


Targeting Neutrophil Extracellular Traps in Gouty Arthritis: Insights into Pathogenesis and Therapeutic Potential

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Abstract: Gouty arthritis (GA) is an immune-mediated disorder characterized by severe inflammation due to the deposition of monosodium urate (MSU) crystals in the joints. The pathophysiological mechanisms of GA are not yet fully understood, and therefore, the identification of effective therapeutic targets is of paramount importance. Neutrophil extracellular traps (NETs), an intricate structure of DNA scaffold, encompassing myeloperoxidase, histones, and elastases - have gained significant attention as a prospective therapeutic target for gouty arthritis, due to their innate antimicrobial and immunomodulatory properties. Hence, exploring the therapeutic potential of NETs in gouty arthritis remains an enticing avenue for further investigation. During the process of gouty arthritis, the formation of NETs triggers the release of inflammatory cytokines, thereby contributing to the inflammatory response, while MSU crystals and cytokines are sequestered and degraded by the aggregation of NETs. Here, we provide a concise summary of the inflammatory processes underlying the initiation and resolution of gouty arthritis mediated by NETs. Furthermore, this review presents an overview of the current pharmacological approaches for treating gouty arthritis and summarizes the potential of natural and synthetic product-based inhibitors that target NET formation as novel therapeutic options, alongside elucidating the intrinsic challenges of these inhibitors in NETs research. Lastly, the limitations of HL-60 cell as a suitable substitute of neutrophils in NETs research are summarized and discussed. Series of recommendations are provided, strategically oriented towards guiding future investigations to effectively address these concerns. These findings will contribute to an enhanced comprehension of the interplay between NETs and GA, facilitating the proposition of innovative therapeutic strategies and novel approaches for the management of GA.

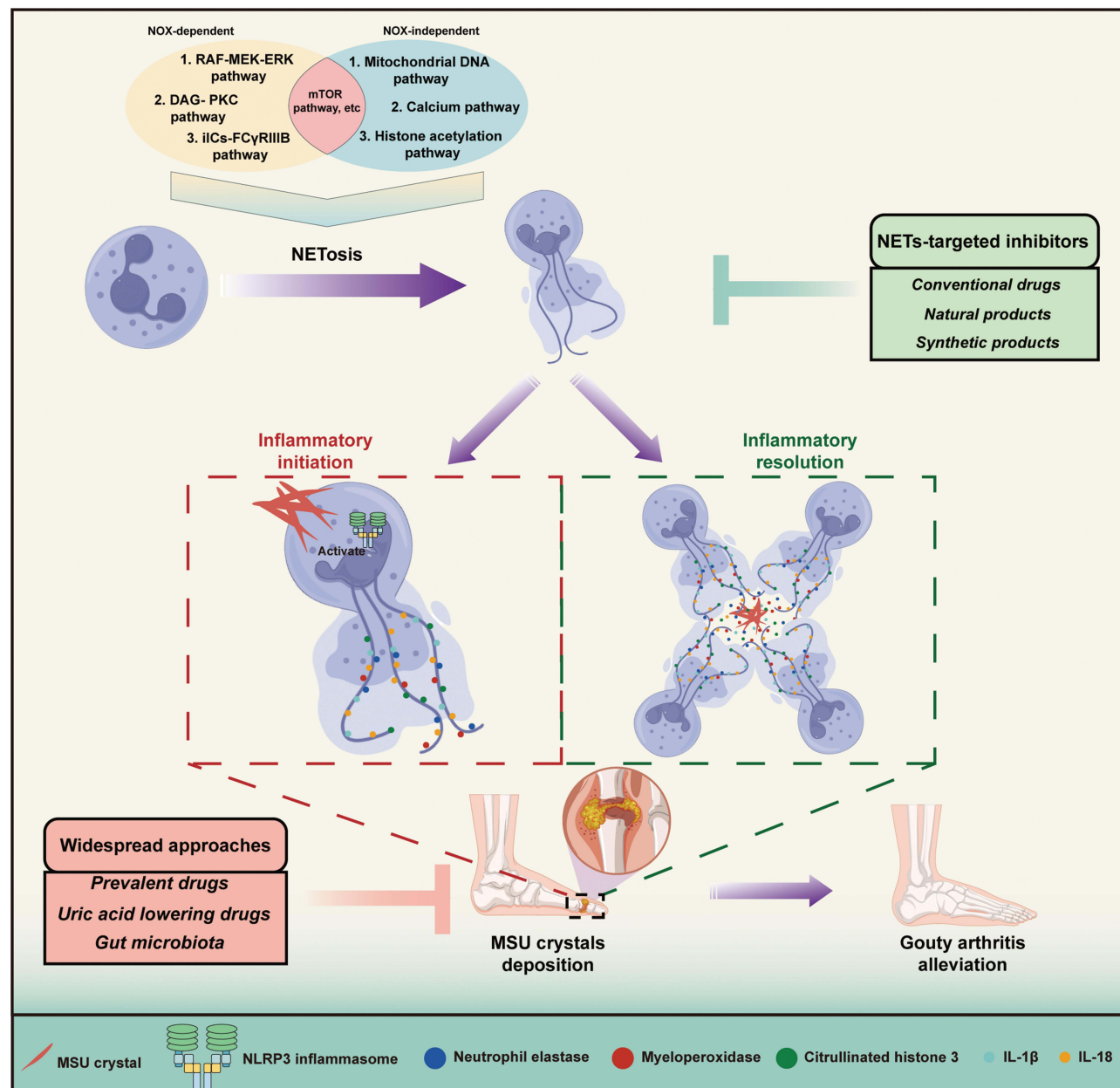
Keywords: neutrophils, neutrophil extracellular traps, gouty arthritis, inflammation, NETs inhibitor

Introduction

Gouty arthritis (GA) is an inflammatory arthritis caused by abnormal monosodium urate (MSU) deposition that occurred in joints and peripheral tissues¹ Ample evidence has suggested that the incidence and prevalence of GA are increasing around the world,² bringing burdens on health systems.³ Patients with GA are characterized by high levels of serum uric acid (UA).⁴ experiencing bursts of symptoms and remission including intense pain, redness and swelling.⁵ Uric acid can accumulate in the bloodstream due to its excessive production or impaired renal excretion, ultimately resulting in the formation of needle-shaped crystals in the joints and adjacent tissues.⁶ Subsequently, the deposition of uric acid crystals in joints triggers an immune response as the body identifies the crystals as foreign substances, thereby initiating an inflammatory cascade that ultimately culminates in the distressing symptoms of gouty arthritis.^{7,8} Nevertheless, the underlying mechanisms behind GA onset and its interaction with the immune system are relatively vague.

Neutrophils are the most abundant immune cells in human and animal peripheral blood.⁹ As the first-line cells in immune system, neutrophils exhibit rapid responsiveness to both microbial and inflammatory cues emanating from damaged tissues. Neutrophils are swiftly mobilized to the site of injury to combat various pathogens, such as microcrystals.¹⁰ In homeostatic

Graphical Abstract



conditions, neutrophils are maintained at a low baseline density.¹¹ However, when tissue damage or infection occurs, neutrophils assume critical roles as immune effectors, with their density increasing dramatically and their recruitment to the damaged site being promptly activated.¹²

Extracellular traps (ETs) are web-like structures containing DNA, histones, and various granule proteins like myeloperoxidase (MPO), released by activated neutrophils and other immune cells to capture and eliminate invading pathogens.¹³ Among them, neutrophils extracellular traps (NETs) have been implicated in different pathological conditions, such as GA.¹⁴ During GA development, NETs are released in response to MSU crystal stimulation, triggering an inflammatory cascade. It seems that inhibiting the formation of NETs, also called NETosis, appears as a feasible approach to alleviate GA.¹⁵ Several studies have also identified various factors that regulate the formation and degradation of NETs.^{16,17} However, evidence also showed that

aggregated NETs (aggNETs), consisting of high-density of NETs, appeared to exert protecting role from inflammation via packing MSU crystals, and the mechanisms of which still remain elusive.¹⁸ Of note, targeting components from NETs offers new avenues for developing NET-based therapies for the GA treatment. In this review, we undertake a comprehensive review of the constituents of NETs and the diverse mechanisms underpinning NETosis, providing a detailed illustration. Additionally, we synthesize current knowledge on clinically available pharmacological interventions for GA and innovatively evaluate natural and synthetic product-based inhibitors that target NET formation as novel therapeutic options. This review aims to illuminate potential therapeutic strategies that leverage our understanding of NETs to combat GA effectively.

NET: The Special Network Structure Released by Neutrophil

NETs have gained a wide range of attention for their production, which is accompanied by the following inflammatory cascade. NETs are reticular skeletons composed of DNA released by neutrophils outside the cell, to which nuclear proteins (eg, histones), cytoplasmic proteins, granular proteins (eg, neutrophil elastase (NE), myeloperoxidase (MPO)), and antimicrobial peptides, etc., are attached (Figure 1). Most of these components exert different effects when they encounter different responses in the immune system.

The extracellular DNA in NETs is derived from the chromatin of the neutrophil nucleus, which is extruded from the cell and released into the extracellular space. The process of NETosis, characterized by the dissolution of the nuclear envelope, decondensation of chromatin and extrusion of DNA, is activated in response to various stimuli.^{19,20} As the major component of NETs, extracellular DNA is considered to be a damage-associated molecular patterns (DAMPs)^{21,22} that induces pro-inflammatory cascades.²³ NETs have been implicated in the pathogenesis of various of inflammatory and autoimmune diseases, including sepsis, rheumatoid arthritis, and lupus.^{24,25} The DNA in NETs can activate different immune cells, such as macrophages and dendritic cells, leading to the release of pro-inflammatory cytokines and amplification of the inflammatory responses.^{26,27} A study²⁸ has shown that the level of cell-free DNA in the plasma of patients with sepsis was positively correlated with mortality, meaning the higher the level of cell-free DNA, the higher the mortality rate of sepsis. Furthermore, DNA has been identified as a key factor in extending the lifespan of neutrophils. Notably, stimulation of neutrophils with mitochondrial DNA has been shown to enhance their activity.²⁹ DNA's significance as the fundamental framework of NETs is undeniable.

Histones are a group of highly basic and conserved proteins located in the nucleus and are a key component of the web-like structures that are released by activated neutrophils. Several histone subtypes, including histones H2, H3, and H4 constitute

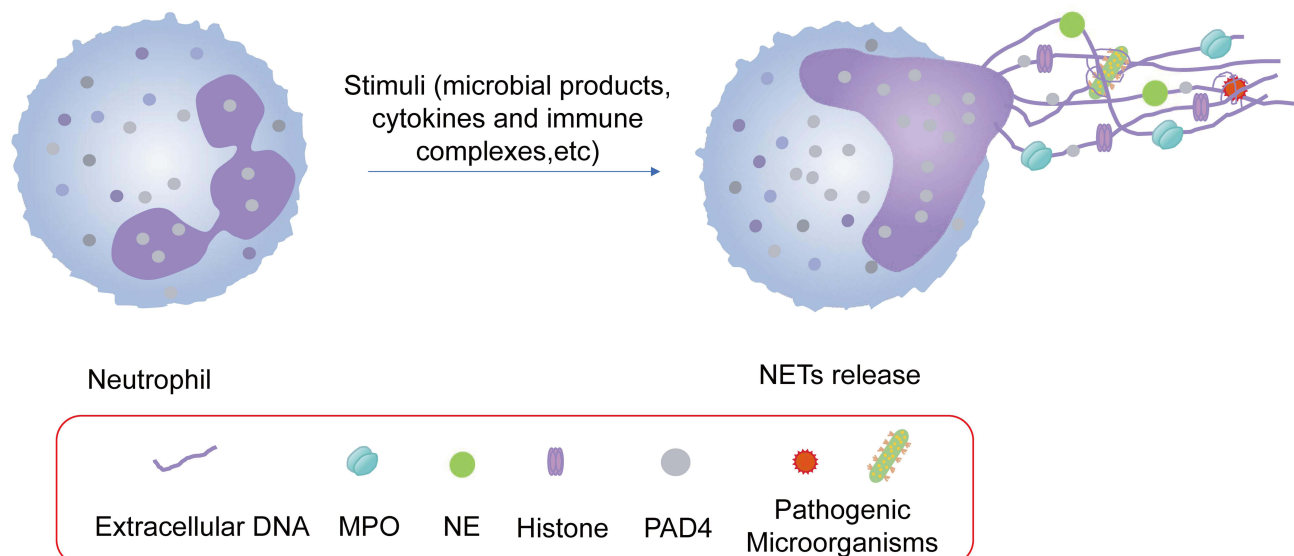


Figure 1 Neutrophils extracellular trap formation. After being stimulated by various inducers including microbial products, cytokines, immune complexes and etc, neutrophils undergo chromatin decondensation and nuclear membrane breakdown, leading to the release of NETs. NETs are mainly DNA-based skeleton structures whose surfaces are inlaid with histones, NE, MPO PAD4. Moreover, NETs are characterized by antibacterial effect through capturing and killing pathogenic microorganisms.

a complex with DNA called nucleosome.³⁰ Histones, a key component of NETs, contribute significantly to the antimicrobial activity of NETs. Specifically, the positively charged amino acids in histones negatively interact with the charged cell membranes of microorganisms, promoting their adherence to the NETs and leading to subsequent elimination.³¹ Not only do histones possess antimicrobial properties, but they also exhibit toxic effects on the host, triggering pro-inflammatory responses upon their release from the nucleus into the extracellular space.^{32,33} The release of histones from dying cells, including from NETs, has been implicated in the pathogenesis of a range of inflammatory and autoimmune diseases, including sepsis, rheumatoid arthritis, and lupus.³⁴ On the one hand, histones can be passively released through the necrotic program to exacerbate inflammatory responses. Moreover, histones can also be actively released during NETosis to exert an antibacterial effect.³⁵ The role of histones as damage-associated molecular patterns (DAMPs) can trigger toll-like receptors (TLRs), activate the NLRP3 inflammasome, and induce calcium influx, leading to the initiation of inflammation in conditions, such as acute pancreatitis.³⁶ In septic mice, histones can be expected to result in endothelial dysfunction, organ failure and even death.³⁷ It must be mentioned that high mobility group box 1 (HMGB1) is a key component of NETs, where it plays an important role in immune response like histones. HMGB1 is a highly conserved nuclear protein present in most mammals. In its capacity as DAMPs, HMGB1 can promote the aggregation of neutrophils at sites of tissue damage and enhance inflammatory responses via interaction with other DAMPs (such as DNA) or pathogen-associated molecular patterns (PAMPs) (such as lipopolysaccharide (LPS)).^{38,39} HMGB1 containing disulfide bonds binds to TLR4, inducing cytokine production by macrophages and also promotes NETs formation by neutrophils.⁴⁰ The relationship between histones and HMGB1 in the context of NETs is complex and involves both synergistic and antagonistic interactions.⁴¹ HMGB1 is known to promote the release of histones from neutrophils and to enhance their ability to bind to bacterial membranes, thereby increasing the efficacy of NETs in killing invading microorganisms.⁴² However, HMGB1 is able to exert both pro-inflammatory and anti-inflammatory effects, depending on the cellular and molecular context when histones begin to activate immune cells and promote inflammation.^{43,44}

Peptidyl arginine deiminase 4 (PAD4) is a calcium-dependent enzyme that plays a critical role in NETs formation. During the process of NETosis, activated neutrophils undergo chromatin decondensation and nuclear membrane breakdown, allowing for the release of NETs composed of DNA, histones, and other proteins.⁴⁵ The citrullination of histones by PAD4 is a crucial step in this process, as it alters their charge and structure, facilitating their release from chromatin and promoting the formation and stability of NETs.^{46,47} PAD4 has been implicated in the pathogenesis of a range of autoimmune and inflammatory diseases.^{48–50} Wesley et al demonstrated that mice lacking PAD4, a key enzyme involved in NETosis, exhibited a significant reduction in NET formation and pro-inflammatory cytokine production, leading to protection against acute kidney injury induced by renal ischemia/reperfusion.⁵¹ Renal function was restored to 48 hours after ischemia/reperfusion, whereas renal function in wild-type mice gradually deteriorated. The PAD-specific inhibitor YW3-56 was used for validation, which indicates that PAD4 plays a key role in ischemia/reperfusion-induced acute kidney injury. It is also a strategic point to differentiate apoptosis⁵² and necrosis.^{53,54}

MPO is present in specific tissues within neutrophils, monocytes, and macrophages. The majority (95%) of MPO found in the bloodstream originates from neutrophils, making changes in its levels a reflection of alterations in neutrophil functionality. Some studies^{55,56} have found that MPO in NETs was biologically active and exhibited bactericidal ability after binding to DNA in NETs, where it generated hypochlorous acid and other reactive oxygen species (ROS) that contributed to the microbicidal activity of NETs.⁵⁷ MPO is also involved in the modification of histones, which helps increase the antimicrobial activity of NETs.⁵⁸ Simultaneously, by utilizing hydrogen peroxide (H₂O₂) and chloride ions, MPO has the ability to generate hypochlorite, which effectively contributes to the eradication of bacteria. This evidence demonstrates that MPO can enhance the bactericidal efficacy of NETs, while also causing tissue damage. Therefore, MPO may act as an antigen in the pathogenesis of anti-neutrophil cytoplasmic antibody-associated vasculitis.⁵⁹ Meanwhile, MPO has emerged as a key player in the pathogenesis of numerous inflammatory conditions, such as vasculitis, systemic lupus erythematosus, and acute pancreatitis.^{60–62} By producing ROS and other substances, MPO can inflict tissue damage and exacerbate inflammation, thereby contributing to the pathophysiology of these diseases.⁶³ Recent studies have shown that inhibiting MPO activity with a specific inhibitor is capable of suppressing the formation of NETs.^{57,64} Collectively, these findings underscore the importance of MPO as a critical component of NETs in mediating their antimicrobial activity, but also emphasize the need to regulate its activity to prevent its contribution to inflammatory disease pathogenesis.

Neutrophil elastase (NE) represents a serine protease variant prominently housed within the azurophilic granules of neutrophils, exerting a pivotal role as a fundamental constituent within NETs. The presence of both NE, as well as MPO, within neutrophils is notable, as these enzymes are extensively associated with the fiber network of NETs.⁶⁵ During NETosis, translocation of NE to the nuclear membrane is required for chromatin decondensation, whereas MPO can bind to chromatin and enhance chromatin dedensification.^{66,67} In addition to degrade extracellular matrix proteins and inflammatory mediators such as cohesin, various membrane proteins and IL-8, etc., NE is capable of inhibiting tissue factors and promoting the formation of vascular fibrin.^{66,68,69} Studies have shown that the formation of NETs involved with MPO and NE depends on the different types of stimuli. Parker⁵⁹ et al found that the generation of phorbol 12-myristate 13-acetate (PMA)-stimulated NETs required the involvement of MPO without the need for *Staphylococcus aureus* or *E. coli*. Conversely, *Leishmania* parasites⁷⁰ induced the generation of NETs that required NE without the need for MPO and ROS. As a potent inducer of NETs, PMA is widely used in the research about NETs based on the different kinds of signaling pathways, enzymes, and ROS requirements.⁷¹ Another inducer of NETs is the MSU crystal. In gout, MSU crystal is the main precipitated form of urate in the blood, at the same time, is also an important participant in activating the neutrophils, which facilitates the release of NETs.⁷² It is worth noting that, unlike PMA, the release of NETs induced by MSU crystal is uncertain if the ROS involvement is needed. According to the in vivo research from Davidson and her companions,⁷³ NET formation induced by MSU crystals was independent of ROS production. The same findings were also found in other studies. Tasiy et al found that under the activation of MSU crystals, NETosis was found to be independent of endogenous ROS, but under the control of PAD4.⁷⁴ Conversely, NETosis caused by MSU crystals in vitro was found to be in an ROS-dependent manner. What's more, inhibiting ROS through different anti-oxidants restrained NETosis caused by MSU crystals.¹ This controversial view deserves a deeper exploration, which may provide insights into the progression of NETosis in diseases.

The Formation of NETs: Vital NETosis and Suicidal NETosis

The formation process of NETs is called NETosis which is a novel type of programmed cell death distinguished from neutrophil apoptosis, necrosis and pyroptosis. The concept of NETosis was firstly purposed by Brinkman and his colleagues in 2004.⁷⁵ They offered the opinion that neutrophils were activated and released extracellular meshwork, called NETs, that was decorated with granule proteins and chromatin, leading to degrade virulence factors and kill bacteria. Following an extensive span of nearly two decades devoted to research, there has been a discernible augmentation in the comprehension of NETosis, leading to a more comprehensive and profound knowledge of this biological process. The process of NETosis can be triggered by various stimuli, such as microbial products, cytokines, and immune complexes, and can occur via distinct mechanisms, mainly involving vital and suicidal NETosis that depend on the viability of neutrophils.^{19,45,76}

The most striking difference between NETosis and other modes of death is the changes in the nucleus and the release of NETs. Upon stimulation, the nuclear membrane of neutrophils disintegrates into vesicles, while chromatin begins to decondense. Then, antimicrobial peptides are released from intracellular particles to adhere to lost chromatin. Simultaneously, upon the disruption of the plasma membrane, a diverse array of intracellular components, including proteins, DNA, and other entities, are extruded into the extracellular milieu. Notably, this process does not entail the condensation of chromatin, commonly known as chromatin pyknosis. Although researches have proved that the formation of NETs improved the body's defense mechanism, NETs are believed to be capable of causing the body to break the pro- and anti-inflammation balance.^{77,78} Apoptosis is cell shrinkage accompanied by DNA rupture and nucleus condensation. The whole cell forms apoptotic bodies through blebbing and other methods. Of note, the process of apoptosis does not cause a rupture of the plasma membrane and the release of NETs, which is the biggest difference between apoptosis and NETosis.⁷⁹ Additionally, neither inflammatory mediators nor inflammatory responses occur in this process. Cell necrosis leads to the swelling and rupture of cells, including the processes like the swelling of various intracellular organelles such as the nucleus, the rupture of the plasma membrane, the release of cellular contents, and the triggering of inflammation.⁷⁹ What distinguishes with NETosis is that in the process of cell necrosis, DNA is barely released. Fuchs et al⁸⁰ performed live cell imaging of NETs to reveal the difference between NETosis, apoptosis and pyroptosis. Pyroptosis is typified by cellular shrinkage, accompanied by DNA fragmentation and degradation, which is similar with the apoptotic process.⁸¹ Pyroptosis is distinguished by cell necrosis accompanied by the process of cell swelling, rupturing and releasing intracellular inflammatory substances, which induces inflammation in the body without production and release of NETs structure.^{82,83}

Vital NETosis, also known as non-lytic NETosis or “vivacious NETosis”, is a relatively new type of NETosis that was first described in 2011.⁸⁴ Unlike suicidal NETosis, vital NETosis does not result in the death of the neutrophil, but rather, the release of NETs while the neutrophil remains alive and functional.⁸⁵ During vital NETosis, the neutrophil undergoes significant morphological changes, including the formation of nuclear lobes and the extrusion of chromatin into the extracellular space, which is accompanied by the release of antimicrobial molecules such as myeloperoxidase and neutrophil elastase. Vital NETosis has been suggested to play a role in promoting wound healing and preventing excessive inflammation, as it can limit the spread of bacterial infections and promote tissue regeneration.⁸⁶

On the other hand, suicidal NETosis, also known as lytic NETosis or “suicidal NETosis”, is a more well-known and characterized type of NETosis that results in the death of the neutrophil.⁸⁷ Suicidal NETosis is distinguished by the rupture of the nuclear envelope of neutrophils, subsequently leading to the extracellular release of chromatin and granular proteins. This process is mediated by the activation of the NADPH oxidase complex, which produces ROS that lead to DNA damage and histone citrullination, as well as the activation of proteases and endonucleases that degrade the neutrophil’s nuclear and cellular components.⁸⁸ Suicidal NETosis is important for the host defense against various pathogens, including bacteria, fungi, and viruses, but it can also contribute to tissue damage and the development of autoimmune and inflammatory diseases.⁸⁹

In summary, vital and suicidal NETosis are two distinct forms of neutrophil death that contain the process of releasing extracellular traps to fight against pathogens. Vital NETosis is currently considered as a relatively new and less characterized process that enables neutrophils to remain viable and functional. In contrast, suicidal NETosis is a well-established mechanism that results in the death of neutrophils and is critical for host defense against infections.

The Underlying Mechanism of NETs Formation

According to the formation mechanism of NETs, it is mainly divided into three pathways: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NADPH oxidase 2, NOX)-dependent, -independent and others (Figure 2).

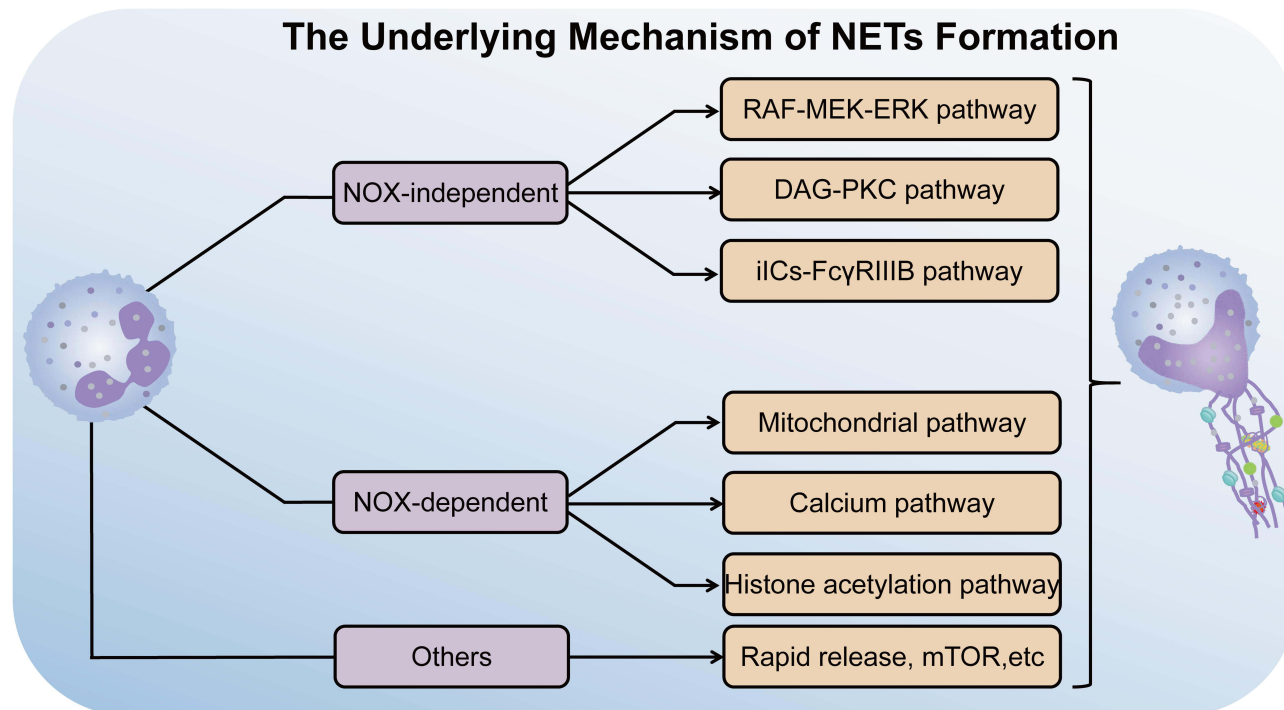


Figure 2 The underlying mechanism of NETs formation. The mechanism of NETs formation can be classified into NOX-dependent pathway, NOX-independent pathway and others. The NOX-dependent pathway to induce NETs formation contains RAF-MEK-ERK pathway, DAG-PKC pathway and iICs-FcγRIIIB Pathway. On the other hand, NETs formation is caused by NOX-independent pathway including the mitochondrial DNA pathway, Calcium pathway and histone acetylation pathway. In some special cases, NETs formation can be performed through neither NOX-dependent pathway nor NOX-independent pathway, as referred to rapid release of NETs formation. On the contrary, pathway like the mTOR pathway is involved in NETs formation by either NOX-dependent pathway or NOX-independent pathway.

NOX-Dependent Formation of NETs

The NADPH oxidase complex is a critical enzyme involved in the generation of ROS that is necessary for the formation of NETs. It has been reported that NOX-dependent formation of NETs is a critical mechanism in the pathogenesis of various inflammatory and autoimmune diseases.⁹⁰ The NOX-dependent pathway is the predominant mechanism involved in the production of NETs.⁸⁰ Plenty of pro-inflammatory mediators can stimulate neutrophils to generate NETs, such as PMA, LPS, interleukin-6 (IL-6), IL-8, tumor necrosis factor alpha (TNF- α), as well as bacteria, fungi, and chemicals.⁹¹ The activation of NOX by stimuli causes the generation of ROS, including O₂, H₂O₂, and HOCl. In addition to killing microorganisms,⁹² ROS is capable of activating NE and MPO in neutrophils⁹³ and interacting with the nucleus without restraint, in which serine proteases and NE cleave histones to promote chromatin decondensation.^{94,95} Soon afterwards, the nuclear membrane loses integrity, leading to the release of chromatin into the cytosol and subsequently the final form NETs.^{65,69} Here, the pathways involved in the NOX-dependent formation of NETs are illustrated in the following.

RAF-MEK-ERK Pathway

The rapidly accelerated fibrosarcoma (RAF)-mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK) pathway (RAF-MEK-ERK pathway) is one of the mitogen-activated protein kinase pathways. This signaling pathway exhibits the capacity to convey extracellular signals to the nucleus, facilitating interactions with specific transcription factors and subsequently eliciting context-dependent cellular responses.⁹⁶ The RAF-MEK-ERK pathway can be activated by various stimuli, for example PMA and Fc γ receptors (Fc γ RIIb), to induce NETosis.⁹⁷ Some studies^{98,99} have shown that the RAF-MEK-ERK pathway is crucial in the process of PMA-induced NETs generation, while downregulating the expression of apoptotic protein Mcl-1 to prevent cell apoptosis. This downregulation can be blocked with protein kinase C (PKC), cRaf and MEK inhibitors.¹⁰⁰ In addition, this pathway also acts as a vital role in NETs induced by unconventional stimuli. During the process of amoebiasis, neutrophils from the host are stimulated and NETs are induced by amoeba. Study has proved that selective inhibition on RAF and ERK exhibited prevention of *E. histolytica* induced NETs.¹⁰¹ The evidence strongly implies that RAF-MEK-ERK pathway serves as an upstream modulator of NADPH oxidase, thereby implicating its involvement in the formation of NETs through the activation of NADPH oxidase and up-regulation of anti-apoptotic proteins. While the study also showed that the inhibitor targeted NADPH could not block the *E. histolytica*-induced NETs formation,¹⁰¹ which might be explained by that ROS generated by trophozoites and processed by the extracellular MPO during the contact with neutrophils were of relatively importance for *E. histolytica* induced NETosis.¹⁰² These findings shed light on the intricate regulatory mechanisms underlying NETosis and further underscores the interconnectedness of signaling pathways in orchestrating neutrophil functions.

DAG-PKC Pathway

Protein kinase C (PKC) is mainly distributed in the cytoplasm in an inactive state and is activated by diacylglycerol (DAG) and then transferred to the cell membrane to participate in the formation of NOX complex, leading to the increased generation of ROS and the sequent formation of NETs.^{103–105} The DAG-PKC pathway can be considered as an upstream signaling pathway for NOX-dependent formation of NETs.¹⁰⁵ DAG mimicking PMA can stimulate neutrophils to form NETs,¹⁰⁶ therefore, leading to the association between PKC signaling and NETs formation via NADPH oxidase-ROS pathway and PKC. Gray et al¹⁰⁶ used LY333531 to inhibit PKC β (LY333531 is a specific potent inhibitor of PKC β that is a subtype of the PKC family), and the results showed the respiratory burst activated by NADPH oxidase was inhibited, and the formation of NETs was also suppressed. The findings of this study provide corroborative evidence for the involvement of the DAG-PKC pathway in the formation of NETs. Notably, among the PKC isoforms, PKC β emerges as the primary regulatory isoform governing the process of NET generation.

iICs-Fc γ RIIb Pathway

Immobilized immune complexes (iICs) are immune complexes that consist of autoantibodies and self-antigens, and Fc gamma receptor IIb (Fc γ RIIb) is a receptor expressed on neutrophils that binds to the Fc portion of IgG antibodies.¹⁰⁷ Upon binding of iICs to Fc γ RIIb receptors which presents on the surface of neutrophils, a signaling cascade is initiated, enhancing the induction of NETosis and subsequent formation of NETs in an ROS release-dependent manner.¹⁰⁷ Alemán et al¹⁰⁸ demonstrated that

activation of FcγRIIIB receptors using specific antibodies effectively induces the generation of NETs, akin to the stimulatory effect observed with PMA. These findings highlight the iICs-FcγRIIIB pathway as a significant contributor to the mechanistic landscape of NET formation. Upon binding of iICs to FcγRIIIB, a plethora of downstream signaling cascades is triggered. Among these, the activation of protein kinases, particularly the Src family kinases, plays a pivotal role in phosphorylating and activating subsequent downstream effectors. Notably, this includes the guanine nucleotide exchange factor Vav1, which, upon activation, serves as a crucial mediator engaging the small GTPase Rac, thereby facilitating its activation. Consequently, Rac activation leads to the initiation of the NOX complex, ultimately enhancing the production of ROS. This robust ROS generation contributes significantly to the formation of NETs, thus substantiating the integral involvement of this signaling pathway in the NETosis process.¹⁰⁷ Alongside the involvement of the Src family kinases and Vav1, a constellation of additional downstream signaling pathways is likely implicated in the activation of the NOX complex and subsequent ROS production. These pathways encompass the RAF-MEK-ERK pathway and the DAG-PKC pathway.^{100,109} These pathways can be activated by various signals, including cytokines, growth factors, and G protein-coupled receptors, and can activate the NOX complex and the subsequent formation of NETs.

NOX-Independent Formation of NETs

While the canonical pathway of NET formation involves the production of ROS by the NOX complex, NETs can also be formed through NOX-independent mechanisms. The exact signaling pathways for NOX-independent formation of NETs are still not fully understood, but several mechanisms have been proposed.^{110,111} Here are some of the proposed signaling pathways for NOX-independent formation of NETs.

Mitochondrial DNA (mtDNA) Pathway

The release of mtDNA can activate the immune system and contributes to the pathogenesis of several diseases, including autoimmune diseases, cancer, and cardiovascular diseases.^{112–114} It should be noted that mtDNA can serve as a DAMP to promote the formation of NETs.¹¹⁵ The pathway for mtDNA release involves several steps. Initially, a critical event in the cellular milieu involves the occurrence of mitochondrial permeability transition, culminating in the release of mitochondrial constituents, such as mtDNA, into the cytoplasmic compartment. This process significantly contributes to the intricate dynamics of cellular homeostasis and underscores the pivotal role played by mitochondria in orchestrating essential cellular functions. Second, the mtDNA is recognized by the cGAS-STING pathway, which triggers the production of type I interferons and other inflammatory cytokines. Finally, the mtDNA can directly activate the NLRP3 inflammasome, which leads to the production of IL-1β and other pro-inflammatory cytokines.¹¹⁶ According to Yousefi et al, their understanding suggests that the release of mtDNA is a regulated process that enables neutrophils to use their DNA as a weapon against microbes. In addition, it has been proposed that mitochondrial DNA may exert immunomodulatory effects on other immune cells.^{113,117} Furthermore, one of the responsible underlying mechanisms entails the belief that SIRT1 possesses the capacity to stimulate the initiation of mitochondrial permeability transition pore channels, facilitating the liberation of mitochondrial DNA and consequently giving rise to the formation of mitochondria-dependent vital NETs, as opposed to the conventional citrullinated histone H3-dependent NETs.¹¹⁸ It is implied that neutrophil-SIRT1-vital NET pathway may be a potential strategy to prevent tumor metastasis.

Calcium Pathway

The calcium pathway refers to the signaling cascade triggered by the release of intracellular calcium ions in response to stimuli such as LPS.¹¹⁹ In the context of NET formation, calcium signaling has been shown to activate the NOX-independent pathway. This pathway is triggered by the calcium-activated potassium channel of small conductance (SK channel), which is the major calcium activated potassium channel known to be present on neutrophils. Among the SK channel members (SK1, SK2 and SK3), SK3 is expressed predominantly on neutrophils and the knockdown of SK3 in differentiated HL-60 (dHL-60) cells reduces the NETs formation induced by ionomycin-mediated NOX-independent NETosis. Ionomycin is a natural calcium ionophore produced by a different gram-positive bacteria *Streptomyces globatus* and has proved the ability to induce NOX-independent NETosis.¹¹⁰ On the other hand, David et al raised the conclusion that NOX-independent NETosis caused by calcium ionophores required the mitochondrial ROS, which

was regulated by the NOX2 enzyme.¹¹⁰ However, evidence also present the correlation between calcium pathway and NOX-dependent NETosis. Calcium is a vital regulator of NETosis, as it activated enzymes and signaling pathways that involve in the production of ROS, which are essential for NOX-dependent NETosis.¹⁹ One of the calcium dependent-enzymes is PAD4, which modified histones and chromatin decondensation.¹²⁰ Also, calcium is deemed as a regulator of NADPH oxidase, the main source of ROS in neutrophils.¹²¹

Histone Acetylation Pathway

Histone acetylation is a post-translational modification that loosens the chromatin structure and affects gene expression.¹²² Histone deacetylases (HDACs) are enzymes that removes acetyl groups from histones and modulates chromatin condensation. According to the study,¹²³ histone acetylation (particularly H4K8) and spontaneous NETosis at baseline were increased by agents that block HDAC activity and enhance histone acetylation, known as HDAC inhibitors. Also, the same situation happened to the NETosis induced by PMA, A23187, or LPS in an additive manner. In the further investigation, inhibition or knockdown of HDAC1, HDAC2, HDAC3, or HDAC6 increased histone acetylation and spontaneous NETosis at baseline. And the inhibition of HDAC was beneficial for the elevation of chromatin decondensation during NETosis induced by PMA. Interestingly, there was a performance that increased in mitochondrial ROS production and caspase-3 activation, both of which were associated with NOX-independent NETs formation, displayed by the inhibition of HDAC. What's more, it is showed that the neutrophil death form was available to be switched from NETosis to apoptosis with HDAC inhibitors in dose-dependent manner.¹²⁴ The evidence mentioned above implied that histone acetylation may be involved in NOX-independent NETs formation by multiple pathways and deserves attention of researchers in the field of NETs. Despite the researchers' partial comprehension of the histone acetylation process, there is currently a limited repertoire of drugs targeting NETosis through modulation of histone acetylation. Consequently, there is still great potential for drug development in this specific domain.

Others

Apart from NOX-independent and NOX-dependent way for the formation of NETs, some methods are found to be classified into neither of them, as referred to rapid release of NETs formation. With the in-depth study of NETs, it has been found that neutrophils can continue to remain active after the clearance of NETs and come into play with antibacterial effects. This process only takes 5–60 minutes, which is different from the release of suicidal NOX-dependent NETosis or NOX-independent NETosis lasting more than 3 hours.¹²⁵ At the end of this process, neutrophils eventually rupture and die. Yipp et al^{45,126} proposed that the formation of nuclear DNA in rapid NETosis was dependent on the binding of LPS. Under the action of gram-negative bacteria, generally, LPS binds to TLR4 on the platelet surface to generate NETs in a NOX-independent manner. For gram-positive bacteria, TLR2 and complement receptor 3 are required. After a few minutes of *S. aureus* stimulation, neutrophils were observed under a microscope to depolymerize their chromosomes, forming beads of DNA strands and nucleosomes. Late chromosomes are excreted in the form of nuclear vesicles, and neutrophils appear to be anucleate. Parts of chromatin and dense granules are excreted from cells by exocytosis to form NETs. The process mentioned above only takes 10 minutes. Anucleate neutrophils are still chemotactic, and this is manifested by hyperpolarization and poly pseudopodia crawling. They still have the function of degranulation and engulfing NETs released by bacteria. It is worth mentioning that according to the research, process of rapid release of NETs did not require the participation of NOX.^{127–129}

On the other hand, mechanistic target of rapamycin (mTOR) signaling pathway is not solely included in the NOX-independent and NOX-dependent pathway, as it can potentially play a role in both pathways. mTOR is a serine/threonine protein kinase that regulates cellular growth, proliferation, and survival.^{130,131} mTOR signaling plays an important role in the regulation of autophagy, which is a conserved catabolic process that degrades cellular components and organelles, leading to NETs formation.¹³² Neutrophils are highly metabolically active cells that require significant amounts of energy to perform their functions.¹³³

It has been reported that inhibiting the mTOR signaling pathway with rapamycin enhanced both autophagy and NETs formation, while activating the mTOR signaling pathway with insulin suppressed both processes in neutrophils isolated from health donors. And blocking autophagy with 3-MA or chloroquine showed a decrease in spontaneous NETs

formation.¹³⁴ It is suggested that spontaneous NETs formation is negatively regulated by the mTOR signaling pathway. However, a different trend in the relationship among mTOR, autophagy and NETs formation was found. Two β -lactam antibiotics, meropenem and ceftazidime/tazobactam, induced the activation of mTOR signaling pathway and inhibited autophagy in neutrophils and HL-60 cells. Moreover, they showed that blocking the mTOR signaling pathway with rapamycin or inhibiting β -lactamase with clavulanic acid attenuated the NETs formation induced by the antibiotics.¹³⁵ Another study displayed that inhibiting mTOR pathway with rapamycin or Torin 1 enhanced autophagy and reduced NETs formation in PMA-stimulated neutrophils, while activating the mTOR pathway with insulin-suppressed autophagy and increased NETs formation in LPS-stimulated neutrophils. Also, blocking autophagy with 3-MA or bafilomycin A1 increased NETs formation in PMA-stimulated neutrophils, while enhancing autophagy with trehalose decreased NETs formation in LPS-stimulated neutrophils.¹³⁶ Based on the aforementioned evidence, mTOR signaling may regulate NET formation by modulating autophagy, although the underlying mechanism appears to depend on the specific stimuli. Nonetheless, the exact mechanisms and implications of this regulation remain elusive and warrant further investigation.

In summary, various mechanisms do exist to achieve the purpose of NETs formation through specific stimuli. It is supposed to be noted that some pathways mentioned above possess the interaction with each other, rather than being independent, suggesting an objective and noticeable view that the classification used to distinguish either NOX-dependent pathway or NOX-independent pathway is relative.

Regulatory Role of NETs in GA Inflammation

GA is a common disease associated with inflammation. The main cause of GA can be attributable to a disorder of purine metabolism leading to excessive production or insufficient excretion of UA. GA attack usually occurs late at night accompanied by joint pain, swelling, redness and fever. Symptoms can cause discomfort or tingling in the joints and can get worse within 24 hours.^{5,137} In most cases, the acute manifestations of inflammation tend to subside spontaneously within a few days to weeks, without any notable residual effects. This observation suggests the existence of efficacious mechanisms that effectively mitigate acute inflammation.¹³⁸

According to the pathogenesis of gout, there are 4 stages of gout, including asymptomatic hyperuricemia, acute gouty arthritis, intercritical gout and chronic tophaceous gout.¹³⁹ GA is typically associated with the acute stage of gout. Nonetheless, there is a significant information that not all patients with gout will experience GA, and GA attacks will not only occur in the acute gouty arthritis stage but also in the chronic tophaceous gout stage.^{137,140} As reported that GA was the most commonly associated with the acute stage of gout and was the most common presenting symptom of gout causing inflammatory symptoms.^{141,142} Also, the chronic tophaceous gout stage is the most advanced stage of the disease and is characterized by the presence of tophi, which are deposits of urate crystals in the soft tissues with the less severe inflammation compared to the early stage.¹³⁹ It is implied that there must be some physiological and pathological activity that acts as a regulator of inflammation.

Among different targets, NET is a crucial role in regulating inflammation.^{92,143} As is mentioned above, neutrophils can be activated through various mechanisms and pathways leading to the releasing of NETs. During the process of GA, stimuli like MSU crystals are beneficial for the recruitment of neutrophils and the generation of NETs, which is accompanied by the release of DAMPs such as histones, activate the immune system to release pro-inflammatory cytokines around joints resulting in an inflammatory environment.^{74,144,145} Herein, it is necessary to figure out the regulatory role of NETs in GA inflammation.

Inflammatory Initiation

GA is caused by the deposition of monosodium urate (MSU) crystals in joints, which triggers a strong inflammatory response. The MSU crystals are recognized by the innate immune system, which activates resident macrophages and recruits neutrophils to the joint resulting in the accompanying inflammation.¹⁴⁶ When the crystals accumulate in the joint, they trigger an inflammatory response by activating the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, a large protein complex that regulates the immune response. The activation of the NLRP3 inflammasome leads to the secretion of pro-inflammatory cytokines such as interleukin-1 beta (IL-1 β) and IL-18, which causes the characteristic pain, redness, and swelling associated with gout. The activation of the NLRP3 inflammasome and

subsequent release of IL-1 β and IL-18 are triggered by innate immune cells like macrophages and dendritic cells, which recognize danger signals and respond by initiating the inflammatory response.^{147,148} During the process of GA, it is widely regarded that MSU stimulate macrophages to produce IL-1 β and IL-18 via the NLRP3 inflammasome, which in turn leads to the recruitment of neutrophils and subsequent release of NETs. Meanwhile, neutrophils then undergo a process of activation and degranulation, leading to the release of their intracellular contents, including ROS, citrullinated histones, and granule enzymes, which together promote the formation of NETs.¹⁴⁹ Also, neutrophils can release NLRP3 inflammasomes through a process called phagocytosis, which is the ingestion of foreign particles such as urate crystals.^{95,150} The urate crystals are recognized by the neutrophils and internalized through the formation of phagosomes. The phagosomes then fuse with lysosomes to form phagolysosomes, which contain enzymes and ROS that can degrade the urate crystals.^{151–154} This process results in the release of DAMPs, such as mitochondrial DNA and ATP, which can activate the NLRP3 inflammasome and bring about the generation of IL-1 β and IL-18, ultimately promoting NET formation.^{18,155,156}

NETs are deemed to be a vital role in initiating GA inflammation. Excessive formation of NETs is a sign of increased GA flare and a critical factor that contributes to GA pathology.¹⁵⁷ NETs can cause tissue damage and trigger inflammation in GA in multiple ways. First, the histones and granular proteins from NETs are capable of activating the complement system and promoting the recruitment of other immune cells, such as monocytes and macrophages, to the site of inflammation.^{34,158,159} The recruited immune cells further propagate the inflammation by secreting pro-inflammatory cytokines, such as IL-1 β , TNF- α , and IL-6.^{15,137,160} What's more, NETs can directly foster inflammation by various pro-inflammatory molecules such as histones, DNA, and granule proteins that can activate the NLRP3 inflammasome.^{84,161} (Figure 3) For example, histones from NETs directly activate the NLRP3 inflammasome by disrupting lysosomal membranes and releasing cathepsins into the cytosol, which in turn activates the NLRP3 inflammasome that assembles with ASC and pro-caspase-1 followed by the step to cleave the pro-caspase-1 into caspase-1 and finally the release of IL-1 β (Figure 3).³³ Last but not least, NETs can cause mitochondrial damage, resulting in the release of mtDNA, which can activate the NLRP3 inflammasome in the diabetic wound.^{162,163} In the recent years, NETs have been treated as a potential target for the treatment of gout. Compelling evidence suggests that loganin exerts its inhibitory effects on NLRP3 inflammasomes by repressing mitochondrial stress in macrophages.¹⁶⁴ However, similar finding has not been seen in studies of MSU crystals induced NETosis, leading to a valuable direction that needed explored. An interaction between macrophages and neutrophils has been used as a method for the treatment of gout. Ji Hye Jeong and his colleges proposed that synovial fluid macrophages were capable of clearing NETs by means of enhancing engulfment of MSU crystals without inducing any immunological response.¹⁵ Taken together, it is believed that NETs function as an intrinsic alarming that mainly initiates the inflammasome activation and elicits the inflammatory response in GA.

Inflammatory Resolution

In the chronic stage of gout, the symptoms that occurring at the beginning of gout are relieved and the inflammation around the joints is reduced.¹⁶⁵ Inflammation during GA can expand without limitation, leading to joint necrosis and even death if there is no regulatory mechanism.^{166,167} There is no doubt that multiple mechanisms are utilized to prevent endless inflammation from happening. Evidence has showed that several key mechanisms are involved in inflammatory regression during gouty arthritis.^{157,168–170} Among them, NETs serve as a vital role in inflammation resolution in the form of aggregation, referred to as aggNETs. Here, we describe an inflammatory resolution that is regulated by NET.

The process of aggNETs formation begins when neutrophils are exposed to uric acid crystals. The crystals stimulate neutrophils to release NETs, which then aggregate and entrap the crystals within the web-like structure of the NETs.¹⁴⁹ The aggregation of NETs and uric acid crystals leads to the formation of aggNETs that is capable of entrapping and degrading MSU crystals.^{149,171} In the research from Christine et al, neutrophils recruited to the site of inflammation and underwent oxidative burst caused by MSU crystals, which led to the formation of NETs.¹⁷¹ As neutrophils recruited to the site of inflammation, aggregation of NETs occurred under a high density of neutrophils and then resulted in the degradation of cytokines and chemokines. In another study, aggNETs displayed it anti-inflammation via the protection from antiproteases *in vivo* and *in vitro*.¹⁷² Actually, the process of manifesting anti-inflammatory function by aggNETs needs the involvement of neutrophil serine proteases (NSPs). As a kind of neutrophils granule, NSPs contain NE, proteinase 3 (PR3) and cathepsin G. In the context of gouty arthritis, NSPs are believed to

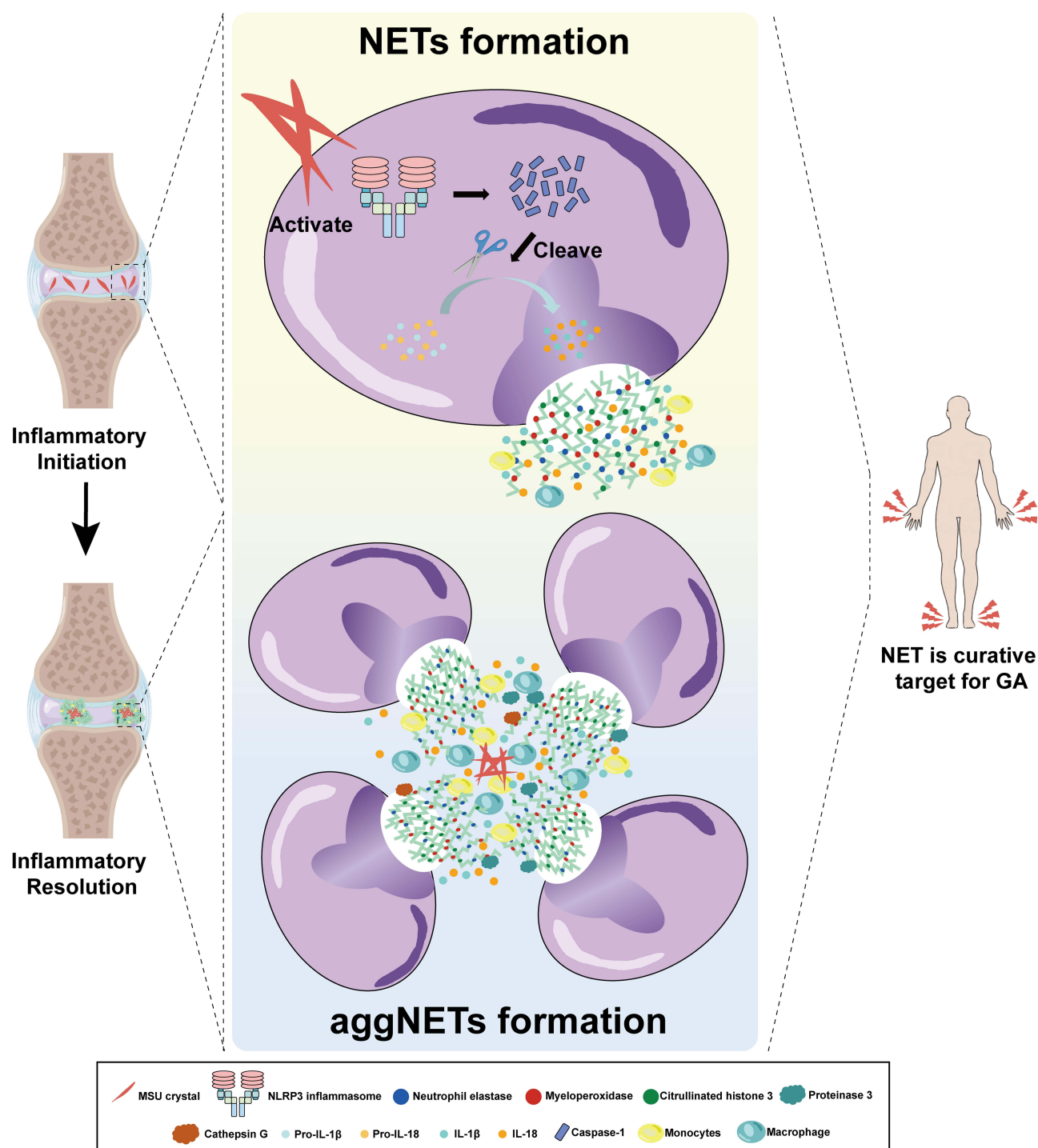


Figure 3 Regulatory role of neutrophil extracellular traps in gouty arthritis. MSU crystal deposition is formed at the joints due to high serum uric acid concentration, leading to the appearance of inflammatory response of neutrophils. During the early period of gouty arthritis, the NLRP3 inflammasome, which is activated by MSU crystals directly or indirectly, is responsible for turning pro-caspase-1 into the mature form of caspase-1 after the assembly of NLRP3. Pro-IL-1 β and pro-IL-18 are transformed into IL-1 β and IL-18 with the help of caspase-1, exhibiting pro-inflammatory effects and promoting the generation of NETs, which are accompanied by adherence to neutrophil elastases, myeloperoxidases and citrullinated histone 3. Additionally, macrophages and monocytes recruited by MSU crystals contribute to the generation of IL-1 β and IL-18. With the recruitment of neutrophils, increasing numbers of neutrophils cause the aggregation of NETs, as referred to aggNETs. aggNETs manifest anti-inflammatory function by neutrophils serine proteases including NE, proteinase 3, cathepsin G and neutrophil serine proteases. At the same time, anti-inflammation is associated with the process of aggNETs engulfing and degrading MSU crystals.

contribute to the resolution of inflammation. It has been shown that neutrophils release elastase and PR3 together with aggNETs, which can then engulf and degrade MSU crystals, thus preventing further activation of the NLRP3 inflammasome and subsequent release of pro-inflammatory cytokines like IL-1 β and IL-18 (Figure 3).^{173–175} Moreover, NSPs have been found to directly degrade and inactivate a range of pro-inflammatory cytokines, including TNF- α , IL-1 β , IL-6, and chemokines such as IL-8.¹⁷¹ Other than that, a report from Jasmin et al showed that the cytotoxic effect of histones on epithelial cells could be weakened by aggNETs's function of sequestering and degrading histones, and the process of which required the participation of serine proteases, NE and PR3.¹⁷⁶ The research suggested that the resolution of inflammation induced by histones resulted from degradation and detoxification of aggNETs. Collectively, aggNETs with the effector NSPs can exert anti-inflammatory effects through various mechanisms, including the degradation and inactivation of pro-inflammatory cytokines and chemokines. In recent times, the concept of NSP has received limited attention in the context of gout treatment. Researchers have instead directed their focus towards investigating distinct components of NSP for in-depth study and exploration. This shift in research emphasis signifies a renewed interest in understanding the intricate roles played by individual NSP constituents and their potential implications for therapeutic interventions in the management of gout and related inflammatory disorders.

Subsequently, the focus of attention falls on the formation of aggNETs, as its formation is currently viewed differently. It is believed that the release and degradation of inflammatory mediators is a dynamic balance.¹⁷⁷ NSPs bonding to aggNETs cause the resolution of inflammation, which implies that the ability to degrade inflammatory mediators is greater than the ability to release them. According to the research by Jonas et al,¹⁷² peak generation and maximum supernatant concentrations of cytokines and chemokines appeared at a neutrophil density of $20\text{--}40 \times 10^6/\text{mL}$ during the stimulation of MSU crystals. The study also found that the contents of inflammatory cytokines and chemokines began decreasing at higher densities of neutrophils with the help of NSPs. Moreover, maximum release of inflammatory mediators and chemokines occurred at a neutrophil density of $20\text{--}40 \times 10^7/\text{cm}^3$ in arthritis mice model induced by MSU crystals, whereas the contents of the mediator reduced at higher density of neutrophils.¹⁷² The evidence mentioned above suggests the neutrophil induced inflammation is a self-limiting condition containing a dynamic process of releasing and degrading inflammatory mediator and chemokines, and more importantly, the formation of aggNETs seems to depend on the density of neutrophils. While in another study, the authors held the opinion that aggNETs formation depended on ROS *in vivo* and *in vitro*. In this study, it turned out that neutrophils had a weaker ability to degrade inflammatory mediators and generate aggNETs when compared to normal.¹⁷¹ In line with this, ROS-deficient *Ncf1*^{−/−} mice which was characterized by the inability to produce ROS and represent mice model of CGD, showed a reduction in NETs aggregation and an increase in inflammatory mediators during the stimulation by MSU crystals when compared to air pouches of wild-type mice. It is assumed that the number of neutrophils only determines the size of the aggregates and then the aggNETs formation starts at $50 \mu\text{g}/\text{mL}$ of MSU crystals in a dose-dependent manner. Moreover, the level of neutrophils does not appear to be a critical factor, as NETs aggregation can still be induced by other stimuli such as ATP or lactoferrin, even at low neutrophil concentrations ($5 \times 10^6/\text{mL}$).¹⁷¹ There exists uncertainty regarding the underlying mechanism for the formation of aggNETs that requires clarification.

NETs as Therapeutic Target for GA Treatment

The evidence we mentioned above strongly illustrates the close connection between GA and NETs. With the increasing research on GA in recent years, well-established anti-GA drugs and decoction have been widely used in the gout population.^{178–181} In addition to this, raising number of pharmaceutical agents are presently undergoing development to counteract GA in accordance with its pathogenesis. NETs have emerged as a pivotal target in the context of GA, and their inhibition has garnered considerable interest as an attractive therapeutic strategy for managing this condition. Consequently, an increasing array of drugs aimed at modulating NETs formation are currently under exploration and development, signifying the potential for novel and promising therapeutic avenues in GA treatment.

Clinical Approaches for GA Prevalent Drugs

According to the *American College of Rheumatology Guideline for the Management of Gout*¹⁸² published in 2020, first-line drugs commonly used in clinical practice for GA attack involve colchicine, non-steroidal anti-inflammatory drugs (NSAID) and glucocorticoid. Colchicine has been a medicine widely used in GA for a long history.^{183,184} The incidence of adverse reactions is

high since the therapeutic dose of colchicine is close to the toxic dose.¹⁸⁵ For patients with acute gout attacks, it is recommended to accept colchicine treatment in the first dose of 1 mg, 0.5 mg after 1 h, and 0.5 mg after 12 h, with a frequency of once a day or twice a day.¹⁸⁶ Small doses of colchicine combined with NSAID also are strategies recommended to cure acute GA attacks.^{187,188} Glucocorticoids are appropriate for the patients suffering GA attacks while having trouble with drug administration orally.¹⁸² In addition, lowering uric acid drugs, including allopurinol, benzbromarone and febuxostat are also vital treatments for gout. However, both advantages and disadvantages exist in drug administration. Studies have shown that side effects, including liver functional disorder and leukopenia, occur after taking allopurinol for a long time.¹⁸⁹ Seriously, allopurinol could cause severe hypersensitivity in patients with a positive HLA-B*5801 allele.¹⁹⁰ Benzbromarone is suitable for alleviating GA and hyperuricemia combined with acute GA, however, its unsatisfactory clinical efficacy limits its application.¹⁹¹ And clinical data suggested that a small number of patients might also be intolerable due to severe gastrointestinal adverse reactions, liver and kidney damage after benzbromarone administration.^{191,192} The use of febuxostat remains controversial because cardiovascular or all-cause mortality increased after long-term administration in adults over the age of 65, while the opposite result was proposed according to long-term cardiovascular safety of febuxostat compared with allopurinol in patients with gout.^{193,194} Surgery presents a comparatively lower occurrence of adverse effects on organs when contrasted with pharmacological interventions, rendering it a viable option that patients with gout are willing to consider. Also, surgical therapy is an effective approach to improve the quality of life of patients.¹⁹⁵ The principal objective of minimally invasive arthroscopic surgery is to carefully insert a needle into the high-pressure region of the tophi, allowing for precise and closed cutting. This procedure facilitates the removal of excessive metabolites from the affected tissue and subsequently clears out the accumulated metabolites, resulting in the effective alleviation of joint cavity pressure. This meticulous approach accomplishes the immediate treatment goal with a focus on preserving tissue integrity and promoting patient recovery.¹⁹⁶ Indeed, the widespread adoption and promotion of minimally invasive arthroscopic surgery are warranted due to its pronounced efficacy in facilitating rapid joint function recovery, minimizing trauma, and alleviating inflammation and pain. Consequently, patients experience a notable enhancement in their quality of life following the surgical intervention. Nevertheless, it is essential to acknowledge the inherent limitations of this surgical approach, particularly concerning the rehabilitation and overall quality of life of elderly patients. Exploring and addressing these limitations will be crucial in optimizing patient outcomes and ensuring the successful application of this surgical technique in the broader context of clinical practice.

Uric Acid Lowering Drugs

Uric acid-lowering drugs are a class of pharmaceutical agents specifically designed to mitigate elevated levels of uric acid in the bloodstream, a condition commonly associated with hyperuricemia and gout. Xanthine oxidase (XO) inhibitors represent a prominent class of pharmaceutical agents extensively employed as anti-gout drugs. XO is an enzyme that plays a critical role in the generation of uric acid, the end product of purine metabolism.¹⁹⁷ Purines are nitrogenous bases found in DNA, RNA, and many cellular metabolites, and they can also be obtained from dietary sources such as meat and seafood.^{198–201} Purines are broken down into uric acid through a series of enzymatic reactions, with XO being the final enzyme involved in this process. XO catalyzes the oxidation of hypoxanthine to xanthine and then the oxidation of xanthine to uric acid, using molecular oxygen as the electron acceptor. This process leads to the ROS generation such as superoxide and hydrogen peroxide as byproducts, which can contribute to oxidative stress and damage in the body.^{202–204} Actually, XO inhibitors can be divided into three subsets including purines (such as allopurinol), non-purines (such as febuxostat) and natural inhibitors (such as quercetin).²⁰⁵ Allopurinol is a purine analog that acts as a suicide inhibitor of XO, meaning it irreversibly binds to the enzyme and prevents it from generating uric acid.^{206,207} Febuxostat, on the other hand, is a non-purine XO inhibitor that selectively targets the enzyme and has a longer half-life than allopurinol. Both drugs have been shown to effectively lower uric acid levels and prevent gout attacks.²⁰⁵ Nevertheless, the research of non-purine part of XO inhibitors attracts most attention because it possesses an interaction with several amino acids in the important domain called Mo-Pt that is a crucial domain for generating uric acid from substrates catalyzed by XO.²⁰⁸ It is necessary to note that a survey of the recent patent literature reveals that the predominant focus of research has been on the development of non-purine and natural XO inhibitors.²⁰⁵ To be added, different kinds of side effects like hypersensitivity can be caused by purines part of XO inhibitors.^{209–211} Besides, natural inhibitors have been a hot point of research for the gout treatment. Quercetin acts early on as a natural polyphenolic

flavonoid compound and was first reported for its XO inhibitory effect in 1999.²¹² Recently, 1,4-dicaffeoylquinic acid, as a novel ingredient, is isolated from the leaves of *Artemisia selengensis* and is found to possess better XO inhibitory potential than allopurinol.²¹³ Song and his companion proposed the davallialactone extracted from *Sanghuangporus vaninii* potently inhibited XO and possessed the potential to be developed into a medicine for gout.²¹⁴ At the same time, another similar research raised the finding that the anti-gouty arthritis effect could be proved in vivo through an important medicinal and edible fungus in China, *Phellinus igniarius* which was constituted of davallialactone.²¹⁵ Up to now, various kind of natural product exhibiting XO inhibitory activity have been found, leading to attract more and more attention from their potential for gout treatment.

Uricosuric agents can reduce serum uric acid levels in patients with hyperuricemia and gout by improving the clearance of uric acid in the kidneys. They achieved this by blocking the reabsorption of uric acid at the proximal tubule of the kidney via the inhibition of the urate transporter 1 (URAT1). The two main classes of uricosuric agents can be divided into probenecid and benzbromarone. Probenecid exerts its pharmacological action through competitive inhibition of URAT1, a urate transporter responsible for the reabsorption of uric acid in the proximal renal tubules. In contrast, benzbromarone exhibits dual inhibitory effects by targeting both URAT1 and xanthine oxidase, an enzyme crucially involved in the biosynthesis of uric acid. The distinct mechanisms of action of these two drugs hold significance in the context of hyperuricemia management and gout treatment, offering clinicians versatile options to modulate uric acid levels and mitigate the risk of gout-related complications. The suitable initial dose of probenecid was recommended for 250 mg for twice a day and the maximum dose could reach up to 1 mg twice a day.^{216,217} And benzbromarone's administration had been specified at a dose of 100–200 mg/day for once a day.^{218–220} Other uricosuric agents are under investigation include sulfinpyrazone, losartan, and topiroxostat. There is possibility that association between each other may exist, such as aspirin and diuretics, which can reduce their effectiveness.^{221,222} In clinical practice, uricosuric agents are often used in combination with xanthine oxidase inhibitors, such as allopurinol or febuxostat, to achieve optimal control of serum uric acid levels.^{223,224} The selection of an appropriate therapeutic approach is contingent upon various factors, including the severity of hyperuricemia, the presence of comorbidities, and the individual patient's response to treatment. These critical considerations underscore the necessity for personalized and tailored management strategies to optimize patient outcomes and ensure effective control of hyperuricemia and its associated complications.^{225,226} While uricosuric agents are generally well-tolerated, it is crucial to acknowledge that benzbromarone and probenecid may be associated with certain adverse effects. Benzbromarone has been linked to potential hepatic toxicity and cardiovascular risk, whereas probenecid may exhibit significant central nervous system toxicity and hypersensitivity syndrome, particularly when administered at higher dosages.^{227–231} These observations highlight the importance of vigilant monitoring and cautious dose adjustments to mitigate the risk of adverse reactions, ensuring the safe and effective utilization of these agents in the management of hyperuricemia and gout. As such, a balanced assessment of risks and benefits is essential to optimize patient outcomes and uphold safety in clinical practice.

Gut Microbiota

Due to the fact that the intestine is responsible for one third of uric acid excretion, gut microbiota possesses an important role in gout and hyperuricemia treatment because the function of eliminating uric acid cannot be ignored.^{232,233} The gut microbiota refers to the complex ecosystem of microorganisms that reside in the human gut, including bacteria, viruses, fungi, and protozoa. It plays a crucial role in regulating the immune system and maintaining host health. Several studies have shown that alterations in the gut microbiota may contribute to the development of gouty arthritis. For example, dysbiosis of gut microbiota has been associated with increased production of uric acid and inflammation, which are the key features of the disease.^{234,235} Modulating the gut microbiota through dietary interventions or probiotics has emerged as a potential therapeutic strategy for gouty arthritis. It has been reported that fisetin possessed the ability to decrease the content of uric acid through modulating the changes in the gut microbiota *Bacteroides*, and *Firmicutes* in hyperuricemia mice model.²³⁶ According to several cohort studies, evidence showed a higher risk of suffering from gout occurred to the people having western diet when compared to those of having Mediterranean diet.²³⁷ Prebiotic fiber and probiotics have been shown to increase the abundance of beneficial gut bacteria, such as *Bifidobacterium* and *Lactobacillus*, and decrease the abundance of pathogenic bacteria.²³⁸ These changes can be utilized for restoring a healthy gut microbiota and reducing inflammation as well as uric acid production.

In addition to dietary interventions, fecal microbiota transplantation (FMT) has been explored as a potential treatment for gouty arthritis. FMT involves the transfer of fecal material from a healthy donor to a recipient, with the aim of restoring a healthy gut microbiota. According to the reported findings, the administration of Qu-Zhuo-Tong-Bi decoction exhibited a notable impact on the gut microbiota composition, leading to a significant increase in the abundance of *Allobaculum* and *Candidatus saccharimonas*. Concurrently, FMT from mice subjected to Qu-Zhuo-Tong-Bi decoction treatment demonstrated a beneficial effect in alleviating the hyperuricemia and gout-like condition in the Uox-KO mouse model.²³⁹ Similarly, another classic Chinese herbal medicine, Si-Miao decoction, exerted an improvement in repairing intestinal pathology and restored the abundance of phylum *Proteobacteria* and genus *Helicobacter* via FMT.²⁴⁰ A preliminary pilot study investigating the application of FMT in patients with gouty arthritis demonstrated encouraging findings. Notably, the study revealed noteworthy reductions in serum uric acid levels alongside evident improvements in clinical symptoms.²⁴¹ These initial results warrant further investigation in larger-scale clinical trials to validate the therapeutic potential of FMT as a potential intervention in the management of hyperuricemia and gout-related manifestations. The observed positive outcomes underscore the need for more comprehensive and rigorous research to explore the potential role of FMT in ameliorating gouty arthritis and its associated metabolic abnormalities.

NETs-Targeted Inhibitors

Conventional Drugs

Even though the prevailing drugs used for the treatment of gout were not originally designed to specifically target NETs, advancements in comprehending their mechanisms of action have revealed their capacity to partially inhibit NETs formation. This emerging insight highlights an ancillary effect of these drugs and raising the importance of considering their broader impacts on neutrophil biology and inflammation, augmenting the potential for repurposing existing medications for the management of gout and related conditions. DNase (deoxyribonuclease) is an enzyme that occurs naturally in the body and is involved in the breakdown of DNA.^{242,243} It is produced by a variety of organisms, including bacteria, fungi, and animals, and still draws plenty of attraction even though it has been investigated in NETs study for years. Regarding the suppression of NETs, DNase is quite different from other NETs inhibitors since the focus of DNase is managed to extrude NETs instead of participating in the formation of NETs. The extracellular exposure of DNA in NETs makes them susceptible to degradation by DNase, which enzymatically cleaves the DNA backbone, resulting in the disassembly of the intricate web-like structures. This process facilitates the efficient clearance of NETs and their associated microorganisms, effectively curbing excessive inflammation and mitigating potential tissue damage. The breakdown of NETs by DNase plays a vital role in maintaining immune homeostasis and averting inflammatory overactivation, highlighting its significance as a regulatory mechanism in immune responses.²⁴⁴ The mechanism by which DNase degrades NETs involves the cleavage of the phosphodiester bonds that link the nucleotides in DNA. DNase complexes, composed of three enzymes including DNase I, DNase II, and DNase1L3, are able to bind to DNA molecules and cut the phosphodiester bonds between the nucleotides, leading to the degradation of the DNA backbone and the disassembly of the NETs.²⁴⁵ It's worth noting that DNase is specific to extracellular DNA resulting in the expose of residual histones and neutrophils serine protease to host, possibly causing local inflammation and further tissue injury.²⁴⁶

Colchicine is a first-line drug highly recommended for the management of acute gout attacks, primarily owing to its exceptional therapeutic efficacy.¹⁸² Although colchicine was not originally intended to target NETs as a gout treatment, study had shown that colchicine impeded the production of NETs via reducing NOX2/ROS production and calcium influx.²⁴⁷ As early as 2018, scientific reports indicated that colchicine exerted an inhibitory effect on the ability of circulating neutrophils in the bloodstream of Behçet's disease patients to form NETs.²⁴⁸ A recent study claimed that colchicine suppressed the production of NETs in patients with acute coronary syndrome post-percutaneous coronary intervention by restoring cytoskeletal dynamics.²⁴⁹ In addition, Apostolidou et al found that colchicine was capable of inhibiting the release of IL-1 β in neutrophils and IL-1 β activity in NETs.²⁵⁰ The evidence mentioned above suggest that the curative effect of colchicine on GA treatment may be targeting NETs.

As another member of first-line drug NSAIDs, ibuprofen is mainly used to treat rheumatic and rheumatoid arthritis.^{21,251} Victoria et al demonstrated that ibuprofen was able to alleviate the illness and inhibit NETs formation in bovine respiratory syncytial virus infection,²⁵² which indicated the potential of ibuprofen in GA treatment by targeting

NETs. At present, there are very few studies on the association between NSAIDs and NETs. It may be due to the clear pharmacological effects of NSAIDs and the lack of development potential.

Glucocorticoids are a class of medications that have potent anti-inflammatory and immunosuppressive effects.^{253,254} They have been used for many years for treating acute GA flares, as they can rapidly reduce pain and inflammation in affected joints.²⁵⁵ Glucocorticoids exert their therapeutic effects by modulating the immune response, leading to the suppression of inflammatory cytokine production, such as IL-1 and TNF- α , both of which play pivotal roles in the pathogenesis of GA. Additionally, glucocorticoids have been observed to impact NETs formation, further contributing to their anti-inflammatory properties and potential relevance in the management of GA. According to Amandine et al, glucocorticoids could reduce the formation of NETs in the lungs of the asthmatic horses either *in vivo* or *in vitro*.²⁵⁶ Despite the absence of specific literature reports on the effect of glucocorticoids in inhibiting NETs formation in the context of GA, the available evidence suggests that this mechanism holds the most plausible potential.

Natural Products

Natural products have been employed for medicinal applications spanning centuries, and their significance persists as a cornerstone in contemporary medicine. These biologically derived substances hold substantial therapeutic potential, contributing to the development of pharmaceutical agents and serving as a valuable resource for drug discovery and medical intervention. The enduring utilization of natural products underscores their enduring relevance and ongoing impact in addressing a diverse array of health conditions, highlighting their continued importance as an invaluable asset in the realm of modern healthcare practices.²⁵⁷

Resveratrol is a natural polyphenolic compound found in a variety of plant species, including grapes, peanuts, and berries. It contains numerous potential health benefits, including anti-inflammatory, antioxidant, and anti-aging properties.²⁵⁸ Resveratrol has been extensively studied for its potential effects on cardiovascular disease, diabetes, neurological disorders, and aging.^{258,259} According to the previous study, resveratrol showed the potential to cleave DNA in NETs and reduce the production of pro-inflammatory cytokines by neutrophils, suggesting its inhibitory effect on NETs formation.²⁶⁰ Additionally, evidence displayed that the patients with severe COVID-19 had an increased number of activated neutrophils that released NETs spontaneously, whereas the situation was improved by resveratrol by suppressing the formation of NETs.²⁶¹ Recently, it is reported that resveratrol could improve GA *in vitro* and *in vivo* by inhibiting the activation of NLRP3 inflammasomes by triggering the Pink1/Parkin pathway to promote mitophagy.²⁶² While the exact mechanisms linking resveratrol to gout and NETs are still under investigation, the cumulative evidence suggests that this natural compound holds therapeutic potential in managing both gout-related inflammation and the complex interplay of NETs in the pathogenesis of the disease.

Quercetin is a natural polyphenolic flavonoid and a kind of plant pigment found in various fruits, vegetables, grains and food sources, including onions, apples, berries, grapes, tea, and red wine.²⁶³ It is also available in supplement form. Pharmacologically, it has potent antioxidant and has anti-inflammatory properties.^{264,265} Clinical studies for the treatment of hyperuricemia showed that the growing content of plasma uric acid in healthy males could be significantly reduced by daily supplementation of quercetin (500 mg), for 4 weeks.²⁶⁶ Further study has shown the anti-GA effect of quercetin *in vivo* by suppressing the activation of the nuclear factor- κ B (NF- κ B) pathway and inflammasome.²⁶⁷ Moreover, recent study has revealed that quercetin exerted its ability to inhibit the activation and infiltration of neutrophils and suppress autophagy, thus led to the restrain of NETs formation in rheumatoid arthritis.²⁶⁸ The available evidence discussed above provides compelling indications of the inhibitory effect of quercetin on the process of GA. Therefore, conducting in-depth investigations into the use of quercetin as a treatment modality for GA represents a valuable avenue of study, with the potential to shed light on its clinical efficacy and mechanistic underpinnings.

Curcumin is a naturally occurring polyphenolic compound found in the rhizome of the turmeric plant, which has been used for centuries in traditional medicine for its anti-inflammatory, antioxidant, and anticancer properties. It is a bright yellow-orange pigment with a characteristic taste and odor, commonly used as a spice and food coloring agent.²⁶⁹ In recent years, curcumin has been investigated for its potential anti-GA properties. Study showed that curcumin was capable of improving the GA characteristics induced by MSU crystals, including joint swelling, inflammatory cell infiltration and MPO activity, and had influence on suppressing the NLRP3 activity and the activation NF- κ B signaling pathway.²⁷⁰ Meanwhile, curcumin demonstrated its ability to inhibit the release of NETs induced by polybrominated diphenyl ethers. The underlying mechanism

involved the modulation of ROS burst by interfering with Nrf2, a transcription factor central to the regulation of oxidative stress responses.²⁷¹ Curcumin also can alleviate hepatic ischemia-reperfusion injury by inhibiting the formation of NETs resulting from the inhibition of MEK/ERK pathway.²⁷² Therefore, targeting NETs may be one of the possible mechanisms of curcumin on the GA treatment. It should be also noted the fact that the application of curcumin is limited due to its rapid degradation, poor aqueous solubility, and low bioavailability.^{273,274} Modifying curcumin to strengthen the therapeutic effect becomes a strategy. For instance, curcumin-loaded tetrahedral framework nucleic acids were synthesized to deliver curcumin, which exhibited better drug stability, biocompatibility, ease of uptake, higher tissue utilization and a better anti-inflammatory effect when compared to free curcumin *in vitro* and *in vivo*.²⁷⁵ This is a worthwhile approach for other natural products that are not well bioavailable and thus limit their application.

Besides, another natural product from *Andrographis paniculate* called andrographolide possesses the potential to be a clinical drug for GA treatment. Recent research has proposed that andrographolide could attenuate the symptoms of rheumatoid arthritis by reducing the infiltration of neutrophils and NETosis *in vivo*. What's more, andrographolide exhibited the ability in balancing NETosis and apoptosis. The NETosis induced by autophagy could be suppressed and the apoptosis induced by lipopolysaccharide-activated neutrophils was enhanced within the administration of andrographolide *in vitro*. These findings imply that andrographolide has considerable potential for being NETs inhibitor and further a strategy for GA treatment.²⁷⁶

In conclusion, utilizing natural products for the treatment of gouty arthritis by targeting NETs shows promising potential. The research and studies conducted in this area suggest that certain natural compounds possess anti-inflammatory and NETs-modulating properties, which could help alleviate the symptoms and progression of gouty arthritis. By targeting NETs, these natural products may disrupt the inflammatory cascade, reduce tissue damage, and decrease the frequency and intensity of gout flares. Furthermore, the use of natural products in gout treatment could offer a more holistic and potentially safer approach, as they are often associated with fewer side effects compared to conventional medications. However, it is important to acknowledge that more extensive research is needed to establish the efficacy, safety, and long-term benefits of these natural products in treating gouty arthritis via NETs targeting. Additionally, personalized treatment plans considering individual variations in response to natural compounds should be explored. Despite the promising results, natural products should not replace standard medical treatments for gouty arthritis. Instead, they can be considered as complementary therapies or adjuncts to conventional medications to enhance overall treatment outcomes.

Synthetic Products

Purinoreceptor, also called P2 purinoreceptor, is a category of nucleotide receptor. Among the families of purinergic receptors, the metabotropic receptors (P2Y) family is strongly associated with immune cell pathology and physiological activity. Under normal conditions,²⁷⁷ several P2Y receptors such as P2Y2, P2Y4 and P2Y6 receptors are involved in regulating the level of Ca^{2+} , K^+ , Cl^- and Na^+ . Whereas P2Y receptors act a vital role in immune cell recruitment, proliferation and differentiation.²⁷⁸ A specific inhibitor of the P2Y6 receptor called MRS2578 was used to verify the influence on neutrophil activation and aggNETs formation induced by gout associated MSU crystals. According to this research, the study revealed that suramin and PPADS, both recognized as general P2Y receptor blockers, as well as MRS2578, effectively inhibited the formation of NETs induced by MSU crystals. Attractively, even though it is the main receptors expressed in neutrophils, the P2Y2 receptor is barely involved in NETs generation due to the finding that the P2Y2 receptor antagonist called AR-C25118925XX had no influence on NETs release. On the other hand, the formation of aggNETs could be attributed to the P2Y6/store-operated calcium entry/IL-8 axis that plays a role in neutrophil migration.²⁷⁹ This mechanism was further elucidated in another study, Su-Hyun et al proposed that during the intracellular endosomal trafficking of the P2Y6 receptor induced by MSU crystals, neutrophil recruitment was enhanced on the basis of the IL-8 expression supported by endosome-dependent signaling.²⁸⁰ Numerous studies also reported the significant role of P2Y6 receptor expression on cells such as microglial and macrophage in different diseases.^{281,282} These evidences suggest a strong potential for MRS2578 to be further developed by targeting P2Y6 receptors for GA treatment. By selectively inhibiting or activating the P2Y6 receptor, at the same time, it may be possible to influence NET release and subsequently mitigate the inflammatory response and tissue damage characteristic of gout flares. This novel therapeutic strategy holds the potential to offer a more targeted and precise treatment option for gout patients, possibly leading to improved symptom management and disease outcomes.

LDC7559 is a newly discovered small molecule that has mighty potential to be developed. In accordance with Gabriel et al, LDC7559 was chosen through a chemical screening and had the ability to combined with gasdermin D (GSDMD) with rarely effect on the activity of MPO, NE and NOX. Besides, LDC7559 binding to GSDMS N terminus thus causing decrease in NETs formation suggested the specificity of LDC7559 to GSDMD. GSDMD, a pore-forming protein, serves as a crucial mediator of pyroptosis and plays a significant role in the process of NETosis. Its involvement in NETosis stems from its essential function in the release of chromatin structures into the extracellular space. GSDMD influences cell membrane stability and nuclear expansion, facilitating the extracellular release of chromatin and contributing to the formation of NETs.²⁸³ Also, inhibition of GSDMD became a therapeutical target to block NETs formation during sepsis.²⁸⁴ It has been reported that GSDMD-dependent NETs formation was associated with various stimuli such as LPS or cytosolic Gram-negative bacteria (*Salmonella AsifA* and *Citrobacter rodentium*)²⁸⁵ and PMA. To date, comprehensive research on the involvement of GSDMD in MSU-induced NETosis is yet to be established, presenting an area that significant attention. The current lack of evidence necessitates focused investigations to elucidate the potential role of GSDMD in the process of NETosis triggered by MSU.

Metformin is a synthetic product having a close connection with GA. While its chemical structure is based on a natural compound called guanidine, the compound itself is synthesized in a laboratory setting rather than being extracted from natural sources.²⁸⁶ Growing studies have found the effect of metformin on different diseases such as Covid-19 and gout.^{287,288} In various pathological conditions, metformin has been shown to directly impede the activity of the mitochondrial respiratory chain complex I. This specific inhibition results in a reduction of adenosine triphosphate (ATP) levels, concomitant with an elevation in adenosine monophosphate (AMP) concentrations. The altered AMP/ATP ratio subsequently activates adenosine monophosphate-activated protein kinase (AMPK), a pivotal metabolic regulator. This cascade of events, involving the inhibition of complex I and subsequent AMPK activation, constitutes a fundamental mechanism underlying Metformin's therapeutic effects across diverse disease contexts.^{289,290} As an inhibitor of mTOR signaling, metformin resulted in the reduction of cell death and pro-inflammatory cytokines, which might be the main reason for the lower frequency of gout attacks occurred to patients with gout.²⁹¹ Despite being discovered over fifty years ago, metformin still holds significant research value because metformin owns immunoregulating effects through suppressive effects on NETosis and NETs formation.²⁹² In summary, the use of metformin as a potential inhibitor of NETs formation and NETosis shows promise in the treatment of GA. further research and clinical trials are necessary to fully understand the effectiveness and safety of metformin in targeting NETs during the GA process, paving the way for a more targeted and personalized treatment for individuals suffering from this debilitating condition.

Conclusions and Future Perspectives

In short, NET is a web like structure based on DNA accompanied with components such as NE, PAD4 and MPO. NETs can be stimulated and formed by various pathways in different diseases and perform distinct functions in response to immune system and inflammatory environment. During the process of GA, NETs exert both pro- and anti-inflammatory effects. With the stimulation of MSU crystals, inflammation onset caused by NETs is mainly induced by the activation of NLRP3 inflammasomes, leading to the release of mature inflammatory cytokines (IL-1 β and IL-18). With increased inflammation and neutrophil recruitment, aggregation of NETs occurs causing the formation of aggNETs that contributes to the anti-inflammatory effect by engulfing and degrading MSU crystals together with the components such as NSPs. At the same time, we summarize the prevalent drugs for GA and describe NETs-targeted inhibitors naturally and synthetically that possess potential for development into clinical applications.

Neutrophil is a type of white blood cell with short lifespan and unable to expand or frozen in *vitro*. According to the research, neutrophils are more fragile than other blood cells and can only survive in *vitro* for up to 5 days maximum under certain condition.^{293,294} It is of great difficulty to conduct the research of NETs in-depth, and growing studies replace neutrophils with differentiated HL-60 cells via dimethyl sulfoxide or all-trans retinoic acid.^{295–297} HL-60 cells are differentiated human promyelocytic leukemia cells that can be used for laboratory research on blood cell formation and physiology. Nevertheless, it is of inefficiency for dHL-60 to generate NETs compared to neutrophils, and differences including the dynamics pattern of the cytoskeleton, endomembrane, phagocytosis abilities and calcium influx exist between dHL-60 and neutrophils.^{298–300} Considering current research, there exists an urgent need to optimize the in *vitro* conditions for neutrophil survival to extend their lifespan, or to develop a dHL-60 cell model that accurately mimics the physiological environment of neutrophils.

Even though the natural and synthetic products have their own advantages on inhibiting NETs, it is necessary to solve the challenges before being applied in the clinic. The integration of natural products in drug discovery has encountered difficulties in the form of practical hurdles for screening, isolation, characterization, and optimization. As a result, their utilization has decreased over time. In addition, natural products may possess a multiplicity of targets, which can lead to low bioavailability, poor pharmacokinetics, or toxic effects.^{257,301} On the other hand, synthetic products exhibit drawbacks including but not limited to high expense, instability, and potential immunogenicity. Synthetic compounds may also lack specificity and biological relevance toward NET-targeted inhibition. Moreover, they may elicit undesirable side effects or resistance mechanisms because of their interactions with other pathways or molecules.³⁰² The evidence mentioned above lead to the attraction should be given to investigate more thoroughly the context-dependent functions and mechanisms of NETs in GA and different stages of gout, as well as their interactions with other immune cells or molecules. Additionally, future research on NETs-targeted inhibitors is also supposed to focus on evaluate more carefully the safety and efficacy of natural and synthetic products as NET-targeted inhibitors in preclinical and clinical studies, as well as their potential adverse effects on normal neutrophil functions and immune responses.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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