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Literature Review

Unraveling NETosis: From Immune Mechanisms to Therapeutic Innovations

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Abstract: Neutrophil Extracellular Traps (NETs) are integral to immune responses, serving both protective and pathological roles in inflammatory diseases. Despite increasing recognition of NETs, significant gaps remain in understanding the nuanced mechanisms of NETosis and its implications for health and disease. This review systematically explores NETosis—the formation of NETs—by elucidating the molecular signaling pathways and cellular mechanisms involved. We highlight the distinction between classical suicidal NETosis and vital NETosis, providing detailed discussion of key regulatory molecules and events, including the roles of reactive oxygen species (ROS) and peptidyl arginine deiminase 4 (PAD4). Furthermore, we analyze the dual nature of NETosis: its protective role in pathogen clearance versus its contribution to tissue damage and autoimmunity. We examine regulatory influences of environmental factors, cytokines, and pathogens on NET formation. Finally, we discuss emerging therapeutic strategies targeting NETosis, focusing on inhibition of essential molecular components responsible for NET formation and the potential repurposing of existing therapies. This comprehensive review enhances understanding of NETosis mechanisms and their pathophysiological implications, ultimately guiding future research and therapeutic interventions across various inflammatory and autoimmune disorders.

Keywords: Neutrophil Extracellular Traps, NETosis, PAD4, Innate Immunity, Autoimmunity, Inflammation, Therapeutic Targets, Neutrophil Activation

Introduction

Neutrophils are the most abundant type of myeloid white blood cells in circulation, accounting for 50-70 percent of all leukocytes. These cells originate from blood forming stem cells in the bone marrow, where they undergo differentiation and maturation. Once fully developed, neutrophils are released into the bloodstream, where they typically survive for several hours up to three days (Wu *et al*., 2020). After this period, neutrophils either return to the bone marrow or are phagocytized in peripheral tissues. They play a vital role in host defense by rapidly migrating to sites of inflammation to eliminate pathogens and facilitate the inflammatory response (Rosales, 2018).

The primary function of neutrophils is closely linked to their phagocytic capabilities. Their granules are filled with various substances essential for eradicating microbes and one key mechanism they employ is the formation of Neutrophil Extracellular Traps (NETs) (Othman *et al*., 2022). These NETs consist of decondensed chromatin mixed with antimicrobial proteins, representing a novel aspect of neutrophil function (Gierlikowska *et al*., 2021; Manfredi *et al*., 2018). This groundbreaking process, initially described by Takei and further clarified by Arturo Zychlinsky, is known as NETosis and is associated with a distinct form of cell death (Papayannopoulos and Zychlinsky, 2009). Recent research categorizes NETosis into two forms: Classical (suicidal) NETosis, which results in complete neutrophil death and vital NETosis, which allows neutrophils to continue their phagocytic activity (Conceição-Silva *et al*., 2021; Poto *et al*., 2022; Wang *et al*., 2023; Tan *et al*., 2021 Korabecna and Tesar, 2017).

Despite significant advancements in understanding NETosis, key knowledge gaps persist regarding its regulation and implications in various inflammatory conditions (Wang *et al*., 2024; Zhao *et al*., 2021; Brinkmann and Zychlinsky, 2012). The existing literature, while extensive as evidenced by roughly 3,000 publications on the subject in the PubMed database often presents conflicting data regarding its mechanisms and roles (Jariwala and Laxer, 2021). Our study addresses these gaps by detailing the processes and pathways involved in both types of NETosis, comparing them with other Programmed Cell Death (PCD) types.

Importantly, this review goes beyond mere characterization by connecting the role of NETosis to the development of certain disorders, thereby providing insights that extend current knowledge in the field. By identifying and analyzing biochemical pathways and mechanisms that have yet to be fully understood, our study aims to highlight the significance and novelty of examining NETosis as a critical process not only in neutrophil functionality but also in broader immune responses.

This comprehensive overview of NETosis and its role in inflammation enhances our understanding of immune mechanisms and offers potential therapeutic insights into managing inflammatory diseases.

Neutrophil Extracellular Traps

Neutrophil Extracellular Traps (NETs) are composed of altered chromatin that creates a mesh of DNA strands with openings approximately 200 nanometers wide. These structures are enriched with bactericidal proteins derived from the neutrophil's nucleus, cytoplasm and granules (Chen and Boskovic, 2024). Important proteins present in NETs include histones, actin, α-actinin, S100 calcium-binding proteins A8, A9 and A12, along with neutrophil elastase and myeloperoxidase (Speziale and Pietrocola, 2021).

Additionally, other immune cells such as eosinophils, mast cells, basophils and macrophages can generate extracellular DNA traps when activated. This process was first termed Programmed Cell Death (PCD), which is separate from both necrosis and apoptosis (Hajibabaie *et al*., 2023). The concept of "vital NETosis" was introduced when researchers found that this process could occur without damaging the cell (Rada, 2019).

Double-Edge Effects of NETosis

In Brinkmann *et al*., and his team were the pioneers in detailing how activated neutrophils release DNA into the extracellular space to trap and eliminate pathogens (Brinkmann *et al*., 2004). This process, referred to as NETosis, is triggered by a range of pathogens and is crucial for the destruction of fungi, bacteria and even the suppression of certain viruses, underscoring its vital role in innate immunity (Chamardani and Amiritavassoli, 2022). Remarkably, neutrophils can also produce NETs under sterile conditions. Additionally, cytotoxic proteins associated with NETs can stimulate platelets and potentially harm host cells (Herre *et al*., 2023; Islam and Takeyama, 2023).

Patients with autoimmune disorders often exhibit autoantibodies against proteins released during NETosis, indicating that NETosis can have both beneficial and detrimental effects (Schönrich and Raftery, 2016; Kaplan and Radic, 2012).

While functional changes associated with NETosis have been documented, research into the key cellular mechanisms underlying this process is still in its early stages (Inozemtsev *et al*., 2023). We need to better understand how NETosis is initiated both inside and outside the cell. In particular, further investigation is required into the processes of chromatin decondensation, changes in nuclear shape, membrane disintegration and remodeling, as well as the subsequent release of cellular components. Below, we present the currently available information on these cellular developments (Thiam *et al*., 2020a; Poli and Zanoni, 2023).

The Two Types of NETosis

Modern literature describes two types of NETosis: (a) "Suicidal" NETosis, which involves NADPH oxidase (NOX) activity and (b) "Vital" NETosis, which occurs in the absence of such activity (Xiang *et al*., 2023; Li *et al*., 2024).

Suicidal NETosis was first characterized after neutrophils were stimulated to produce NETs using Phorbol Myristate Acetate (PMA). This stimulation, along with microcrystals of cholesterol or (Auto)antibodies, triggers the process (James *et al*., 2024; Guillotin *et al*., 2023. In response, calcium ions ($Ca^{2}+$) are released from the endoplasmic reticulum, activating protein kinase enzymes (Gupta *et al*., 2014). This activation then leads to the NOX complex being engaged, resulting in the production of various Reactive Oxygen Species (ROS), which are critical for suicidal NETosis. For example, these ROS initiate the degeneration of cytoplasmic granules containing Myeloperoxidase (MPO) and Neutrophil Elastase (NE) (Lin *et al.*, 2024). Additionally, ROS facilitate the migration of NE into the nucleus, where it cleaves histones to promote chromatin decondensation. The necessary histone H3 citrullination is concurrently enhanced by the activation of Peptidyl Arginine Deiminase 4 (PAD4) (Li *et al.*, 2023). The granule proteins and decondensed chromatin then combine to form NETs, which exit the cell following membrane rupture. This form of NETosis invariably leads to neutrophil destruction.

Several key molecules are involved in NET formation. PAD4 is essential for chromatin modification, the breakdown of cytoplasmic granules and the release of nuclear DNA into the cytoplasm (Volkov *et al.*, 2021). Research by Sprenkeler and colleagues has shown that successful DNA release during NETosis requires active and complete actin polymerization, along with functioning myosin II (Sprenkeler *et al.*, 2022). Cyclin-Dependent Kinases (CDK) also play an important role; NETs are produced only upon activation of CDK4 and CDK6, indicating that nuclear membrane rupture is closely linked to the cell cycle (Ding *et al.*, 2020). Studies suggest that in certain disorders, such as trauma and systemic lupus erythematosus, mitochondrial DNA, rather than nuclear DNA, may serve as the primary material for NETs (Nikiforov *et al.*, 2023). Mitochondrial ROS may facilitate calcium influx, though their specific role remains unclear. Furthermore, concentrations of CO_2 and bicarbonate (HCO_3^-) may affect the performance of NETosis and pH can influence glucose metabolism in neutrophils during suicidal NETosis (Halfon *et al.*, 2024; Jiao *et al.*, 2023).

The second type, vital NETosis, occurs in the absence of NADPH oxidase activity. Unlike suicidal NETosis, which lasts for about 3-4 h, vital NETosis lasts only around half an hour. The activation mechanisms for the two types vary significantly (Blagov *et al.*, 2023; Poznyak *et al.*, 2023). Vital NETosis can be induced by stimuli such as active platelets, microbial exposure and complement proteins, without NOX involvement in the process (Yipp and Kubes, 2013; Ravindran *et al.*, 2019). Upon activation, calcium ions enter neutrophils, stimulating PAD4, which facilitates histone H_3 citrullination. This process reduces the electrostatic bonds between DNA and histones, leading to chromatin decondensation and vesicular release of chromatin along with granule proteins and histones from the cells (Szatmary *et al.*, 2018). Importantly, this process spares the neutrophil's outer membrane, allowing the cells to survive (Wang *et al.*, 2009; Leshner *et al.*, 2012; Zhang *et al.*, 2023).

In suicidal NETosis, the N-terminal ends of histones are cleaved by neutrophil elastase, a feature that may help differentiate between the two types of NETosis (Zhu *et al.*, 2023). However, it is evident that further research into the mechanisms governing both processes is necessary (von Köckritz-Blickwede and Winstel, 2022).

Mechanisms of NETosis

A pivotal event during NETosis is the migration of proteins from granules into the cytosol. Azurophilic granules harbor a structure called the "asurosome," composed of eight proteins, including the serine proteases Neutrophil Elastase (NE), azurocidin, and cathepsin G, along with Myeloperoxidase (MPO), which generates hypochlorous acid from chloride and hydrogen peroxide (H_2O_2) (Arnhold, 2020). Research indicates that Reactive Oxygen Species (ROS) prompt the azurosome to dissociate from a membrane-bound complex, permitting its entry into the cytosol through an MPO-dependent mechanism (Orekhov *et al.*, 2023). Interestingly, MPO's enzymatic activity is not essential at this initial stage; instead, it functions primarily as an intracellular receptor for H_2O_2, but its role becomes crucial later in NETosis as it activates NE via hypochlorite anions (Herre *et al.*, 2023).

NE and other serine proteases contribute to NETosis by degrading cytoskeletal components, which is necessary for the process (Parker and Winterbourn, 2013; Akong-Moore *et al.*, 2012). Following this, these proteases relocate to the nucleus, where they act on the nuclear lamina and histones, leading to chromatin breakdown and the disintegration of the nuclear envelope. Notably, the release of proteins from granules during NETosis exhibits similarities to the ROS-dependent permeabilization of lysosomal membranes, which can cause the release of cathepsins during various forms of necrotic cell death (Kutay and Hetzer, 2008).

The translocation of Peptidyl Arginine Deiminase 4 (PAD4) from the nucleus to the cytoplasm is essential for the process of histone citrullination, which promotes chromatin decondensation. Disruption of PAD4 function impairs chromatin decondensation and NETosis triggered by Shigella flexneri or calcium ionophores. Neutrophils deficient in PAD4 do not produce NETs when stimulated with PMA (Zhou *et al.*, 2018). PAD enzymes are activated by calcium ions and H_2O_2, which facilitate histone citrullination. Furthermore, the processes involving histone citrullination and NETosis related to lipopolysaccharides rely on the integrity of microtubules (Green and Thompson, 2023). Thus, PAD4 activation appears to depend on multiple converging factors. While histones may also undergo acetylation during NETosis, the precise role of this modification remains ambiguous. Ultimately, the combination of chromatin decondensation and the proteolytic breakdown of the nuclear lamina leads to the nuclear membrane's disintegration and the release of chromatin into the cytoplasm (Lee *et al*., 2023).

Recent studies suggest that the activation of Cyclin-Dependent Kinases (CDKs), which drive cell cycle progression, may initiate NETosis. During this process, components of the mitotic apparatus, including the phosphorylation of nuclear lamin and centrosome separation, might be repurposed to dismantle the nuclear membrane (Konig and Andrade, 2016; Zhu *et al*., 2021).

In the final stages of NETosis, pores develop in the plasma membrane, allowing chromatin release and subsequent NET formation. Granular proteins attach closely to the decondensed chromatin through electrostatic interactions. Gasdermin D (GSDMD) is involved in forming these pores, facilitating the release of the chromatin complex, similar to its function during pyroptosis in macrophages (Hu *et al*., 2023). Unlike pyroptosis, where GSDMD activation is mediated by caspase-1 and caspase-4/5, in NETosis, NE primarily cleaves and activates GSDMD, facilitating pore formation in both the plasma and nuclear membranes (Weindel *et al*., 2023; Zhu *et al*., 2024). GSDMD activation in neutrophils may also result from non-canonical inflammasome pathways, although the implications for NETosis are still being studied (Shen *et al*., 2022; Zahid *et al*., 2021).

Another member of the gasdermin family, gasdermin E, is activated by caspase-3, which induces mitochondrial permeabilization and mediates apoptosis alongside secondary necrosis (Rogers and Alnemri, 2019). Additionally, MLKL, a pseudokinase not related to the gasdermin family, becomes activated by receptor-interacting protein kinases during necroptosis and may also contribute to membrane disruption. The activation of MLKL in neutrophils could further promote the formation and release of NETs (Kesavardhana *et al*., 2020; Stoess *et al*., 2023).

Physiological Modulation of Netosis

In a physiological environment, NETosis can be influenced by factors such as pH, the bicarbonate-to-carbonic acid ratio, and oxygen levels. A decreased CO_2/HCO_3^- ratio and moderate alkalinity enhance the degree of NETosis induced by agents like PMA, lipopolysaccharides, calcium ionophores, or monosodium urate microcrystals (Li *et al*., 2018). An increase in the pH level within the neutrophil cytoplasm raises calcium concentrations, accelerating ROS production from both NOX and mitochondria (Maueröder *et al*., 2016). Conversely, lowering the medium pH hampers NETosis, potentially due to the suppression of glycolysis. At low pH, the likelihood of the mitochondrial permeability transition pore opening is significantly reduced, which can lead to decreased mitochondrial ROS (mtROS) production (Kent *et al*., 2021; Napolitano *et al*., 2021). It is suggested that this pH dependence of NETosis ensures its maximal activation at the periphery of inflammation, providing antimicrobial protection while suppressing NETosis in the acidic center of the lesion to prevent excessive tissue damage (Vorobjeva *et al*., 2021; 2020).

The influence of hypoxia on NETosis remains contentious.

The regulatory role of HIF-1α, a central player in the hypoxic response, has significant implications for NETosis, the process by which neutrophils expel DNA traps to combat pathogens. Eliminating HIF-1α results in a notable decrease in NETosis, while pharmacological agents that stabilize HIF-1α enhance this process (Mitroshina and Vedunova, 2024). Interestingly, hypoxia can reduce NETosis induced by PMA, though this effect does not occur with Staphylococcus aureus and is independent of HIF-1α. Furthermore, the osmotic environment surrounding the cells plays a crucial role; a hypertonic solution can inhibit both Reactive Oxygen Species (ROS) production and NETosis. However, restoring NETosis is possible by supplementing with hydrogen peroxide (H_2O_2) when ROS levels are inadequate (Reyes *et al*., 2021; Chen *et al*., 2022).

NETosis is predominantly activated by proinflammatory cytokines, while anti-inflammatory agents can counteract this response. For instance, prostaglandin E_2 aids in resolving inflammation by increasing cyclic Adenosine Mono-Phosphate (cAMP) levels, which subsequently suppresses NETosis (Gierlikowska *et al*., 2022; Shishikura *et al*., 2016). Moreover, activated Protein C (aPC), a serine protease with multiple benefits including antithrombotic and anti-inflammatory properties, also inhibits NETosis. This inhibition occurs through its interaction with the endothelial protein C receptor and other pathways, as well as its ability to cleave histones within NETs. InterLeukin-10 (IL-10) is another anti-inflammatory cytokine known to inhibit NET formation. In cord blood, specific peptides have been identified that selectively inhibit NET development without disrupting other neutrophil functions like phagocytosis; a notable example is the "Neonatal Net Inhibitory Factor" (nNIF) (Mutua and Gershwin, 2021). Experiments in animal models have shown that administering nNIF intravenously can protect against bacterial sepsis and systemic inflammation caused by Lipo Poly Saccharides (LPS) (Healy *et al*., 2017).

Pathogens deploy various strategies to evade the antimicrobial effects of NETs. Many secrete lytic enzymes, especially endonucleases, which effectively degrade NETs. Furthermore, several bacteria, including

Mycobacterium tuberculosis and Pseudomonas aeruginosa, along with specific fungi from the Aspergillus family, develop protective membranes that help shield them from the damaging effects of NETs (Baz *et al*., 2024). For instance, Pseudomonas aeruginosa coats itself with sialic acids, which trigger the formation of IL-10 that inhibits NETosis. This mechanism is similarly utilized by the Human Immunodeficiency Virus (HIV), whose virions induce dendritic cells to produce IL-10, thereby shielding the virus from lytic destruction by NET enzymes. Additionally, the Hepatitis B virus hampers NETosis by reducing ROS production in neutrophils through its envelope and core proteins (Storisteanu *et al*., 2017; Domínguez-Díaz *et al*., 2021).

Open Question in the Pathogenesis

The reason why the activation of the same cell membrane receptors can stimulate both phagocytosis or degranulation, and NETosis remains unclear. One hypothesis suggests that the size of the microorganism plays a key role: Neutrophils are more likely to form NETs when a pathogen is too large to be ingested. Interestingly, NETosis can also be triggered by various extracellular and intracellular pathogens, including viruses.

For instance, Candida albicans have been shown to provoke NETosis even after being internalized by neutrophils (Schultz *et al*., 2022). Research suggests that younger neutrophils, which possess a greater number of granules, may be more susceptible to undergoing NETosis compared to their older counterparts, who have fewer granules. This observation raises the question of whether the quantity of granules affects the ability to generate NETs (Wigerblad and Kaplan, 2023). However, this idea is challenged by findings indicating that older neutrophils circulating in the bloodstream appear to have a higher propensity for NET formation. Additionally, low-density granulocytes found in patients with Systemic Lupus Erythematosus (SLE) or psoriasis exhibit spontaneous NETosis despite having a reduced granule count. This evidence implies that granule quantity may not be the critical factor influencing the NETosis process (Tay *et al*., 2020; Reshetnyak and Nurbaeva, 2023).

Investigating the factors that trigger NETosis in response to various stimuli, particularly whether the concentration of the stimulus or the degree of cellular activation is the key factor, could yield important insights into the mechanisms that initiate NETosis (Guiducci *et al*., 2018).

Unresolved Inquiries Regarding PAD4 in NETosis

The involvement of PAD4 in NETosis is well documented in both animal models and human cells; however, several critical questions remain unresolved. What are the mechanisms that activate PAD4 during NETosis in pathophysiological contexts in vivo (Song *et al*., 2023)? What specific concentration of Ca^{2+} is necessary for the activation of PAD4, and how is this concentration achieved in living organisms? Is PAD4 subject to Post-Translational Modifications (PTMs) that may influence its activation threshold (Rossetti *et al*., 2023)? Furthermore, does PAD4 facilitate chromatin decondensation exclusively through histone citrullination, or are there other substrates of PAD4 that contribute to this process?

Exploring these questions will not only deepen our comprehension of chromatin decondensation during NETosis but will also shed light on the fundamental mechanisms that govern DNA-histone interactions (Estúa-Acosta *et al*., 2019).

Unresolved Inquiries Regarding Primary Granule Resident Proteases in Netosis

Although there is evidence indicating that proteolytic enzymes are involved in NETosis, several details remain unclear. How do Neutrophil Elastase (NE) and cathepsin G gain access to chromatin during this process, and at what point does this occur? Is there a nuclear localization signal that enables them to cross into the nucleus? Do they enter the nucleus before or after the nuclear membrane is disrupted or made permeable (Kasperkiewicz *et al*., 2020)? Of particular interest is NE's strong binding affinity for DNA, which appears to inhibit its proteolytic activity. This leads to the question of how NE is able to cleave histone H_4 in the presence of DNA. Investigating the behaviors of NE, Proteinase 3 (PR3), and cathepsin G through live-cell imaging during NETosis could yield important insights into their individual functions in this process (Liang *et al*., 2022).

Unresolved Inquiries Regarding DNA Release into the Cytosol

Despite widely acknowledged evidence that decondensed chromatin must disrupt the nuclear lamina and envelope for NETosis to occur, much remains unclear about the underlying mechanisms. What initiates the formation of gaps in the lamin meshwork during NETosis? If these gaps result from local disassembly, which enzymes facilitate the necessary protein modifications for this process? Can the mechanical forces generated by chromatin decondensation trigger the disruption of both the nuclear lamina and membranes?

What is the kinetics of the inner and outer nuclear membranes, as well as the lamin network, during active NETosis? If decondensed chromatin is enclosed in vesicles that emerge from the nuclear membranes, what is the organization of these vesicles? Furthermore, if these vesicles contain both the inner and outer nuclear membranes, how do they merge with the plasma membrane?

High-resolution live-cell imaging will be essential for elucidating the mechanisms of DNA release during active NETosis (Thiam *et al*., 2020b).

Unresolved Inquiries Regarding Cytoskeletal Breakdown

Beyond providing structural support for cells, the cytoskeleton is vital to numerous signaling pathways, such as mechanotransduction, cellular polarity, organelle transport, and force generation. As a result, the degree of cytoskeletal disassembly during NETosis prompts several critical inquiries:

- Is there a polarization of the plasma membrane in cells that are undergoing NETosis?
- How are organelles arranged during the NETosis process?
- What mechanisms are responsible for generating the forces required to complete NETosis?
- How does NETosis occur under physiological conditions where cells must adjust to the stiffness and architecture of the extracellular matrix?

New insights and theoretical frameworks regarding force generation will be important for elucidating the dynamics of NETosis in vivo (Uray and Uray, 2021).

Therapy of NETosis

The evidence linking NETosis to various disorders has sparked extensive research into diverse therapeutic strategies. One approach involves using medications to inhibit NETosis. This includes anti-cytokine treatments aimed at reducing neutrophil accumulation at sites of inflammation and preventing their activation, as well as inhibitors targeting key components of the NETosis process, such as Neutrophil Elastase (NE), PAD4 and Gasdermin D (GSDMD). Another strategy focuses on degrading NETs or mitigating their harmful effects (Mutua and Gershwin, 2021).

Anti-cytokine treatments targeting IL-1β are commonly employed in autoimmune and inflammatory disorders to potentially address excessive NETosis. Currently, clinical trials are underway for Anakinra, a recombinant IL-1β receptor antagonist, as a prospective treatment for COVID-19. Regarding NETosis inhibitors, most experimental data has been gathered on NE inhibitors. The first NE inhibitor, sivelestat, was approved for treating acute respiratory distress syndrome in Japan and South Korea; however, meta-analyses of clinical data have not consistently supported its efficacy. Clinical studies on a new generation of NE inhibitors have only recently commenced (Cesta *et al*., 2023). Trials involving PAD4 and GSDMD inhibitors are still in the preclinical phase.

There is considerable interest in repurposing existing medications to inhibit NETosis. For example, disulfiram, used in alcoholism therapy, inhibits GSDMD activation and has shown protective effects in animal models of lethal LPS-induced sepsis. Notably, GSDMD also plays a crucial role in the pyroptosis pathway, with studies suggesting its involvement in inhibiting macrophage pyroptosis. In vitro studies have shown that microtubule inhibitors, such as colchicine, can also inhibit NETosis. Clinical trials are currently investigating the efficacy of colchicine against COVID-19 (Hu *et al*., 2020; Zhuang *et al*., 2022).

Among the medications aimed at degrading NETs, DNase I and anti-histone antibodies have been extensively investigated. Recombinant DNase I has proven effective in nearly all NETosis-associated disease models. Specifically, the introduction of DNase I significantly improves outcomes in animal experiments involving acute respiratory distress syndrome and chronic obstructive pulmonary disease. In patients with cystic fibrosis, inhalation of DNase has optimized lung function, while NE has facilitated phlegm dissolution, enhancing accessibility for DNase treatment. Clinical trials of DNase variants resistant to actin, such as PRX-110, are progressing through Phase II and showing promising results. There is hope that DNase will not only liquefy phlegm but also halt the progression of ARDS, as observed in murine studies (Chen *et al*., 2023; Gollomp *et al*., 2020).

Studies using the mitochondria-targeted antioxidant SkQ1 have demonstrated its potential anti-NETosis efficacy. In a murine model of systemic inflammatory response syndrome, SkQ1 mitigated the lethal effects of the inflammatory cytokine TNF. MitoQ, an antioxidant structurally similar to SkQ1, inhibited NETosis in a murine model of lupus (SLE). These findings suggest that medications utilizing mitochondria-targeted antioxidants are likely to be developed soon to treat conditions associated with excessive NETosis, particularly in the context of COVID-19 (Fedorov *et al*., 2022).

Limitations

While this review provides a comprehensive overview of NETosis, several limitations should be considered:

- Variability across studies: The mechanisms and functions of NETosis can vary significantly across different studies due to variations in experimental conditions, such as cell types, stimuli, and assays used. This variability can affect the reproducibility of findings and complicate the generalization of results.
- Focus on specific mechanisms: Although we aim to cover the main pathways involved in NETosis,

certain less-studied mechanisms may not be fully addressed. This oversight could limit our understanding of the complete landscape of NETosis and its implications in various physiological and pathological contexts.

- Limited clinical data: The translation of NETosis research into clinical applications is still in its infancy. Many therapeutic approaches targeting NETosis are based on preclinical studies or small-scale clinical trials. Larger, multicentric studies are needed to validate these findings and determine their clinical relevance.
- Complexity of the immune response: NETosis does not occur in isolation; it is part of a complex immune response involving various cell types and signaling pathways. This interconnectedness means that alterations in NETosis may have broader implications that are not fully captured in standalone studies.
- Potential for overemphasis: There is a risk of overemphasizing the role of NETosis in certain diseases without acknowledging other contributing factors and pathways. A more balanced perspective is essential to avoid attributing disease causation solely to NETosis.
- Evolution of knowledge: The field of NETosis is rapidly evolving, with new discoveries emerging regularly. As such, this review may not encompass the latest research findings published after the cutoff date, limiting its applicability to the most current understanding of the topic.

Recognizing these limitations is crucial for contextualizing the findings presented in this review and for guiding future research in the area of NETosis.

Conclusion

In summary, the comprehensive exploration of NETosis has unveiled a complex array of molecular pathways and functional implications associated with this intriguing biological process. The formation and release of Neutrophil Extracellular Traps (NETs) have been shown to play crucial roles in a wide range of physiological and pathological conditions, from host defense and immune regulation to the pathogenesis of inflammatory, autoimmune, and thrombotic disorders.

The multifaceted involvement of NETs in innate immunity, inflammation, and thrombosis emphasizes their significant impact on human health. NETs have the ability to capture and neutralize pathogens, regulate immune responses, and modulate inflammatory processes, highlighting their essential contribution to maintaining immune homeostasis and host defense. Moreover, emerging evidence linking aberrant NET formation to various diseases, including sepsis, autoimmune disorders, vascular diseases, and cancer, underscores the clinical relevance of understanding NETosis.

Importantly, identifying potential therapeutic targets and strategies aimed at modulating NET formation and activity holds great promise for developing novel interventions. Harnessing the physiological and pathological insights of NETs to create targeted therapies for conditions characterized by dysregulated NETosis presents an exciting avenue for future research and clinical applications.

However, despite significant progress in elucidating the molecular mechanisms of NETosis, many fundamental questions and challenges remain. Further investigations are needed to unravel the precise regulatory networks governing NET formation, including the intricate interplay between various signaling pathways, epigenetic modifications, and environmental cues. Additionally, gaining a deeper understanding of the context-dependent roles of NETs in different disease states, as well as their interactions with other immune cells and vascular components, is essential for leveraging the therapeutic potential of targeting NETosis.

In conclusion, this thorough exploration of NETosis has not only enhanced our understanding of neutrophil biology and immunology but also holds great promise for developing innovative therapeutic approaches. By advancing our knowledge of NETosis and its implications for human health and disease, we can aspire to translate these insights into clinical applications, ultimately improving patient outcomes and addressing unmet medical needs.

Author's Contributions

All authors equally contributed to this research.

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