Neutrophils and NETs in kidney disease

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Abstract

Neutrophils, conventionally regarded as a homogeneous immune cell population, have emerged as a heterogeneous group of cells with distinct gene profiles and immune properties. Activated neutrophils release a spectrum of bioactive substances, including cytokines, chemokines, proteolytic enzymes, reactive oxygen species and neutrophil extracellular traps (NETs), which are composed of decondensed DNA and antimicrobial proteins. NETs have a pivotal role in innate immunity, including in preventing the ascent of uropathogenic bacteria into the kidneys, as they efficiently trap pathogenic microorganisms. However, although indispensable for defense against pathogens, NETs also pose risks of self-damage owing to their cytotoxicity, thrombogenicity and autoantigenicity. Accordingly, neutrophils and NETs have been implicated in the pathogenesis of various disorders that affect the kidneys, including acute kidney injury, vasculitis, systemic lupus erythematosus, thrombotic microangiopathy and in various aetiologies of chronic kidney disease. Pathological alterations in the glomerular vascular wall can promote the infiltration of neutrophils, which can cause tissue damage and inflammation through their interactions with kidney-resident cells, including mesangial cells and podocytes, leading to local cell death. Targeting neutrophil activation and NET formation might therefore represent a new therapeutic strategy for these conditions.

Introduction

Neutrophils account for ~70% of all leukocytes in peripheral blood and ~1 billion neutrophils per kg of body weight are produced in the bone marrow (BM) every day[1]. Most neutrophils are retained in the BM owing to interactions between CXC-chemokine ligand 12 (CXCL12) expressed in the reticular tissues surrounding the BM vasculature and CXC-chemokine receptor 4 (CXCR4) expressed on the surface of neutrophils[2]. Granulocyte colony-stimulating factor (G-CSF) regulates the mobilization of neutrophils from the BM into the bloodstream by downregulating neutrophil expression of CXCR4.

Under physiological conditions, most blood neutrophils are replaced by new cells in 6–8 hours, without engaging vascular endothelial cells[3]. However, in the presence of pathogenic microorganisms, pattern recognition receptors such as Toll-like receptors (TLRs) are activated in macrophages and dendritic cells, leading to the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and IL-1β. These mediators activate vascular endothelial cells and circulating leukocytes, including neutrophils[4] (BOX 1). Activated neutrophils bind to the vascular endothelium through interactions between selectins and their ligands, which retain circulating neutrophils at sites of inflammation. Specifically, P- and E-selectins expressed on activated vascular endothelial cells bind to glycoproteins such as P-selectin glycoprotein ligand-1 (PSGL-1) on activated neutrophils, which initiates rolling of neutrophils along the endothelium[5]. Thereafter, neutrophil integrins, including LFA-1 (composed of CD11a and CD18) and Mac-1 (composed of CD11b and CD18), bind to intercellular adhesion molecule-1 (ICAM-1) on vascular endothelial cells, facilitating firm adhesion between the two cell types[6], which is crucial for neutrophil tissue infiltration. The lifespan of circulating neutrophils is extended by 2–3 times following tissue infiltration[1], which favors pathogen elimination. However, this extension can also increase the risk of prolonged local inflammation.

Activated neutrophils have various effector functions, including phagocytosis, degranulation, production of reactive oxygen species (ROS) and the release of neutrophil extracellular traps (NETs)[7]. These activities are essential for eliminating pathogenic microorganisms and are involved in subsequent tissue repair[2]. However, NETs can also exert harmful effects, such as self-injury, induction of thrombosis and promotion of autoimmunity, especially when NET generation and degradation are not regulated[8,9].

In systemic autoimmune diseases, such as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), IgA vasculitis (IgAV) and systemic lupus erythematosus (SLE), autoimmune reactions can lead to glomerulonephritis and, occasionally, kidney interstitium disorders. Acute kidney injury (AKI) and chronic kidney disease (CKD) are also associated with transient and persistent inflammation, respectively, and when transient inflammation is not resolved adequately, leading to undesirable fibrosis, AKI can progress to CKD. Importantly, neutrophils can have a crucial role in these autoimmune and inflammatory conditions[10].

In this Review, we explore the multifaceted roles of neutrophils in kidney disease, underscoring the dynamic landscape of neutrophil biology and examining current understanding of their pathogenic roles, with a particular focus on NETs. We also discuss the potential of neutrophil targeting as a novel therapeutic strategy for kidney disease.

Neutrophil heterogeneity

Neutrophils were initially considered to be a relatively homogeneous cell population but multiple studies have uncovered their phenotypic and functional diversity under physiological and pathological conditions. One study highlighted differences between aged neutrophils and those that are newly released from the BM[11]. Aged neutrophils lose CD62L (L-selectin) expression and acquire expression of CD11b and CXCR4, which directs them back to the BM, where they are eliminated by macrophages[12]. Of note, aged

neutrophils are characterized by increased integrin activation and an enhanced ability to form NETs[13].

Additional neutrophil subpopulations defined by functional diversity of Fc receptors, as well as the expression of CD177 and olfactomedin 4 (OLFM4) have also been reported. For example, approximately 20% of neutrophils have reduced reactivity to IgG immune complexes, which is mediated by Fc receptors, resulting in diminished bactericidal, phagocytic and chemotactic functions[14]. Although these neutrophil subpopulations are separable by differential density centrifugation, the origin, including an association with cellular senescence, has not been determined. CD177 (also known as HNA-2 or NB1), is typically expressed in 40–60% of neutrophils in most individuals but this frequency can range from 0–100%[15,16]. The rise in CD177 expression on neutrophils is induced by G-CSF[17] and elevated levels of $CD177^+$ neutrophils are associated with various pathological conditions, including severe bacterial infections, AAV and SLE[17,18]. However, the exact functional role of CD177 and the immunological significance of $CD177^+$ and $CD177^-$ neutrophils remain unclear, because CD177 expression and its interaction with proteinase 3 (PR3) differs between humans and mice. In humans, the binding of CD177 to PR3, which is a major autoantigen in AAV, has a crucial role in disease pathogenesis[19]. By contrast, the PR3–CD177 interaction is weaker in mice, owing to structural differences and lower PR3 expression, limiting the applicability of murine models to human conditions[20]. As for the neutrophil granule protein OLFM4, it was initially identified as a gene transcript highly induced by G-CSF in myeloid stem cells and is expressed in 20–25% of neutrophils[21]. Of note, in people with sepsis, a higher frequency of $OLFM4^+$ neutrophils in peripheral blood is linked to a greater risk of organ failure and mortality[22]. Studies in OLFM4-deficient mice reported enhanced intracellular killing of *Staphylococcus aureus* and *Escherichia coli*, suggesting that OLFM4 might have an immunosuppressive role[23].

Peripheral blood neutrophils are also divided into two subgroups according to their density. Following density-gradient centrifugation, normal-density granulocytes (NDGs) are found in the polymorphonuclear cell layer, whereas low-density granulocytes (LDGs) accumulate in the mononuclear cell layer[24,25].

Origin of LDGs

LDGs have a segmented, banded or myelocyte-like nucleus, which reflects the heterogeneity of this population of neutrophils, at varying degrees of maturation[26,27]. Gene analyses showed that various serine proteases and bactericidal proteins, which are abundantly expressed in the promyelocytic stage of neutrophil differentiation, are increased in LDGs compared with NDGs[28]. Because neutrophil progenitors are low-density cells and their cellular density gradually increases during granulocyte maturation, LDGs are considered to be immature neutrophil progenitors released prematurely from the BM. This hypothesis is consistent with the high frequency of circulating LDGs in stem cell donors treated with G-CSF[27].

Under inflammatory conditions, various cytokines and stimuli induce granulopoiesis, which might promote the mobilization of immature LDGs from the BM to the bloodstream. One study found that the proportion of LDGs increased when peripheral blood neutrophils from healthy volunteers were stimulated with the bacteria-derived compound *N*-formylmethionyl-leucyl-phenylalanine (fMLP) *in vitro* to model inflammation[29]. Another study suggested that LDGs in SLE originate from precursor cells different from those that give rise to NDGs[30]. Further studies are needed to clarify whether NDGs and LDGs originate from the same precursors and whether they differentiate in the BM or the periphery. The factors that determine whether cells differentiate into NDGs or LDGs also remain unclear.

Function of LDGs

The number of LDGs in the bloodstream increases in various conditions, including infections, cancers and autoimmune diseases[31]. LDGs are characterized by low CD14 and positive CD15 expression, and can be divided into two groups based on the expression of CD10, a marker that distinguishes mature and immature neutrophils[27,32]. In SLE, $CD10^-$ LDGs have an immature phenotype with a less segmented nucleus, higher transcriptional activity with increased expression of thousands genes, including cell cycle genes, compared with $CD10^+$ LDGs[33]. By contrast, $CD10^+$ LDGs have a multilobulated nucleus and are considered relatively mature. These cells also express higher levels of type I interferon (IFN)-related genes and have enhanced chemotaxis, NET formation and phagocytosis compared with $CD10^-$ LDGs[33].

LDGs have diverse roles, depending on the disease context. In autoimmune diseases such as SLE, LDGs have a proinflammatory effect through multiple mechanisms, including enhanced cytokine release, as well as granule exocytosis leading to the release of proteolytic enzymes, compared with NDGs[26]. These processes collectively contribute to tissue damage, amplification of the inflammatory response and disease progression. In AAV, LDG levels increase during the active phase of the disease (defined by a Birmingham vasculitis activity score (BVAS) ≥ 2) compared with the remission state (BVAS = 0) after treatment[34], and the expression of *MPO* and *PRTN3*, which encode major ANCA antigens, is higher in LDGs than in NDGs[35]. LDG counts also correlate positively with disease activity in SLE[36]. Furthermore, compared with NDGs, LDGs are characterized by increased oxidative stress and a heightened ability to activate vascular endothelial cells, further exacerbating inflammation and vascular damage[26,33]. By contrast, in people with cancer, LDGs seem to have an immunosuppressive function, weakening immune surveillance and promoting tumour progression; these cells are also known as myeloid-derived suppressor cells[37]. LDGs in people with cancer are also heterogeneous

regarding their surface markers, including CD10[37]. These conflicting findings highlight the complexity of LDGs and the need for further research to fully elucidate their roles in different pathological settings.

NETs and NET formation

NETs comprise extracellular DNA mixed with antimicrobial proteins, including neutrophil elastase, myeloperoxidase (MPO) and histones, and are released from activated neutrophils[7]. NET formation does not always result in cell death. Lytic (suicidal) NET formation results in cell death characterized by nuclear decondensation and DNA unraveling, which contrasts with the nuclear condensation and DNA fragmentation observed in apoptotic cell death. NET formation can also be non-lytic (vital), whereby genomic or mitochondrial DNA are released, in some cases through the vesicular transport system, but the cell remains viable[10,38]. The heterogeneity of neutrophils, combined with various types of stimulation underlie the diversity in NET formation.

NETs released from NDGs

Several hours after being stimulated by various substances, including lipopolysaccharide (LPS), cytokines, immune complexes, crystals and phorbol 12-myristate 13-acetate (PMA), NDGs release decondensed genomic DNA combined with cytoplasmic and nuclear proteins. It is important to note that PMA-induced NETs do not completely reflect the formation of NETs *in vivo*, as PMA is not a physiological NET inducer. Diverse pathways, including the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived ROS-dependent pathway[39], the peptidylarginine deiminase 4 (PAD4)-dependent pathway[40], and the caspase-dependent pathway[41], have been identified as mediators of NET formation, and these pathways are often interconnected. In some instances, NDGs release genomic DNA through the vesicular transport system within one hour, resulting

in non-lytic (vital) NET formation[42].

NETs released from LDGs

Compared with NDGs, LDGs have an enhanced ability to form NETs spontaneously[26,43]. In SLE, the spontaneous NET formation of LDGs requires the activation of mitochondrial ROS (mtROS), as it is decreased by the mitochondria-targeted superoxide dismutase mimetic MitoTEMPO, although the underlying mechanism has not been clarified[31,44]. By contrast, in tuberculosis, NET formation in LDGs requires NADPH oxidase-derived ROS activation, which facilitates the conversion of NDGs to LDGs[45]. The precise mechanisms governing NET formation in LDGs remain unclear and require further investigation.

Duality of NETs

Pathogens are captured by the DNA framework of NETs[7]. Initially, it was believed that NET-releasing neutrophils killed bacteria in blood through bactericidal proteins derived from the neutrophil cytoplasm that were bound to the extracellular DNA. However, additional work demonstrated that NET-dependent bacteria killing required not only the MPO enriched in the NETs but also additional hydrogen peroxide, which can be supplied by viable neutrophils[46,47]. Importantly, in blood, NETs induce activation of platelets[48] and coagulation factors[49], resulting in thrombus formation[50]. Thrombi initiated by immune cells, including NET-forming neutrophils, are termed immunothrombi, which primarily function to contain pathogens and prevent their dissemination throughout the bloodstream[51]. Notably, in severe cases of COVID-19, SARS-CoV-2 induces NET formation directly and indirectly, and subsequent immunothrombus formation. Although these processes aim to contain viral particles within the clots, excessive immunothrombi formation can cause microvascular occlusion and result in organ failure[52–54]. Of note, although NETs contribute to immunothrombus formation, no evidence suggests that they

have a substantial role in haemostasis. Immunothrombi formed through NET activity differ from physiological thrombi, which are essential for haemostasis and the prevention of bleeding[55]. This distinction highlights that NETs are primarily involved in immune defense and pathogen containment, rather than in the regulation of blood clotting.

Despite their important role in the defense against pathogens, excessive NET formation and disordered degradation can adversely affect the body[8,9]. DNA and histones in NETs are DAMPs and can cause inflammation[9]; neutrophils themselves are activated by NET-derived DAMPs via TLR4[56]. NETs can also activate the cyclic GMP–AMP (cGAMP) synthase (cGAS)–stimulator of IFN genes (STING) pathway, contributing to inflammatory responses[44]. Following recognition of cytoplasmic DNA, cGAS synthesizes cGAMP, which binds to and activates STING[44]. This process leads to the production of type I IFNs, IL-6 and other inflammatory cytokines that have a substantial role in immune responses, including in autoimmunity[44]. In addition, histones have strong cytotoxic activity and are involved in vascular endothelial injury[57,58]. Consequently, NET responses must be highly localized and limited, with adequate regulation of NET formation and removal, to avoid adverse effects such as damage to the surrounding tissue or excessive immune activation and inflammation.

DNA, the main component of NETs, is degraded by the endonuclease DNase I, which is produced primarily in the digestive system, including the pancreas[59,60]. NETs are also systematically eliminated by macrophage efferocytosis in a cytochalasin D-dependent manner[61]. Of note, preprocessing of NETs by DNase I facilitated their clearance by macrophages[61]. Therefore, DNase I and macrophages are key effectors of NET degradation.

NET detection

Currently, no gold standard exists for the detection of NETs[62]. Histologically, PAD4-

related NET deposition in tissues can be confirmed by simultaneous staining of neutrophil markers such as neutrophil elastase and MPO, along with the PAD4 product citrullinated histone H3 (Cit-H3)[40] and DNA (**Fig. 1**). However, the detection of mtROS-induced NET formation requires the use of live cell imaging with mitochondria specific dyes, such as MitoTracker, combined with ROS-specific fluorescent probes, such as MitoSox, which is specific for superoxide[63]. For NETs formed through the vesicular transport system, extracellular vesicles must be extracted from extracellular fluids by ultracentrifugation, and NET-related molecules, including neutrophil elastase, MPO and Cit-H3, in the vesicles are detected by immunoblotting[64]. In liquid samples, such as serum, DNA–neutrophil elastase, DNA–MPO or DNA–histone complexes derived from NETs can be detected using enzyme-linked immunosorbent assay (ELISA)[65]. Although these assays measure the degradation products of NETs rather than NETs themselves, the complexes that they detect are widely used as surrogate markers for NETs. Flow cytometry (FCM) can also be used to detect emerging NETs, typically by first gating on NDGs, based on forward and side scatter profiles, and measuring extracellular DNA staining with reagents such as SYTOX Green and neutrophil markers (for example, MPO in humans and Ly-6G in mice)[66]. NDGs that are positive for extracellular DNA and neutrophil markers are identified as NET-emerging cells, although suicidal NET formation cannot be fully detected as the cells would have already lysed. Of note, imaging FCM can enable automated and quantitative assessments of NETs[67,68].

Crosstalk between neutrophils and kidney parenchymal cells

Under physiological conditions, neutrophils predominantly reside in the BM, spleen, and lungs, with few cells found in the kidneys[69]. In the lungs, neutrophils are predominantly localized within blood vessels, forming a defense niche in collaboration with the vascular endothelium to protect against bloodstream infections[70]. Although similar evidence is

lacking for the kidneys, through which neutrophils typically only pass through, disruptions in physiological homeostasis can lead to neutrophil activation and subsequent interaction with kidney parenchymal cells, through which they can contribute to the pathogenesis of kidney disease. Below we examine the interactions between neutrophils and glomerular cells.

Neutrophil infiltration into glomerular capillaries

The glomerulus comprises endothelial cells, a basement membrane, mesangial cells and podocytes. Although these endothelial cells are fenestrated, their fenestrae measure approximately 70–100 nm, which is substantially smaller than the diameter of a neutrophil (~16 μm) and therefore prevents their passage under physiological conditions[71]. Additionally, the negatively-charged glycocalyx (composed of glycoproteins and glycosaminoglycan) on the endothelial lumen repels neutrophils, as their surface is also negatively charged, largely owing to sialic acid residues[72] (**Fig. 2a**).

In certain pathological conditions such as thin basement membrane disease (whereby the glomerular basement membrane (GBM) thins from the normal 300–400 nm to 150–250 nm) or in chronic glomerulonephritis (for example, IgA nephropathy), red blood cells (8 μm in diameter) can cross the barrier, resulting in haematuria, although it remains uncommon for larger leukocytes to pass through. However, under pathological conditions such as crescentic glomerulonephritis (observed, for example, in AAV), the integrity of the capillary wall becomes compromised, resulting in leukocyturia[73]. Neutrophil infiltration into the mesangium and capillary walls has been reported, for example, in postinfectious glomerulonephritis[74], suggesting that endothelial injury allows neutrophils to breach the glomerular filtration barrier and interact with mesangial cells and podocytes.

Sialic acid residues have a crucial role in maintaining the electrostatic barrier,

preventing neutrophils from interacting with the endothelium under physiological conditions. However, during inflammation or injury, sialic acid undergoes glycan modifications such as desialylation, which causes conformational changes in adhesion molecules and enhances neutrophil infiltration across the endothelial barrier[75]. These modifications facilitate neutrophil adhesion to the endothelium, enabling interactions with mesangial cells and podocytes, thereby promoting inflammation (discussed below).

Under physiological conditions, glomerular capillaries respond to high shear stress by inducing the conformational unfurling of von Willebrand factor (vWF), increasing its susceptibility to cleavage by a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13 (ADAMTS13), which helps to prevent clotting[76]. However, under pathological conditions such as preeclampsia and thrombotic microangiopathy (TMA), including Shiga toxin-producing *Escherichia coli* (STEC)-associated haemolytic uremic syndrome (HUS), activated complement promotes neutrophil adhesion to blood vessels, which renders glomerular endothelial cells especially vulnerable to thrombotic injury[77]. Additionally, Shiga toxin stimulates the release of ultra-large vWF multimers, to which platelets adhere preferentially[78].

Interaction between neutrophils and mesangial cells

Once neutrophils cross the glomerular endothelial barrier, they interact with mesangial cells (**Fig. 2b**). One study highlighted the role of lipoxygenase products — leukotriene B4 (LTB4) and LTD4 — in neutrophil adhesion to mesangial cells[79]. In vitro, exposure of neutrophils to LTB4 promoted their adhesion to cultured mesangial cells via the CD18 glycoprotein on neutrophils, whereas LTD4 promoted neutrophil–mesangial cell adhesion via the mesangial cells. Lipoxins (LXA4 and LXB4) attenuated LTD4-induced responses but not those triggered by LTB4. These findings suggest that lipoxygenase-derived eicosanoids regulate neutrophil adhesion through dual mechanisms: LTB4 promotes

direct neutrophil adhesion via CD18, whereas LTD4 enhances neutrophil adherence to mesangial cells indirectly by acting on mesangial cells[79].

In immune complex-mediated glomerulonephritis, neutrophils infiltrate the mesangium via complement receptors. They are recruited from the circulation by C3a and C5a, which are generated during complement activation. These complement fragments act as potent chemoattractants, guiding neutrophils toward sites of immune complex deposition. Once in the glomeruli, neutrophils interact with mesangial cells primarily through complement receptor 3, facilitating adhesion and triggering downstream inflammatory responses[80]. Additionally, in a mouse model of diabetic kidney disease (DKD), NETs contribute to glomerular endothelial cell (GEC) injury, leading to GEC pyroptosis, a form of inflammatory cell death, which might contribute to subsequent mesangial expansion[81]. Mesangial cells also have an important role in inflammatory responses and extracellular matrix remodeling in conditions such as lupus nephritis and IgA nephropathy. These cells respond to neutrophil-derived cytokines, including IL-6 and TNF, through cytokine receptors that mediate inflammatory signaling and extracellular matrix deposition. Neutrophils, along with macrophages and dendritic cells, coordinate cytokine production, amplifying the inflammatory response[10]. Mesangial cells also express Fc receptors with high affinity for immune complexes, with a role in immune complex recognition and clearance. These cells help maintain glomerular homeostasis by using Fc receptor-mediated uptake and degradation to remove circulating immune complexes that pass through the glomerular capillaries. However, under pathological conditions such as SLE and IgA nephropathy, this Fc receptor-dependent mechanism can also activate complement pathways, leading to oxidative stress and mesangial proliferation[82]. *In vitro* studies that specifically address the direct impact of NETs on mesangial cells are currently lacking.

Neutrophil–podocyte crosstalk in glomerular injury

After neutrophils pass through the GBM, they might also come into contact with podocytes (**Fig. 2c**). Although no specific receptor facilitating direct interaction between neutrophils and podocytes has been identified, under certain conditions, such as crescentic glomerulonephritis, podocytes are exposed to IFN-γ, which is released by infiltrating immune cells, including T cells and macrophages. Podocytes stimulated by IFN-γ express ICAM-1, which could bind to neutrophil integrins[83]. In experimental acute glomerulonephritis, crosstalk between neutrophils and podocytes promoted disruption of the glomerular filtration barrier, with neutrophil granule products damaging the podocyte cytoskeleton and leading to proteinuria[84].

Podocytes, in turn, release cytokines that enhance neutrophil chemotaxis and exocytosis in pathological conditions associated with podocyte injury[85]. In a mouse model of podocyte injury induced by hyperhomocysteinemia, podocyte-derived inflammatory exosomes — whose release was driven by NLR family pyrin domain containing 3 (NLRP3) inflammasome activation — promoted neutrophil glomerular infiltration[86]. Additionally, in a bisphenol-A-induced CKD model, podocyte damage induced by NETs occurred both in vitro and in vivo. This process was mediated by bisphenol-A-induced NET formation via histone citrullination, leading to podocyte injury and contributing to proteinuria[87]. However, one study reported that podocytes can also exert anti-inflammatory effects by modulating IL-6 signaling, thereby reducing neutrophil recruitment[88]. This study utilized an in vitro co-culture system where human podocytes and GECs were cultured on opposite sides of a porous insert to investigate neutrophil recruitment under inflammatory conditions. Podocytes primarily released soluble IL-6, which exerted immunomodulated effects through the activation of suppressor of cytokine signaling 3, thereby downregulating chemokine expression in GECs and reducing neutrophil adhesion[88]. Although many studies indicate that podocytes can contribute to

inflammation by releasing pro-inflammatory cytokines, their ability to regulate immune responses through IL-6 signaling suggests a context-dependent role. The discrepancy between findings might be attributed to differences in experimental conditions, disease models, or inflammatory stimuli, highlighting the complexity of podocyte-mediated immune modulation in glomerular diseases.

Neutrophils and NETs in kidney disease

Neutrophils have been implicated in the pathogenesis of various kidney diseases, including AKI, small-vessel vasculitis, SLE, TMA and CKD. Activated neutrophils and NETs have a crucial role in initiating injury and inflammation in these pathological conditions.

AKI

AKI is a multifaceted pathological condition characterized by both intrarenal and systemic inflammation. Intrarenal inflammation might be driven by conditions such as hypovolemia, shock and severe infections. Conversely, AKI can also lead to systemic inflammation as demonstrated in basic and clinical studies linking AKI with increased systemic cytokine levels and immune dysregulation[89,90]. Clinically, the neutrophil-to-lymphocyte ratio (NLR) has been identified as an independent risk factor for AKI in patients immediately after gastrointestinal and hepatobiliary surgery[91]. Although neutrophils contribute to intrarenal inflammation, this elevated NLR likely reflects systemic inflammation rather than direct neutrophil-mediated injury.

In an animal model of ischaemia–reperfusion injury (IRI), tubular epithelial cells initially underwent programmed necrosis (necroptosis) through pathways involving receptor-interacting protein kinase (RIPK) and cyclophilin D (CypD) in response to hypoxia[92]. Subsequently, DAMPs such as histones and high mobility group box 1

(HMGB1) released from necrotic tubules induced NET release in a PAD4-dependent manner, which exacerbated tubular injury by promoting inflammation, oxidative stress, and endothelial dysfunction, ultimately impairing kidney function. Notably, NET formation was not triggered by hypoxia, rather, it was driven by DAMPs derived from hypoxia-induced necrotic tubules[89,93]. In addition, extracellular DNA derived from necrotic tubular epithelial cells can activate platelets. This activation promotes platelet–granulocyte interactions and the formation of NETs, which can further promote platelet activation. As proof, inhibition of platelet activation mitigated experimental AKI induced by IRI[94].

Consistently, a similar phenomenon was observed in experimental and clinical sepsis-associated AKI[95]. In this study, NETs were detected in mice with cecal ligation and puncture-induced sepsis, and the combination of recombinant human DNase and antibiotics attenuated sepsis-related organ damage and mortality by regulating NET formation[95]. Furthermore, circulating NETs were identified in people with sepsis, and their abundance was positively associated with disease severity[95].

AKI is also associated with the subsequent development of remote organ failure, particularly acute respiratory distress syndrome (ARDS). AKI and lung injury are characterized by the activation of inflammatory signaling pathways, increased vascular permeability, microvascular thrombosis and infiltration of immune cells[96]. In experimental models, AKI-associated damage to distant organs such as the lungs, was at least partly mediated by IRI-induced cellular necrosis and neutrophil activation accompanied by NET formation in the kidneys. Kidney injury also increased levels of circulating histones, as well as neutrophil activation and NET release in the lungs and other distant organs[89,96] (**Fig. 3a**).

In COVID-19, AKI is a common and severe complication[97] that might involve both direct and indirect kidney damage caused by SARS-CoV-2. Clinically, SARS-CoV-

2 spike protein and nonstructural protein 8 were detected in kidney tissues obtained at autopsy from people with severe COVID-19, suggesting active viral replication in the kidneys[98]. By contrast, in *ex vivo* studies, SARS-CoV-2 did not infect immortalized kidney tubular epithelial and glomerular endothelial cells. However, the virus caused direct kidney tubular necrosis through TLR4, TLR3 and IL-1 receptor (IL-1R) signaling pathways[99]. Moreover, SARS-CoV-2 infects or activates neutrophils via angiotensin-converting enzyme 2 (ACE2), inducing the formation of NETs in a PAD4-dependent manner. These NETs might contribute to vascular injury and coagulation in the kidneys[99–102] (**Fig. 3b**).

Furthermore, LDGs are increased in patients with severe COVID-19 compared with healthy individuals[103]; in severe disease, these cells tend to form NETs and also have transcriptional characteristics associated with neutrophil recruitment and activation[104]. Thus, LDGs might contribute to the progression of multiorgan failure, including AKI, in people with COVID-19.

Small-vessel vasculitis

Neutrophils have a central role in the pathogenesis of small-vessel vasculitis, including AAV and IgAV. Necrotizing and crescentic glomerulonephritis (NCGN) and immune complex-related glomerulonephritis are characteristic of AAV and IgAV, respectively.

AAV

AAV is a systemic small-vessel vasculitis characterized by the presence of ANCA in the serum and rapidly progressive glomerulonephritis. AAV includes diseases such as microscopic polyangiitis (MPA), predominantly associated with MPO-ANCA, and granulomatosis with polyangiitis (GPA), predominantly associated with PR3-ANCA. Although MPA and GPA exhibit distinct clinical manifestations, NCGN is a common

feature of both diseases[105,106]. PAD4-related NET deposition in the glomeruli is a characteristic feature of AAV[65,107].

The pathogenesis of AAV comprises four steps: neutrophil priming; binding of ANCA to primed neutrophils; excessive activation of neutrophils and NET formation triggered by ANCA binding; and vascular endothelial injury and impaired NET removal[60]. Inflammatory cytokines such as TNF and IL-1β, as well as complement C5a, bind to their respective receptors on neutrophils, promoting the expression of ANCA antigens on the plasma membrane[108]. When ANCA bind to their antigens, the antibody Fc portion binds to FcγR on neutrophils, triggering ROS production via phosphorylation of SYK[109]. This process requires adherence of neutrophils to vascular endothelial cells through integrins[68]. In addition to ANCA[65], NET formation in AAV is induced by a combination of proinflammatory cytokines and/or DAMPs[110], the endogenous TLR4 agonist calprotectin[56], granulocyte–monocyte-CSF (GM-CSF) and other serum factors[111]. Studies in murine models have confirmed a causal relationship between ANCA-induced NETs and NCGN, in a process that involves deficiencies in RIPK1, RIPK3 and mixed-linage kinase domain-like protein (MLKL)-dependent necroptosis[112]. CypD is also involved in ANCA-mediated NET formation via ROS suppression[113]. Furthermore, the expression of semaphorin 4D on neutrophils, which normally binds to plexin B2 on vascular endothelial cells to inhibit NET formation, is attenuated in people with AAV owing to shedding by TNFα-converting enzyme constitutionally expressed on neutrophils; thus, NETs are easily formed in people with AAV[114]. By contrast, DNase I activity in the serum decreases[115] and NETs formed in tissues acquire DNase I resistance in people with AAV[116]. When NETs fail to be degraded by DNase I and persist in tissues, neutrophil cytoplasmic proteins such as MPO can be recognized as autoantigens, leading to the production of ANCA[117]. MPO is more biologically active when bound to extracellular substances such as NET-derived DNA[47,118], and is likely shielded from endogenous inhibitors that would

normally clear up these enzymes after degranulation[47]. This effect creates a vicious cycle whereby ANCA induce NETs, which, in turn, promote further ANCA production, with a central role in the pathogenesis of AAV.

IgAV

Although the pathogenesis of IgAV is not fully understood, immunological abnormalities and environmental factors, including infections, might be involved[119]. In ~50% of cases, an upper respiratory tract infection is observed before the onset of IgAV[120]. In addition, serum levels of galactose-deficient IgA1 are increased in people with IgAV compared with healthy individuals, and IgA with aberrant glycosylation is thought to be involved in the pathogenesis of this disease[121]. Current data suggest that IgA, probably produced in response to certain pathogens, binds to the Fcα receptor I on the neutrophil plasma membrane, which subsequently induces ROS-mediated NET formation[122,123]. Accordingly, PAD4-related NET deposition has been reported in the glomeruli of people with IgAV[124].

SLE

In SLE, immune complexes containing ribonucleoproteins as antigens induce NET formation in LDGs through FcγRs expressed on the neutrophil plasma membrane. Accordingly, NET deposition is observed in kidney biopsy tissue from people with lupus nephritis[28]. NETs associated with SLE are morphologically and functionally different from those observed in AAV[125] (**Fig. 4**). In SLE, NETs are more likely to be released from LDGs, which are found in greater numbers than in healthy individuals. In addition, NETs in SLE are enriched with mtDNA, driven by mtROS activation. These mtDNA-enriched NETs contribute to chronic inflammation by promoting the expression of inflammatory cytokines and genes related to type I IFN signaling[26,36,44]. The factors driving mtDNA

release or nuclear DNA release in NET formation remain unknown.

Some people with SLE have reduced serum DNase I activity owing to genetic polymorphisms or the existence of autoantibodies that inhibit DNase I activity, resulting in impaired NET degradation[59]. Autoantibodies that inhibit NET degradation by DNase I include anti-DNase I antibodies[126] and anti-NET antibodies that mask the action site of DNase I[115,127]. Impaired degradation of NETs leads to their persistence in tissues, which exacerbates the pathology of SLE[128].

TMA

TMA is a group of diseases with three clinical characteristics: thrombocytopenia, haemolytic anaemia and damage to organs such as the kidneys and brain. Currently, TMA is subdivided into ADAMTS13-deficient TMA, infection-associated TMA, complement-associated TMA and secondary TMA. However, the common underlying pathology across all forms is vascular endothelial cell damage. DNA–histone complexes, which are soluble indicators of NETs are elevated in the serum of people with TMA compared with that of healthy individuals[129].

In addition, DNase I activity was reduced in the serum of people with TMA compared with that of healthy individuals, and impaired degradation of NETs is involved in the pathogenesis of TMA[130]. In particular, a negative correlation between NET degradation ability and serum levels of blood urea nitrogen and creatinine was shown in cases of STEC-HUS[131]. Histones derived from NETs activate platelets directly or via TLRs, and induce platelet aggregation[132]. In addition, DNA and serine proteases derived from NETs induce factor XII activation and tissue factor activation, respectively, resulting in thrombus formation[133,134]. Platelets activated by vascular endothelial cell injury also release HMGB1 and induce NET formation[135], which further promotes the formation of thrombi. These findings suggest that the vascular endothelial cell damage caused by NETs,

as well as the circulatory disorders caused by thrombi induced by NETs and activated platelets, promote kidney damage in TMA.

Of note, kidney damage after transplantation can be caused by rejection, TMA, or medication. Rejection is broadly classified into T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR)[136]. Pathologically, ABMR is characterized by the binding of donor antibodies to vascular endothelial cells, complement activation and thrombi formation[136]. The neutrophil immune response in allograft rejection occurs mainly on the vascular endothelium. Elevated levels of circulating NETs have been observed, and NETs have been also detected in the glomeruli and peritubular capillaries of kidney biopsy specimens in people with acute ABMR, suggesting their involvement in kidney damage associated with ABMR[137].

CKD

CKD is a multifaceted condition characterized by sustained kidney dysfunction lasting over three months. The 2024 KDIGO clinical practice guideline stratifies CKD based on glomerular filtration rate (GFR) and albuminuria[138], both of which are strong predictors of mortality across all stages[139]. CKD is intricately linked to chronic uraemia-associated immune dysfunction, which significantly increases the risk of various adverse outcomes. For example, people with CKD are more susceptible to cancer and infections[140,141] and often exhibit dysregulated immune responses, which contributes to an increased risk of atherosclerotic vascular complications[142,143]. Accordingly, the NLR, which is recognized as an inflammation marker, correlated positively with mortality in people with advanced CKD[144].

Uric acid, which is a metabolic product of purine nucleotides, is elevated in people with CKD compared with healthy individuals owing to decreased renal clearance[145,146]. Crystalline uric acid has immunostimulatory effects and is involved in

gout arthritis, whereas elevated serum uric acid levels inhibit the activation of β2 integrin, resulting in decreased neutrophil migration and phagocytosis[147]; this effect might contribute to the immunodeficiency observed in people with CKD. Fibroblast growth factor 23, which is elevated in CKD, has been shown to inhibit β2 integrin activation in neutrophils, impairing their ability to adhere to vascular endothelium and migrate into tissues, thereby reducing infection resistance[148]. Additionally, CKD-associated uremia impairs glucose uptake in neutrophils, leading to decreased ROS production and impaired bactericidal function owing to aberrant glycogen synthase kinase-3β activation[149]. These findings suggest that neutrophil dysfunction contributes to the immunodeficiency observed in CKD.

AKI to CKD transition

The transition from AKI to CKD involves a complex cascade of pathological processes that continue to cause kidney damage and dysfunction. Although the exact underlying mechanisms are not fully elucidated, several key processes have been noted. AKI drives an inflammatory response characterized by the infiltration of immune cells, including neutrophils, into kidney tissues, which, if unresolved, contributes to ongoing tissue injury and inflammation[89]. Persistent inflammation leads to the activation of profibrotic pathways, including those involving transforming growth factor-β, which promotes the accumulation of extracellular matrix components and leads to kidney fibrosis[150].

As discussed earlier, neutrophils have a pivotal role in the persistent inflammation and cell death observed in AKI. Pyroptosis, a form of inflammatory cell death primarily affecting macrophages, not only promotes AKI but also contributes to progression towards CKD[151]. In a unilateral ureteral obstruction mouse model, gasdermin D (GSDMD) in neutrophils initiated NET formation, and these GSDMD-dependent NETs further promoted kidney fibrosis and inflammation[152]. These data suggest that pyroptosis

might contribute to the development of CKD, including kidney fibrosis, both directly and indirectly. *Ex vivo* studies also suggest that anti-inflammatory macrophages (also termed M2 macrophages) acquire proinflammatory properties in response to NETs[153].

Atherosclerosis

CKD is associated with several risk factors that promote the development of atherosclerosis, including uraemia-related factors, hypertension, dyslipidemia, type 2 diabetes mellitus, inflammation, oxidative stress and mineral disorders. Neutrophils and monocytes seem to be chronically activated by uraemic toxins, such as *p*-cresol and indoxyl sulfate, and are involved in the development of arteriosclerosis in patients with CKD. Specifically, indoxyl sulfate activates monocytes, leading to the production of proinflammatory cytokines via enhanced expression of arachidonic acid metabolism-related genes[154]. Serum from people with CKD, which contains uraemic toxins, induced neutrophil activation and cytokine production in vitro[155–158]. In a clinical setting, the number of circulating neutrophils correlated with the arteriosclerosis score, and neutrophils spontaneously undergo NET formation in people with CKD, particularly those receiving dialysis[139]. The abundance of NETs, represented by neutrophil elastase activities and serum nucleosome levels is also associated with vascular endothelial dysfunction, as assessed by flow-mediated dilation of the brachial artery, which contributes to cardiovascular disease[139]. Meanwhile, LDGs are reported to be increased in people with CKD compared with healthy individuals, similar to findings in people with SLE. Notably, the $CD14^{-}CD16^{-}CD15^{+}$ LDG subpopulations, which exhibit an immature profile, are increased in people with CKD and correlate positively with the aortic calcification score, suggesting a potential role in CKD-associated atherosclerosis[159]. Additionally, in people with CKD, these cells express higher levels of *DEF3*, which is a marker of early granulopoiesis, relative to healthy individuals. *DEF3* expression also

correlates positively with the calcification score in people with CKD. In healthy males, high DEF3 levels might be associated with lower body mass and LDL levels, as well as increased insulin sensitivity, indicating a potentially beneficial metabolic role. These findings suggest that the effects of DEF3 might differ between healthy individuals and those with CKD[160].

Crystal nephropathy

Crystal nephropathy, which arises from the accumulation of crystals such as calcium oxalate and uric acid in kidney tubules, causes inflammation, tissue damage and kidney dysfunction. Various factors, including hydration status, medication usage, metabolic abnormalities and systemic diseases, can trigger this accumulation[161]. The presence of crystals elicits an inflammatory response, drawing neutrophils to the affected sites, where they release inflammatory chemokines and form NETs, worsening tissue damage and inflammation. This cascade contributes to the progression of crystal nephropathy into CKD[162]. In autoinflammatory diseases such as Behçet's disease, inflammatory bowel disease, gout and adult-onset Still disease, NETs are known to promote the activation of the NLRP3 inflammasome and IL-1β production via the release of DAMPs, causing inflammation[9]. A similar mechanism is thought to contribute to the development of crystal nephropathy, whereby various crystalline components activate inflammasomes, and both kidney immune and non-immune cells cooperate to induce autoinflammatory pathologies and kidney damage[10,161].

Hyperoxaluria is characterized by the development of nephrolithiasis, which can lead to CKD. Urate crystals can also accumulate in the kidney tubular lumen and cause nephrolithiasis. Calcium oxalate crystals activate the NLRP3 inflammasome pathway in dendritic cells in the kidneys, causing neutrophil infiltration and damage to kidney tubular epithelial cells[163]. Similarly, macrophage phagocytosis of urate crystals activates the

NLRP3-caspase-1 inflammasome pathway, resulting in IL-1β production, neutrophil migration and inflammation[164]. Both types of urinary crystals can induce the formation of NETs directly[165]. In addition, urate crystals trigger RIPK3–MLKL-dependent necroptosis in kidney tubular epithelial cells[166], and necrotic cell-derived DAMPs can further activate the innate immune response.

Experimental studies have demonstrated that injection of cholesterol crystals into the renal artery can induce TMA through the formation of thrombi. This process involves the activation of neutrophils and vascular endothelial cells[167]. GSDMD is involved in the crystal-induced TMA and consequently contributes to ischaemic tissue infarction and organ failure. Mechanistically, GSDMD drives neutrophil necrosis, maturation and tissue recruitment[168].

DKD

DKD is the most common primary cause of kidney failure and is treated with drugs such as renin–angiotensin–aldosterone system inhibitors and sodium–glucose co-transporter 2 inhibitors. However, the prevention of kidney failure and cardiovascular events, which are common complications of DKD, remains challenging[169,170]. Type 2 diabetes mellitus is often complicated by microangiopathy owing to chronic inflammation involving immune cell activation and neutrophils exposed to high concentrations of glucose release NETs in vitro[171,172]. This process was compromised by an inhibitor of the polyol pathway, which converts glucose to fructose, and is highly active in conditions of hyperglycaemia. Additionally, NET components such as DNA have been implicated in the prolongation of wound healing in people with diabetes, where NETs are induced chronically[173,174]. PAD4-dependent NET deposition has also been demonstrated on the glomerular capillary walls of people with DKD, indicating its potential role in kidney pathology[81]. However, some studies reported that, compared with neutrophils from healthy individuals, those from

people with diabetes have a decreased ability to form NETs in response to the NET inducers such as LPS and PMA, which might increase susceptibility to infections[175]. This discrepancy might arise from differences in disease stage, immune signaling or experimental approach. Further research is needed to clarify the potential role of NETs in diabetes complications and compromised immunity.

Neutrophil-targeting therapies for kidney disease

Several drugs targeting neutrophil activation for the treatment of kidney disease are currently in development, including clinical trials of C5a inhibitors[176], and preclinical studies of ROS inhibitors[177], PAD inhibitors[178], CypD inhibitors[113], Syk inhibitors[179], Btk inhibitors[180] and Cathepsin C (CatC) inhibitors[181] (**Table 1**). In addition to targeting neutrophils, supplementation with exogenous DNase is a promising approach to digest NETs in conditions characterized by impaired NET degradation[182]. Of note, targeting neutrophils must be balanced with potential unwanted side effects regarding immunity to infection.

C5a inhibitors

When ANCA were transferred into wild-type mice, the recipients developed NCGN[183]. However, ANCA passive transfer did not cause NCGN in C5-deficient nor factor B-deficient mice, whereas C4-deficient recipients still developed the disease[184]. These findings suggest that the alternative pathway, rather than the classical pathway of complement activation is involved in ANCA-induced neutrophil activation and the development of ANCA-induced NCGN. Interestingly, C6-deficiency did not affect the development of ANCA-induced NCGN, indicating that binding of C5a to the C5a receptor (C5aR), which is expressed on neutrophils, is essential for NCGN, rather than formation of C5b–9, also referred to as the membrane attack complex (MAC)[185]. This

hypothesis was confirmed as ANCA-induced NCGN did not occur in C5aR-deficient mice, and a C5aR antagonist ameliorated ANCA-induced NCGN in mice expressing human C5aR[185].

Accordingly, the ADVOCATE study demonstrated that, compared with the high-dose glucocorticoids (GCs) traditionally used in the treatment of AAV, the C5aR antagonist avacopan induced remission as effectively and maintained it for a longer period[176]. However, in this study, GCs were administered to two-thirds of people in the avacopan group to prevent the worsening of vasculitis. Therefore, whether remission in AAV can be achieved with avacopan alone, without concurrent GC use, remains unclear. In addition, people with severe kidney or respiratory failure were not enrolled in this study, thus further investigation is needed to verify the therapeutic efficacy of avacopan in those with severe AAV. Furthermore, as long-term evidence data are lacking, the optimal duration of treatment with avacopan remains unknown. An ongoing phase II clinical trial (IXPLORE) is evaluating the efficacy of the anti-C5a antibody vilobelimab in people with AAV[186].

ROS inhibitors

As described earlier, distant organ injury such as ARDS is a serious concern in people with AKI. Circulating NET-forming neutrophils are associated with mitochondrial oxidative stress and a deficiency of the mitochondrial antioxidant enzyme isocitrate dehydrogenase 2 (IDH2) exacerbated lung injury after IRI-induced AKI in mice[177]. Treatment with Mito-TEMPO, a mitochondria-specific antioxidant, attenuated kidney IRI-induced lung injury, with greater attenuation observed in IDH2-deficient mice compared with wild-type mice[177].

PAD inhibitors

Enhanced NET formation was observed in the New Zealand mixed 2328 (NZM) murine model of lupus[178]. In these animals, NETs activate plasmacytoid dendritic cells and serve as a source of autoantigens, including the ortholog of human cathelicidin, LL37, which is externalized in NETs. Treatment with Cl-amidine, a pan-PAD inhibitor, inhibited NET formation in NZM mice and significantly altered circulating autoantibody profiles and complement levels, while reducing glomerular IgG deposition. Moreover, Cl-amidine increased the differentiation capacity of BM endothelial progenitor cells, improved endothelium-dependent vasorelaxation, and markedly delayed arterial thrombosis induced by photochemical injury[178]. These findings suggest that PAD inhibition might improve SLE pathology, including immune complex-mediated glomerulonephritis, by reducing neutrophil activity.

CypD inhibitors

ANCA-induced lytic (suicidal) NET formation is crucially involved in the pathogenesis of AAV. CypD has an important role in cell death by opening the mitochondrial permeability transition pores (mPTPs)[187]. Pharmacological and genetic inhibition of CypD could suppress ANCA-induced NET formation by reducing ROS and cytochrome c release from the mitochondria in mice[113]. RNA-sequencing followed by analyses of upstream regulators revealed the importance of intracellular calcium (a CypD activator) and cyclosporin (a CypD inhibitor) in ANCA stimulation, indicating that the CypD-dependent opening of mPTPs is associated with ANCA-induced neutrophil activation and NET formation[113]. Consistent with this hypothesis, genetic deletion of CypD in murine models of AAV ameliorated NCGN by inhibiting CypD-dependent NET formation[113].

Syk inhibitors

Syk, a 72-kDa cytoplasmic non-receptor protein tyrosine kinase, has an important role in

signal transduction in various cell types, including neutrophils[179]. Syk is phosphorylated during ANCA-induced neutrophil activation *in vitro*. Treatment with fostamatinib, a Syk inhibitor, rapidly resolved urinary abnormalities, significantly improved kidney and pulmonary pathology, and preserved kidney function *in vivo*[188]. In addition, IRI-induced AKI exacerbated murine lupus nephritis, because Syk-enhanced NET formation in the kidneys promoted the production of anti-dsDNA antibodies, which was attenuated by a Syk inhibitor[189].

Btk inhibitors

Btk is an enzyme expressed in leukocytes other than T and plasma cells and is involved in signal transduction mediated by the B-cell receptor and FcγRs[190,191]. Btk inhibitors might suppress autoantibody production by inhibiting B-cell differentiation into antibody-producing plasma cells and reducing FcγR-mediated neutrophil activation[192]. Tirabrutinib, a Btk inhibitor, suppressed MPO and anti-MPO–immune complex-induced NET formation *in vitro* and ameliorated experimental ANCA-associated NCGN and pulmonary haemorrhage by reducing NET-forming neutrophils, although it did not decrease ANCA titer in rat models[180]. Although the reason behind the lack of ANCA suppression in this model remains unclear, this study suggests that Btk might be a therapeutic target in AAV.

CatC inhibitors

CatC is a lysosomal cysteine dipeptidyl aminopeptidase that cleaves a dipeptide at the *N*-terminal of neutrophil serine proteases (NSPs), which are initially present as inactive zymogens, to convert NSPs into active enzymes during the early stages of neutrophil maturation in the BM[193]. Neutrophils derived from patients with Papillon–Lefevre syndrome (PLS), who lack CatC activity, cannot release NETs[194]. Targeting CatC to

prevent the maturation and activation of neutrophil elastase might be a novel strategy to suppress NET formation. People with PLS develop periodontitis but not severe infectious diseases[195], suggesting that the specific inhibition of CatC might be a relatively safe treatment option for NET-associated diseases that avoids severe immunosuppression. A CatC inhibitor successfully inhibited MPO and MPO-ANCA-immune complex-induced NET formation *in vitro* and ameliorated experimental ANCA-associated NCGN and pulmonary haemorrhage by reducing NET-forming neutrophils in rats[181].

Supplementation with exogenous DNase

As DNase I is a physiological degrader of NETs[59] and serum DNase I activity is decreased in people with both AAV and SLE[115], supplementation with exogenous DNase I is expected to alleviate NET-mediated pathology. Inhalation of recombinant human DNase I cleared NETs and reduced lung injury in a pristane-induced lupus mouse model[182]. The addition of heparin to DNase I also restored the sensitivity of COVID-19-induced NETs to DNase I *in vitro*[196], but clinical trials of recombinant human DNase I in people with SLE did not show improvements in lupus nephritis[197]. These findings suggest that NETs formed under disease conditions are possibly more resistant to DNase I than those formed under normal conditions. DNase1L3, an isoform of DNase I, has a higher ability to degrade chromatin-bound DNA compared to DNase I[198] and might effectively degrade protein-associated DNA structures, such as NETs, which are resistant to cleavage by DNase I. The therapeutic potential of both DNase I and DNase1L3 in mitigating NET-mediated pathology warrants further investigation.

Conclusions

Here we summarized the multifaceted roles of neutrophils and NETs in the pathogenesis of several conditions affecting the kidneys. Neutrophils are a heterogeneous population

that can be activated through a variety of stimuli and they have multiple effector functions, including NET release, that can be protective but can also contribute to the complex pathophysiology of diseases. Of note, much of the current evidence on neutrophil biology in kidney disease comes from preclinical studies using murine models, which have a much lower peripheral blood neutrophil count than humans. Despite these limitations, targeting neutrophils is considered a promising therapeutic strategy for various neutrophil-mediated inflammatory disorders, including kidney disease, and some strategies have already been implemented in clinical practice. International collaborative clinical studies are expected to demonstrate the real-world efficacy of neutrophil-targeting therapies in improving kidney disease outcomes.

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All authors researched data for the article, made substantial contributions to discussions of the content and wrote, reviewed or edited the manuscript before submission.

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Peer review information

TBC

Key points

- Neutrophils, the most abundant leukocytes in peripheral blood, become activated in response to various stimuli. Activated neutrophils are involved in several physiological and pathological conditions, including inflammation, immune responses and wound healing.
- Among the biological substances released from activated neutrophils, neutrophil extracellular traps (NETs) are essential to prevent the spread of pathogenic microorganisms. However, excessive NET formation and their impaired degradation can lead to tissue damage, microcirculation disorders and autoantibody production.
- NET formation is not always accompanied by cell death, neutrophil responses to stimuli vary depending on the type of stimulus and the neutrophil subset. In kidney disease, dysregulated NET formation can contribute to chronic inflammation and tissue injury.
- Neutrophils and NETs are involved in the pathogenesis of various kidney diseases and new therapeutic strategies targeting neutrophils and NETs might have a role in the treatment of these conditions.

Table 1. Potential neutrophil-associated therapeutic targets

Target	Drugs	Research findings	References
C5a receptor	Avacopan	Remission induction in patients with AAV instead of high-dose GCs Maintenance of remission for a longer time compared to GCs, potentially offering nephroprotective benefits	176
C5a	Vilobelimab	Phase II clinical trial ongoing for patients with AAV	186
ROS	(preclinical)	Attenuation of kidney IR-induced lung injuries, with greater effects in Idh2-deficient mice than in wild-type mice	177
PAD	(preclinical)	Inhibition of NET formation in NZM mice, significantly altering circulating autoantibody profiles and complement levels while reducing glomerular IgG deposition	178
CypD	Cyclosporin A	Amelioration of NCGN in ANCA passive transfer models	113
Syk	(preclinical)	Resolution of urinary abnormalities, significant improvement in renal and pulmonary pathology, and preservation of renal function in active immune AAV models Attenuation of murine lupus nephritis exacerbated by IR-induced AKI	188 189
Btk	(preclinical)	Suppression of MPO and anti-MPO-IC-induced NET formation *in vitro* and amelioration of experimental AAV	180
CatC	(preclinical)	Inhibition of MPO-ANCA-induced NET formation *in vitro* and amelioration of experimental AAV	181

AAV, ANCA-associated vasculitis; AKI, acute kidney injury; ANCA, anti-neutrophil cytoplasmic autoantibody; Btk, Bruton's tyrosine kinase; CatC, cathepsin C; CypD, cyclophilin D; GCs, glucocorticoids; IC, immune complex; Idh2, isocitrate dehydrogenase 2; IR, ischaemia–reperfusion; MPO, myeloperoxidase; NCGN, necrotizing and crescentic glomerulopnephrits; NET, neutrophil extracellular trap; NZM, New Zealand mixed 2328; PAD, peptidylarginine deiminase; ROS, reactive oxygen species; Syk, spleen tyrosine kinase.

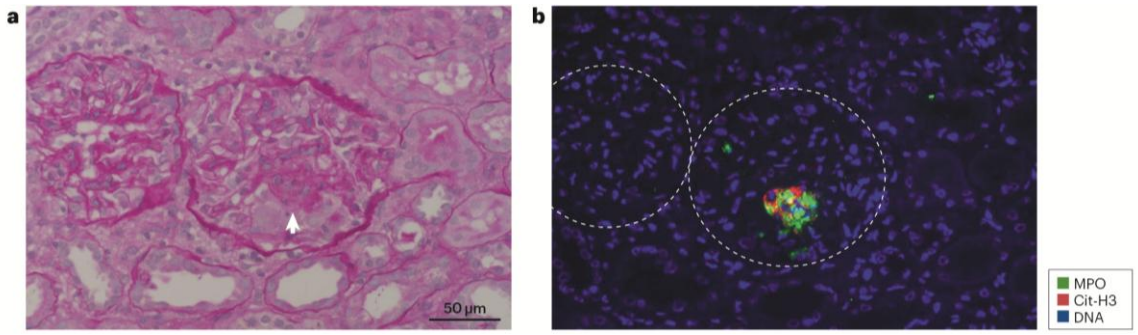


Fig. 1. Deposition of PAD4-related NETs in the glomerulus

Staining of a kidney biopsy specimen from an individual diagnosed with myeloperoxidase (MPO) specific–anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (MPO–AAV) to identify neutrophil extracellular traps (NETs). A| Periodic acid-Schiff staining and the arrowhead indicates an area of segmental necrosis. B| NETs can be detected in the area of glomerulosclerosis, as evidenced by positive staining for MPO and the peptidylarginine deiminase 4 (PAD4) product citrullinated histone H3 (Cit-H3). The dashed lines demarcate glomeruli. Scale bar, 50 µm.

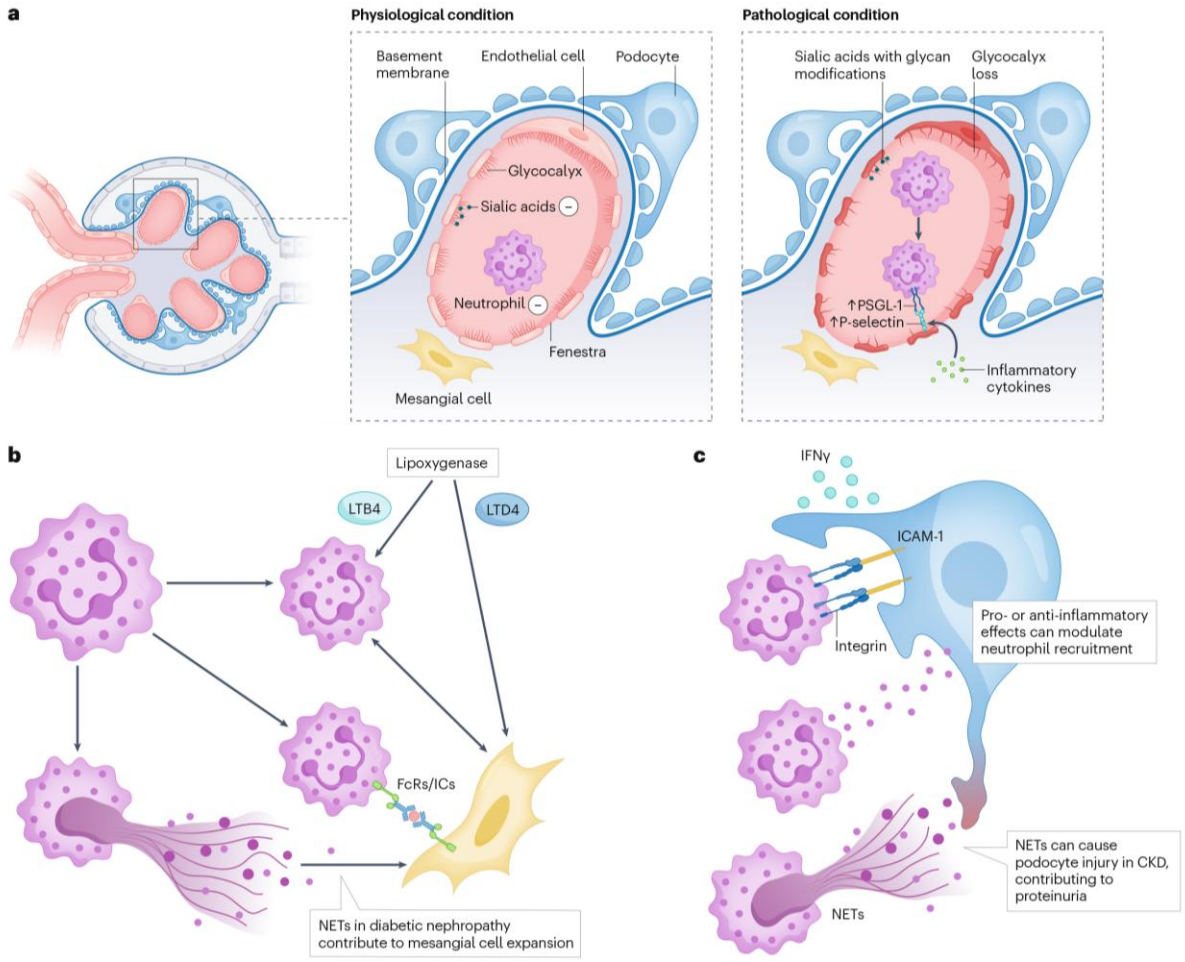


Fig. 2. Crosstalk between neutrophils and kidney parenchymal cells

A| Neutrophil infiltration into the glomerular capillary. Glomerular endothelial cells contain fenestrae measuring approximately 70–100 nm through which neutrophils cannot typically pass owing to the size restriction and charge barriers. However, under pathological conditions such as crescentic glomerulonephritis (for example, anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV)), the integrity of the capillary wall becomes compromised, resulting in leukocyturia. The interactions between P-selectin and E-selectin on glomerular endothelial cells and glycoproteins such as P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils might facilitate neutrophil passage from the blood into the kidney tissue. B| Interaction with mesangial cells. Once neutrophils cross the glomerular endothelial barrier, they interact with mesangial cells. Leukotriene B4 (LTB4) acts on neutrophils, whereas LTD4 targets mesangial cells, promoting these interactions. In addition, neutrophils and mesangial cells also interact through Fcγ receptors (FcγRs) and immune complexes (ICs). Neutrophil extracellular traps (NETs) further contribute to mesangial expansion. C| Interaction with podocytes. After neutrophils pass through the glomerular basement membrane, they might contact podocytes. IFNγ induces the expression of intercellular adhesion molecule-1 (ICAM-1) on podocytes, which could bind to integrins expressed on neutrophils. Neutrophil granules

damage the podocyte cytoskeleton and NETs can further contribute to podocyte injury, causing proteinuria. Although podocytes can release cytokines that enhance neutrophil chemotaxis they can also exert anti-inflammatory effects by modulating IL-6 signaling and reducing neutrophil recruitment.

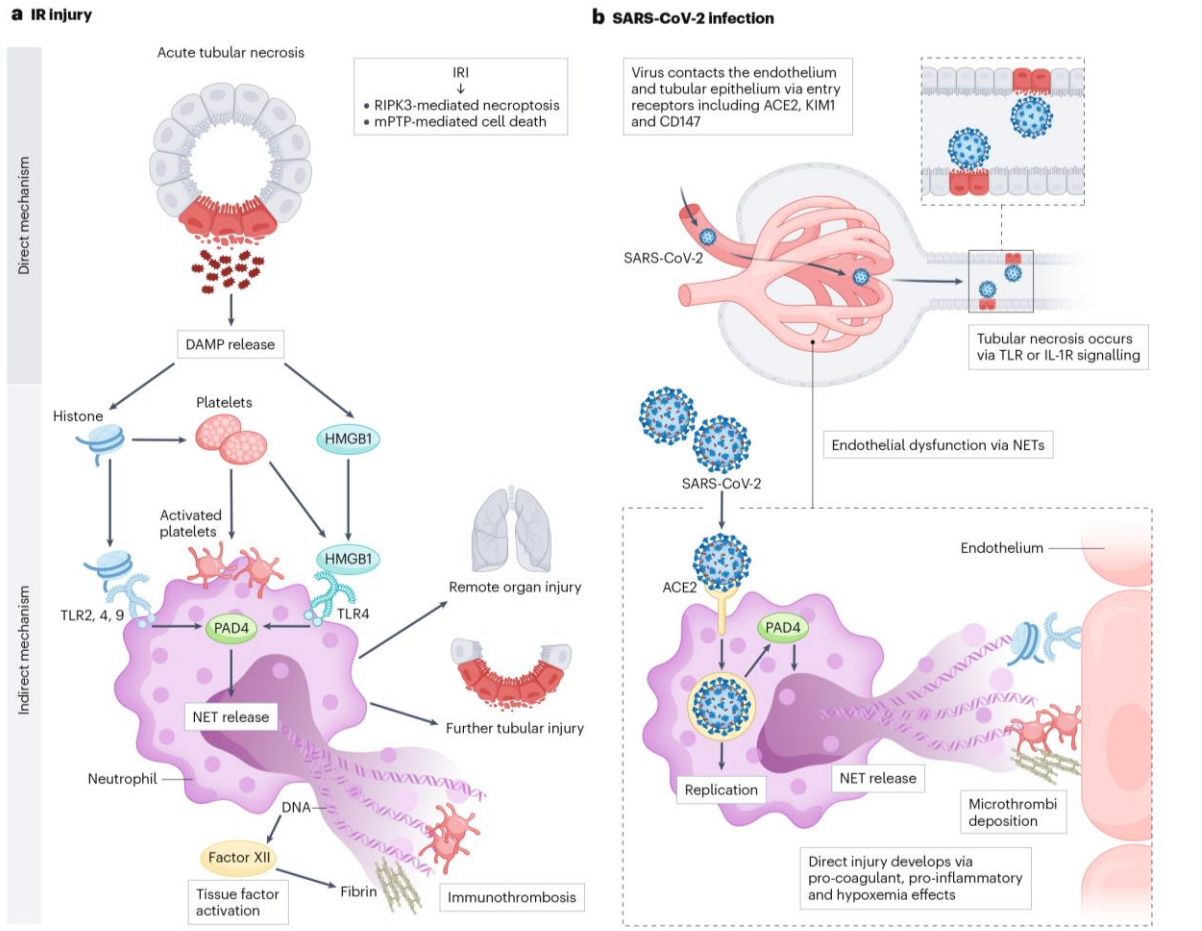
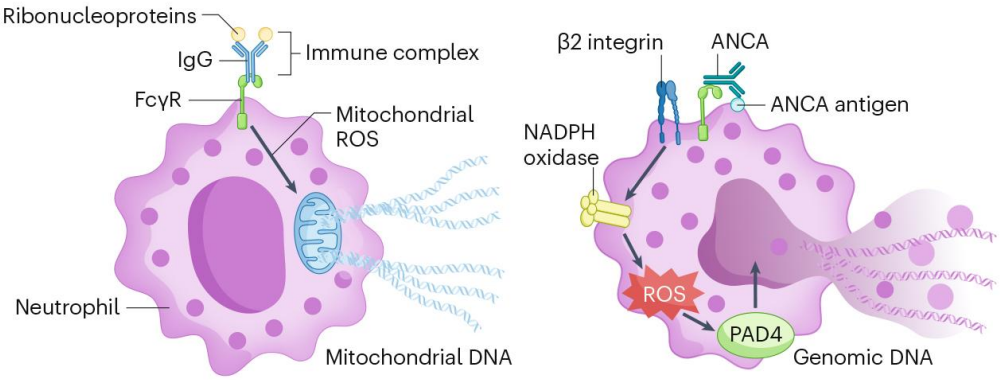


Fig. 3. Role of NETs in AKI

A| In kidney ischaemia–reperfusion injury (IRI), tubular epithelial cells initially undergo programmed necrosis (necroptosis) through pathways involving the receptor-interacting protein kinase 3 (RIPK3) and cyclophilin D (CypD) in response to hypoxia. Subsequently, damage-associated molecular patterns (DAMPs) such as histones and high mobility group protein B1 (HMGB1) released from the necrotic tubules induce neutrophil extracellular traps (NETs) in a peptidylarginine deiminase 4 (PAD4)-dependent manner, leading to further tubular disorders and distant organ failure such as acute respiratory distress syndrome. B| In COVID-19, SARS-CoV-2 might directly infect or injure glomerular endothelial and renal tubular epithelial cells via angiotensin-converting enzyme 2 (ACE2). Consequently, tubular necrosis occurs via Toll-like receptor (TLR) and IL-1 receptor (IL-1R) signaling pathways. Moreover, SARS-CoV-2 infects or activates neutrophils via ACE2, inducing NETs in a PAD4-dependent manner. These NETs can result in glomerular endothelial dysfunction and intravascular coagulation, ultimately contributing to the development of acute kidney injury (AKI). mPTP, mitochondrial permeability transition pore.



Disease	SLE	AAV
Neutrophil subset	LDG	NDG
Trigger	Immune complexes containing ribonucleoproteins	ANCA binding to cognate antigen
ROS production	Mitochondria	NADPH oxidase
Extracellular DNA	Mitochondrial DNA (predominantly)	Genomic DNA
NET formation	Non-lytic (vital)	Lytic (suicidal)
Effect on pathology	• Chronic inflammation • Anti-dsDNA antibody production	• Vascular endothelial injury • ANCA production

Fig. 4. Formation of NETs in SLE and AAV

In systemic lupus erythematosus (SLE), immune complexes containing ribonucleoproteins bind to Fcγ receptors (FcγRs) on the plasma membrane of low-density granulocytes (LDGs), which mainly release mitochondrial DNA under the influence of mitochondrial reactive oxygen species (ROS). This release leads to non-lytic (vital) neutrophil extracellular trap (NET) formation, which contributes to chronic inflammation by promoting the expression of inflammatory cytokines and genes related to type I interferon (IFN) signaling, as well as the production of anti-double stranded DNA (dsDNA) antibodies. By contrast, in anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV), ANCAs bind to their respective antigens expressed on the plasma membrane of normal-density granulocytes (NDGs), while the Fc portion of ANCA binds to FcγRs. FcγRs and the bystander β2 integrin cooperate to induce ROS production under the action of NADPH oxidase. These ROS promote the translocation of peptidylarginine deiminase 4 (PAD4) from the cytoplasm to the nucleus, where genomic DNA is decondensed by PAD4 and finally released outside the cell, resulting in lytic (suicidal) NET formation. This process contributes to vascular endothelial injury and ANCA production, driving an ANCA–NET vicious cycle.

BOX 1. Neutrophil activation

Neutrophils express a large number of cell-surface receptors crucial for recognizing pathogens and sensing inflammatory environments. These receptors include TLRs and C-type lectins, various cytokine and complement receptors, G-protein-coupled chemokine and chemoattractant receptors, and Fc receptors such as Fcγ receptors (FcγRs). In addition, neutrophils express adhesion receptors such as selectins, selectin ligands and integrins. Binding of these receptors initiates diverse signal transduction pathways, triggering a variety of immune reactions.

TLRs and C-type lectins

TLRs and C-type lectins recognize damage-associated molecular patterns (DAMPs) derived from injured cells and pathogen-associated molecular patterns (PAMPs), which induce the activation of mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) pathways.

Cytokine and complement receptors

Cytokine and complement receptors also activate the MAPK and NF-κB pathways. In addition, they stimulate the Janus kinase (JAK)–signal transducers and activators of transcription (STAT) signaling cascade and, in some cases, activate caspases that induce apoptosis.

G-protein-coupled receptors

G-protein-coupled receptors transmit parallel signals through phospholipase Cβ, leading to increased cytoplasmic Ca^{2+} concentrations and activation of protein kinase C. They also activate phosphatidylinositol 3-kinase (PI3K), which drives the AKT–extracellular signal-regulated kinase (ERK) pathways.

FcγRs and adhesion molecules

FcγRs bind to the Fc region of immunoglobulins, whereas adhesion molecules mediate cell–cell and cell–matrix interactions. When engaged by their respective ligands, these receptors initiate spleen tyrosine kinase (SYK)-mediated signaling cascades, involving downstream activation of Bruton's tyrosine kinase (BTK) and activating PI3K, MAPK and NF-κB signaling pathways.