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Role of Neutrophils Extracellular Traps in Acute kidney injury

Neama Ahmed Abd El-Aziz[1], Hesham Abd El Halim[2], Hesham Kamal Habeeb Keryakos[3], Amel Mahmoud Kamal El-din[4]

[1]Assistant Lecturer of Internal Medicine, Faculty of Medicine, Suez University.
[2]Professor of Internal Medicine, Faculty of Medicine, Minia University.
[3]Assistant Professor of Internal Medicine, Faculty of Medicine, Minia University.
[4]Professor of clinical pathology, Faculty of Medicine, Minia University.

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ABSTRACT
Acute kidney injury (AKI) results in the abrupt loss of kidney function, leading to the retention of waste products, electrolyte disturbances, and volume status changes. The term AKI has replaced acute renal failure because smaller changes in kidney function without overt failure can result in significant clinical consequences and increased morbidity and mortality. Changes in kidney function are detected by a change in biomarkers, the most common biomarker being serum creatinine (SCr). Serum creatinine is an imperfect biomarker for recognizing AKI, given that an increase in SCr often lags (48–72 hours) behind the onset of injury. So, we are searching for a new biomarker for AKI as Uncontrolled inflammatory and immune responses are often involved in the development of acute and chronic forms of renal injury. Neutrophils are innate immune cells recruited early to sites of inflammation, where they produce pro-inflammatory cytokines and release mesh-like structures comprised of DNA and granular proteins known as neutrophil extracellular traps (NETs), which are potentially toxic, contribute to glomerular injury, activate autoimmune processes, induce vascular damage, and promote kidney fibrosis. Evidence from multiple studies suggests that an imbalance between production and clearance of NETs is detrimental for renal health. So many studies aimed at modulating NET-associated processes could have a therapeutic impact on a plenty of inflammatory diseases that target the kidney. In this article we summarize the role of NETs in the pathogenesis of acute kidney injury and their mechanisms of tissue damage.

Keywords: kidney; AKI; ischemia, Autoimmunity; Neutrophils; NETosis; NETs.

INTRODUCTION

Mehta et al.,**(1)** defining AKI as an increase in the serum creatinine level of 0.3 mg/dl (26.5 mmol/litre) or more within 48 hours.serum creatinine level that has increased by at least 1.5 times the baseline value within the previous 7 days, urine volume of less than 0.5 ml per kilogram of body weight per hour for 6 hours.
In 2004, the Acute Dialysis Quality Initiative published the risk, injury, failure, loss, end-stage (RIFLE) criteria. The RIFLE classification isbased on changes in two markers: SCr and urinary output. The classification includes three graded stages of AKI – risk, injury, and failure – with two outcomes: loss of kidney function greater than 4 weeks and end-stage renal disease greater than 3 months **(2).**
The RIFLE-defined period for change in SCr or urinary output was 7 days.Two significant concerns were observed by clinicians and investigators following the implementation of the RIFLE categorization. First, patients who had AKI by SCr had worse outcomes than those who got AKI by urine output criteria. Second, the specified change in GFR (i.e., a 50% increase) did not correspond to the specified change in SCr value.

RIFLE Classification

	RIFLE		**AKIN**	**RIFLE/AKIN**
Category	**SCr or ↓ GFR**	**Stage**	**Increase in SCr**	**Urinary Output Change**
Risk	1.5-fold ↑ SCr 25% ↓ GFR	1	1.5- to 1.9-fold ↑ SCr or ↑ SCr ≥ 0.3 mg/dL	< 0.5 mL/kg/hr for 6–12 hr

Injury	2-fold ↑ SCr 50% ↓ GFR	2	2- to 2.9-fold ↑ SCr	< 0.5 mL/kg/hr for ≥ 12 hr
Failure	3-fold ↑ SCr or SCr> 4 mg/dL with acute risk > 0.5 mg/dL or 75% ↓ GFR	3	3-fold SCr or SCr> 4 mg/dL with acute risk > 0.5 mg/dL or RRT	< 0.3 mL/kg/hr for ≥ 24 hr or anuria for ≥ 12 h

AKI = acute kidney injury; RRT = renal replacement therapy.
Information from:**(3).**

Biomarkers

Serum creatinine is a well-known marker of kidney function and not a sensitive kidney injury marker, given that it may lag 48–72 hours from the time of injury. In the definition and staging of AKI, kidney damage biomarkers will probably take the role of SCr and are required to enhance AKI detection. Kidney damage biomarkers, such as tissue inhibitor of metalloproteinase-2 (TIMP-2), liver-type fatty acid binding protein (L-FABP), insulin-like growth factor binding protein 7 (IGFBP-7), neutrophil gelatinase-associated lipocalin (NGAL), interleukin (IL)-18, and kidney injury molecule-1 (KIM-1), may be increased prior to an increase in SCr, improving the detection of kidney damage without functional change **(4,5).**

Damage and functional biomarkers working together could improve AKI detection, differential diagnosis, and treatment.KIM-1, the biomarker, is a type 1 transmembrane protein that is not highly expressed in healthy kidneys. It contributes to the phagocytosis of apoptotic cells and debris and is up-regulated during ischemia injury. Renal healing and remodeling are affected by this. After controlling for other variables, a 1-unit rise in normalized KIM-1 was linked to an OR of 12.4 (95% CI, 1.2–119) for the occurrence of acute tubular necrosis (ATN), demonstrating the specificity of this biomarker **(6)**.

This biomarker may aid in the subsequent adjudication of drug-induced kidney damage and serves as a diagnostic discriminator.

The 25-kDa protein known as neutrophil gelatinase-associated lipocalin belongs to the lipocalin family. It is found in the urine three hours after damage, peaks six hours after injury, and is up-regulated following ischemia or nephrotoxic AKI. Through improved renal tubule cell proliferation and apoptosis inhibition, NGAL reduces kidney damage. Urine and plasma NGAL concentrations peaked six hours after heart surgery in the TRIBE-AKI research, which involved 1219 adults **(7)**. Higher mortality, longer hospital stays, and a higher risk of AKI were linked to elevated postoperative concentrations (within 6 hours of arrival in the intensive care unit). Patients who may be more susceptible to kidney damage can be identified by neutrophil gelatinase-associated lipocalin.

Future research must ascertain how earlyInterleukin-18 is a pro-inflammatory cytokine formed in the proximal tubule. Urine IL-18 concentrations are elevated within the first 6 hours post-AKI and peak 12–18 hours post-injury. Interleukin-18 plays a role in the inflammation that exacerbates tubular necrosis. Elevated urinary concentration of IL-18 post-cardiac surgery is an early marker of AKI and an independent predictor of dialysis or death in critically ill patients **(7).**

A 14-kDa protein found in the proximal tubule, liver-type fatty acid binding protein is a sign of renal hypoxia. L-FABP can identify AKI and predict the need for RRT and in-hospital mortality in patients at risk for AKI, according to a meta-analysis **(8).**

In Japan, liver-type fatty acid binding protein is authorized as a tubular biomarker to help detect AKI in severely ill patients before their SCr rises. One mechanism linked to the pathogenesis of AKI is the induction of cell cycle arrest by insulin-like growth factor binding protein 7 and TIMP-2. When combined, these biomarkers predicted stage 2 or stage 3 AKI with an AUC of 0.8, which is better than existing biomarker prediction models. Future studies will delineate the most appropriate biomarker for risk assessment, differential diagnosis, and causality assessment and prognosis. The TRIBE-AKI consortium study data have provided preliminary data on the biomarker concentration ranges in various subpopulations and preliminary information on the ability to predict AKI (**9,10,11**). However, the relationship between biomarker changes to mechanisms of injury over time requires delineation to best assess use.

RISK FACTORS

Risk factors associated with acute kidney injury (AKI) encompass several elements, including advanced age, pre-existing comorbidities, proteinuria, exposure to nephrotoxins, significant surgical procedures, sepsis, fluid resuscitation, and overall volume status. While the likelihood of developing AKI increases with age, it is noteworthy that older individuals are less frequently candidates for renal replacement therapy **(12)**.

Comorbidities such as chronic kidney disease (CKD), diabetes mellitus, hypertension, coronary artery disease, heart failure, liver dysfunction, and chronic obstructive pulmonary disease contribute to the risk of AKI.

Additionally, the presence of proteinuria, particularly in patients with a glomerular filtration rate (GFR) exceeding 60 mL/min/1.73 m² or an elevated urinary albumin-to-creatinine ratio, has been linked to a heightened risk of AKI. Hospitalized patients, particularly those in critical condition, frequently encounter exposure to various nephrotoxins and contrast agents. This patient demographic commonly receives medications such as antimicrobials, non-steroidal anti-inflammatory drugs (NSAIDs), and proton pump inhibitors. The incidence of acute kidney injury (AKI) is notably higher following cardiac surgeries, while it occurs less frequently in patients undergoing non-cardiac procedures. Sepsis is a prevalent risk factor for the onset of AKI, and the occurrence of AKI significantly heightens the risk of mortality. The selection of fluids for resuscitation may contribute to the risk of developing AKI, as hydroxyethyl starch has been linked to a greater likelihood of AKI compared to crystalloids **(13)**. Additionally, high-volume resuscitation with crystalloids poses a higher risk of AKI than balanced salt solutions, primarily due to the harmful effects associated with chloride loading. Furthermore, fluid overload and interventions aimed at managing volume overload can exacerbate the risk of AKI.

Introducing a new biomarker

Neutrophils are the first cellular effectors to be recruited at sites of infection and tissue damage; where they play a significant role in inflammation, immune cell recruitment, pathogen clearance, and tissue repair. Neutrophils execute their functions through four main mechanisms: phagocytosis, degranulation, cytokine production, and neutrophil extracellular trap (NET) formation (**14,15**).

A successful host defense requires efficient neutrophil activation that, in the case of sterile inflammation, is triggered by danger signals known as damage-associated molecular patterns (DAMPs). The innate immune system recognizes DAMPs after their release from damaged cells, or due to chemical andproteolytic modifications secondary to tissue injury. In the kidney, neutrophil infiltration and DAMPsreleased by necrotic renal cells amplify intrarenal inflammation and tissue damage(**16,17).**

DAMPs alsoenhance tubular injury by stimulating neutrophil receptors to induce activation of the enzyme peptidylarginine deiminase 4 (PAD4), chromatin decondensation, and NET formation (**18).** NETs are also asource of autoantigens that induce production of anti-neutrophil cytoplasmic antibodies (ANCAs).

Neutrophil Extracellular Traps

NETs are intricate extracellular networks composed of DNA, histones, and proteins sourced from polymorphonuclear granules. These structures serve to localize immune effectors and assist in restricting the spread of pathogens. Among the molecular components that enhance the microbicidal capabilities of NETs are neutrophil elastase (NE), myeloperoxidase (MPO), cathepsin G, lactoferrin, pentraxin 3, gelatinase, proteinase 3, and peptidoglycan binding proteins. Notably, NE and cathepsin G are also involved in the processing of interleukin 1 and interleukin 36, highlighting the significance of NET formation in the activation of cytokines and the inflammatory response (**19**).

Prior to the release of NETs into the extracellular environment, several critical changes occur, including the disruption of the nuclear membrane,decondensation of cytoplasmic chromatin, and rupture of the plasma membrane. In this context, NETs are produced from dying neutrophils through a distinct cell death mechanism known as NETosis, which is separate from both apoptosis and necrosis. In the case of suicidal NETosis, the release of NETs results in cell death**(20).**

Conversely, vital NETosis allows for the formation of NETs while preserving cell viability. The specific type of NETosis that neutrophils undergo is largely influenced by the molecular characteristics of the stimuli encountered**(21).**

Recent proteomic studies have shown that stimulation of neutrophils with phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, or Escherichia coli lipopolysaccharide leads to the generation of NETs with varying protein compositions and post-translational modifications, which likely correlate with their biological roles**(22).**

Vital NETosis, in contrast, is characterized by the absence of plasma membrane damage or cellular lysis, as the release of NETs occurs via the budding of nuclear vesicles containing DNA. Neutrophils that experience vital NETosis become anuclear while preserving the integrity of their plasma membrane, motility, and the capacity for phagocytosis. The activation of toll-like receptors (TLRs) and complement factor 3 serves as partial triggers for this process. Unlike suicidal NETosis, vital NETosis is a more rapid and predominantly oxidant-independent pathway **(23).**

Nonetheless, recent findings have identified a reactive oxygen species (ROS)-dependent mechanism that involves mitochondrial DNA, which leads to vital NETosisin neutrophils that have been primed with granulocyte-macrophage colony-stimulating factor and subsequently stimulated with lipopolysaccharide **(24).**

Autophagy, a fundamental catabolic process that protects cells from damage during stress and cytotoxic challenges, also plays a role in regulating NET formation. However, studies examining the impact of autophagy inhibitors and activators on NET formation have yielded conflicting results. Research conducted by Itakura A. et

al. and Park S.Y. et al. demonstrated that the induction of autophagy in neutrophils through rapamycin is sufficient to trigger NET formation, even in the absence of additional priming factors. Conversely, McInturff A.M. et al. reported that rapamycin diminishes LPS-dependent NET formation. Additionally, evidence suggests that mTOR-dependent regulation of NET formation occurs through post-transcriptional modulation of hypoxia-inducible factor 1 alpha expression. Furthermore, the application of wortmannin, a phosphatidylinositol 3-kinase inhibitor that disrupts autophagosome formation, results in neutrophil apoptosis rather than NETosis in response to PMA and lipopolysaccharide (**25**).

Despite recent progress, the signaling mechanisms governing NET formation remain largely uncharacterized. Further research is essential to elucidate the distinct molecular pathways that regulate NETosis and their implications for neutrophil-mediated biological functions in both health and disease **(26).**

Neutrophil Extracellular Traps in Renal Disease

Acute Kidney Injury

Acute kidney injury (AKI) is a prevalent reason for nephrology consultations and is associated with significant mortality. It is defined by a swift reduction in glomerular filtration rate, which is linked to diminished renal blood flow, inflammation, or nephrotoxic effects**(27).**

The pathological manifestations of AKI typically include injured renal tubules, impaired renal vasculature, heightened inflammation, and infiltration of immune cells**(28-30).**

While neutrophils are recognized as key players in pro-inflammatory responses, the precise mechanisms by which they contribute to AKI remain a topic of discussion. Recent findings suggest that the release of neutrophil extracellular traps (NETs) plays a role in the development of AKI, particularly following ischemia-reperfusion injury and hemolytic uremic syndrome**(28-33).**

Ischemic AKI leads to increased levels of circulating and localized NETs and histones, along with elevated expression of PAD4 in the affected kidneys**(34-36).** Research by Raup-Konsavage W.M. et al. has shown that cells expressing PAD4 are predominantly neutrophils that cluster in peritubular capillaries, interstitial spaces, and renal tubules following ischemia-reperfusion injury**(30,31**). NETs are implicated in inducing death of tubular epithelial cells, facilitating clot formation in peritubular capillaries through interactions between platelets and neutrophils, and priming additional neutrophils for NETosis**(18,32).** These processes contribute to sustained hypoxia and exacerbate tissue injury. Notably, inhibiting PAD4 through pharmacological or genetic methods has been shown to confer protection against AKI in animal models by reducing inflammation and NET formation. Additionally, the degradation of NETs using DNase I or anti-histone IgG has been found to mitigate renal injury, highlighting the critical role of NET formation in the pathogenesis of ischemic AKI**(18,31).** Furthermore, tubular necrosis and NET formation can lead to dysfunction in remote organs, a frequent complication of severe AKI, due to the release of circulating histones and cytokines**(18).**

In HUS, a vascular disease caused by the Shiga toxin of enterohemorrhagic bacteria, neutrophils mediate an inflammatory response that is essential for the progression of hemolytic anemia, thrombocytopenia, and acute renal failure characteristic of the disease. NETs promote renal failure during HUS by contributing to the inflammatory response and thrombosis in the microvasculature. Furthermore, plasma from HUS patients contains increased levels of circulating cell free-DNA and nucleosomes compared to healthy subjects **(33).**

Neutrophils from HUS patients also exhibit a higher capacity to undergo spontaneous NETosis.

Lupus Nephritis

NETosis is a pathogenic feature of Systemic Lupus Erythematosus (SLE) frequently associated with active periods of the disease and production of autoantibodies**(37).** Remarkably, defective NET removal in the sera of SLE patients correlates with signs of active nephritis such as proteinuria, decreased albumin levels, and lower creatinine clearance rates (**38,39**).

Neutrophils from patients with SLE spontaneously generate NETs that are more oxidized and immunogenic than those released by neutrophils from healthy subjects **(40).**

Moreover, high levels of NETs in the skin, kidney, and bone marrow reinforce their direct role in SLE-associated organ dysfunction**(41).**

The presence of NETs in renal biopsies from SLE patients with nephritis further supports their contribution to kidney damage **(42).**

DNase I mutations are present in some patients with SLE and could partially explain NET persistence in the sera of these patients **(43).** Apoptotic or necrotic material from NETs is considered the primary source of self-antigens responsible for promoting an autoimmune state and exacerbating tissue injury **(44).** Autoantibodies contribute to NET persistence by blocking their degradation and increasing complement activation, whereas the presence of NET components and autoantibodies directed against them tightly reflects SLE activity **(45,46).**

Immune complexes binding matrix metalloproteinase 2, a NET component, enhance NET release and matrix metalloproteinase 2 activity in serum from SLE patients [47]. Similarly, the LL37 peptide found in NETs associates with the immunogenic response to cell-free DNA in these patients **(48).**

Citrullinated histone H1 and anti-citrullinated H1 autoantibodies present in SLE patients also correlate with disease activity **(49).**

Additionally, SLE patients possess a distinct neutrophil subpopulation known as low-density granulocytes **(50).** Low-density granulocytes are prone to pro-inflammatory cytokine release and spontaneous NET formation. These cells stimulate the production of interferon alpha (IFN-α), a central factor in the development of SLE pathogenesis, in plasmacytoid dendritic cells (pDCs) **(48,51)**. DNA and NET-associated proteins released by low-density granulocytes activate TLR9 in pDCs to induce IFN-α production **(48).** Moreover, neutrophils on NETosis alone produce IFN-α in response to circulating chromatin, LL37, and high mobility group box 1 **(52,53).** IFN-α is cytotoxic to endothelial cells as it can disrupt endothelial differentiation and angiogenesis **(50).** Therefore, IFN-α-mediated effects on mature endothelial cells and their precursors are harmful to the renal vasculature during SLE flares. The concerted activity of neutrophils and pDCs amplify immune dysregulation, inflammation, and tissue damage via IFNα **(51).** Besides their effects over pDCs, NET components also activate B cells, and in return, anti-DNA complexes can further boost the activity of pDCs and B cells, creating a detrimental autoimmune cycle **(54).**

Additionally, excessive NET production by SLE neutrophils is linked to hypoxia, upregulation of the stress-response protein DNA Damage Inducible Transcript 4 (DDIT4/REDD1), and autophagy **(55).** NETs released by active SLE neutrophils are rich in tissue factor and interleukin 17A; molecules that promote thrombosis, inflammation, tissue injury, and fibrosis in target organs including the kidney. In patients with lupus nephritis Frangou E. et al. observed NETs comprised of tissue factor within glomeruli and the tubulointerstitial compartment in proximity to the Bowman's capsule; suggesting a possible role for these NETs in capsule disruption and crescent formation **(55).**

Small Vessel and ANCA-Associated Vasculitis

ANCAs against MPO and proteinase 3 are diagnostic tools in small vessel vasculitis (SVV), a systemic autoimmune disease. Both autoantigens are NET components and participate in a harmful feedback circuit responsible for increased cell adhesion, complement activation, and NET production **(56,57).**Blood samples of SVV patients have higher levels of MPO and DNA; whereas NETs are associated with strong neutrophilic infiltrates in biopsies from patients with active disease **(58).**Additionally, patients with active SVV, present high serum levels of IFN-α, a cytokine related to pDCs activated by NET components **(58).**Although studies to determine the longitudinal relationship of NETs with SVV are still on the works, it is evident that patients with active SVV have higher levels of circulating NETs and an increased propensity to polymorphonuclear cell death in comparison with patients in remission and healthy controls **(59).**

ANCA-associated vasculitis (AAV) belongs to a group of immune vasculitides defined by necrotizing inflammation of small vessels and circulating ANCAs **(60).** Although neutrophils from patients with AAV are less likely to undergo apoptosis, they exhibit spontaneous NET formation **(61).** As in SLE, there is evidence of NETs in kidney biopsies of AVV patients **(58,62).** AAV comprise three diseases: granulomatosis with polyangiitis, microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis **(63).** MPA is an ANCA-associated pathology that affects small vessels, particularly in renal glomeruli **(64).** NET components, notably MPO, act as autoantibodies that increase circulating ANCAs and promote the subsequent development of MPA **(65).** Conversely, immunoglobulins from MPA patients further induce NET release, and their ability to do so correlates with ANCAs affinity for MPO. Additionally, DNAse I activity is lower in sera from MPA patients, a feature that impairs adequate NET degradation **(66).** NET release and persistence are also responsible for the renal damage present in up to 90% of the patients with MPA **(61).** Although it has been largely assumed that ANCAs induce NET formation in neutrophils, Kraaij T. et al. recently demonstrated that uncontrolled NET formation in AAV patients is independent of ANCA levels in serum **(62).** Their findings also indicate that NETosis is higher in patients positive for MPO-ANCAs rather than PR3-ANCAs which suggests that neutrophils and NETs might have different roles in granulomatosis with polyangiitis versus MPA pathogenesis. Moreover, excessive NETosis in patients with AAV associates with active clinical disease instead of severe infection; highlighting the role of NETs in autoimmunity **(62).**

Mechanisms of NET-Associated Tissue Injury

Although the release of neutrophil extracellular traps (NETs) serves as a legitimate innate defense mechanism, the components of NETs lack specificity, and their actions may inadvertently lead to tissue damage and the development of autoimmunity. As a result, an imbalance in the production or clearance of NETs can have harmful effects on renal function. Recent research indicates that NETs may possess toxic properties, with their presence in the kidneys contributing to glomerular injury**(28).** Neutrophil-derived enzymes can directly trigger apoptosis in endothelial cells and degrade the basement membrane, while tubulointerstitial damage can diminish glomerular blood flow, fostering a NETotic environment. Additionally, epithelial tubular cells respond to hypoxia and kidney injury by releasing histones, which activate neutrophils to produce more NETs, perpetuating a pro-inflammatory cycle that further damages endothelial cells. Indirectly, NETs can exacerbate vascular injury

by activating the alternative complement pathway and contribute to kidney fibrosis through the induction of endothelial to mesenchymal transition (EndMT)**(67).**

Therapeutic Interventions

The mechanisms associated with NETs that lead to tissue injury may play a significant role in reducing the severity of renal diseases. Given that the composition of NETs and their release mechanisms are influenced by the distinct pathogenesis of each disease, a universal therapeutic approach may not be effective in preventing NET formation while preserving the neutrophil-mediated immune response against infections. Therefore, it is essential to tailor therapeutic strategies to the specific type of disease. Currently, the therapeutic potential of novel selective inhibitors of PAD4, an enzyme essential for protein citrullination and NET formation, is under investigation in preclinical models related to cancer-associated kidney injury and autoimmune diseases. It is anticipated that the effectiveness of several of these compounds will soon be evaluated in phase I/II clinical trials. The formation of NETs and the production of ANCA are also relevant factors in this context. Furthermore, the administration of PAD4 inhibitors such as 2-chloramidine, YW3-56, and GSK484 prior to ischemia-reperfusion or in animal models of cancer-related kidney injury has been shown to significantly reduce renal damage, necrosis, congestion, and systemic inflammation. Despite these advancements, the ongoing development of more potent, effective, and safe PAD4-specific inhibitors for clinical application is vital to bridge the gap between experimental findings in animal models and their translation to human subjects.In addition to PAD4 inhibitors, the impacts of the platelet inhibitor clopidogrel and the neutrophil elastase inhibitor sivelestat have been examined both in vitro and in vivo. Clopidogrel-mediated platelet inhibition has been shown to diminish NET formation and mitigate kidney injury following ischemia-reperfusion in murine models. Conversely, sivelestat has been observed to lower proteinuria, creatinine levels, and glomerular damage in a rat model of nephritis. Furthermore, inhibitors that target nitric oxide production and oxidative stress have also been found to reduce NET release, indicating their potential utility in alleviating NET-related renal damage in vivo.The dysregulation of interferon (IFN) production is affected by NET release and the subsequent activation of plasmacytoid dendritic cells (pDCs). Rontalizumab, a humanized IgG monoclonal antibody that neutralizes IFN-α, was administered to lupus patients in a phase one clinical trial, which confirmed the antibody's safety profile. In the context of systemic lupus erythematosus (SLE) and lupus nephritis, NET degradation, rather than formation, plays a critical role in disease pathogenesis. In experimental SLE models, treatment with DNase I and dexamethasone resulted in reduced proteinuria and serum creatinine levels, alongside improved renal histopathology. Therefore, therapeutic strategies aimed at enhancing NET degradation, such as DNase I, warrant further investigation to mitigate NET-related tissue damage in lupus nephritis and acute kidney injury (AKI). Additionally, intravenous human sulfo-immunoglobulin (IVIG-S) therapy, derived from γ-globulins of healthy blood donors, has been shown to decrease NET formation both in vitro and in vivo, as well as reduce ANCA-MPO titers and the development of ANCA-associated vasculitis (AAV) in rat models. While IVIG therapy has been effectively utilized in treating autoimmune diseases, agammaglobulinemia, and severe infections in humans, further prospective clinical trials are necessary to establish its efficacy in patients with AAV.

CONCLUSIONS

The domain of NETosis has experienced significant expansion since 2004, when Brinkmann V. et al. characterized NETs as extracellular structures composed of chromatin and granule proteins released by neutrophils, which possess the capability to bind bacteria. Since that time, NETosis has been the subject of extensive investigation in vitro, in various animal models, and in human diseases. While the findings from these studies vary widely, it has become clear that NETosis transcends a mere host-defense mechanism (**68**). It is implicated in pathophysiological conditions linked to sterile inflammation and autoimmunity, particularly in its role in targeting the kidneys, thereby contributing to both acute and chronic renal injuries. Mechanisms associated with NETs are involved in conditions such as acute kidney injury (AKI), hemolytic uremic syndrome (HUS), lupus nephritis, small vessel vasculitis (SVV), and ANCA-associated vasculitis (AAV)**(45).** However, the processes governing NET formation and clearance remain incompletely understood. Furthermore, the modulation of neutrophils and NETosis in less thoroughly investigated pathological states, such as chronic kidney disease and renal transplantation, warrants further exploration. Consequently, there is an urgent need for studies that examine how factors such as dialysis, transplantation, immunosuppression, and elevated levels of donor-specific antibodies influence neutrophil biology and their potential to cause NET-mediated tissue damage (**69**). The formation of neutrophil extracellular traps (NETs) represents a significant therapeutic target in the treatment of various human disorders, particularly renal diseases. Gaining insights into the molecular mechanisms that govern NETosis and its interplay with apoptosis will facilitate the development of customized therapeutic approaches, thereby minimizing the potential for adverse side effects. Additionally, it is crucial to consider the interactions between neutrophils, NETs, and other cell types, including platelets and endothelial cells, as this crosstalk influences biological outcomes in both healthy and diseased states.

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