

Role and Therapeutic Targeting Strategies of Neutrophil Extracellular Traps in Inflammation

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Abstract: Neutrophil extracellular traps (NETs) are large DNA reticular structures secreted by neutrophils and decorated with histones and antimicrobial proteins. As a key mechanism for neutrophils to resist microbial invasion, NETs play an important role in the killing of microorganisms (bacteria, fungi, and viruses). Although NETs are mostly known for mediating microbial killing, increasing evidence suggests that excessive NETs induced by stimulation of physical and chemical components, microorganisms, and pathological factors can exacerbate inflammation and organ damage. This review summarizes the induction and role of NETs in inflammation and focuses on the strategies of inhibiting NETosis and the mechanisms involved in pathogen evasion of NETs. Furthermore, herbal medicine inhibitors and nanodelivery strategies improve the efficiency of inhibition of excessive levels of NETs.

Keywords: neutrophil extracellular traps, inflammation, targeted inhibition, nanotherapy, herbal medicine

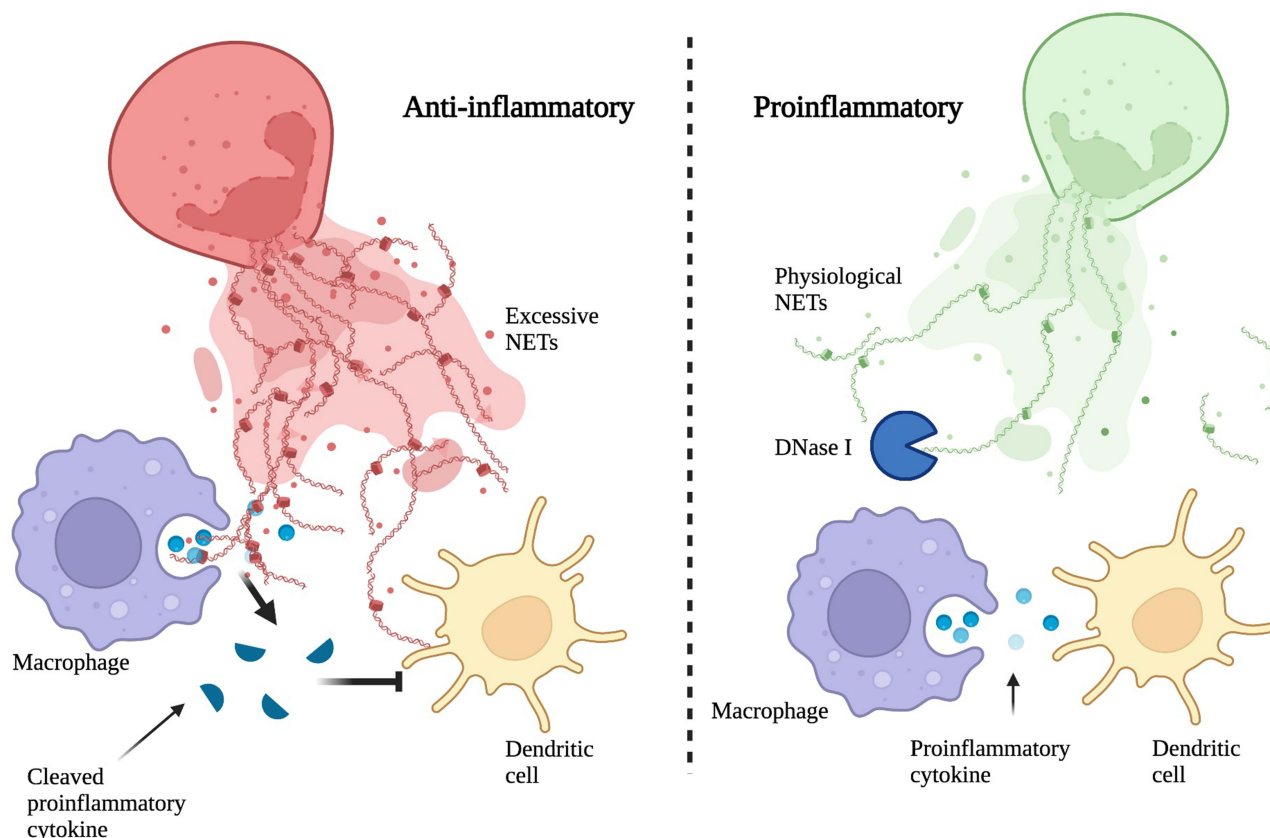
Introduction

The discovery of neutrophil extracellular traps (NETs) has opened a new chapter in our understanding of the nature and function of neutrophils. In addition to previously identified mechanisms of phagocytosis, degranulation, production of reactive oxygen species (ROS), and cytokines, neutrophils use an additional effector function—production of NETs—to modulate immune responses.¹ NETs were first discovered by Takei et al² in 1996 who determined that they were involved in a novel process of phorbol 12-myristate 13-acetate (PMA)-induced programmed neutrophil cell death, and activated a molecular pathway that differed from that of apoptosis and necrosis. In 2004, Brinkman et al³ provided a more comprehensive description and named this process NETosis. In addition to chemical agents such as PMA, various stimuli in vivo or in vitro—including pathogens, inflammatory mediators, and cell damage products—have been found induce NETs formation through different effector mechanisms.⁴ Despite the heterogeneity of activation of the NETosis signaling pathway by different stimuli, the stimulus ultimately leads to a general outcome involving chromatin and protein binding, reorganization of the intracellular membrane, and finally release of NETs into the extracellular space.^{5,6} These stimuli activate NETosis by binding to pattern recognition receptors (such as Toll-like receptor [TLR], C-type lectin receptor, and nucleotide-binding oligomerization domain-like receptor), complement receptors, Fc receptors, chemokine receptors, and other receptors.^{7,8}

NETosis mainly includes three pathways (Figure 1). The most widely characterized mechanism is the formation of suicidal NETs.^{9,10} PMA stimulates the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) and the production of ROS in neutrophils through the protein kinase C (PKC) and Raf-MEK-ERK signaling pathways.¹¹ The activation of myeloperoxidase (MPO) and ROS production promote the translocation of neutrophil elastase (NE) from azurophilic granules to the nucleus, and relocated NE initiates chromatin

Graphical Abstract

Role of NETs in inflammation



decondensation in the nucleus.¹² Peptidylarginine deiminase IV (PAD4) promotes nucleosome histone cleavage and chromatin decondensation through citrullination.^{13,14} PAD4 and cell cycle proteins jointly regulate nuclear membrane rupture,¹⁵ and actin leads to plasma membrane rupture.^{16,17} Finally, with fragmentation of the cell membrane, the decondensed chromatin is mixed with granule proteins, which are released into the extracellular space to form NETs.

Compared to the relatively lengthy period (3 to 4 hours) required for the formation of suicidal NETs, *Staphylococcus aureus* (*S. aureus*) rapidly stimulates neutrophil release of NETs in a very short time of 5 to 60 minutes. This rapid and novel mechanism of NETs formation is called “Vital NETosis”.¹⁸ In this pathway, NOX activity is not required and nuclear DNA-laden vesicles are extruded without disrupting the plasma membrane, which preserves neutrophil function.^{18,19} A mechanism independent of ROS, NE, and PAD4 has been reported. Gram-negative bacteria and lipopolysaccharide (LPS) induce NETosis through the formation of gasdermin D (GSDMD) via a caspase-11-dependent mechanism.²⁰

NETs are always accompanied by inflammation as a part of the immune response. Many stimuli that cause inflammation induce NETosis, whereas NETs promote the onset and worsening of inflammation, as seen in sepsis, diabetes, and rheumatoid arthritis (RA).^{21–23} Therefore, the inhibition of NETosis is an important approach for the treatment of inflammatory diseases. Although many recent reviews have described the inhibition of NETs formation, an evaluation of their advantages and disadvantages and effective therapeutic targeting strategies is lacking.^{7,24–27} To this

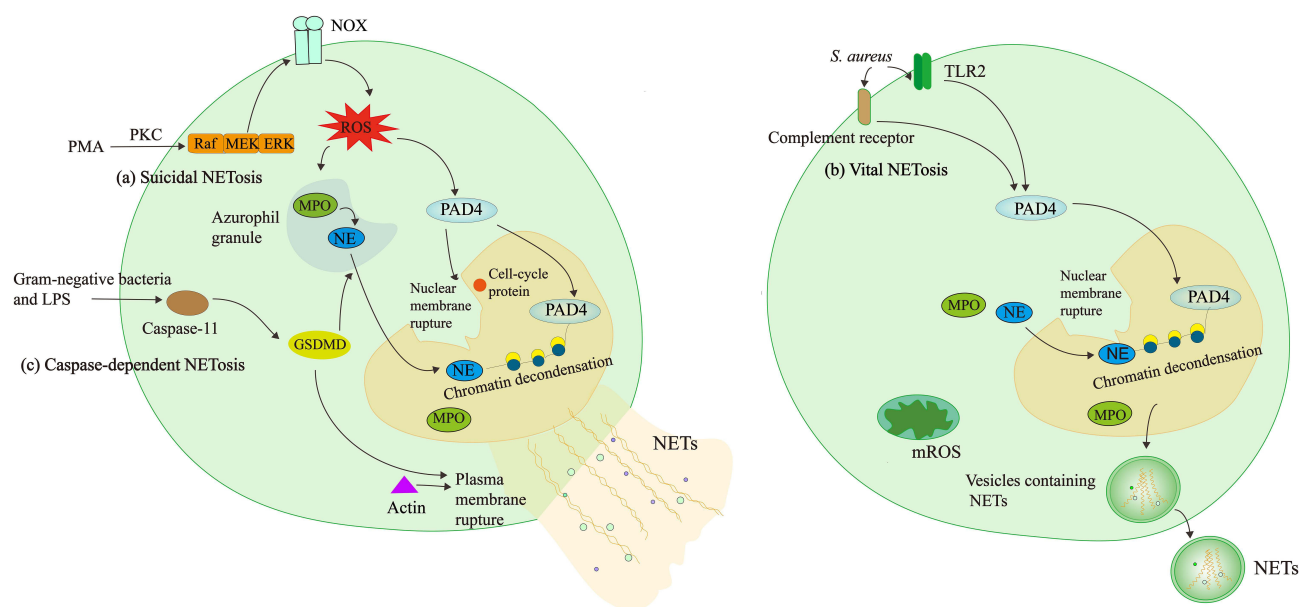


Figure 1 Mechanisms of NETs formation.

Abbreviations: NETs, neutrophil extracellular traps; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; NOX, NADPH oxidase; ROS, reactive oxygen species; MPO, myeloperoxidase; NE, neutrophil elastase; PAD4, peptidylarginine deiminase IV; LPS, lipopolysaccharide; GSDMD, gasdermin D; TLR, toll-like receptor; NETs, neutrophil extracellular traps.

end, in this review, we include a description of biomimetic NETs and targeting herbal medicines to the database of NETs inhibition and nanotargeting therapy to improve the efficiency of targeting NETs.

Stimuli Inducing Inflammatory NETosis

Lots of stimuli are known to induce NETosis, such as chemical reagents, activated platelets, and microorganisms.⁴ Some of these stimuli are identified in NETosis induction in vitro, while others are identified following the study of the pathogenesis of inflammation, which more clearly revealed the role of NETosis in inflammation. Below, we discuss the stimuli triggering NETosis and the role of NETs in inflammation to provide more comprehensive background information to better explain the strategy of NETs-related therapy.

Physical and Chemical Components

As a common activator of PKC, PMA was the first chemical identified to initiate NETosis in a NOX-dependent manner. Interestingly, NETs produced by the calcium ionophore A23187 stimulated NETosis in an alternative NOX-independent pathway, which appeared to be extremely like the former in protein composition.⁴ The generation of NETs stimulated by external nonphysiological factors may aggravate inflammation. For example, cigarette smoke extract was shown to induce the formation of NETs and airway inflammation in mice in an ROS-dependent manner.²⁸ There is no doubt that NETs induced by physiological factors are more closely related to inflammation. Eosinophils and eosinophil-generated Charcot-Leyden crystals can directly induce neutrophil activation, increase the activity of NE and cathepsin G, form NETs, and eventually aggravate the local inflammatory circulation in chronic neutrophilic rhinosinusitis.^{29,30} The NETs-related protein LL-37 also induces NETosis to sustain an auto-inflammatory cycle.³¹

Microorganisms

In addition to the common microbial virulence factor LPS, invading bacteria, viruses, and fungi are inducers of NETs that further mediate inflammation development. Neutrophils can selectively induce the formation of NETs according to the size of invading microorganisms. NETs target large pathogens, while phagocytosis targets smaller pathogens, which sequester NE to reduce unnecessary NETosis.³² The resulting NETs have an obvious dual effect. NETs trap and kill bacteria,³ fungi,³³ viruses,³⁴ and parasites³⁵ to stop the spread of infections. Conversely, NETs induced by

microorganisms can promote the development of inflammation and deteriorate health. Active severe acute respiratory syndrome-associated coronavirus-2 (SARS-CoV-2) virus has been reported to invade through the angiotensin converting enzyme 2 (ACE2) and the transmembrane serine protease 2 pathway, and can also down-regulate the ACE2 receptor to promote angiotensin imbalance, further promoting NETs release, pulmonary epithelial cell death and thrombosis.^{36,37} The symbiotic fungus *Candida albicans* exposed to skin colonization in mice also significantly activates Th17 cells and increases neutrophil activation and NETosis, and can also aggravate the inflammation of psoriatic dermatitis.³⁸ Furthermore, *Pseudomonas aeruginosa* biofilm on the ocular surface expresses a high level of bacterial type-3 secretion and promotes NETosis. The resulting NETs barrier “dead zone” limits bacteria to the external environment of the cornea to prevent brain invasion while causing severe keratitis.³⁹

Pathological Factors

High pH,⁴⁰ hypertonicity,⁴¹ hyperglycemia,⁴² disorders of NETs degradation, and other pathological factors are also vital for the induction of NETosis. Due to different disease contexts, symptomatic treatment may help to more deeply explore the impact that the unique microenvironment of various diseases plays on NETosis. Unstable tear film and hyperosmotic characteristics of dry eye syndrome can explain the existence of excessive NETs on the ocular surface of patients with dry eyes.⁴³ Compared to non-diabetic patients, hyperglycemia increases the rate of NETosis and the release of NETs in patients with type 2 diabetes.⁴² There may be inflammatory factors and other stimuli in the inflammatory environment that induce NETosis and aggravate inflammation. The colonic inflammatory environment of patients with ulcerative colitis releases interleukin (IL)-1 β and locally regulated thrombus tissue factor in the development and DNA damage responses 1 protein to promote NETosis.⁴⁴ These mechanisms suggest that the factors that lead to the overproduction of NETs, and failure to degrade NETs in time will also cause inflammation related to NETs. In patients with severe dry eye syndrome, lacrimal nuclease deficiency causes environmental DNA (eDNA) and NETs to accumulate in the anterior corneal tear film and bind to cathelicidin, which re-enters the ocular surface of cells to activate the type I interferon (IFN) response.⁴⁵ Previous studies have shown that alveolar macrophage-mediated apoptosis of apoptotic neutrophils also contributes to persistent NETs-mediated inflammation.⁴⁶

Pro-Inflammatory Activities of NETs

Although neutrophils release NETs to capture pathogens, persistent infections, and the inflammatory environment result in excessive production of NETs, which mediate various inflammatory diseases. A wide range of inflammatory responses, including local inflammation of the lung, intestine, eye, nose, mouth, and skin, and autoimmune inflammation are involved. NETs mediate inflammation primarily by activating the inflammasome, via crosstalk with damage-associated molecular patterns (DAMPs), or by amplifying the inflammatory response with immune cells or immune factors that are related to autoimmunity and occlusive arterial disease (Figure 2).

NETs Activation of the Inflammasome

Inflammasomes activated by NETs are important initiators of inflammation in the immune response.^{47,48} NETs activate the inflammasome in resident cells, such as monocytes or macrophages, in patients with high eDNA asthma, resulting in the secretion of IL-1 β .⁴⁹ NETs also activate the inflammasome in lupus macrophages and lead to the release of the inflammatory cytokine IL-18, which aggravates the inflammatory response.⁵⁰ Furthermore, recent studies had found that NETs regulate the assembly of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome in neutrophils through PAD4, and the formation of the NLRP3 inflammasome further promotes NETosis.⁵¹

NETs Crosstalk with DAMPs

DAMPs are endogenous molecules that induce and enhance aseptic inflammation. Under conditions of injury or hypoxia, DAMPs activate the human immune response, worsen the inflammatory response, and cause tissue damage.⁵² The main components of NETs, such as histones, granules proteins, high-mobility group box 1 (HMGB1) and DNA, are DAMPs that can trigger inflammation.⁵³ DAMPs have also been indicated to induce NETosis. HMGB1 released from lung cells

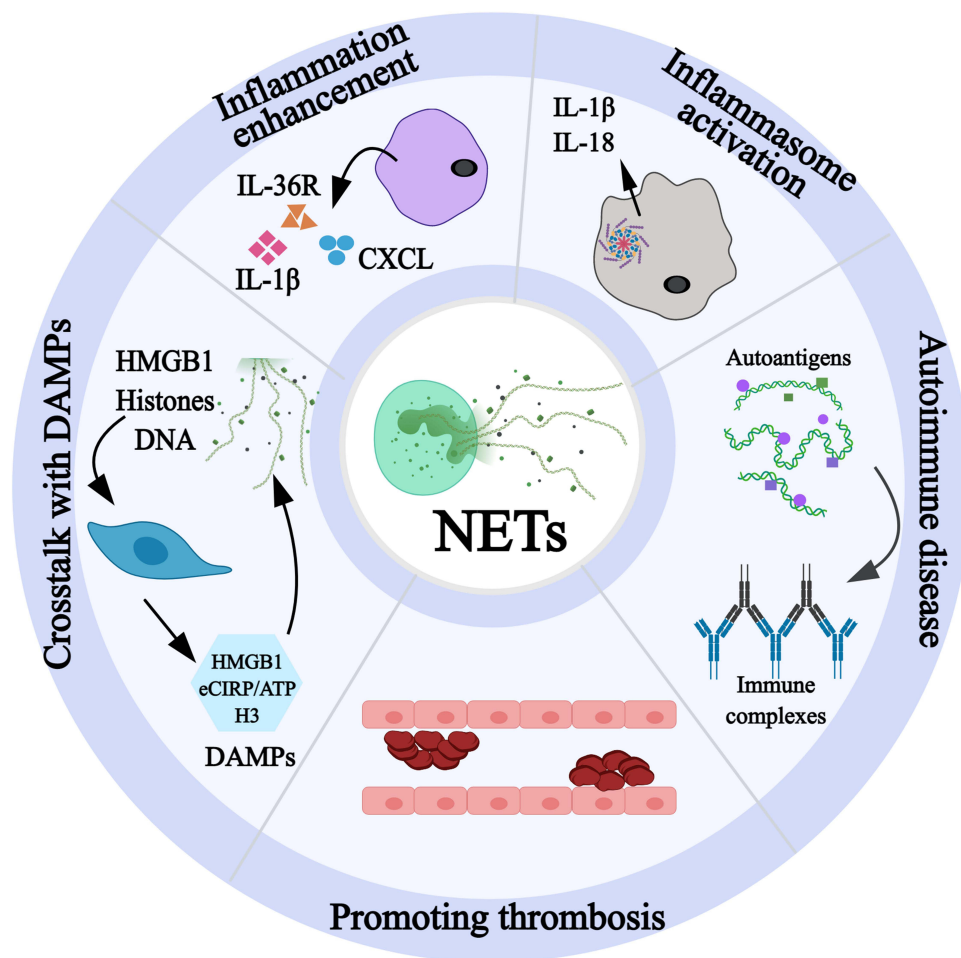


Figure 2 Pro-inflammatory activities of NETs.

Abbreviations: NETs, neutrophil extracellular traps; HMGB1, high-mobility group box 1; CXCL, C-X-C motif chemokine ligand; eCIRP, extracellular cold-inducible RNA-binding protein; H3, histone 3; IL, interleukin.

from patients with coronavirus disease 2019 (COVID-19) is a key DAMP that can cause NETosis and then may lead to persistent inflammation.⁵⁴

NETs Enhance Inflammation

NETs amplify inflammation by interacting with immune or tissue cells, such as inflammatory cells and epithelial cells. NETs stimulate macrophages to secrete IL-1 β , which in turn produces more NETs that amplify tissue damage.⁵⁵ NETs also amplify inflammation by stimulating airway and alveolar epithelial cells to express C-X-C motif chemokine ligand (CXCL)-1, CXCL-2, and CXCL-8 via the TLR4/nuclear transcription factor- κ B (NF- κ B) pathway.⁵⁶ Recently, NETs have also been found to interact directly with keratinocytes and activate a TLR4/IL-36R crosstalk, which then activates the myeloid differentiation factor 88 (MyD88)/NF- κ B signaling pathway to induce the production of chemokine lipocalin 2 and the pro-inflammatory factor IL-36 γ , thus amplifying the cascade leading to psoriatic dermatitis.⁵⁷

NETs Mediate the Autoimmune Reaction

NETs are considered to play a central role in the autoimmune response.²⁷ The imbalance of NETosis leads to persistence production of NETs and constitutes the main source of autoantigens, including LL-37, and dsDNA, histones.^{58,59} These autoantigens lead to the accumulation and activation of immune complexes. Unlike systemic lupus erythematosus (SLE) for nuclear antigens, patients with anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) specifically present ANCA targeting MPO or proteinase 3 (PR3), while patients with RA showed higher levels of anti-

citrullinated protein antibody (ACPA).^{60,61} In addition to classic autoimmune diseases such as SLE, AAV, and RA, more and more inflammation has been found to be related to NETs-mediated autoimmunity. The emergence of autoantibodies related to NETs in inflammation such as dry eye disease,⁶² psoriasis,⁶³ and COVID-19⁶⁴ shows the occurrence of an autoimmune reaction.

NETs Induce Thrombosis and Occlusion

NETs mediate platelet activation, coagulation, and thrombosis.⁶⁵ The complete NETs stent-platelet-thrombin axis is capable of promoting intravascular thrombin activation and microvascular thrombosis in sepsis.⁶⁶ In the plasma-induced NETs model of COVID-19, SARS-CoV-2 activates complement C3 to drive platelet/NETs and induce the formation of NETs enriched with tissue factor, which in turn activates endothelial cells to express tissue factor, thus increasing their procoagulant activity, and further activates platelets to aggravate the inflammatory cycle.⁶⁷ Platelet factor 4 coagulation factor signaling in platelets binds to NETs, making them robust and resistant to DNase and leads to microthrombosis in patients with COVID-19.⁶⁸ The negative effect of NETs is not only embodied in inflammation induced by residues of NETs but also in inflammation induced by occlusion of arteries caused by aggregated NETs (aggNETs).⁶⁹ Aggregation of NETs in the secretions of allergic eye disease leads to meibomian gland duct occlusion and acinar atrophy in mice, resulting in excessive evaporative xerophthalmia.⁷⁰ Although aggNETs aggravate ocular surface inflammation under pathological conditions, aggNETs also remove eye closure inflammation. AggNETs promote cleansing of the ocular surface and eliminate ocular inflammation by degrading inflammatory mediator cytokines and chemokines.⁷¹

Inhibition Strategies of NETs

NETosis is a continuous and complex process, PAD4 enzymes, histone, MPO and other components play their respective roles, all of which are indispensable. These components not only participate in NETosis and make up NETs but also mediate various types of NETs-related inflammation, as seen in cystic fibrosis (CF) and asthma.⁷² The composition of these proteins in NETs is positively correlated with the severity of most inflammatory responses.

The main components of NETs, namely DNA, histones, and soluble proteins, are detected in the sputum of patients with CF, and their levels positively correlate with the severity of lung disease.^{53,69} Compared to healthy controls, the levels of NETs components (eDNA, PAD4, NE, MPO, and LL-37) are elevated in patients with chronic obstructive pulmonary disease (COPD).^{73–76} Therefore, inhibition of NETs is an important strategy for the treatment of inflammatory diseases. Below, we summarize the classic, most recent nonspecific and specific targeting strategies for the inhibition of NETs, including those of specific of eDNA, PAD4, NETs-derived enzymes (NE, histone, citrullinated histone, MPO, and GSDMD), as well as nonspecific ROS, components of the complement system, chemokines, and antibiotics (Table 1).

Microorganisms have developed mechanisms such as degrading DNA, altering the surface structure to increase resistance to NETs, and inhibiting NETosis to evade NETs to promote proliferation and spread of microbes.¹²³ For example, *S. aureus*, a classic representative bacteria, escapes NETs by cleaving extracellular DNA, micrococcal nuclease, extracellular adhesion protein, and fibronectin binding protein B to block the protease activity of NETs.¹¹⁴ The 3'-nucleotidase/nuclease mediates *Candida*¹¹⁵ and *Leishmania*¹²⁴ escape from NETs. Furthermore, pneumococci can adjust its surface charge to negative by secreting pneumococcal surface protein A, which repel negatively charged NETs.¹¹⁹

These mechanisms of natural microbial infections to inhibit NETs, have inspired researchers to develop a new strategy for anti-inflammatory agents. Proteins that mimic pathogens to escape NETs may contribute to the discovery of new drugs that effectively inhibit NETs. For example, nucleases secreted by pathogens that degrade NETs and various similar proteins can be considered potential drugs to modulate NETs-associated inflammation.¹²⁵ Another important example is recombinant *Trichinella spiralis*, which secretes serine protease 1 (TsSERP1). The latter inhibits the formation of human NE and NETs and alters the production of human pro-inflammatory cytokines and chemokines released by neutrophils.¹²⁶

Herbal medicine has been widely used in the treatment of NETs-related inflammatory diseases due to its high safety and remarkable curative effect.^{127,128} However, due to the complex components and multi-target effects of herbal medicine, it is not easy to explain its specific mechanisms of action.¹²⁹ Fortunately, with the development of separation technology and analytical equipment, many active components of herbal medicines have been reported to beneficially

Table 1 Anti-NETs Therapy

Mechanisms		Potential Drugs	Ref.
eDNA degradation	–	DNase I Staphylococcal nuclease	[77,78]
PAD inhibitors	PAD4 inhibitors	CI-amidine, GSK484/199, YW3-56, roflumilast, tanimilast, BMS-P5	[79–85]
	PAD2 inhibitors	AFM32a	
ROS/NADPH inhibitors	–	Metformin, DPI, vitamin C, NAC, (+)-borneol	[86,87,87–89]
Granule protein inhibitors	Citrullinated histone inhibitor	CitH3 mAb (4 Cit), tACPA	[90–95]
	Histone inhibitor	SPA, rTM, HIPE, CHIP, heparin	
	MPO inhibitor	Luminol, PF-1355, AZM198, PA-dPEG24	[96–99]
	NE inhibitor	AZD 9668, sivelestat, SLPI, BAY 85–8501	[100–103]
GSDMD inhibitors	–	Disulfiram, LDC7599	[104,105]
Complement and CXCL inhibitors	Complement C5 inhibitor	OmCI	[106,107]
	Complement C1 inhibitor	Reparixin	
Inhibitors of NETs formation and release	–	Antibiotic (azithromycin, chloramphenicol, gentamicin, ceftriaxone, erythromycin)	[108–111]
	–	Tetrahydroisoquinolines, hydroxychloroquine	[112,113]
Simulation of immune evasion of NETs by pathogen	Cleaves eDNA	3'-nucleotidase/nuclease, immunoglobulin-like protein A, DNase-like protein, surface lipoprotein MHO-0730, Staphylococcal nuclease	[114–118]
	Resists NETs	Pneumococcal surface protein A, leukocidins PVL and HlgAB	[119,120]
	Inhibits NETosis	Multifunctional virulence factor TcpC (PAD4 inhibitor), calreticulin, HBV C and E protein (ROS inhibitor), extracellular adhesion protein, and fibronectin binding protein B	[114,121,122]

Abbreviations: NETs, neutrophil extracellular traps; PAD, peptidylarginine deiminase; DPI, diphenyleneiodonium chloride; NAC, N-acetylcysteine; CitH3, citrullinated histone H3; tACPA, therapeutic anti-citrullinated protein antibody; SPA, small polyanion; rTM, recombinant thrombomodulin; HIPE, histone inhibitory peptide; CHIP, cyclical histone H2A interference peptide; MPO, myeloperoxidase; NE, neutrophil elastase; SLPI, secretory leukocyte protease inhibitor; GSDMD, gasdermin D; CXCL, C-X-C motif chemokine ligand; OmCI, ornithodoros moubata complement inhibitor; HBV, hepatitis B virus; ROS, reactive oxygen species.

modulate NETs-related inflammation through different mechanisms (Table 2). For example, flavonoid luteolin and carnosic acid inhibits NETs production by inhibiting the Raf1-MEK-1-Erk pathway and ROS production.^{130,131} Salvianolic acid B and 15,16-dihydrotanshinone I in *Salvia miltiorrhiza* significantly inhibit the formation of early NETs by inhibiting the activity of MPO and NADPH oxidase, respectively.¹³² The main components of the Xuebijing Injection (Approved No. Z20040033), safflower and *Ligusticum Chuanxiong* hort., contain safflor yellow A, hydroxysafflor yellow A, anhydrosafflor yellow B and senkyunolide I, respectively. Xuebijing Injection has been shown to significantly inhibit NETosis.^{133,134} Some of the active compounds of herbal medicines such as terpenoid triptolide and andrographolide have been shown to be effective in inhibiting NETs, but specific mechanisms are not yet known. Herbal medicine is a treasure for researchers looking to discover active molecules in the treatment of inflammatory diseases related to NETs.

Inhibition of PAD4

PAD4 is a key enzyme in the formation of NETs in vivo. It involves the citrullination of histone and induction of chromatin decondensation. PAD4 disorders have been shown to be associated with a variety of inflammation types and has led to the emergence of an autoimmune response and an increase in inflammation. More and more studies have indicated that the emergence of ACPA is caused by PAD4-mediated imbalance of citrullination of extracellular proteins, which is the core element of inflammatory autoimmune disease in RA.¹⁵⁴ In the peripheral joints and blood of patients with RA, inflammatory cytokines and ACPA autoantibodies promote NETosis and keep the autoimmune circulation active.⁶¹ Citrulline histones derived from NETs on the ocular surface also stimulate ACPA production. These autoantibodies not only induce the release of pro-inflammatory cytokines by interacting with Fc receptors on activated neutrophils and dendritic cells but also stimulate NETosis and activate the complement

Table 2 Active Ingredients of Herbal Medicine and Their Effects on NETs

Effective Components	Active Ingredients	Dosage Form	Models	Routes or Dose of Administration	Mechanism	Types of Inflammation	Ref.
Glycosides	Chikusetsusaponin V	Injection	C57BL/6 mice; primary neutrophils	i.p. (200, 100, 50 mg/kg); 100 μ M	Reduces NETs-related inflammation via Caspase-1-HMGB1	Acetaminophen-induced liver injury	[135]
	Glycyrrhizin	Injection	C57BL/6 mice	i.p. (15 mg/kg)	Inhibits NETosis and improves lung function via HMGB1/TLR9/MyD88	Sepsis-induced ARDS	[136]
	PEGylated nanoparticle albumin-bound steroidal ginsenoside derivatives	Nanoparticles	Plasma of patients	50 μ g/mL	Modulates histone H4 levels and the cytokine storm via NF- κ B and SREBP2 signaling pathways	COVID-19	[137]
	Polydatin	Injection	DBA/1 or BALB/c mice; human or mouse neutrophils	i.p. (45 mg/kg); 0, 50, 75, 100, 125, 150 μ g/mL	Inhibits NETs formation	RA/SLE	[138,139]
	Forsythiaside B	Injection	Rats	i.v. (20, 40, 80 mg/kg)	Inhibits PAD4	Sepsis	[140]
	Luteolin	Injection	C57BL/6 mice; human neutrophils	i.p. (50 mg/kg); 3, 10, 30 μ M	Inhibits Raf1-MEK-1-Erk pathway, ROS production and NETosis	Inflammatory arthritis	[130]
	15,16-dihydrotanshinone I	Solution	Human neutrophils	20 μ M	Inhibits NOX activity	Neutrophil inflammation	[132]
	Quercetin	Injection	C57BL/6 mice	i.p. (30 mg/kg)	Inhibits expression of CitH3, PAD4 and autophagy	RA	[141]
	Safflor yellow A, Hydroxysafflor yellow A, and Anhydrosafflor yellow B	Injection	C57BL/6J mice; HL-60	i.p. (5×10^{-5} mol/kg); 160 mg/L	Inhibits the Raf/MEK/ERK signaling pathway	ALI	[142]
	Zingerone	Injection	C57 mice; human and mouse neutrophils	i.p. (25, 50 mg/kg)	Inhibits Nrf2-mediated ROS	Sepsis	[143]
Phenolic acids	Salvianolic acid B	Solution	Human neutrophils	200 μ M	Inhibits MPO activity	Neutrophil inflammation	[132]
Quinones	Emodin	Injection	C57BL/6 mice	i.p. (30 μ g/kg)	Inhibits NETosis and NETs-related MPO and NE release	RA	[144]
	Tanshinone IIA	Injection	C57BL/6 mice; mouse neutrophils	i.p. (30 mg/kg); 0, 5, 10, 25 μ M	Inhibits NETs-associated MPO and NE release	RA	[145]

Terpenoids	Andrographolide	Injection	C57BL/6 mice	i.p. (25, 50 mg/kg)	Decreases levels of PAD4, CitH3, and NETosis	RA	[146]
	Triptolide	Solution	Human neutrophils	0.2 μ M	Suppresses NETosis in an ROS independent manner	NETs related inflammation	[147]
	The total terpenoids of <i>Celastrus orbiculatus</i>	Solution	Human neutrophils	0, 5, 10, 20, 40, 80 μ g/mL	Inhibits NETs-associated DNA and granular proteins complexes in ROS dependent manner	Neutrophil inflammation	[148]
	Senkyunolide I	Injection	C57BL/6 mice; mouse neutrophils	i.p. (36 mg/kg); 100 nM	Reduces MPO-DNA level	Sepsis-induced lung injury	[133]
Alkaloid	Tetrandrine	Injection	C57BL/6 mice	i.p. (6 mg/kg)	Alleviates neutrophil activities, and decreases MPO and NE	RA	[149]
	Tetramethylpyrazine	Injection	Rats; mouse neutrophils	i.p. (40 mg/kg); 0, 5, 10, 20, 40 μ M	Downregulates expression of NETs-DNA, H3cit and NOX	Hepatic IRI	[150]
Phenolics	6-Gingerol	Injection	C57BL/6 mice; human neutrophils	i.p. (20 mg/kg); 0.1, 1, 10, 100 μ M	Attenuates NETosis partially dependent on inhibition of PDE activity, cAMP and PKA	SLE	[151]
Reduning Injection	Iridoid, lignans, etc.	Injection	C57BL/6j mice	i.p. (5, 10 mL/kg)	Downregulates MAPK pathways to inhibit PAD4 and NETosis	ALI	[152]
Kan-Lu-Hsiao-Tu-Tan	Baicalin, baicalein, etc.	Solution	Imiquimod-induced psoriasis animal; human neutrophils	Topical use (10 mg/mL); 1, 3, 10 μ g/mL	Inhibits formation of ROS and NETs	Psoriasis	[153]

Abbreviations: NETs, neutrophil extracellular traps; HMGB1, high-mobility group box 1; TLR9, toll-like receptor 9; MyD88, myeloid differentiation factor 88; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SREBP2, sterol regulatory element-binding protein 2; MEK-1, mitogen-activated protein kinase kinase-1; Erk, extracellular signal-regulated kinase; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; NOX, NADPH oxidase; CitH3, citrullinated histone H3; PAD, peptidylarginine deiminase; Nrf2, nuclear factor erythroid 2-related factor 2; MPO, myeloperoxidase; NE, neutrophil elastase; ALI, acute lung injury; IRI, intestine reperfusion injury; cAMP, cyclic adenosine monophosphate; PDE, phosphodiesterase; PKA, protein kinase A; MAPK, mitogen-activated protein kinase; i.p. intraperitoneal; etc., et cetera.

system, thus creating a self-perpetuating cycle of chronic inflammation on the ocular surface of dry eye syndrome.⁶² ACPA may form an immune complex with citrullinated antigens released during NETosis to promote inflammation. Furthermore, monoclonal ACPA also induces tenosynovitis, pain, and bone loss in mice by relying on PAD4-mediated citrullination.¹⁵⁵ In the mouse model of double hemorrhagic shock and septicemia with double injury, compared to wild-type animals, the lack of PAD4 leads to reduced organ dysfunction and improved survival.

Several studies have shown that PAD4 inhibitors Cl-amidine and BB-Cl-amidine reduced NETosis and organ damage in SLE and improved septic survival in mice.^{81,156,157} In addition to PAD4 inhibitors, the PAD2 inhibitor AFM32a also significantly reduces pro-inflammatory cytokines and NETosis in the endotoxic shock mouse model. Furthermore, the Reduning Injection (Approved No. Z20050217), a traditional herbal medicine (THM) composed of gardenia jasmine and *Artemisia annua*, down-regulates MAPK pathways to inhibit respiratory diseases related to PAD4 and NETosis such as ALI.¹⁵² However, the expression of PAD4 is not limited to the NETosis of neutrophils, but also to the transcriptional regulation of genes,¹⁵⁸ regulation of stem cell differentiation,¹⁵⁹ and inhibition of ROS to reduce bacterial killing.¹⁶⁰ Extra discretion should be exercised due to the multifunctional activity of PAD4, which may cause side effects of simple inhibition.

Reduction of Histones and Citrullinated Histones

Histone not only participates in NETosis but also induces neutrophils to form NETs through TLR.¹⁶¹ The accumulation of histones, NE, and MPO leads to the destruction of the epithelium, endothelium, and connective tissue, causing additional chronic tissue damage, and worsening of function.^{21,162,163} Exaggerated NETs and histones cause epithelial pouch ulcers to promote bacteremia and endotoxemia, which in turn lead to the formation of low-grade systemic inflammation, a key factor in the deterioration of systemic diseases.¹⁶⁴ Histones also promote the deterioration of inflammation. Histone derived from NETs bridges the extracellular domain S2 of the spike protein in host cells, and sialic acid improves the proximity of the virus and the cell membrane to increase the infectivity of SARS-CoV-2.³⁶ In the colon of mice with experimental colitis, histones derived from NETs may alter intestinal permeability and barrier function by downregulating the expression of adhesion proteins and tight junction proteins and epithelial cell apoptosis.¹⁶⁵

Recombinant thrombomodulin blocks the accumulation of histones and NETs in the lungs by binding to circulating histones.⁹² Meara et al⁹¹ developed a small polyanion (SPA) mCBS-like heparin to replace histones from NETs but retaining the integrity of NETs so as to avoid the inflammatory effects of antibacterial protein, thereby inhibiting histone and NETs-mediated inflammation. Compared with histones, PAD4-mediated citrullinated histone is largely a specific marker of the process of NETs formation. Eight core histone components (two each from H2A, H2B, H3, and H4) and DNA comprise the nucleosome, the basic tissue unit of the NETs genome.¹⁶⁶ In one study, the monoclonal antibody CitH3 (4Cit) mAb specifically recognized citrullinated H3R26 to neutralize citrullinated histone H3 and attenuate the formation of NETs and the pro-inflammatory response during endotoxic shock.⁹⁵ However, this antibody targets all citrullinated histones H3 and not just NETs. A recent study shows that monoclonal antibody tACPA specifically binds to the histone citH2A and citH4 (Cit3) citrulline on NETs chromatin to inhibit NETs-mediated inflammatory diseases.⁹⁰ Some researchers believe that this specific recognition of NETs nucleosomes may be based on the binding of tACPA to form “clips” that prevent nucleosomes from spreading and act as highly specific markers conducive to phagocytosis.¹⁶⁷ Cit3 may become a new target for directing drugs to NETs for its specific relationship on citrullinated histones.

Down-Regulation of MPO and NE Activity

MPO and NE regulate NETosis by synergistically driving chromatin denudation, which is a marker of NETs formation in inflammation.¹⁶⁸ Of these, the MPO-DNA complex and citrullinated histone H3 are considered to be specific markers of NETs.¹⁶⁹ Plasma levels of the MPO-DNA complex can be used to predict the severity of COVID-19 infection and organ dysfunction in patients with septic shock.^{169,170} Interestingly, the excessive production of hypochlorite HOCl, a MPO-mediated bactericidal oxidant related to NETosis in the intestine of patients with Crohn's disease, can lead to an increase

in inflammation.¹⁷¹ Previous studies have shown that MPO in neutrophils is also an autoimmune target of inflammation associated with NETs. Patients with AAV specifically exhibit ANCA targeting MPO or PR3,⁶⁰ and induced NETosis is believed to be independent of IgG ANCA trigger and rich in citrulline histones.¹⁷² Excess NETs produced by ANCA stimulation leads to vasculitis and participates in the formation of ANCA itself, thus promoting a vicious circle of autoimmune AAV.¹⁷³ The inhibition of MPO released by neutrophils and monocytes by AZM198 can reduce neutrophil degranulation and NETosis.⁹⁸ Furthermore, the complement C1 peptide inhibitor PA-dPEG24 and ganoderma lucidum polysaccharides peptides can also block the MPO pathway associated with NETs formation.^{99,174}

NE is a serine protease, which is very important in NETosis. NE is reported to be associated with severe systemic and multiple organ damage in COVID-19 and can be used as an independent predictor of multiple organ injury in patients with COVID-19.¹⁷⁵ Knockout and inhibition of NE in mice can prevent the formation of extracellular neutrophil traps and can increase the resistance of mice to toxic shock.^{101,176} The NE inhibitor sivelestat has been listed as a drug for the treatment of ARDS in Japan and Korea.¹⁷⁷ Several NE inhibitors are in clinical trial pipelines. For example, the highly selective and reversible NE inhibitor AZD9668 can improve lung capacity and can reduce inflammatory levels in patients with bronchiectasis, CF, and COPD.^{100,178,179} Furthermore, tanshinone IIA extracted from the THM *Salvia miltiorrhiza* Bunge inhibited the release of NETs due to MPO and NE and reduced RA related inflammation in mice.¹⁴⁵

Degrading of DNA

The extracellular neutrophil trap is considered a network formed by externalization of biological chromatin and the formation of a large extracellular DNA scaffold. As the main component of NETs, the DNA of NETs are closely related to many inflammatory conditions.¹⁸⁰ Elevated extracellular DNA in the sputum leads to impaired lung function and poor control of symptoms in exacerbated airway inflammation in patients with severe asthma.^{181–183} NETs in SLE are rich in oxidized mtDNA-induced inflammatory type I IFN.¹⁸⁴ In addition, recent studies have found that externalized small RNA in NETs also induces a type I IFN response, which promotes vascular inflammation of lupus.¹⁸⁵ Higher concentrations of NETs-DNA are not only associated with the aggravation of inflammation such as asthma, inflammatory bowel disease, and immune thrombus but they also have strong associations with autoimmune inflammation.^{186–188} Some anti-dsDNA antibodies in active SLE disease inhibit NETs digestion and stimulate the type I IFN response or NF-κB activity to amplify inflammation.¹⁸⁶

Considered a safe tool for degrading DNA, the Food and Drug Administration (FDA) has approved DNase I for the clinical treatment of CF and SLE.^{189,190} A recent study on the treatment of COVID-19 has reported that nebulized human DNase improves respiratory function and reduced acute symptoms in patients.⁷⁷ In addition, dual-active DNase with DNaseI and DNaseII activity will degrade NETs-DNA more rapidly and effectively.¹⁹¹ In terms of THM, the total terpenoids of *Celastrus orbiculatus* prevented the formation of the NETs-DNA complex and acted as an anti-inflammatory agent for suicidal NETosis.¹⁴⁸

Blocking of GSDMD

NETosis is dependent on GSDMD, which promotes NE translocation to the nucleus. GSDMD also causes the nucleus to expand and induces the formation of pores in the plasma membrane.¹⁰⁵ Direct activation of GSDMD by the SARS-CoV-2 virus induces excessive NETs production, and worsens tissue and organ damage in patients.¹⁹² Moreover, the aseptic inflammatory environment in patients with sickle cell disease also promotes the activation of GSDMD, thereby increasing NETs in the liver. NETs translocate from the liver through hepatopulmonary embolism and acute inflammatory lung injury.¹⁹³ Therefore, the knockout and inhibition of GSDMD will help reduce inflammation in conditions such as COVID-19, ALI, and ARDS.^{192–194}

Through chemical screening, it was found that the specific binding of LDC7559 to GSDMD reduced the activation of pathological inflammatory bodies and NETosis.¹⁰⁵ Furthermore, disulfiram can also covalently inhibit GSDMD activity to effectively reduce NETs production and prevent organ damage and systemic inflammation during sepsis.¹⁰⁴

Regulation of ROS

ROS is necessary for the formation of NADPH-dependent NETs. It interacts with NETosis in a multidimensional manner and participates in most inflammatory cascades.^{195,196} The uncontrolled increase of ROS under oxidative stress leads to increased NETs and inhibition of T cells in the immune system, which will eventually lead to an aggravation of severe COVID-19 inflammation.¹⁹⁷ Metformin, a ROS inhibitor, is an antidiabetic drug, which reduces the activation of NOX in neutrophils and inactivates the NETosis response, thereby improving the pathological state of diabetes.⁸⁸ ROS is a common pathway for many THM to inhibit NETs, including luteolin, triptolide, hesperetin and Kan Lu Hsiao Tu Tan (KLHTT).^{130,147,153,198} Although ROS inhibitors (DPI, vitamin C, luteolin, and NAC) are beneficial for reducing NETosis, appropriate drug delivery systems for them are still lacking.

Other Inhibitors

Calcitonin is not only the main antifungal component of NETs but it is also related to the diagnosis and progression of NETs-related CF and other diseases. Serum calprotectin levels can be used as an indicator of the prediction of key future clinical events in CF, including lung deterioration and decreased lung function.¹⁹⁹ Interestingly, recent studies have found that S100A9 increases ROS production to promote NETs formation, thus increasing white blood cell recruitment in patients with abdominal sepsis. It should be mentioned that the S100A9 inhibitor ABR-238901 reduces lung damage in abdominal sepsis.²⁰⁰ HMGB1 is the DAMP that activates NETs and is part of the release of cellular contents during NETosis. NETs induced by HMGB1 promote intestinal ischemia/reperfusion-induced ALI.²⁰¹ The THM Chikusetsusaponin V and glycyrrhizin inhibit NETs formation in APAP-induced liver injury and sepsis-induced ARDS by regulating HMGB1-related mechanisms.^{135,136,202}

In addition to antibacterial activity, antibiotics play an immunomodulatory role with neutrophils.¹⁰⁹ Many previous studies have shown that antibiotics such as gentamicin, azithromycin, and chloramphenicol interfere with the formation of NETs.^{108,203} The combination therapy of antibiotics is beneficial to rationally target the dual activity of NETs. For example, the combination therapy of imipenem and ceftriaxone. The early use of imipenem promotes NETs to fully resist bacteria, and later use of ceftriaxone inhibits NETs to prevent inflammatory damage caused by NETs.¹¹⁰

MicroRNA (miRNA) may regulate the formation of NETs. Inhibition of miRNA genes, miR-155 and miR-223, miR-146a has been shown to reduce inflammation mediated by the formation of NETs.^{204–206} Furthermore, intrapulmonary delivery of miR-146a can inhibit diffuse alveolar hemorrhage by reducing NETosis.²⁰⁶ Therefore, targeted inhibition of miRNA exosomes or extracellular vesicles or miRNA delivery is beneficial to inhibition of NETosis.²⁰⁷

Nanodelivery Systems

Since NETs play a key role in inflammation, inhibition of NETs has proven to be effective in modulating inflammation. However, NETs inhibition remains a challenge, even in the application of NETs inhibitors. A nano drug delivery system may represent a potential solution.²⁰⁸ Nanodelivery systems and their active targeting strategy would greatly improve the stability and effectiveness of NETs inhibitors. In this section, we summarize recent nano-drug delivery strategies for targeting of NETs (Table 3). Nanomaterial-based NETs systems lead to: (1) improved pharmacokinetics behavior and therapeutic efficiency of drugs, such as extension of the half-life of DNase I and the efficacy of the NE inhibitor sivelestat and (2) to the improved biodistribution of drugs. Nanocarriers with a targeting moiety delivers drug specific to NETs, such as delivering PAD4 inhibitor GSK484 to the intimal inflammatory area to inhibit NETs (Figure 3). It is worth noting that some nanomaterials have the potential to activate the inflammatory pathway by promoting NETosis,^{209,210} thus, discretion is advised when selecting nanocarrier preparations.

The encapsulation of drugs by nanocarriers shows significant advantages, to improve the stability, safety, and efficacy of drugs, especially for nucleic acids and enzymes. A major disadvantages of DNase I is its short half-life in plasma, which hinders its efficacy in inhibiting NETosis and antiinflammation. Melanin-like nanospheres and poly (lactic acid-glycolic acid copolymer) nanoparticles were developed to increase the enzyme stability and half-life of DNase I. The excellent bioadhesion properties of polydopamine contribute to its long-term activity.^{211,212} A highly hydrophilic microgel loaded with DNase-I (DNase-I MG) digests NETs more quickly and efficiently than the free enzyme.²¹⁴

Table 3 Nano-Drugs for NETs Inhibition

Nanodelivery Systems	Pharmacological Compounds	Objects of Administration	Routes and Dose of Administration	Effects	Disease	Ref.
Poly (dopamine) poly (ethylene glycol) nanoparticles	DNase I	C57BL/6 mice;	i.v. (100 units)	Improved the short half-life of DNase I	COVID-19 patients with sepsis	[211]
DNase-I coated melanin-like nanospheres (DNase-I pMNS)		human neutrophils				
Positively charged dimethylamino-modified polydopamine nanoparticles		C57BL/6 mice;	i.v. (100 units)	Improved the short half-life of DNase I	COVID-19	[212]
DNase I functional microgel		neutrophils				
		SD rats	Arterial injection (2.5 mg/kg)	Superior combining ability of cfDNA	RA	[213]
			0.6 mg/mL	Improved bioavailability	NETs-mediated inflammation	[214]
Fc-modified monoclonal antibodies binding to PF4-NETs complex (KKO)		Human neutrophils			Sepsis	
Poly(3-Hydroxybutyrate) Microspheres	Cl-amidine	WT, Cxcl4 ^{-/-} , hPF4 ⁺ mice	i.v. (5 mg/kg)	Enhanced DNase resistance, limiting toxic NETs degradation products		[215]
		SK-BR-3 cell line	1 mg/mL	Increased drug release	Cancer and NETs associated inflammation	[216]
Multilayer vesicles cross-linked between bilayers	Sivelestat	BALB/c mice	i.p. (50 mg/Kg)	Improved efficacy	Endotoxic shock	[101]
Sialic acid chain-modified nanoparticles	Sialic acid	Human neutrophils	10 µg/mL	Inhibited ROS, improved efficacy	NETs-related pathologies	[217]
Monoclonal antibodies 2C5-modified micelles	-	Neutrophils	5 µg/mL	Specifically targeted intact nucleosomes	NETs-mediated inflammation	[218]
Calcium alginate encapsulation	Staphylococcal nuclease	C57BL/6 mice	i.g. (25,75 mg/kg)	Targeted the colon	Ulcerative colitis	[78]
Collagen IV-targeted nanoparticles	GSK484	ApoE ^{-/-} mice	Perfusion (4 mg/kg)	Regions of endothelial cell sloughing and collagen IV-rich basement membrane exposure	Endothelial damage	[219]
Mesenchymal stromal cell-derived extracellular vesicles	-	C57BL/6 mice	i.v. (50 µg/100 µL)	Delivered to functional mitochondria of neutrophils in the liver	Liver ischemia-reperfusion injury	[220]
Photo-cross-linking silk hydrogel system	Metformin	C57BL/6 mice	Topical use (0.2 mL)	Regions of diabetic trauma	Diabetic wound	[221]
Nec-1/PDA@Pt-Fe ₃ O ₄ nanocarrier	Nec-1	C57BL/6 mice	i.p. (6 mg/kg)	Inhibited receptor-interacting protein 1 kinase activity	Lupus nephritis	[222]
PLGA-LPV@M	Lopinavir	BALB/c mice; human neutrophils	i.v. (100µg/ mL); 0, 0.5, 1 mg/mL	Neutralized inflammatory cytokines and suppressed neutrophils	COVID-19	[223]

Abbreviations: NETs, neutrophil extracellular traps; COVID-19, coronavirus disease 2019; RA, rheumatoid arthritis; ROS, reactive oxygen species.

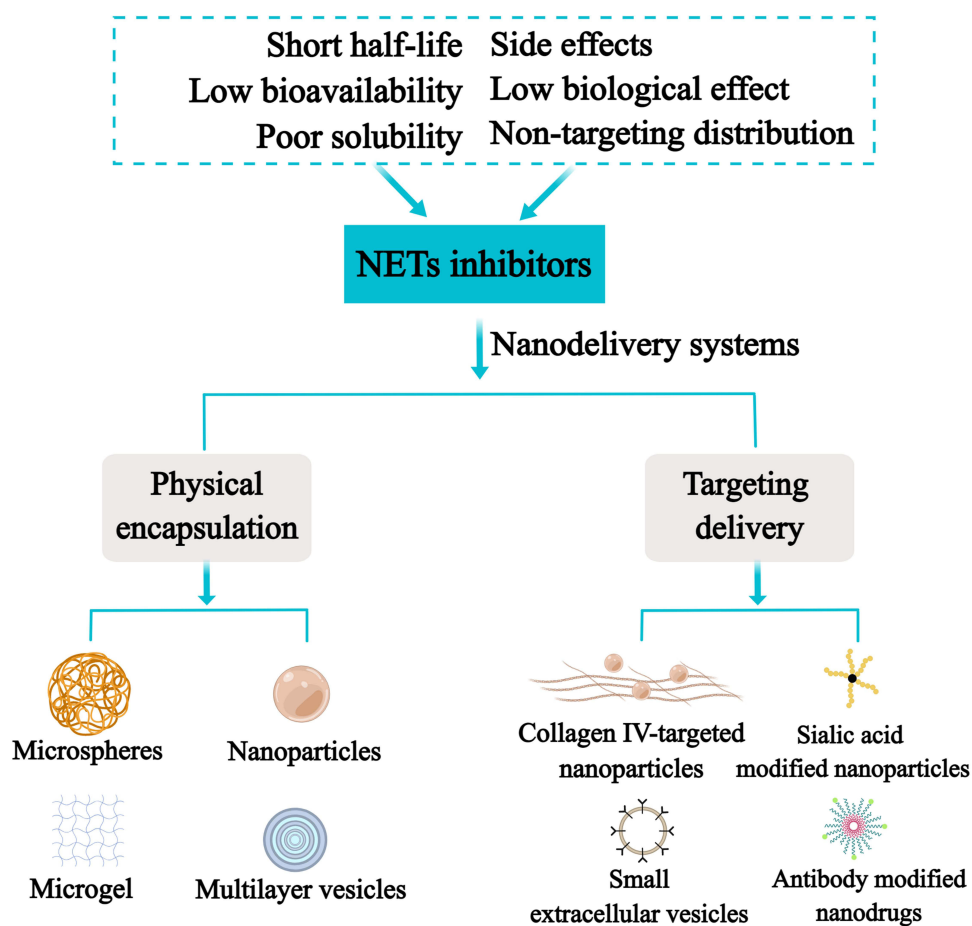


Figure 3 Successful nanocarriers developed for inhibition of NETs.

Abbreviation: NETs, neutrophil extracellular traps.

Similar long-term and safe drug release was achieved by poly (3-hydroxybutyrate) microspheres loaded with the Pan-PAD4 inhibitor Cl-amidine.²¹⁶ Meanwhile, nanocarrier delivery not only increased stability but also played an incredible role in the curative effect. Free sivelestat is not effective in the mouse model of endotoxic shock; however, interbilayer cross-linked multilayer vesicles incorporating this NE inhibitor prevented the formation of NETs and showed lowered pulmonary inflammation in animals in the context of endotoxic shock.¹⁰¹

Nanocarriers conjugated with targeting moieties based on the ligand-receptor molecular interaction significantly improved biodistribution and reduced side effects. Systemic administration of inhibitors may cause serious side effects due to its wide expression throughout the body. Molinaro et al²¹⁹ developed collagen IV-targeted nanoparticles to deliver the PAD4 inhibitor GSK484 to intimal lesions. Metformin with antioxidant and anti-inflammatory activity is loaded onto mesoporous silicon nanoparticles and gene delivery combined with antibacterial dressing carriers to heal diabetic wounds through dual controlled release of polarized macrophages and inhibition of NETosis.^{22,221} Nano-targeted neutrophils interfere with their function to inhibit NETosis. Nanoparticles decorated with sialic acid residue dimer can to easily bind to the neutrophil siglec-5 to reduce ROS production.²¹⁷ Mesenchymal stem cell-derived apoptotic vesicles not only interact with positively charged NETs-DNA medium but also clear NETs in sepsis by converting neutrophil NETosis into apoptosis through the Fas pathway.²²⁴ Extracellular vesicles derived from mesenchymal stromal cells induce functional mitochondrial transfer, fusion, and repair of neutrophil mitochondrial function in liver ischemia-reperfusion injury models, thereby inhibiting ROS production and significantly reducing NETosis.²²⁰ Another targeting strategy for therapeutic manipulation of NETs is based on active targeting of NETs ligands. A Fc-modified monoclonal antibody KKO combined with the PF4-NETs complex has been developed to enhance resistance to DNase; thus retaining NETs-

mediated bacterial capture, reducing the release of the toxic degradation product of NETs, and improving the mouse model of septicemia.²¹⁵ The micellated monoclonal antibody 2C5 is specific to the intact nucleosome of NETs and can also be used to deliver DNase I aimed at NETs to treat inflammation.^{218,225}

Future Perspectives

The research focus of NETs has recently shifted from infectious inflammation to aseptic inflammation. NETs may act as a key link in inflammation, acting as both the “cause” and the “effect” in many inflammatory conditions. For example, smoking has been known to be the main cause of COPD, but now cigarette extract has been found to aggravate COPD inflammation by inducing NETosis. This is not only conducive to the discovery of the mechanism of inflammation but also indicates a new path to the treatment of inflammatory diseases. Further research has revealed that NETs have pro-inflammatory effects, which activate the inflammasome, bind to DAMPs, amplify inflammation, and participate in autoimmunity and thrombosis. The components of NETs play an important role in the promotion of inflammation, so inhibiting the generation of NETs and the degradation of its components may be an important strategy to modulate inflammation.

NETs inhibitors are constantly under the spotlight as novel drug agents, and the advantages outweigh the disadvantages. By targeting key components in different stages of NETosis (eg, eDNA and PAD4), we summarize potential targeted treatment. Some proteins secreted by pathogens also act as NETs inhibitors, so they are also classified as potential drugs. For example, recombinant TsSERP1 from *Trichinella spiralis* inhibits the formation of human NE. Furthermore, the active components of THM have a significant inhibitory effect on NETosis. Literature regarding the inhibition of NETs has described important breakthroughs and has stimulated further research. The first is the specific targeting of therapeutic citrullinated histones for nucleosomes CitH2A and CitH4, which is based on the binding of tACPA to form a “clip” for the specific recognition of NETs nucleosomes. This suggests that more in-depth research is needed to identify strategies for highly specific NETs. Second, the combination with antibiotics in the NETs-associated bactericidal strategy, that is, an early use to promote NETosis, followed by later use to inhibit NETosis, to effectively retain the germicidal effect without causing uncontrolled inflammation. Furthermore, the Fc-modified KKO monoclonal antibody combined with the PF4-NETs complex retains the bactericidal effect of NETs and reduces the release of toxic degradation product from NETs by improving DNase resistance. There is an urgent need to systematically analyze the balanced effects of NETs in the fight against pathogens and in promoting inflammation. Finally, inhibition and regulation of gene-based miRNA is a new target for the inhibition of NETosis, which needs to be studied further. In summary, to optimize the treatment and management of NETs, it may be necessary to identify more efficient inhibition targets and drugs or to use combination therapy that does not weaken the immune system.

Conclusions

NETs are involved in the development of many diseases. NETs play an active role by phagocytizing pathogens as an initial defense mechanism, however, excessive NETosis could be destructive because of the release of enzymatic proteins causing non-specific activity leading to tissue pathology during inappropriate inflammatory responses.

Overall, this review focuses on the mechanisms involved in NETs formation, as well as the pro-inflammatory effects of NETs. Since patients of many diseases may benefit from anti-NETs therapy, we also collected information on herbal medicines related to NETs and outlined potential strategies for the management of NETs, including the inhibition of PAD4, reduction of histones, down-regulation of MPO and NE activity. Additionally, to improve the efficacy of NETs inhibitors, nanocarriers and targeting therapy can be employed. Considering the physicochemical characteristics and the in vivo behavior of NET inhibitors, improving the curative effect of targeting NETs by nanocarriers is a promising strategy. Nonetheless, the research is at the preliminary stages, and it is necessary to design and further investigate smart drug delivery systems to ensure the improvement in the efficacy and control of NETs without other detrimental effects.

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Disclosure

The authors declare no competing interest in this work.

References

1. Tan C, Aziz M, Wang P. The vitals of NETs. *J Leukoc Biol*. 2021;110(4):797–808. doi:10.1002/JLB.3RU0620-375R
2. Takei H, Araki A, Watanabe H, Ichinose A, Sendo F. Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate (PMA) accompanied by changes different from typical apoptosis or necrosis. *J Leukoc Biol*. 1996;59(2):229–240. doi:10.1002/jlb.59.2.229
3. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303(5663):1532–1535. doi:10.1126/science.1092385
4. Petretto A, Bruschi M, Pratesi F, et al. Neutrophil extracellular traps (NET) induced by different stimuli: a comparative proteomic analysis. *PLoS One*. 2019;14(7):e0218946. doi:10.1371/journal.pone.0218946
5. Du C, Cai N, Dong J, et al. Uncovering the role of cytoskeleton proteins in the formation of neutrophil extracellular traps. *Int Immunopharmacol*. 2023;123:110607. doi:10.1016/j.intimp.2023.110607
6. Sollberger G, Tilley DO, Zychlinsky A. Neutrophil extracellular traps: the biology of chromatin externalization. *Dev Cell*. 2018;44(5):542–553. doi:10.1016/j.devcel.2018.01.019
7. Huang J, Hong W, Wan M, Zheng L. Molecular mechanisms and therapeutic target of NETosis in diseases. *MedComm*. 2022;3(3):e162. doi:10.1002/mco2.162
8. Chen T, Li Y, Sun R, et al. Receptor-mediated NETosis on neutrophils. *Front Immunol*. 2021;12:775267. doi:10.3389/fimmu.2021.775267
9. Cooper PR, Palmer LJ, Chapple ILC. Neutrophil extracellular traps as a new paradigm in innate immunity: friend or foe?. *Periodontol*. 2000. 2013;63(1):165–197. doi:10.1111/prd.12025
10. Schoen J, Euler M, Schauer C, et al. Neutrophils' extracellular trap mechanisms: from physiology to pathology. *Int J Mol Sci*. 2022;23(21):12855. doi:10.3390/ijms232112855
11. Hakkim A, Fuchs TA, Martinez NE, et al. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. *Nat Chem Biol*. 2011;7(2):75–77. doi:10.1038/nchembio.496
12. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol*. 2010;191(3):677–691. doi:10.1083/jcb.201006052
13. Wang Y, Li M, Stadler S, et al. Histone hypercitullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J Cell Biol*. 2009;184(2):205–213. doi:10.1083/jcb.200806072
14. Zhu D, Zhang Y, Wang S. Histone citrullination: a new target for tumors. *Mol Cancer*. 2021;20(1):90. doi:10.1186/s12943-021-01373-z
15. Amulic B, Knackstedt SL, Abu Abed U, et al. Cell-Cycle Proteins Control Production of Neutrophil Extracellular Traps. *Dev Cell*. 2017;43(4):449–462.e5. doi:10.1016/j.devcel.2017.10.013
16. Thiam HR, Wong SL, Qiu R, et al. NETosis proceeds by cytoskeleton and endomembrane disassembly and PAD4-mediated chromatin decondensation and nuclear envelope rupture. *Proc Natl Acad Sci U S A*. 2020;117(13):7326–7337. doi:10.1073/pnas.1909546117
17. Reis LR, Souza Junior DR, Tomasin R, Bruni-Cardoso A, Di Mascio P, Ronsein GE. Citrullination of actin-ligand and nuclear structural proteins, cytoskeleton reorganization and protein redistribution across cellular fractions are early events in ionomycin-induced NETosis. *Redox Biol*. 2023;64:102784. doi:10.1016/j.redox.2023.102784
18. Pilsczek FH, Salina D, Poon KKH, et al. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to *Staphylococcus aureus*. *J Immunol*. 2010;185(12):7413–7425. doi:10.4049/jimmunol.1000675
19. Yipp BG, Petri B, Salina D, et al. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat Med*. 2012;18(9):1386–1393. doi:10.1038/nm.2847
20. Chen KW, Monteleone M, Boucher D, et al. Noncanonical inflammasome signaling elicits gasdermin D-dependent neutrophil extracellular traps. *Sci Immunol*. 2018;3(26). doi:10.1126/sciimmunol.aar6676
21. Zhang H, Wang Y, Qu M, et al. Neutrophil, neutrophil extracellular traps and endothelial cell dysfunction in sepsis. *Clin Transl Med*. 2023;13(1):e1170. doi:10.1002/ctm2.1170
22. Chu Z, Huang Q, Ma K, et al. Novel neutrophil extracellular trap-related mechanisms in diabetic wounds inspire a promising treatment strategy with hypoxia-challenged small extracellular vesicles. *Bioact Mater*. 2023;27:257–270. doi:10.1016/j.bioactmat.2023.04.007
23. McHugh J. Carbamylated NETs promote bone erosion in RA. *Nat Rev Rheumatol*. 2023;19(4):193.
24. Chamardani TM, Amiritavassoli S. Inhibition of NETosis for treatment purposes: friend or foe?. *Mol Cell Biochem*. 2022;477(3):673–688. doi:10.1007/s11010-021-04315-x
25. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol*. 2018;18(2):134–147.
26. Mutua V, Gershwin LJ. A review of neutrophil extracellular traps (NETs) in disease: potential anti-NETs therapeutics. *Clin Rev Allergy Immunol*. 2021;61(2):194–211.
27. Fousert E, Toes R, Desai J. Neutrophil extracellular traps (NETs) take the central stage in driving autoimmune responses. *Cells*. 2020;9(4):915. doi:10.3390/cells9040915

28. Zou Y, Chen X, He B, et al. Neutrophil extracellular traps induced by cigarette smoke contribute to airway inflammation in mice. *Exp Cell Res*. 2020;389(1):111888. doi:10.1016/j.yexcr.2020.111888
29. Gevaert E, Delemarre T, De Volder J, et al. Charcot-Leyden crystals promote neutrophilic inflammation in patients with nasal polyposis. *J Allergy Clin Immunol*. 2020;145(1):427–430.e4. doi:10.1016/j.jaci.2019.08.027
30. Delemarre T, Holtappels G, De Ruyck N, et al. A substantial neutrophilic inflammation as regular part of severe type 2 chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2021;147(1):179–188.e2. doi:10.1016/j.jaci.2020.08.036
31. Cao Y, Chen F, Sun Y, et al. LL-37 promotes neutrophil extracellular trap formation in chronic rhinosinusitis with nasal polyps. *Clin Exp Allergy*. 2019;49(7):990–999. doi:10.1111/cea.13408
32. Branzk N, Lubojemska A, Hardison SE, et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat Immunol*. 2014;15(11):1017–1025.
33. He Y, Liu J, Chen Y, Yan L, Wu J. Neutrophil extracellular traps in *Candida albicans* infection. *Front Immunol*. 2022;13:913028. doi:10.3389/fimmu.2022.913028
34. Saitoh T, Komano Y, Saitoh Y, et al. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. *Cell Host Microbe*. 2012;12(1):109–116. doi:10.1016/j.chom.2012.05.015
35. Guimarães-Costa AB, Nascimento MTC, Froment GS, et al. *Leishmania amazonensis* promastigotes induce and are killed by neutrophil extracellular traps. *Proc Natl Acad Sci U S A*. 2009;106(16):6748–6753. doi:10.1073/pnas.0900226106
36. Veras FP, Pontelli MC, Silva CM, et al. SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. *J Exp Med*. 2020;217(12). doi:10.1084/jem.20201129
37. Pastorek M, Dúbrava M, Celec P. On the origin of neutrophil extracellular traps in COVID-19. *Front Immunol*. 2022;13:821007. doi:10.3389/fimmu.2022.821007
38. Hurabielle C, Link VM, Bouladoux N, et al. Immunity to commensal skin fungi promotes psoriasiform skin inflammation. *Proc Natl Acad Sci U S A*. 2020;117(28):16465–16474. doi:10.1073/pnas.2003022117
39. Thanabalasuriar A, Scott BNV, Peiseler M, et al. Neutrophil extracellular traps confine *Pseudomonas aeruginosa* ocular biofilms and restrict brain invasion. *Cell Host Microbe*. 2019;25(4):526–536.e4. doi:10.1016/j.chom.2019.02.007
40. Khan MA, Philip LM, Cheung G, et al. Regulating NETosis: increasing pH promotes NADPH oxidase-dependent NETosis. *Front Med*. 2018;5:19. doi:10.3389/fmed.2018.00019
41. Mazzitelli I, Bleichmar L, Melucci C, et al. High salt induces a delayed activation of human neutrophils. *Front Immunol*. 2022;13:831844. doi:10.3389/fimmu.2022.831844
42. Farhan A, Hassan G, Ali SHL, et al. Spontaneous NETosis in diabetes: a role of hyperglycemia mediated ROS and autophagy. *Front Med*. 2023;10:1076690. doi:10.3389/fmed.2023.1076690
43. Tibrewal S, Ivanir Y, Sarkar J, et al. Hyperosmolar stress induces neutrophil extracellular trap formation: implications for dry eye disease. *Invest Ophthalmol Vis Sci*. 2014;55(12):7961–7969. doi:10.1167/iovs.14-15332
44. Angelidou I, Chrysanthopoulou A, Mitsios A, et al. REDD1/Autophagy pathway is associated with neutrophil-driven IL-1 β inflammatory response in active ulcerative colitis. *J Immunol*. 2018;200(12):3950–3961. doi:10.4049/jimmunol.1701643
45. Sonawane S, Khanolkar V, Namavari A, et al. Ocular surface extracellular DNA and nuclease activity imbalance: a new paradigm for inflammation in dry eye disease. *Invest Ophthalmol Vis Sci*. 2012;53(13):8253–8263. doi:10.1167/iovs.12-10430
46. Kapellos TS, Bassler K, Aschenbrenner AC, Fujii W, Schultze JL. Dysregulated functions of lung macrophage populations in COPD. *J Immunol Res*. 2018;2018:2349045. doi:10.1155/2018/2349045
47. Singh P, Kumar N, Singh M, et al. Neutrophil extracellular traps and NLRP3 inflammasome: a disturbing duo in atherosclerosis, inflammation and atherothrombosis. *Vaccines*. 2023;11(2):261. doi:10.3390/vaccines11020261
48. Paget C, Doz-Deblauwe E, Winter N, Briard B. Specific NLRP3 inflammasome assembling and regulation in neutrophils: relevance in inflammatory and infectious diseases. *Cells*. 2022;11(7):1188. doi:10.3390/cells11071188
49. Lachowicz-Scroggins ME, Dunican EM, Charbit AR, et al. Extracellular DNA, neutrophil extracellular traps, and inflammasome activation in severe asthma. *Am J Respir Crit Care Med*. 2019;199(9):1076–1085. doi:10.1164/rccm.201810-1869OC
50. Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. *J Immunol*. 2013;190(3):1217–1226. doi:10.4049/jimmunol.1202388
51. Münzer P, Negro R, Fukui S, et al. NLRP3 inflammasome assembly in neutrophils is supported by PAD4 and promotes NETosis under sterile conditions. *Front Immunol*. 2021;12:683803. doi:10.3389/fimmu.2021.683803
52. Block H, Rossaint J, Zarbock A. The fatal circle of NETs and NET-associated DAMPs contributing to organ dysfunction. *Cells*. 2022;11(12):1919. doi:10.3390/cells11121919
53. Denning NL, Aziz M, Gurien SD, Wang P. DAMPs and NETs in sepsis. *Front Immunol*. 2019;10:2536. doi:10.3389/fimmu.2019.02536
54. Cicco S, Cicco G, Racanelli V, Vacca A. Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs): two potential targets for COVID-19 treatment. *Mediators Inflamm*. 2020;2020:7527953. doi:10.1155/2020/7527953
55. Chen X, Li Y, Qin L, He R, Hu C. Neutrophil extracellular trapping network promotes the pathogenesis of neutrophil-associated asthma through macrophages. *Immunol Invest*. 2021;50(5):544–561. doi:10.1080/08820139.2020.1778720
56. Wan R, Jiang J, Hu C, et al. Neutrophil extracellular traps amplify neutrophil recruitment and inflammation in neutrophilic asthma by stimulating the airway epithelial cells to activate the TLR4/ NF- κ B pathway and secrete chemokines. *Aging*. 2020;12(17):16820–16836. doi:10.18632/aging.103479
57. Shao S, Fang H, Dang E, et al. Neutrophil extracellular traps promote inflammatory responses in psoriasis via activating epidermal TLR4/ IL-36R crosstalk. *Front Immunol*. 2019;10:746. doi:10.3389/fimmu.2019.00746
58. Villanueva E, Yalavarthi S, Berthier CC, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J Immunol*. 2011;187(1):538–552. doi:10.4049/jimmunol.1100450
59. Chen S-Y, Wang C-T, Chen C-Y, et al. Galectin-3 mediates NETosis and acts as an autoantigen in systemic lupus erythematosus-associated diffuse alveolar haemorrhage. *Int J Mol Sci*. 2023;24(11).
60. Sangaletti S, Tripodo C, Chiodoni C, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood*. 2012;120(15):3007–3018. doi:10.1182/blood-2012-03-416156

61. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci Transl Med.* 2013;5(178):178ra140. doi:10.1126/scitranslmed.3005580
62. Kwon J, Surenkhuu B, Raju I, et al. Pathological consequences of anti-citrullinated protein antibodies in tear fluid and therapeutic potential of pooled human immune globulin-eye drops in dry eye disease. *Ocul Surf.* 2020;18(1):80–97. doi:10.1016/j.jtos.2019.10.004
63. Yamamoto T. Psoriasis and connective tissue diseases. *Int J Mol Sci.* 2020;21(16):5803. doi:10.3390/ijms21165803
64. Torres-Ruiz J, Absalón-Aguilar A, Nuñez-Aguirre M, et al. Neutrophil extracellular traps contribute to COVID-19 hyperinflammation and humoral autoimmunity. *Cells.* 2021;10(10):2545. doi:10.3390/cells10102545
65. Thakur M, Junho CVC, Bernhard SM, Schindewolf M, Noels H, Döring Y. NETs-induced thrombosis impacts on cardiovascular and chronic kidney disease. *Circ Res.* 2023;132(8):933–949. doi:10.1161/CIRCRESAHA.123.321750
66. McDonald B, Davis RP, Kim S-J, et al. Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood.* 2017;129(10):1357–1367. doi:10.1182/blood-2016-09-741298
67. Skendros P, Mitsios A, Chrysanthopoulou A, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J Clin Invest.* 2020;130(11):6151–6157. doi:10.1172/JCI141374
68. Middleton EA, X-Y. He, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood.* 2020;136(10):1169–1179. doi:10.1182/blood.2020007008
69. Yaykasli KO, Schauer C, Muñoz LE, et al. Neutrophil extracellular trap-driven occlusive diseases. *Cells.* 2021;10(9):2208. doi:10.3390/cells10092208
70. Mahajan A, Hasiková L, Hampel U, et al. Aggregated neutrophil extracellular traps occlude Meibomian glands during ocular surface inflammation. *Ocul Surf.* 2021;20. doi:10.1016/j.jtos.2020.12.004
71. Mahajan A, Grüneboom A, Petru L, et al. Frontline Science: aggregated neutrophil extracellular traps prevent inflammation on the neutrophil-rich ocular surface. *J Leukoc Biol.* 2019;105(6):1087–1098. doi:10.1002/JLB.HI0718-249RR
72. Keir HR, Chalmers JD. Neutrophil extracellular traps in chronic lung disease: implications for pathogenesis and therapy. *Eur Respir Rev.* 2022;31(163):210241. doi:10.1183/16000617.0241-2021
73. Jiang D, Wenzel SE, Wu Q, Bowler RP, Schnell C, Chu HW. Human neutrophil elastase degrades SPLUNC1 and impairs airway epithelial defense against bacteria. *PLoS One.* 2013;8(5):e64689. doi:10.1371/journal.pone.0064689
74. Thulborn SJ, Mistry V, Brightling CE, Moffitt KL, Ribeiro D, Bafadhel M. Neutrophil elastase as a biomarker for bacterial infection in COPD. *Respir Res.* 2019;20(1):170. doi:10.1186/s12931-019-1145-4
75. Wright TK, Gibson PG, Simpson JL, McDonald VM, Wood LG, Baines KJ. Neutrophil extracellular traps are associated with inflammation in chronic airway disease. *Respirology.* 2016;21(3):467–475. doi:10.1111/resp.12730
76. Uddin M, Watz H, Malmgren A, Pedersen F. NETopathic inflammation in chronic obstructive pulmonary disease and severe asthma. *Front Immunol.* 2019;10:47. doi:10.3389/fimmu.2019.00047
77. Fisher J, Mohanty T, Karlsson CAQ, et al. Proteome profiling of recombinant DNase therapy in reducing NETs and aiding recovery in COVID-19 patients. *Mol Cell Proteomics.* 2021;20:100113. doi:10.1016/j.mcpro.2021.100113
78. Dong W, Liu D, Zhang T, You Q, Huang F, Wu J. Oral delivery of staphylococcal nuclease ameliorates DSS induced ulcerative colitis in mice via degrading intestinal neutrophil extracellular traps. *Ecotoxicol Environ Saf.* 2021;215:112161. doi:10.1016/j.ecoenv.2021.112161
79. Lewis HD, Liddle J, Coote JE, et al. Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. *Nat Chem Biol.* 2015;11(3):189–191. doi:10.1038/nchembio.1735
80. Liang Y, Pan B, Alam HB, et al. Inhibition of peptidylarginine deiminase alleviates LPS-induced pulmonary dysfunction and improves survival in a mouse model of lethal endotoxemia. *Eur J Pharmacol.* 2018;833:432–440. doi:10.1016/j.ejphar.2018.07.005
81. Knight JS, Subramanian V, O'Dell AA, et al. Peptidylarginine deiminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. *Ann Rheum Dis.* 2015;74(12):2199–2206. doi:10.1136/annrheumdis-2014-205365
82. Totani L, Amore C, Piccoli A, et al. Type-4 phosphodiesterase (PDE4) blockade reduces NETosis in cystic fibrosis. *Front Pharmacol.* 2021;12:702677. doi:10.3389/fphar.2021.702677
83. Schioppa T, Nguyen HO, Salvi V, et al. The PDE4 inhibitor tanimistat restrains the tissue-damaging properties of human neutrophils. *Int J Mol Sci.* 2022;23(9):4982. doi:10.3390/ijms23094982
84. Wu Z, Deng Q, Pan B, et al. Inhibition of PAD2 improves survival in a mouse model of lethal LPS-induced endotoxic shock. *Inflammation.* 2020;43(4):1436–1445. doi:10.1007/s10753-020-01221-0
85. Li M, Lin C, Deng H, et al. A novel peptidylarginine deiminase 4 (PAD4) inhibitor BMS-P5 blocks formation of neutrophil extracellular traps and delays progression of multiple myeloma. *Mol Cancer Ther.* 2020;19(7):1530–1538. doi:10.1158/1535-7163.MCT-19-1020
86. Ostafin M, Pruchniak MP, Ciepiela O, Reznick AZ, Demkow U. Different procedures of diphenyleiiodonium chloride addition affect neutrophil extracellular trap formation. *Anal Biochem.* 2016;509:60–66. doi:10.1016/j.ab.2016.05.003
87. Kirchner T, Hermann E, Möller S, et al. Flavonoids and 5-aminosalicylic acid inhibit the formation of neutrophil extracellular traps. *Mediators Inflamm.* 2013;2013:710239. doi:10.1155/2013/710239
88. Menegazzo L, Scattolini V, Cappellari R, et al. The antidiabetic drug metformin blunts NETosis in vitro and reduces circulating NETosis biomarkers in vivo. *Acta Diabetol.* 2018;55(6):593–601. doi:10.1007/s00592-018-1129-8
89. Chen H, Xu X, Tang Q, et al. (+)-Borneol inhibits the generation of reactive oxygen species and neutrophil extracellular traps induced by phorbol-12-myristate-13-acetate. *Front Pharmacol.* 2022;13:1023450. doi:10.3389/fphar.2022.1023450
90. Chirivi RGS, van Rosmalen JWG, van der Linden M, et al. Therapeutic ACPA inhibits NET formation: a potential therapy for neutrophil-mediated inflammatory diseases. *Cell Mol Immunol.* 2021;18(6):1528–1544. doi:10.1038/s41423-020-0381-3
91. Meara CHO, Coupland LA, Kordbacheh F, et al. Neutralizing the pathological effects of extracellular histones with small polyanions. *Nat Commun.* 2020;11(1):6408. doi:10.1038/s41467-020-20231-y
92. Hayase N, Doi K, Hiruma T, et al. Recombinant thrombomodulin prevents acute lung injury induced by renal ischemia-reperfusion injury. *Sci Rep.* 2020;10(1):289. doi:10.1038/s41598-019-57205-0
93. Wichapong K, Silvestre-Roig C, Braster Q, Schumski A, Soehnlein O, Nicolaes GAF. Structure-based peptide design targeting intrinsically disordered proteins: novel histone H4 and H2A peptidic inhibitors. *Comput Struct Biotechnol J.* 2021;19:934–948. doi:10.1016/j.csbj.2021.01.026

94. An S, Raju I, Surenkhuu B, et al. Neutrophil extracellular traps (NETs) contribute to pathological changes of ocular graft-vs.-host disease (oGVHD) dry eye: implications for novel biomarkers and therapeutic strategies. *Ocul Surf*. 2019;17(3):589–614. doi:10.1016/j.jtos.2019.03.010
95. Deng Q, Pan B, Alam HB, et al. Citrullinated histone H3 as a therapeutic target for endotoxic shock in mice. *Front Immunol*. 2019;10:2957. doi:10.3389/fimmu.2019.02957
96. Björnsdóttir H, Welin A, Michaëlsson E, et al. Neutrophil NET formation is regulated from the inside by myeloperoxidase-processed reactive oxygen species. *Free Radic Biol Med*. 2015;89:1024–1035. doi:10.1016/j.freeradbiomed.2015.10.398
97. Zheng W, Warner R, Ruggeri R, et al. PF-1355, a mechanism-based myeloperoxidase inhibitor, prevents immune complex vasculitis and anti-glomerular basement membrane glomerulonephritis. *J Pharmacol Exp Ther*. 2015;353(2):288–298. doi:10.1124/jpet.114.221788
98. Antonelou M, Michaëlsson E, Evans RDR, et al. Therapeutic myeloperoxidase inhibition attenuates neutrophil activation, ANCA-mediated endothelial damage, and crescentic GN. *J Am Soc Nephrol*. 2020;31(2):350–364. doi:10.1681/ASN.2019060618
99. Hair PS, Enos AI, Krishna NK, Cunnion KM. Inhibition of immune complex complement activation and neutrophil extracellular trap formation by peptide inhibitor of complement C1. *Front Immunol*. 2018;9:558. doi:10.3389/fimmu.2018.00558
100. Stockley R, De Soya A, Gunawardena K, et al. Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. *Respir Med*. 2013;107(4):524–533. doi:10.1016/j.rmed.2012.12.009
101. Okeke EB, Louttit C, Fry C, et al. Inhibition of neutrophil elastase prevents neutrophil extracellular trap formation and rescues mice from endotoxic shock. *Biomaterials*. 2020;238:119836. doi:10.1016/j.biomaterials.2020.119836
102. Watz H, Nagelschmitz J, Kirsten A, et al. Safety and efficacy of the human neutrophil elastase inhibitor BAY 85-8501 for the treatment of non-cystic fibrosis bronchiectasis: a randomized controlled trial. *Pulm Pharmacol Ther*. 2019;56:86–93. doi:10.1016/j.pupt.2019.03.009
103. Majewski P, Majchrzak-Gorecka M, Grygier B, Skrzyszowska-Moncznik J, Osiecka O, Cichy J. Inhibitors of serine proteases in regulating the production and function of neutrophil extracellular traps. *Front Immunol*. 2016;7:261. doi:10.3389/fimmu.2016.00261
104. Silva CMS, Wanderley CWS, Veras FP, et al. Gasdermin D inhibition prevents multiple organ dysfunction during sepsis by blocking NET formation. *Blood*. 2021;138(25):2702–2713. doi:10.1182/blood.2021011525
105. Sollberger G, Choidas A, Burn GL, et al. Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. *Sci Immunol*. 2018;3(26). doi:10.1126/sciimmunol.aar6689
106. Garcia CC, Weston-Davies W, Russo RC, et al. Complement C5 activation during influenza A infection in mice contributes to neutrophil recruitment and lung injury. *PLoS One*. 2013;8(5):e64443. doi:10.1371/journal.pone.0064443
107. Alsabani M, Abrams ST, Cheng Z, et al. Reduction of NETosis by targeting CXCR1/2 reduces thrombosis, lung injury, and mortality in experimental human and murine sepsis. *Br J Anaesth*. 2022;128(2):283–293. doi:10.1016/j.bja.2021.10.039
108. Bystrzycka W, Manda-Handzik A, Sieczkowska S, Moskalik A, Demkow U, Ciepiela O. Azithromycin and chloramphenicol diminish neutrophil extracellular traps (NETs) release. *Int J Mol Sci*. 2017;18(12):2666. doi:10.3390/ijms18122666
109. Manda-Handzik A, Bystrzycka W, Sieczkowska S, Demkow U, Ciepiela O. Antibiotics modulate the ability of neutrophils to release neutrophil extracellular traps. *Adv Exp Med Biol*. 2017;944:47–52.
110. Duan Z, Xie T, Chu C, et al. De-escalation antibiotic therapy alleviates organ injury through modulation of NETs formation during sepsis. *Cell Death Discov*. 2021;7(1):345. doi:10.1038/s41420-021-00745-0
111. Zhang H, Qiu SL, Tang QY, et al. Erythromycin suppresses neutrophil extracellular traps in smoking-related chronic pulmonary inflammation. *Cell Death Dis*. 2019;10(9):678. doi:10.1038/s41419-019-1909-2
112. Zhang S, Zhang Q, Wang F, et al. Hydroxychloroquine inhibiting neutrophil extracellular trap formation alleviates hepatic ischemia/reperfusion injury by blocking TLR9 in mice. *Clin Immunol*. 2020;216:108461. doi:10.1016/j.clim.2020.108461
113. Martinez NE, Zimmermann TJ, Goosmann C, et al. Tetrahydroisoquinolines: new inhibitors of neutrophil extracellular trap (NET) formation. *Chembiochem*. 2017;18(10):888–893. doi:10.1002/cbic.201600650
114. Speziale P, Pietrocola G. Staphylococcus aureus induces neutrophil extracellular traps (NETs) and neutralizes their bactericidal potential. *Comput Struct Biotechnol J*. 2021;19:3451–3457. doi:10.1016/j.csbj.2021.06.012
115. Afonso M, Mestre AR, Silva G, et al. Candida Extracellular Nucleotide Metabolism Promotes Neutrophils Extracellular Traps Escape. *Front Cell Infect Microbiol*. 2021;11:678568. doi:10.3389/fcimb.2021.678568
116. Kumar A, Varma VP, Sridhar K, et al. Deciphering the Role of Leptospira Surface Protein LigA in Modulating the Host Innate Immune Response. *Front Immunol*. 2021;12:807775. doi:10.3389/fimmu.2021.807775
117. Zonta YR, Dezen ALO, Della Coletta AM, et al. Releases a DNase-like protein that degrades NETs and allows for fungal escape. *Front Cell Infect Microbiol*. 2020;10:592022. doi:10.3389/fcimb.2020.592022
118. Cacciotto C, Dessi D, Cubeddu T, et al. MHO_0730 as a surface-exposed calcium-dependent nuclease of mycoplasma hominis promoting neutrophil extracellular trap formation and escape. *J Infect Dis*. 2019;220(12):1999–2008. doi:10.1093/infdis/jiz406
119. Martinez PJ, Farhan A, Mustafa M, et al. PspA facilitates evasion of pneumococci from bactericidal activity of neutrophil extracellular traps (NETs). *Microb Pathog*. 2019;136:103653. doi:10.1016/j.micpath.2019.103653
120. Bhattacharya M, Berends Evelien T. M., Chan R, et al. Staphylococcus aureus biofilms release leukocidins to elicit extracellular trap formation and evade neutrophil-mediated killing. *Proc Natl Acad Sci U S A*. 2018;115(28):7416–7421. doi:10.1073/pnas.1721949115
121. Ou Q, Fang J-Q, Zhang Z-S, et al. TpcC inhibits neutrophil extracellular trap formation by enhancing ubiquitination mediated degradation of peptidylarginine deiminase 4. *Nat Commun*. 2021;12(1):3481. doi:10.1038/s41467-021-23881-8
122. Shao S, Hao C, Zhan B, et al. Trichinella spiralis calreticulin S-domain binds to human complement C1q to interfere with C1q-mediated immune functions. *Front Immunol*. 2020;11:572326. doi:10.3389/fimmu.2020.572326
123. Ríos-López AL, González GM, Hernández-Bello R, Sánchez-González A. Avoiding the trap: mechanisms developed by pathogens to escape neutrophil extracellular traps. *Microbiol Res*. 2021;243:126644. doi:10.1016/j.micres.2020.126644
124. Silva-Oliveira G, Linhares-Lacerda L, Mattos TRF, et al. Laminin triggers neutrophil extracellular traps (NETs) and modulates NET release induced by Leishmania amazonensis. *Biomedicines*. 2022;10(3):521. doi:10.3390/biomedicines10030521
125. Janssen L, Muller HS, Martins Vd P. Unweaving the NET: microbial strategies for neutrophil extracellular trap evasion. *Microb Pathog*. 2022;171:105728. doi:10.1016/j.micpath.2022.105728

126. Kobpornchai P, Reamtong O, Phuphisut O, Malaitong P, Adisakwattana P. Serine protease inhibitor derived from *Trichinella spiralis* (TsSERP) inhibits neutrophil elastase and impairs human neutrophil functions. *Front Cell Infect Microbiol.* 2022;12:919835. doi:10.3389/fcimb.2022.919835
127. Oswal M, Varghese R, Zagade T, Dhatrak C, Sharma R, Kumar D. Dietary supplements and medicinal plants in urolithiasis: diet, prevention, and cure. *J Pharm Pharmacol.* 2023;75(6):719–745. doi:10.1093/jpp/rgac092
128. Karati D, Varghese R, Mahadik KR, Sharma R, Kumar D., Aazza, S. Plant bioactives in the treatment of inflammation of skeletal muscles: a molecular perspective. *Evid Based Complement Alternat Med.* 2022;2022:4295802. doi:10.1155/2022/4295802
129. Sharma R, Jadhav M, Choudhary N, et al. Deciphering the impact and mechanism of Trikatu, a spices-based formulation on alcoholic liver disease employing network pharmacology analysis and in vivo validation. *Front Nutr.* 2022;9:1063118. doi:10.3389/fnut.2022.1063118
130. Yang S-C, Chen P-J, Chang S-H, et al. Luteolin attenuates neutrophilic oxidative stress and inflammatory arthritis by inhibiting Raf1 activity. *Biochem Pharmacol.* 2018;154:384–396. doi:10.1016/j.bcp.2018.06.003
131. Tsai Y-F, Yang S-C, Hsu Y-H, et al. Carnosic acid inhibits reactive oxygen species-dependent neutrophil extracellular trap formation and ameliorates acute respiratory distress syndrome. *Life Sci.* 2023;321:121334. doi:10.1016/j.lfs.2022.121334
132. Tao L, Xu M, Dai X, et al. Polypharmacological profiles underlying the antitumor property of salvia miltiorrhiza root (danshen) interfering with NOX-dependent neutrophil extracellular traps. *Oxid Med Cell Longev.* 2018;2018:4908328. doi:10.1155/2018/4908328
133. Zha Y-f, Xie J, Ding P, et al. Senkyunolide I protect against lung injury via inhibiting formation of neutrophil extracellular trap in a murine model of cecal ligation and puncture. *Int Immunopharmacol.* 2021;99:107922. doi:10.1016/j.intimp.2021.107922
134. Shang T, Zhang Z-S, Wang X-T, et al. Xuebijing injection inhibited neutrophil extracellular traps to reverse lung injury in sepsis mice via reducing Gasdermin D. *Front Pharmacol.* 2022;13:1054176. doi:10.3389/fphar.2022.1054176
135. Liu J, Jiang M, Jin Q, et al. Modulation of HMGB1 release in APAP-induced liver injury: a possible strategy of Chikusetsusaponin V targeting NETs formation. *Front Pharmacol.* 2021;12:723881. doi:10.3389/fphar.2021.723881
136. Gu J, Ran X, Deng J, et al. Glycyrrhizin alleviates sepsis-induced acute respiratory distress syndrome via suppressing of HMGB1/TLR9 pathways and neutrophils extracellular traps formation. *Int Immunopharmacol.* 2022;108:108730. doi:10.1016/j.intimp.2022.108730
137. Park HH, Kim H, Lee HS, et al. PEGylated nanoparticle albumin-bound steroidal ginsenoside derivatives ameliorate SARS-CoV-2-mediated hyper-inflammatory responses. *Biomaterials.* 2021;273:120827. doi:10.1016/j.biomaterials.2021.120827
138. Yang F, Luo X, Luo G, et al. Inhibition of NET formation by polydatin protects against collagen-induced arthritis. *Int Immunopharmacol.* 2019;77:105919. doi:10.1016/j.intimp.2019.105919
139. Liao P, He Y, Yang F, et al. Polydatin effectively attenuates disease activity in lupus-prone mouse models by blocking ROS-mediated NET formation. *Arthritis Res Ther.* 2018;20(1):254. doi:10.1186/s13075-018-1749-y
140. He W, Xi Q, Cui H, et al. Forsythiaside B ameliorates coagulopathies in a rat model of sepsis through inhibition of the formation of PAD4-dependent neutrophil extracellular traps. *Front Pharmacol.* 2022;13:1022985. doi:10.3389/fphar.2022.1022985
141. Yuan K, Zhu Q, Lu Q, et al. Quercetin alleviates rheumatoid arthritis by inhibiting neutrophil inflammatory activities. *J Nutr Biochem.* 2020;84:108454. doi:10.1016/j.jnutbio.2020.108454
142. Wang Y-P, Guo Y, Wen P-S, et al. Three ingredients of Safflower alleviate acute lung injury and inhibit NET release induced by lipopolysaccharide. *Mediators Inflamm.* 2020;2020:2720369. doi:10.1155/2020/2720369
143. Zhu Y, Wang D, Luo J, et al. Zingerone inhibits the neutrophil extracellular trap formation and protects against sepsis via Nrf2-mediated ROS inhibition. *Oxid Med Cell Longev.* 2022;2022.
144. Zhu M, Yuan K, Lu Q, et al. Emodin ameliorates rheumatoid arthritis by promoting neutrophil apoptosis and inhibiting neutrophil extracellular trap formation. *Mol Immunol.* 2019;112:188–197. doi:10.1016/j.molimm.2019.05.010
145. Zhang S, Huang G, Yuan K, et al. Tanshinone IIA ameliorates chronic arthritis in mice by modulating neutrophil activities. *Clin Exp Immunol.* 2017;190(1):29–39. doi:10.1111/cei.12993
146. Li X, Yuan K, Zhu Q, et al. Andrographolide ameliorates rheumatoid arthritis by regulating the apoptosis-NETosis balance of neutrophils. *Int J Mol Sci.* 2019;20(20).
147. Guan H, Xie L, Ji Z, Song R, Qi J, Nie X. Triptolide inhibits neutrophil extracellular trap formation. *Ann Transl Med.* 2021;9(17):1384. doi:10.21037/atm-21-3522
148. Tao L, Xu M, Liu Y. The total terpenoids of *Celastrus orbiculatus* (TTC) inhibit NOX-dependent formation of PMA-induced neutrophil extracellular traps (NETs). *Eur J Inflamm.* 2018;16:2058739218805667. doi:10.1177/2058739218805667
149. Lu Q, Jiang H, Zhu Q, et al. Tetrandrine ameliorates rheumatoid arthritis in mice by alleviating neutrophil activities. *Evid Based Complement Alternat Med.* 2022;2022:8589121. doi:10.1155/2022/8589121
150. Liu Y, Qin X, Lei Z, Chai H, Huang Z, Wu Z. Tetramethylpyrazine inhibits neutrophil extracellular traps formation and alleviates hepatic ischemia/reperfusion injury in rat liver transplantation. *Exp Cell Res.* 2021;406(1):112719. doi:10.1016/j.yexcr.2021.112719
151. Ali RA, Gandhi AA, Dai L, et al. Antineutrophil properties of natural gingerols in models of lupus. *JCI Insight.* 2021;6(3). doi:10.1172/jci.insight.138385
152. Yang C, Song C, Liu Y, et al. Re-Du-Ning injection ameliorates LPS-induced lung injury through inhibiting neutrophil extracellular traps formation. *Phytomedicine.* 2021;90:153635. doi:10.1016/j.phymed.2021.153635
153. Chiang C-C, Cheng W-J, Lin C-Y, et al. Kan-Lu-Hsiao-Tu-Tan, a traditional Chinese medicine formula, inhibits human neutrophil activation and ameliorates imiquimod-induced psoriasis-like skin inflammation. *J Ethnopharmacol.* 2020;246:112246. doi:10.1016/j.jep.2019.112246
154. O'Neil LJ, Oliveira CB, Wang X, et al. Neutrophil extracellular trap-associated carbamylation and histones trigger osteoclast formation in rheumatoid arthritis. *Ann Rheum Dis.* 2023;82(5):630–638. doi:10.1136/ard-2022-223568
155. Krishnamurthy A, Circumaru A, Sun J, et al. Combination of two monoclonal ACPAs induced tenosynovitis, pain and bone loss in mice in a Peptidyl Arginine Deiminase-4 dependent manner. *Arthritis Rheumatol.* 2022.
156. Colón DF, Wanderley CW, Franchin M, et al. Neutrophil extracellular traps (NETs) exacerbate severity of infant sepsis. *Crit Care.* 2019;23(1):113. doi:10.1186/s13054-019-2407-8
157. Biron BM, Chung C-S, O'Brien XM, Chen Y, Reichner JS, Ayala A. Cl-Amidine prevents histone 3 citrullination and neutrophil extracellular trap formation, and improves survival in a murine sepsis model. *J Innate Immun.* 2017;9(1):22–32. doi:10.1159/000448808

158. Wang Y, Wysocka J, Sayegh J, et al. Human PAD4 regulates histone arginine methylation levels via demethylation. *Science*. 2004;306(5694):279–283. doi:10.1126/science.1101400
159. Slade DJ, Horibata S, Coonrod SA, Thompson PR. A novel role for protein arginine deiminase 4 in pluripotency: the emerging role of citrullinated histone H1 in cellular programming. *BioEssays*. 2014;36(8):736–740. doi:10.1002/bies.201400057
160. Zhou Y, An L-I, Chaerkady R, et al. Evidence for a direct link between PAD4-mediated citrullination and the oxidative burst in human neutrophils. *Sci Rep*. 2018;8(1):15228. doi:10.1038/s41598-018-33385-z
161. Li X, Ye Y, Peng K, Zeng Z, Chen L, Zeng Y. Histones: the critical players in innate immunity. *Front Immunol*. 2022;13:1030610. doi:10.3389/fimmu.2022.1030610
162. Saffarzadeh M, Juenemann C, Queisser MA, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS One*. 2012;7(2):e32366. doi:10.1371/journal.pone.0032366
163. Klebanoff SJ, Kinsella MG, Wight TN. Degradation of endothelial cell matrix heparan sulfate proteoglycan by elastase and the myeloperoxidase-H₂O₂-chloride system. *Am J Pathol*. 1993;143(3):907–917.
164. Vitkov L, Muñoz LE, Knopf J, et al. Connection between periodontitis-induced low-grade endotoxemia and systemic diseases: neutrophils as protagonists and targets. *Int J Mol Sci*. 2021;22(9):4647. doi:10.3390/ijms22094647
165. Lin EY-H, Lai H-J, Cheng Y-K, et al. Neutrophil extracellular traps impair intestinal barrier function during experimental colitis. *Biomedicine*. 2020;8(8):275. doi:10.3390/biomedicine8080275
166. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*. 1997;389(6648):251–260. doi:10.1038/38444
167. Radic M. Antibodies clamp down on NET nucleosomes. *Cell Mol Immunol*. 2020;17(9):895–896. doi:10.1038/s41423-020-0467-y
168. Ng H, Havervall S, Rosell A, et al. Circulating markers of neutrophil extracellular traps are of prognostic value in patients with COVID-19. *Arterioscler Thromb Vasc Biol*. 2021;41(2):988–994. doi:10.1161/ATVBAHA.120.315267
169. Zuo Y, Yalavarthi S, Shi H, et al. Neutrophil extracellular traps in COVID-19. *JCI Insight*. 2020;5(11).
170. Maruchi Y, Tsuda M, Mori H, et al. Plasma myeloperoxidase-conjugated DNA level predicts outcomes and organ dysfunction in patients with septic shock. *Crit Care*. 2018;22(1):176. doi:10.1186/s13054-018-2109-7
171. Schroder AL, Chami B, Liu Y, et al. Neutrophil extracellular trap density increases with increasing histopathological severity of Crohn's disease. *Inflamm Bowel Dis*. 2022;28(4):586–598. doi:10.1093/ibd/izab239
172. van Dam LS, Kraaij T, Kamerling SWA, et al. Intrinsically distinct role of neutrophil extracellular trap formation in antineutrophil cytoplasmic antibody-associated vasculitis compared to systemic lupus erythematosus. *Arthritis Rheumatol*. 2019;71(12):2047–2058. doi:10.1002/art.41047
173. Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. *Nat Rev Rheumatol*. 2019;15(2).
174. Lin D, Zhang Y, Wang S, et al. Ganoderma lucidum polysaccharide peptides GL-PPSQ2 alleviate intestinal ischemia-reperfusion injury via inhibiting cytotoxic neutrophil extracellular traps. *Int J Biol Macromol*. 2023;244:125370. doi:10.1016/j.ijbiomac.2023.125370
175. Guéant JL, Guéant-Rodriguez RM, Fromonot J, et al. Elastase and exacerbation of neutrophil innate immunity are involved in multi-visceral manifestations of COVID-19. *Allergy*. 2021;76(6):1846–1858. doi:10.1111/all.14746
176. Tkalecvić J, Novelli M, Phylactides M, Iredale JP, Segal AW, Roes J. Impaired immunity and enhanced resistance to endotoxin in the absence of neutrophil elastase and cathepsin G. *Immunity*. 2000;12(2):201–210. doi:10.1016/S1074-7613(00)80173-9
177. Aikawa N, Kawasaki Y. Clinical utility of the neutrophil elastase inhibitor sivelestat for the treatment of acute respiratory distress syndrome. *Ther Clin Risk Manag*. 2014;10:621–629. doi:10.2147/TCRM.S65066
178. Elborn JS, Perrett J, Forsman-Semb K, Marks-Konczalik J, Gunawardena K, Entwistle N. Efficacy, safety and effect on biomarkers of AZD9668 in cystic fibrosis. *Eur Respir J*. 2012;40(4):969–976. doi:10.1183/09031936.00194611
179. Vogelmeier C, Aquino TO, O'Brien CD, Perrett J, Gunawardena KA. A randomised, placebo-controlled, dose-finding study of AZD9668, an oral inhibitor of neutrophil elastase, in patients with chronic obstructive pulmonary disease treated with tiotropium. *COPD*. 2012;9(2):111–120. doi:10.3109/15412555.2011.641803
180. Aubé F-A, Bidas A, Pépin G. Who and how, DNA sensors in NETs-driven inflammation. *Front Immunol*. 2023;14:1190177. doi:10.3389/fimmu.2023.1190177
181. Abdo M, Uddin M, Goldmann T, et al. Raised sputum extracellular DNA confers lung function impairment and poor symptom control in an exacerbation-susceptible phenotype of neutrophilic asthma. *Respir Res*. 2021;22(1):167. doi:10.1186/s12931-021-01759-z
182. Pham DL, Ban GY, Kim SH, et al. Neutrophil autophagy and extracellular DNA traps contribute to airway inflammation in severe asthma. *Clin Exp Allergy*. 2017;47(1):57–70. doi:10.1111/cea.12859
183. Toussaint M, Jackson DJ, Swieboda D, et al. Host DNA released by NETosis promotes rhinovirus-induced type-2 allergic asthma exacerbation. *Nat Med*. 2017;23(6):681–691. doi:10.1038/nm.4332
184. Lood C, Blanco LP, Purmalek MM, et al. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat Med*. 2016;22(2):146–153. doi:10.1038/nm.4027
185. Blanco LP, Wang X, Carlucci PM, et al. RNA externalized by neutrophil extracellular traps promotes inflammatory pathways in endothelial cells. *Arthritis Rheumatol*. 2021;73(12):2282–2292. doi:10.1002/art.41796
186. Lou H, Wojciak-Stothard B, Ruseva MM, et al. Autoantibody-dependent amplification of inflammation in SLE. *Cell Death Dis*. 2020;11(9):729.
187. Shi C, Yang L, Braun A, Anders HJ. Extracellular DNA—a danger signal triggering immunothrombosis. *Front Immunol*. 2020;11:568513. doi:10.3389/fimmu.2020.568513
188. Maronek M, Gromova B, Liptak R, et al. Extracellular DNA correlates with intestinal inflammation in chemically induced colitis in mice. *Cells*. 2021;10(1):81. doi:10.3390/cells10010081
189. Hodson ME. Aerosolized dornase alfa (rhDNase) for therapy of cystic fibrosis. *Am J Respir Crit Care Med*. 1995;151(3 Pt 2):S70–S74. doi:10.1164/ajrcm/151.3_Pt_2.S70
190. Davis JC, Manzi S, Yarboro C, et al. Recombinant human Dnase I (rhDNase) in patients with lupus nephritis. *Lupus*. 1999;8(1):68–76. doi:10.1191/096120399678847380
191. Englert H, Göbel J, Khong D, et al. Targeting NETs using dual-active DNaseI variants. *Front Immunol*. 2023;14:1181761. doi:10.3389/fimmu.2023.1181761

192. Silva CMS, Wanderley CWS, Veras FP, et al. Gasdermin-D activation by SARS-CoV-2 triggers NET and mediate COVID-19 immunopathology. *Crit Care*. 2022;26(1):206. doi:10.1186/s13054-022-04062-5
193. Vats R, Kaminski TW, Brzoska T, et al. Liver-to-lung microembolic NETs promote gasdermin D-dependent inflammatory lung injury in sickle cell disease. *Blood*. 2022;140(9):1020–1037. doi:10.1182/blood.2021014552
194. Xie J, Zhu CL, Wan XJ, et al. GSDMD-mediated NETosis promotes the development of acute respiratory distress syndrome. *Eur J Immunol*. 2022. doi:10.1002/eji.202250011
195. Raina K, Kumari R, Thakur P, et al. Mechanistic role and potential of Ayurvedic herbs as anti-aging therapies. *Drug Metab Pers Ther*. 2023. doi:10.1515/dmd-2023-0024
196. Stoiber W, Obermayer A, Steinbacher P, Krautgartner WD. The role of reactive oxygen species (ROS) in the formation of extracellular traps (ETs) in humans. *Biomolecules*. 2015;5(2):702–723. doi:10.3390/biom5020702
197. Schönrich G, Raftery MJ, Samstag Y. Devilishly radical NETwork in COVID-19: oxidative stress, neutrophil extracellular traps (NETs), and T cell suppression. *Adv Biol Regul*. 2020;77:100741.
198. Chen F, Chu C, Wang X, et al. Hesperetin attenuates sepsis-induced intestinal barrier injury by regulating neutrophil extracellular trap formation via the ROS/autophagy signaling pathway. *Food Funct*. 2023;14(9):4213–4227.
199. Reid PA, McAllister DA, Boyd AC, et al. Measurement of serum calprotectin in stable patients predicts exacerbation and lung function decline in cystic fibrosis. *Am J Respir Crit Care Med*. 2015;191(2):233–236. doi:10.1164/rccm.201407-1365LE
200. Du F, Ding Z, Rönnow CF, Rahman M, Schiopu A, Thorlacius H. S100A9 induces reactive oxygen species-dependent formation of neutrophil extracellular traps in abdominal sepsis. *Exp Cell Res*. 2022;421(2):113405. doi:10.1016/j.yexcr.2022.113405
201. Zhan Y, Ling Y, Deng Q, et al. HMGB1-mediated neutrophil extracellular trap formation exacerbates intestinal ischemia/reperfusion-induced acute lung injury. *J Immunol*. 2022;208(4):968–978. doi:10.4049/jimmunol.2100593
202. Zhang XL, Wang TY, Chen Z, et al. HMGB1-promoted neutrophil extracellular traps contribute to cardiac diastolic dysfunction in mice. *J Am Heart Assoc*. 2022;11(4):e023800. doi:10.1161/JAHA.121.023800
203. Keir HR, Shoemark A, Dicker AJ, et al. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. *Lancet Respir Med*. 2021;9(8):873–884. doi:10.1016/S2213-2600(20)30504-X
204. Hawez A, Taha D, Algaber A, Madhi R, Rahman M, Thorlacius H. MiR-155 regulates neutrophil extracellular trap formation and lung injury in abdominal sepsis. *J Leukoc Biol*. 2022;111(2):391–400.
205. Liao TL, Chen YM, Tang KT, Chen PK, Liu HJ, Chen DY. MicroRNA-223 inhibits neutrophil extracellular traps formation through regulating calcium influx and small extracellular vesicles transmission. *Sci Rep*. 2021;11(1):15676. doi:10.1038/s41598-021-95028-0
206. Hsieh Y-T, Chou YC, Kuo P-Y, et al. Down-regulated miR-146a expression with increased neutrophil extracellular traps and apoptosis formation in autoimmune-mediated diffuse alveolar hemorrhage. *J Biomed Sci*. 2022;29(1):62. doi:10.1186/s12929-022-00849-4
207. Águila S, de Los Reyes-García AM, Fernández-Pérez MP, et al. MicroRNAs as new regulators of neutrophil extracellular trap formation. *Int J Mol Sci*. 2021;22(4):2116. doi:10.3390/ijms22042116
208. Bonilha CS, Veras FP, de Queiroz Cunha F. NET-targeted therapy: effects, limitations, and potential strategies to enhance treatment efficacy. *Trends Pharmacol Sci*. 2023;44(9):622–634. doi:10.1016/j.tips.2023.06.007
209. Keshavan S, Calligari P, Stella L, Fusco L, Delogu LG, Fadeel B. Nano-bio interactions: a neutrophil-centric view. *Cell Death Dis*. 2019;10(8):569. doi:10.1038/s41419-019-1806-8
210. Fetz AE, Bowlin GL. Neutrophil extracellular traps: inflammation and biomaterial preconditioning for tissue engineering. *Tissue Eng Part B Rev*. 2022;28(2):437–450. doi:10.1089/ten.teb.2021.0013
211. Lee YY, Park HH, Park W, et al. Long-acting nanoparticulate DNase-I for effective suppression of SARS-CoV-2-mediated neutrophil activities and cytokine storm. *Biomaterials*. 2021;267:120389. doi:10.1016/j.biomaterials.2020.120389
212. Park HH, Park W, Lee YY, et al. Bioinspired DNase-I-coated melanin-like nanospheres for modulation of infection-associated NETosis dysregulation. *Adv Sci*. 2021;8(19):e2103748. doi:10.1002/adv.202103748
213. Chen Y, Wang Y, Jiang X, et al. Dimethylamino group modified polydopamine nanoparticles with positive charges to scavenge cell-free DNA for rheumatoid arthritis therapy. *Bioact Mater*. 2022;18:409–420. doi:10.1016/j.bioactmat.2022.03.028
214. Hosseinejad A, Ludwig N, Wienkamp A-K, et al. DNase I functional microgels for neutrophil extracellular trap disruption. *Biomater Sci*. 2021;10(1):85–99. doi:10.1039/D1BM01591E
215. Gollomp K, Sarkar A, Harikumar S, et al. Fc-modified HIT-like monoclonal antibody as a novel treatment for sepsis. *Blood*. 2020;135(10):743–754. doi:10.1182/blood.2019002329
216. Ahmed D, Puthussery H, Basnett P, Knowles JC, Lange S, Roy I. Controlled delivery of pan-PAD-inhibitor Cl-amidine using poly(3--hydroxybutyrate) microspheres. *Int J Mol Sci*. 2021;22(23):12852. doi:10.3390/ijms222312852
217. Bornhöft KF, Viergutz T, Kühnle A, Galuska SP. Nanoparticles equipped with α 2,8-linked sialic acid chains inhibit the release of neutrophil extracellular traps. *Nanomaterials*. 2019;9(4):610. doi:10.3390/nano9040610
218. Mendes LP, Rostamizadeh K, Gollomp K, et al. Monoclonal antibody 2C5 specifically targets neutrophil extracellular traps. *MAbs*. 2020;12(1):1850394. doi:10.1080/19420862.2020.1850394
219. Molinaro R, Yu M, Sausen G, et al. Targeted delivery of protein arginine deiminase-4 inhibitors to limit arterial intimal NETosis and preserve endothelial integrity. *Cardiovasc Res*. 2021;117(13):2652–2663. doi:10.1093/cvr/cvab074
220. Lu T, Zhang J, Cai J, et al. Extracellular vesicles derived from mesenchymal stromal cells as nanotherapeutics for liver ischaemia-reperfusion injury by transferring mitochondria to modulate the formation of neutrophil extracellular traps. *Biomaterials*. 2022;284:121486. doi:10.1016/j.biomaterials.2022.121486
221. Mei J, Zhou J, Kong L, et al. An injectable photo-cross-linking silk hydrogel system augments diabetic wound healing in orthopaedic surgery through spatiotemporal immunomodulation. *J Nanobiotechnology*. 2022;20(1):232. doi:10.1186/s12951-022-01414-9
222. Li M, Wang Y, Han X, Liu Y, Ma M, Zhang L. Multifunctional polydopamine-based nanoparticles for dual-mode imaging guided targeted therapy of lupus nephritis. *Pharmaceutics*. 2022;14(10):1988. doi:10.3390/pharmaceutics14101988
223. Tan Q, He L, Meng X, et al. Macrophage biomimetic nanocarriers for anti-inflammation and targeted antiviral treatment in COVID-19. *J Nanobiotechnology*. 2021;19(1):173. doi:10.1186/s12951-021-00926-0

224. Ou Q, Tan L, Shao Y, et al. Electrostatic charge-mediated apoptotic vesicle biodistribution attenuates sepsis by switching neutrophil NETosis to apoptosis. *Small*. 2022;18(20):e2200306. doi:10.1002/sml.202200306
225. Filipczak N, Li X, Saawant GR, Yalamarty SSK, Luther E, Torchilin VP. Antibody-modified DNase I micelles specifically recognize the neutrophil extracellular traps (NETs) and promote their degradation. *J Control Release*. 2023;354:109–119. doi:10.1016/j.jconrel.2022.12.062

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