-stimulating factor. GM-CSF = granulocyte-monocyte-stimulating factor. IFN = interferon. IgA = immunoglobulin A. IL = interleukin. IRF4 = interferon regulatory factor 4. Reg T lymphocytes = regulatory T lymphocytes. MIF = macrophage migration inhibitory factor. MN = monocyte. NK = natural killers. RORγt = retinoid orphan receptor gamma t. TGF-β = transforming growth factor-beta. Th = T helper lymphocyte. TNF = tumor necrosis factor.

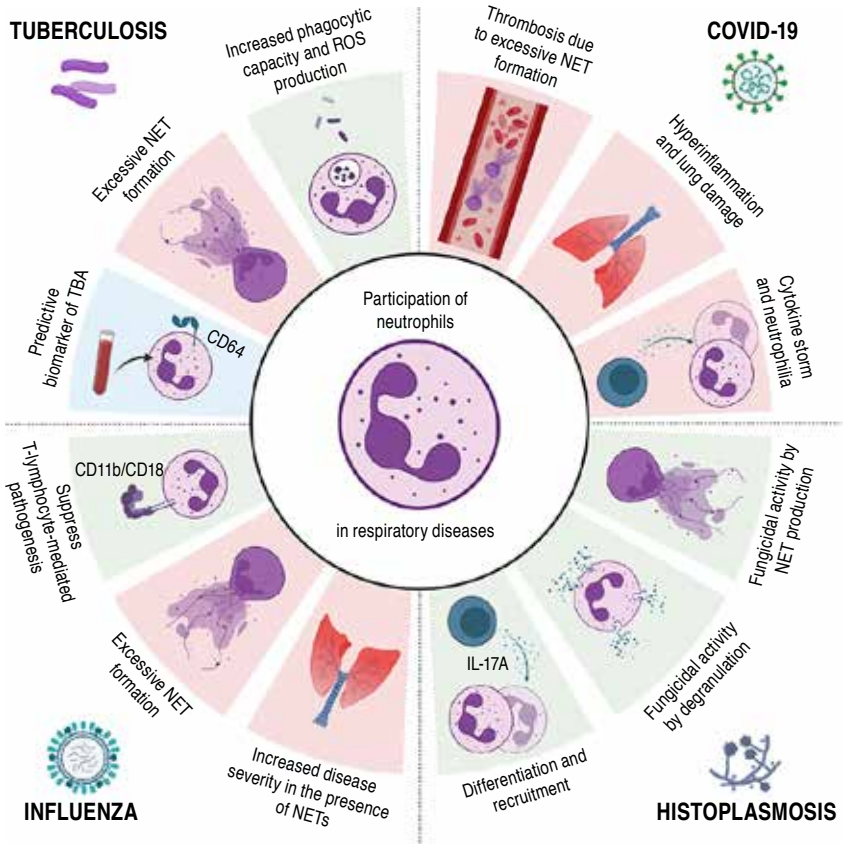

Figure 3:

Participation of neutrophils in respiratory diseases. Neutrophils participate in the elimination of the causative agents of different infectious diseases; however, their rapid response bactericidal mechanisms can cause damage to the organism. In viral diseases such as COVID-19 and influenza, neutrophilia, excessive production of NETs and proinflammatory cytokines cause severe lung damage with poor prognosis, even in COVID-19 excess NETs cause thrombosis with fatal outcomes. Whereas in tuberculosis and histoplasmosis neutrophilia, NET production, phagocytosis, and degranulation are bactericidal mechanisms involved in the pathogenesis of the disease, without causing severe lung damage that is directly responsible for a poor prognosis.
NET = neutrophil extracellular traps ROS = reactive oxygen species. AT = active tuberculosis.

Neutrophils contribute to disease control, and in IAV infection in the murine model, neutrophils and their adhesion molecules (CD11b/CD18) are important in limiting T cell-mediated pathology.[52] However, although the virus induces an innate immune response characterized by neutrophil infiltrate in the lungs and mechanisms that promote resolution of the infection, they also contribute to the pathogenesis of severe disease.[53] Despite tissue damage, lack of neutrophils is associated with increased lung damage.[53,54]

In patients with influenza, neutrophils and NETs are associated with greater severity.[55] In mice co-infected with IAV and *Staphylococcus aureus* there is excessive recruitment of neutrophils to the lungs and NETs, which contribute to severe pulmonary inflammation.[56] Similarly, patients with

severe IAV H1N1 and H7N9 infection have elevated levels of NETs associated with disease severity and poor prognosis.[57]

3.3 Tuberculosis: WHO reported 10.6 million of new cases with tuberculosis and 1.6 million deaths in 2021.[58] *M. tuberculosis* causes tuberculosis (TB) and is transmitted by aerosols. It causes asymptomatic infection (latent TB) in 90-95% and active TB in 5-10% of infected persons, causing mainly pulmonary TB (PTB).[58-61]

Protection depends on the innate and acquired immunity generated. Neutrophils participate in the response to *M. tuberculosis* during early infection, carrying out phagocytosis, production of ROS, cytokines and chemokines.[62] However, although they participate in the immune response, they are not very crucial in the resolution of the disease, probably because they are cells with a short half-life.[63] They participate in macrophage recruitment and promote inflammation and granuloma formation to contain infection.[60,62-64]

They contribute to TB resistance by producing antimicrobial peptides (LL-37 and lipocalin 2) that participate in the elimination of mycobacteria.[65,66] Macrophages phagocytose NETs generated by infection with *M. tuberculosis* and produce IL-1β, IL-6, TNF-α and IL-10, evidencing their participation in the modulation of the immune response.[67] NETs are in the plasma of TB patients and the increase correlates with the severity of the disease.[68,69]

In the search for biomarkers, the FcγR1 receptor (CD64) has been found to be increased on neutrophils and monocytes of patients with active TB, and may be a predictive biomarker of disease.[59,70]

3.4 Pulmonary histoplasmosis: caused by the inhalation of *Histoplasma capsulatum* microconidia or mycelial fragments, it is an endemic mycosis that affects more than 60 countries.[71] With high incidence in North America and in tropical areas of Latin America with temperate, subtropical or humid tropical climates.[72]

Table 2: Toxic effects of NETs on lung epithelium and epithelial cells.

NET components	Cytotoxic effect
DNA fibers	Diffuse alveolar damage and hemorrhage
Histones	Increase the permeability of the endothelium
NE	Destruction of the cytoskeleton of endothelial cells. Affects the integrity of the alveolar barrier. Associated with inflammation and thrombosis by the release of proinflammatory cytokines.
MPO	Involved in epithelial cell apoptosis by the release of ROS

DNA = deoxyribonucleic acid. MPO = myeloperoxidase. NE = neutrophil elastase. NET = neutrophil extracellular traps.

The disease is benign and asymptomatic in immunocompetent individuals, but can progress to acute lung disease, and the severity depends on immune status, time of exposure and virulence of the strain.[73] In the pulmonary alveoli, microconidia develop into yeast and are phagocytosed by macrophages through the CR3 receptor. However, *Histoplasma capsulatum* multiplies and induces apoptosis to spread to other cells.[74] Neutrophils phagocytize opsonized yeasts through their CR1 and CR3 receptors, while non-opsonized yeasts are recognized by CD18 with the release of NETs.[75] Components of azurophilic granules, such as BPI and cathepsin G inhibit yeast growth.[8,76]

Protection depends on cellular immunity; however, IL-17A production promotes granulopoiesis, production and recruitment of neutrophils to the site of infection.[3,4,77] Study of neutrophil subcellular fractions has shown that yeasts promote NETs release and reduce their viability.[78]

THERAPEUTIC STRATEGIES IN COVID-19

Mortality of patients with COVID-19 (with mechanical ventilation) was 24-53%, in part, due to the interference of mucopurulent secretions in ventilation. Neutrophil NETs contribute to the viscosity of secretions and are also present in serum.

There are almost 100 clinical studies involving the use of drugs to inhibit NET formation although only 19 have been completed.[41] Studies have shown that the use of aerosolized **dornase alfa** (Pulmozyme, human recombinant DNAsa 1 and mucolytic) and albuterol decrease the viscosity of secretions by degrading DNA, improving oxygenation and reducing respiratory support in patients.[79-82] In addition, complications are due to the presence of antigen-antibody complexes in the plasma, which interact with FcγRIIA receptors (CD32a) on the neutrophil membrane and favor the formation of NETs. However, **fostamatinib** is a drug that inhibits the Spleen Tyrosine Kinase (SYK) activation pathway associated with these receptors, reducing the formation of NETs.[83] Also, there are controlled studies in COVID-19 patients using **colchicine**, since it interferes in inflammatory pathways inhibiting neutrophil adhesion, mobilization, degranulation and chemotaxis. It decreases cell adhesion molecules, consequently, it reduces migration and interaction of neutrophils with endothelial cells and their recruitment to the site of inflammation. It also prevents microtubule polymerization by inhibiting the formation of the NLRP3 inflammasome by reducing the production of IL-1β which prevents the production of IL-6 and TNF-α, and also inhibits the production of ROS and Nitric Oxide (NO).[84,85]

Additionally, drugs have been proposed to block NET components such as **alvelestat** which inhibits NE involved in inflammation,[86] **metformin** which binds to HMGB1 (High

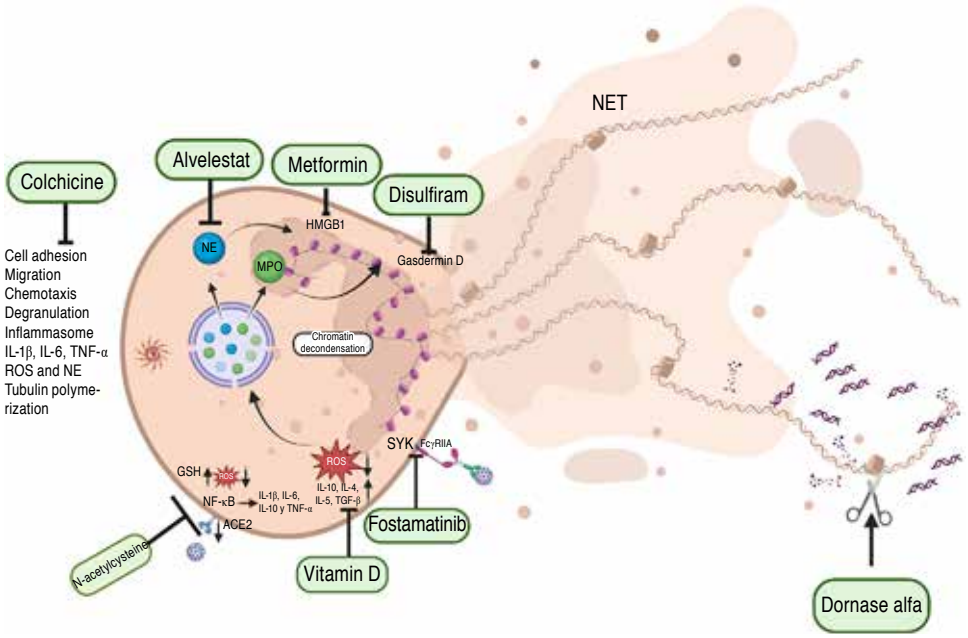

Figure 4:

Therapeutic strategies in patients with COVID-19. Neutrophils are involved in inflammation and thrombosis in patients with COVID-19, and drugs that intervene in different stages of activation have been used to reduce host damage.
(Created with BioRender.com).
GSH = reduced glutathione.
HMGB1 = High Mobility Group Box 1.
MPO = myeloperoxidase. NE = neutrophil elastase. NF-κB = nuclear factor kappa B.
ROS = reactive oxygen species.
TNF-α = tumor necrosis factor alfa.

mobility group box 1) inhibiting inflammation,[87] **disulfiram** which binds to gasdermin D inhibiting its ability to cause membrane pores and promote NET formation in COVID-19 patients reducing inflammation and tissue damage.[88]

The production of ROS is critical as it favors the production of NETs, and the use of **N-acetylcysteine**, which has an antioxidant function favoring the production of reduced glutathione that decreases ROS; and an anti-inflammatory effect by preventing the binding of the SARS-CoV-2 virus to the ACE2 receptor and inhibits the activation of the transcriptional factor NF-κB reducing the production of inflammatory cytokines, have been proposed.[89] **Vitamin D** has been proposed for use because it has anti-inflammatory actions by increasing the production of IL-10, IL-4, IL-5, and TGF-β, and because through antioxidant mechanisms it reduces oxidative stress and ROS production *(Figure 4)*.[90]

CONCLUSIONS

Neutrophils are important in host defense; however, in viral infections such as COVID-19 they are associated with inflammatory events and thrombosis. The knowledge of their mechanisms of action has allowed proposing therapeutic alternatives that can provide a better prognosis to patients with COVID-19. While in tuberculosis and histoplasmosis they have limited participation in the resolution of the disease.

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