Netting Gut Disease: Neutrophil Extracellular Trap in Intestinal Pathology

Kai Chen,1,2 Li-Hua Shao,1 Feng Wang,1 Xiao-Fei Shen,1 Xue-Feng Xia,1 Xing Kang,1 Peng Song,1 Meng Wang,1 Xiao-Feng Lu,1 Chao Wang,1 Qiong-Yuan Hu,1,2 Song Liu,1 and Wen-Xian Guan1

1Department of Gastrointestinal Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China
2Medical School of Nanjing University, Nanjing, China

Correspondence should be addressed to Qiong-Yuan Hu; qiongyuan_hu@foxmail.com, Song Liu; medical.lis@gmail.com, and Wen-Xian Guan; guan_wenxian@sina.com

Received 11 January 2021; Revised 4 July 2021; Accepted 29 September 2021; Published 19 October 2021

Academic Editor: Peeter Karihtala

Copyright © 2021 Kai Chen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Many gut disease etiologies are attributed to the presence of robust inflammatory cell recruitment. The recruitment of neutrophils plays a vital role in inflammatory infiltration. Neutrophils have various antimicrobial effector mechanisms, including phagocytosis, oxidative burst, and degranulation. It is suggested that neutrophils could release neutrophil extracellular traps (NETs) to kill pathogens. However, recent evidence indicates that neutrophil infiltration within the gut is associated with disrupted local immunological microenvironment and impaired epithelial barrier. Growing evidence implies that NETs are involved in the progression of many diseases, including cancer, diabetes, thrombosis, and autoimmune disease. Increased NET formation was found in acute or chronic conditions, including infection, sterile inflammation, cancer, and ischemia/reperfusion injury (IRI). Here, we present a comprehensive review of recent advances in the understanding of NETs, focusing on their effects in gut disease. We also discuss NETs as a potential therapeutic target in gut disease.

1. Introduction

Neutrophils are the first immune cells recruited into the inflammatory sites. They can recognize, phagocytize, and kill pathogens by producing reactive oxygen species, releasing lytic enzymes, and inducing neutrophil extracellular traps (NETs), a process termed “NETosis” [1]. NETs are extracellular structures composed of DNA fibers, chromatin, and granule proteins [1]. NETs not only have bactericidal activity but also play a crucial role in noninfectious conditions, including cancer [2], diabetes [3], thrombosis [4], and autoimmune disease [5]. Recently, NETs were suggested to lead to pathological changes in various gut diseases, including inflammatory bowel disease (IBD) [6, 7], colorectal cancer (CRC) [8, 9], and intestinal ischemia/reperfusion injury (IRI) [10, 11]. However, the relationship between NET formation and gut mucosal barrier remains largely unknown. Here, we describe the latest findings regarding NETs associated with intestinal infection, inflammation, cancer, and IRI. We also discuss how NETs serve as a future therapeutic target in the gut. Targeting NET formation and directly degrading NET structure could be promising novel strategies for therapeutic interventions in gut disease [10, 11].

2. NET Formation

Neutrophils are derived from myeloid progenitor cells in the bone marrow and are recruited to inflammatory tissue through a classical leukocyte recruitment cascade [12]. Once arrive at the inflammatory site, neutrophils could be
activated by various stimuli such as bacteria [13, 14], fungi [15], viruses [16], and platelets [17]. In addition to these physiological materials, nonphysiological small compounds including phorbol myristate acetate (PMA) and calcium ionophores (CaI) could also activate neutrophils and have been used for mechanistic studies [18]. Neutrophils exert many biological functions including chemotaxis, antimicrobial functions, phagocytosis, degranulation, and NET formation [12]. The first publication that described NETs demonstrated NETs are composed of web-like structures of DNA coated with histones, elastase, and myeloperoxidase (MPO) [1]. Under high-resolution scanning electron microscopy (SEM), NETs contain smooth stretches with a diameter of 15 to 17 nm and globular domains of around 25 nm [1].

To date, studies have shown that NETs are formed through two main pathways: lytic NET formation which depends on NADPH oxidase (NOX) and nonlytic NET formation independent of NOX. Lytic NET formation leads to neutrophil death, while the nonlytic pathway could occur without cell death [19, 20]. Fuchs et al. [19] reported that NETosis caused by Staphylococcus aureus (S. aureus) or PMA depended on reactive oxygen species (ROS) produced by NOX. After stimulation by PMA, the nuclei of neutrophils lost their shape, and the chromatin homogenized. The nuclear envelope and granular membranes then decomposed, allowing the NET components to mix. Finally, as the cell membrane broke, NETs were released [19]. The NOX-independent pathway could release NETs without plasma membrane disruption. After NET release, neutrophils are still alive and reserve the ability to phagocytose and chemotaxis [20, 21]. The NOX-independent pathway requires neutrophil Toll-like receptor (TLR) 4 activation. The ability of neutrophils to produce NETs was diminished when treated mice with anti-TLR4 antibody (Ab) or knockout TLR4 [22, 23]. Reactive oxygen species (ROS) appear to promote NET formation whether or not the procedure is mediated by NOX. NOX-independent NET release requires ROS produced in the mitochondria [24]. ROS triggers the dissociation of neutrophil elastase (NE) from a membrane-associated complex into the cytosol and activates its proteolytic activity in a MPO-dependent manner [25]. NE translocates to the nucleus and partially degrades specific histones. Subsequently, MPO synerizes with NE in driving chromatin decondensation [26, 27].

NOX-dependent and NOX-independent NET formations converge to common outcomes, including activation of protein-arginine deiminase type 4 (PAD4), histone modification, chromatin decondensation, and NET release [28]. Histone posttranslational modification (PTM) could regulate chromatin decondensation and subsequent NET formation. Histone citrullination is the driver of chromatin decondensation, generated by PAD4 catalyzed hypercitrullination in the histones [29]. At the beginning of NETosis, the positive charge of histones decreased when chemically modified by enzymes such as PAD4 or NE, thus reducing the counterforces that hold the negatively charged DNA/chromatin together [27, 29]. Another modification of histone is histone acetylation was also suggested to promote NETosis when induced upon stimulation in human neutrophils [30]. Following chromatin decondensation, nuclear and plasma membrane ruptured to extrude NETs in the lytic manner. However, nonlytic NET formation could release NETs via vesicles without cell membrane rupture. Neutrophils without nuclei but have intact cell membranes; namely, “cytoplasts”, retain phagocytosis function [31]. Additionally, it should be noted that NETosis can be driven not only by biochemical signaling but also by material properties. Neubert et al. [32] have found that NETosis is highly organized into three distinct phases with a clear no-return point, determined by the chromatin status. Entropic chromatin swelling is the major physical driving force for cell morphology change and nuclear and plasma membrane rupture.

In summary, the pathways of NET formation have been partially formulated. The mechanisms are implicated, and further studies focusing on NET formation are awaited.

3. NETs and Enterogenic Infections

Microorganisms like bacteria [13, 14] and parasites [33] in the human gut have been proved to stimulate NET formation. The first study that described NETs suggested that NETs have an antibacterial function through sequestering bacteria and delivering a high local concentration of antimicrobial molecules [1]. During infection, NETs could persist for several days and eventually be dismantled by plasma nuclease DNase I [34, 35]. In the gut, NET formation was demonstrated to be a crucial manner of neutrophils inducing innate immune. Previous studies showed that PAD4-dependent NET generation is indispensable for intestinal clearance of Citrobacter rodentium (C. rodentium) [36]. C. rodentium colonized the intestine more rapidly when PAD4 was inhibited [36]. Consistently, Chaaban et al. [37] found that NET inhibition increased mortality, inflammation, and bacterial translocation in the necrotizing enterocolitis (NEC) model, suggesting the importance of neutrophil-mediated NET formation in preventing systemic bacterial dissemination during NEC. Although NETs may be critical in combating specific infections, evidence has showed that dysregulated NET formation could induce pathologies that impair the intestine epithelium barrier. Marin-Esteban et al. [13] developed a coculture model of activated neutrophils with the enterocyte-like Caco-2 cells. The F-actin cytoskeletons of enterocyte-like cells were damaged in the presence of NETs. Crane et al. [38] suggested that NETs could assist enteropathogenic Escherichia coli (E.coli) and Shiga-toxigenic E.coli to remain attached to the intestinal mucosa via DNA strands. These results have implied that NETs may benefit pathogens in the gut more than hurt them under some specific circumstance.

4. NETs and Intestinal Injuries during Sepsis

Sepsis is a systemic disorder with a dysregulated host response caused by infection and is accompanied by multiple organ dysfunctions and a high risk of death [39]. Gut microbiota translocation is suggested as the driver of sepsis and organ injuries [40]. Intestinal barrier dysfunction can lead to bacterial translocation and the release of intestine-derived inflammatory factors, which enter the systemic circulation [41].
Although NETs may exert a protective function in early immune response in sepsis [42], increasing evidence shows that if dysregulated, NETs and the components could contribute to intestinal epithelium destruction during sepsis [43–45]. Abundant neutrophils were activated to release NETs in the gut in lipopolysaccharide- (LPS-) induced sepsis [45]. Elevated serum NETs are associated with intestinal injury in abdominal sepsis patients [44]. Sun et al. [44] suggested that NETs activated endoplasmic reticulum (ER) stress in the lethal septic shock model. TLR9 antagonist administration alleviated NET-induced damage in the intestinal epithelial cell monolayer through ER stress inhibition [44]. Collectively, these findings demonstrated that the release of NETs may lead to intestinal damage during sepsis.

5. NETs and IBD

IBD, including Crohn’s disease (CD) and ulcerative colitis (UC), are characterized by aberrant immunological responses leading to chronic inflammation without tissue regeneration [46]. Recent studies show that elevated plasma NET levels are associated with IBD occurrence in patients and experimental models [6, 47–49]. NET presence has been demonstrated in biopsy samples from IBD patients [49]. Pentraxin (PTX) 3, stored in neutrophil granules, could be released in response to microbial recognition. Released PTX3 can partially localize in NETs [50]. Savchenko et al. [50] reported that the numbers of PTX3 were increased in UC patients. In UC patients, indicating that NET release containing PTX3 may contribute to cell immune defense in inflamed colon tissue of UC patients.

However, in addition to making up a part of immune defense, NETs may serve as a detrimental factor in gut epithelial barrier function and lead to the pathogenesis of mucosal inflammation during IBD. Lin et al. [48] found that NETs could alter the integrity of tight junction and adherent junction proteins, inducing intestinal cell death. Consistently, NET treatment in UC lamina propria mononuclear cells could activate extracellular signal-regulated kinase (ERK) 1/2 pathway, thereby enhancing the production of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [6]. Moreover, IBD is associated with a hypercoagulable state and thromboembolism [51]. NETs are suggested to induce hypercoagulable state and thromboembolic disorders. Degradation of NETs by DNase I could reverse coagulation time and reduce fibrin formation in the active UC group [52]. These data have concluded that NET formation during IBD could ultimately exacerbate mucosal inflammation and NET inhibition has a protective effect on this disorder.

6. NETs and Colorectal Cancer

Neutrophils make up a significant part of the inflammatory cell infiltrate in many models of cancer. These neutrophils infiltrated in the tumor microenvironment, leading to pro- or antitumorogenic functions. These pro- or antitumor effects depend on the type of neutrophils [53]. Specific tumor-mediated signals, such as transforming growth factor-β (TGF-β), are believed to induce the formation of a tumorigenic (N2) phenotype. Neutrophils also show an antitumorogenic (N1) phenotype [53]. Similarly, NETs play a vital role in both inhibition and promotion of cancer progression. Arellaki et al. [54] suggested that NET structure could inhibit growth and induce apoptosis in colon cancer cells in vitro. However, increasing evidence indicates that excessive NET production in tumor microenvironment may facilitate tumor growth, invasion, and metastasis [8, 55, 56].

CRC is the world’s fourth most deadly cancer with almost 900,000 deaths annually [57]. Liver is the most frequent site of CRC metastasis, as most intestinal mesenteric drainage enters through the hepatic portal venous system [57]. Despite early detection and treatment, metastases including lymphatic and distant metastases remain the leading cause of death in CRC patients [58]. Patients with CRC are exposed to increasing risk of venous thrombosis, accompanied with high procoagulant state [59]. Recently, considerable evidence has indicated that NETs are involved in CRC progression and metastatic dissemination, both in animal models and CRC patients. High numbers of blood and intratumor neutrophils in various solid tumors were reported to predict poor clinical outcome [55, 60]. NET levels increased in the circulation of CRC patients compared with healthy volunteers. In addition, enhanced NET production was associated with post-operative complications such as longer hospitalization and increased mortality [8]. In this part, the role of NETs as a detrimental factor in cancer progression was discussed, especially as it relates to CRC, in terms of tumor growth, tumor-associated thrombosis, and liver metastasis.

6.1. NET Production in Tumor Microenvironment. Initially, systemic infection was considered necessary to induce NET formation in cancer. Minor or severe systemic infections in tumor-bearing mice could activate neutrophils and induce NET release [56]. Growing evidence has yet suggested that recruitment of neutrophils and formation of NETs play a crucial role in tumor microenvironment. In various solid tumors, including CRC, the presence of NETs was detected within the tumor microenvironment [61, 62] (Figure 1). Release of the granulocyte colony-stimulating factor (G-CSF) into the bloodstream assists tumors in recruiting neutrophils for NET formation [63]. Cell-free DNA (cf-DNA) derived from cancer cells can activate TLR9 signaling and promote IL-8 secretion in CRC [64]. Through secreting IL-8, cancer cells promoted the release of NETs. Alfaro et al. [55] found that IL-8 derived from tumors contributed to the chemotactic recruitment of granulocytic myeloid-derived suppressor cells (GrMDSC) and induced the formation of NETs in GrMDSC. Moreover, cancer cells could also secrete exosomes to regulate tissue microenvironment. Exosomes derived from cancer cells including CRC cells triggered IL-8 production and stimulated NETosis in neutrophils [60, 65].

Recruitment of neutrophils in tumor microenvironment promotes NET formation and facilitates tumor growth [62]. Compared with healthy volunteers, neutrophils from CRC patients could produce more NETs in vitro [8]. Furthermore, recent studies have suggested that NET-associated proteases play a crucial role in the spread of cancer [66]. Albrengues et al. [66] revealed that two NET-associated proteases, namely,
neutrophil elastase (NE) and matrix metalloproteinase-9 (MMP-9), sequentially cleaved laminin of extracellular matrix (ECM) and promoted ECM degradation. The proteolytically remodeled laminin led to integrin α3β1 signaling activation in cancer cells, inducing the proliferation of dormant cancer cells [66].

6.2. NETs Promote the High Procoagulant Status of CRC. CRC patients face a higher risk of venous thrombosis due to a state of high coagulation. Markers of extracellular DNA traps were detected in the thrombus [67]. An increase of NETs was closely associated with cancer-associated thrombosis, procoagulant status, and blood clot formation [17, 59, 68].

Platelet-neutrophil interactions have been key initiators of NET release [17, 68]. P-selectin on activated platelets can induce platelet-mediated NETosis by binding to P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils [69]. Moreover, platelet-derived high-mobility group box 1
(HMGB1) can induce NETosis through neutrophil TLR4 activation [70]. Zhang et al. [71] reported that platelets from CRC patients could stimulate healthy neutrophils to extrude NETs, which could be inhibited by the depletion of HMGB1.

Platelets initiate the production of NETs. The latter, in return, triggers strong activation of platelets. The reciprocal action sets up a positive feedback loop. NETs provide a return, triggers strong activation of platelets. The reciprocal NETs, which could be inhibited by the depletion of HMGB1. CRC patients could stimulate healthy neutrophils to extrude NETs through neutrophil TLR4 activation [70]. Zhang et al. [71] reported that platelets from CRC patients are more potent to activate platelets by inducing the exposure of phosphatidylserine (PS) on platelets, eventually leading to significantly enhanced procoagulant activity (PCA) [71]. These data suggest that NETs may serve as new therapeutic targets to reverse the thrombotic consequences of CRC.

6.3. NETs in the Metastasis of Colorectal Cancer. In the early stage of cancer progression, neutrophils can accumulate in premetastatic organs in response to factors released by cancer cells [61]. Previous studies indicated that NETs played an important role in cancer metastasis. Several studies in mice and humans have shown that high expression of NETs facilitated cancer metastasis in the liver, lung, and lymph nodes [72, 73]. Initially, NET deposition was observed in organ microvasculature as response to surgical stress or systemic infection in cancers. Tohme et al. [73] reported that NET formation was demonstrated occurred after major liver resection in metastatic CRC patients. The NET biomarker, circulating MPO-DNA, was associated with early metastatic recurrence in colon cancer patients [73]. In a cecal ligation and puncture (CLP) model, Cools-Lartigue et al. [72] demonstrated the microvascular NET deposition in the hepatic sinusoidal spaces. Lung carcinoma cells within DNA webs were associated with increased formation of hepatic micrometastases [72]. Recently, studies have suggested that tumor could drive NET deposition in end organs, with or without surgical stress or major infection [74]. Intravascular NETs display effects on increasing vascular permeability and promoting cancer cell extravasation [75]. In this sense, NETs serve to create a "premetastatic niche".

In the "niche", circulating tumor cells (CTCs) could be sequestered by DNA web of NETs, then lodging in the end organ and tissue, establishing new tumors [76]. The DNA mesh of NETs could trap CTCs but cannot kill or injure these metastasizing cells. Once wrapped around tumor cells, NETs and NET-associated proteins would directly interact with tumor cell membrane. It has been demonstrated that carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a cell adhesion molecule expressed on endothelial cells, could promote liver metastasis of CRC [77]. The most recent study confirmed that CEACAM1 is present on both murine and human NETs. Blocking CEACAM1 on human NETs or knocking it down in mice could decrease the adhesion and migration of tumor cells by more than 50% [78]. CCDC25, a transmembrane protein expressed on CRC cells, is another newly discovered molecule promoting CRC metastasis. This protein senses NET-DNA and then activates the integrin-linked kinase (ILK)-β-parvin pathway to enhance cell mobility [79]. Najmeh et al. [56] identified β1 integrin as an abundant constituent of NETs. Present both in vitro and in vivo, β1 integrins, expressed on both tumor cells and NETs, mediated the adhesion of cancer cells to NETs. In the mechanistic investigations in vitro, NETosis triggered the release of HMGB1 and activated TLR9 pathways in cancer cells, further promoting tumor progression [73]. Following the dissemination and adhesion, CTCs can proliferate to form stable metastatic foci. Neutrophils and NETs, assumed to be sources of tissue factor (TF), could lead to angiogenic activity and facilitate tumor proliferation [54]. Consistently, a previous study found extracellular DNA presented on the surface of cancer cells, increased IL8 production, and facilitated angiogenesis of cancers [80]. Collectively, migration of neutrophils to premetastatic niches and subsequent NET formation allows the entrapment of CTCs, which leads to the formation of metastatic implants (Figure 2).

7. NETs in Intestinal IRI

Ischemia/reperfusion injury is a clinical problem, especially when the injury is involved in the gastrointestinal tract. Intestinal IRI occurs following acute mesenteric ischemia, traumatic or septic shock, burns, and surgical procedures. It can lead to multiple organ failure (MOF) and high mortality in critically ill patients [81–83]. Neutrophils may contribute to IRI in the intestine by forming extracellular traps [10]. Researchers have reported that NET biomarker citrullinated H3 (citH3) was elevated in several organs following IRI, including the kidneys [84], brain [85], liver [86], and myocardial tissues [87]. It has been recognized that NETs may exert harmful effects on these organs when coagulation, inflammation, and cell death are triggered [84–87]. Boettcher et al. [88] found that DNase I treatment could reduce intestinal injury during IRI, indicating that NETs may contribute to the development and progression of intestinal IRI [88]. In this section, we provide an overview of studies on the role of NETs in intestinal IRI. Ascher et al. [89] quantified leukocyte adherence and NET formation in IRI mesenteric venules by intravital imaging. During IRI, TLR4 expression in neutrophils was elevated, responsible for elevated NET formation [10]. NETs exacerbated the intestinal inflammation after IRI and destroyed the cytoskeleton structure of gut epithelial, along with functional integrity of tight junctions [10]. It was demonstrated that DNase I treatment could ameliorate tissue injury, apoptosis, and oxidative stress in the intestine [88]. In a rat model of trauma/hemorrhagic shock, early intravenous tranexamic acid administration attenuated NET formation and prevented disruption of tight junction protein [90]. These data indicate that NETs play a detrimental role in the pathogenesis of intestinal barrier during intestinal IRI. Moreover, as Hayase et al. [91] reported, extracellular histone and NET accumulation exacerbate remote liver injury after intestinal IRI. Administration of recombinant thrombomodulin (rTM) neutralized extracellular histones as well as attenuated liver...
8. NETs as Future Therapeutic Strategies

NETs can be regarded as promising therapeutic targets to improve the clinical outcome in gut diseases. Various therapeutic agents targeting NETs are clinically administered in some conditions and are expected to have a protective effect on gut diseases. Inhibitors of molecules interfering with NET formation have been tested. Activated protein C (APC), a serine protease with anti-inflammatory activities, was confirmed to inhibit NETosis, as a part of anti-inflammatory function [92]. In a nonhuman primate model of E.coli-induced sepsis, pre-treatment with APC abrogated release of MPO from neutrophils, an enzyme essential for NETosis [92]. Given that the enzyme PAD4 plays an important role in NET formation, it may be considered a potential therapeutic target [92–94]. Through inhibiting PAD4, NET release could be markedly reduced. In addition to intervening this enzyme, metabolic intermediates of NETosis could also serve as a therapeutic target. Deng et al. [93] developed a novel monoclonal antibody targeted citH3 generated by PAD2 and PAD4. Following blocked circulating citH3 and reduced NET formation, this antibody attenuated inflammatory responses and ameliorated acute lung injury (ALI). Recombinant thrombomodulin, a novel agent used for the treatment of patients with disseminated intravascular coagulation (DIC) in Japan [95], also displayed an effect on inhibiting NET formation in vitro [96, 97]. Hayase et al. [98] suggested rTM could alleviate liver injury by suppressing hepatic NET accumulation after intestinal IRI, thus improving survival. However, the mechanisms of the rTM-mediated NETosis inhibition are not clear and remain to be determined. It is possible that rTM exerts an inhibitory effect against TLR4-mediated signaling or bind to histones [96, 97].

In addition to interfering with NETosis, direct degradation of NETs is an alternative method. DNase I is an endonuclease that selectively cleaves the phosphodiester bond in DNA, the major structural component of NETs [1]. Intravenous administration of DNase I in the colitis mouse model can restore the mucosal barrier integrity and attenuate intestinal inflammation [48]. Xia et al. [99] developed a practical and clinically applicable delivery system providing long-term expression of DNase I: Human DNase I cDNA was put under the control of a liver-specific promoter, cloned into an adeno-associated virus (AAV) expression cassette. AAV-mediated DNase I reduced NET formation in CRC liver metastases. Moreover, heparin possessed the highest negative charge density of any biological macromolecule. Thus, it could strip positively charged histones from the DNA backbone of NETs to destabilize them [94, 100]. Found that the administration of heparin could restore pathologic changes of ocular graft-vs.-host disease (oGVHD) dry eye induced by NETs. NETs represent a good target for DNase therapy. However, DNase I or heparin does not specifically target NETs but degrades extracellular DNA of any source. As a result, future studies of NET-specific therapies are required.

Previous studies have proved that NETs display a protective effect against infection, and NET inhibition attenuated anti-infection effects of neutrophils. Data implicating the degree to which NETs either inhibit or exacerbate the inflammation progression are controversial. In order to investigate the role of NETs in gut bacteria clearance, Saha et al. [36] challenged PAD4-deficient (PAD4−/−) mice and wild-type (WT) littermates with C. rodentium. They found luminal colonization of C. rodentium in PAD4−/− mice unable to form NETs peaked between 11 and 14 days after infection, whereas WT mice suppressed the infection by 14 days. Moreover, an experiment was conducted to examine the outcome of NET inhibition in NEC model induced by Klebsiella pneumoniae infection [37]. Chloramine treatment inhibited NET formation and increased systemic inflammation, bacterial load, organ injury, and mortality in murine...
NEC [37]. In this sense, inhibition of NETs impaired the capacity of neutrophils defending enterogenic infection.

Although the strikingly different outcomes of NET inhibition in these studies may be derived from distinct animal models and stimuli, they bring contradiction to treatments targeting at NETs. Instead of complete depletion of NETs, new therapies should be developed which preserve the protective function against infection while preventing the excessive inflammation caused by NETs. Interestingly, Van Avondt et al. [101] found a solution through modifying signal inhibitory receptor on leukocyte-1 (SIRL-1). SIRL-1 intervention suppressed NET formation in response to S. aureus stimulation and preserved intracellular antimicrobial defense and ROS generation [101]. The findings provide the possibility to develop some new treating strategies, both attenuating the detrimental effect and retaining the protective effect of NETs.

9. Conclusion

NET-related researches have been shifted from innate immune defense to noninfectious diseases ranging from autoimmune disease to cancer. It is suggested that NETs aggravate inflammation, damage surrounding tissue, promote thrombosis, and facilitate cancer progression. It is essential to understand the role of NETs in gut disease, as neutrophil accumulation and activation are critical mechanisms of pathogenesis in the gut. As we presented in this review, NETs could affect the initiation and progression of IBD, CRC, and intestinal IRI. However, much remains unclear about the specific mechanism of NETs in intestine pathogenesis. Given the multitude of NET compositions, novel NET functions in unknown circumstances in the gut are likely to emerge in the future. Besides, it is not clear whether treatment agents like DNase and PAD4 inhibitors have side effects when targeting some receptors in addition to NETs. Therefore, in order to find the most precise molecule candidates for therapeutic targeting, a better understanding of the mechanisms of NETs in gut disease is needed. Moreover, NETs either present protective functions such as antimicrobial or pose harmful effects. How to balance the beneficial and detrimental effects of NETs would be a key point during novel drug development.

Data Availability

All data generated or analyzed in this study are available from the corresponding authors on reasonable request.

Conflicts of Interest

All authors declare no conflicts of interest.

Authors’ Contributions

Q. -Y. H., S. L., and W. -X. G. conceptualized the study. K. C. wrote the original draft. L. -H. S., F. W., X. -F. S., X. -F. X., X. K., P. S., M. W., X. -F. L., and C. W. contributed to writing, reviewing, and editing the article. S. L. contributed in acquiring funds. W. -X. G. supervised the study. Kai Chen, Li-Hua Shao, Feng Wang, and Xiao-Fei Shen contributed equally to this work.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (81602103 (to S.L.) and 82102294 (to Q. -Y. H.)).

References

[1] V. Brinkmann, U. Reichard, C. Goosmann et al., “Neutrophil extracellular traps kill bacteria,” Science, vol. 303, no. 5663, pp. 1532–1535, 2004.
[2] M. T. Masucci, M. Minopoli, S. del Vecchio, and M. V. Carrieri, “The emerging role of neutrophil extracellular traps (NETs) in tumor progression and metastasis,” Frontiers in Immunology, vol. 11, p. 1749, 2020.
[3] L. Wang, X. Zhou, Y. Yin, Y. Mai, D. Wang, and X. Zhang, “Hyperglycemia induces neutrophil extracellular traps formation through an NADPH oxidase-dependent pathway in diabetic retinopathy,” Frontiers in Immunology, vol. 9, p. 3076, 2019.
[4] C. Shi, L. Yang, A. Braun, and H. J. Anders, “Extracellular DNA—a danger signal triggering immunothrombosis,” Frontiers in Immunology, vol. 11, article 568513, 2020.
[5] E. Fousert, R. Toes, and J. Desai, “Neutrophil extracellular traps (NETs) take the central stage in driving autoimmune responses,” Cells, vol. 9, no. 4, p. 915, 2020.
[6] V. Dinallo, I. Marafini, D. di Fusco et al., “Neutrophil extracellular traps sustain inflammatory signals in ulcerative colitis,” Journal of Crohn’s & Colitis, vol. 13, no. 6, pp. 772–784, 2019.
[7] T. Li, C. Wang, Y. Liu et al., “Neutrophil extracellular traps induce intestinal damage and thrombotic tendency in inflammatory bowel disease,” Journal of Crohn’s & Colitis, vol. 14, no. 2, pp. 240–253, 2020.
[8] J. J. R. Richardson, C. Hendrickse, F. Gao-Smith, and D. R. Thickett, “Neutrophil extracellular trap production in patients with colorectal cancer in vitro,” International Journal of Inflammation, vol. 2017, Article ID 4915062, 11 pages, 2017.
[9] L. Yang, L. Liu, R. Zhang et al., “IL-8 mediates a positive loop connecting increased neutrophil extracellular traps (NETs) and colorectal cancer liver metastasis,” Journal of Cancer, vol. 11, no. 15, pp. 4384–4396, 2020.
[10] S. Wang, T. Xie, S. Sun et al., “DNase-1 treatment exerts protective effects in a rat model of intestinal ischemia-reperfusion injury,” Scientific Reports, vol. 8, no. 1, article 17788, 2018.
[11] A. Caudrillier, K. Kessenbrock, B. M. Gilliss et al., “Platelets induce neutrophil extracellular traps in transmigration-related acute lung injury,” The Journal of Clinical Investigation, vol. 122, no. 7, pp. 2661–2671, 2012.
[12] S. Maas, O. Soehnlein, and J. Viola, “Organ-specific mechanisms of transendothelial neutrophil migration in the lung, liver, kidney, and aorta,” Frontiers in Immunology, vol. 9, p. 2739, 2018.
[13] V. Marin-Esteban, I. Turbica, G. Dufour et al., “Afa/Dr diffusely adhering Escherichia coli strain C1845 induces...
Oxidative Medicine and Cellular Longevity

neutrophil extracellular traps that kill bacteria and damage human enterocyte-like cells,” *Infection and Immunity*, vol. 80, no. 5, pp. 1891–1899, 2012.

[14] L. Vong, C. W. Yeung, L. J. Pinnell, and P. M. Sherman, “Adherent-invasive Escherichia coli exacerbates antibiotic-associated intestinal dysbiosis and neutrophil extracellular trap activation,” *Inflammatory Bowel Diseases*, vol. 22, no. 1, pp. 42–54, 2016.

[15] S. Bruns, O. Kniemeyer, M. Hasenberg et al., “Production of extracellular traps against Aspergillus fumigatus in vitro and in infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin RodA,” *PLoS Pathogens*, vol. 6, no. 4, article e1000873, 2010.

[16] T. Saitoh, J. Komano, Y. Saitoh et al., “Novel neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1,” *Cell Host & Microbe*, vol. 12, no. 1, pp. 109–116, 2012.

[17] J. Cedervall, A. Hamidi, and A.-K. Olsson, “Platelets, NETs and cancer,” *Thrombosis Research*, vol. 164, Supplement 1, pp. S148–S152, 2018.

[18] T. Hoppenbrouwers, A. S. A. Autar, A. R. Sultan et al., “In vitro induction of NETosis: comprehensive live imaging comparison and systematic review,” *PLoS One*, vol. 12, no. 5, article e0176472, 2017.

[19] T. A. Fuchs, U. Abed, C. Goosmann et al., “Novel cell death program leads to neutrophil extracellular traps,” *The Journal of Cell Biology*, vol. 176, no. 2, pp. 231–241, 2007.

[20] S. K. Jorch and P. Kubis, “An emerging role for neutrophil extracellular traps in noninfectious disease,” *Nature Medicine*, vol. 23, no. 3, pp. 279–287, 2017.

[21] I. Slaba, J. Wang, E. Kolaczkowska, B. McDonald, W. Y. Lee, and P. Kubis, “Imaging the dynamic platelet-neutrophil response in sterile liver injury and repair in mice,” *Hepatology*, vol. 62, no. 5, pp. 1593–1605, 2015.

[22] J.-M. Tadie, H.-B. Bae, S. Jiang et al., “HMGB1 promotes neutrophil extracellular trap formation through interactions with Toll-like receptor 4,” *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 304, no. 5, pp. L342–L349, 2013.

[23] Y. Ode, M. Aziz, and P. Wang, “CIRP increases ICAM-1+phenotypes of neutrophils exhibiting elevated eNOS and NETs in sepsis,” *Journal of Leukocyte Biology*, vol. 103, no. 4, pp. 693–707, 2018.

[24] D. N. Douda, M. A. Khan, H. Grasemann, and N. Palaniyar, “SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx,” *Proceedings of the National Academy of Sciences*, vol. 112, no. 9, pp. 2817–2822, 2015.

[25] K. D. Metzler, C. Goosmann, A. Lobojemska, A. Zychlinsky, and V. Papayannopoulos, “A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis,” *Cell Reports*, vol. 8, no. 3, pp. 883–896, 2014.

[26] N. Sorvillo, D. Cherpokova, K. Martinod, and D. D. Wagner, “Extracellular DNA NET-works with dire consequences for health,” *Circulation Research*, vol. 125, no. 4, pp. 470–488, 2019.

[27] V. Papayannopoulos, K. Metzler, A. Hakkim, and A. Zychlinsky, “Neutrophil extracellular and myeloperoxidase regulate the formation of neutrophil extracellular traps,” *The Journal of Cell Biology*, vol. 191, no. 3, pp. 677–691, 2010.

[28] M. Honda and P. Kubis, “Neutrophils and neutrophil extracellular traps in the liver and gastrointestinal system,” *Nature Reviews. Gastroenterology & Hepatology*, vol. 15, no. 4, pp. 206–221, 2018.

[29] Y. Wang, M. Li, S. Jadler et al., “Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation,” *The Journal of Cell Biology*, vol. 184, no. 2, pp. 205–213, 2009.

[30] H. J. Hamam, M. A. Khan, and N. Palaniyar, “Histone acetylation promotes neutrophil extracellular trap formation,” *Biomedicines*, vol. 9, no. 1, p. 32, 2019.

[31] F. Pilczek, D. Salina, K. Poon et al., “A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus,” *Journal of Immunology (Baltimore, Md. : 1950)*, vol. 185, no. 12, pp. 7413–7425, 2010.

[32] E. Neubert, D. Meyer, F. Rocca et al., “Chromatin swelling drives neutrophil extracellular trap release,” *Nature Communications*, vol. 9, no. 1, 2018.

[33] Z. Fonseca, C. Diaz-Godinez, N. Mora et al., “Entamoeba histolytica induce signaling via Raf/MEK/ERK for neutrophil extracellular trap (NET) formation,” *Frontiers in Cellular and Infection Microbiology*, vol. 8, p. 226, 2018.

[34] N. Branzk, A. Lobojemska, S. Hardison et al., “Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens,” *Nature Immunology*, vol. 15, no. 11, pp. 1017–1025, 2014.

[35] A. Hakkim, B. G. Furnrohr, K. Amann et al., “Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis,” *Proceedings of the National Academy of Sciences*, vol. 107, no. 21, pp. 9813–9818, 2010.

[36] P. Saha, B. Yeoh, X. Xiao et al., “PAD4-dependent NETs generation are indispensable for intestinal clearance of Citrobacter rodentium,” *Mucosal Immunology*, vol. 12, no. 3, pp. 761–771, 2019.

[37] H. Chaaban, K. Burge, J. Eckert et al., “Neutrophil extracellular trap inhibition increases inflammation, bacteriaemia and mortality in murine necrotizing enterocolitis,” *Journal of Cellular and Molecular Medicine*, 2020.

[38] J. K. Crane, J. E. Broome, and A. Lis, “Biological activities of uric acid in infection due to enteropathogenic and Shiga-toxigenic Escherichia coli,” *Infection and Immunity*, vol. 84, no. 4, pp. 976–988, 2016.

[39] M. Cecconi, L. Evans, M. Levy, and A. Rhodes, “Sepsis and septic shock,” *The Lancet*, vol. 392, no. 10141, pp. 75–87, 2018.

[40] B. W. Haak and W. J. Wiersinga, “The role of the gut microbiota in sepsis,” *The Lancet Gastroenterology & Hepatology*, vol. 2, no. 2, pp. 135–143, 2017.

[41] E. A. Deitch, “Gut-origin sepsis: evolution of a concept,” *The Surgeon*, vol. 10, no. 6, pp. 350–356, 2012.

[42] W. Meng, A. Paunel-Görgülü, S. Flohé et al., “Depletion of neutrophil extracellular traps in vivo results in hypersusceptibility to polymicrobial sepsis in mice,” *Critical Care*, vol. 16, no. 4, 2012.

[43] G. Camicia, R. Pozner, and G. de Larrañaga, “Neutrophil extracellular traps in sepsis,” *Shock*, vol. 42, no. 4, pp. 286–294, 2014.

[44] S. Sun, Z. Duan, X. Wang et al., “Neutrophil extracellular traps impair intestinal barrier functions in sepsis by regulating TLR9-mediated endoplasmic reticulum stress pathway,” *Cell Death & Disease*, vol. 12, no. 6, 2021.
X. Gao, S. Hao, H. Yan, W. Ding, K. Li, and J. Li, “Neutrophil extracellular traps contribute to the intestine damage in endotoxemic rats,” Journal of Surgical Research, vol. 195, no. 1, pp. 211–218, 2015.

T.-C. Liu and T. S. Stappenbeck, “Genetics and pathogenesis of inflammatory bowel disease,” Annual Review of Pathology, vol. 11, no. 1, pp. 127–148, 2016.

Y. Gottlieb, R. Elhasid, S. Berger-Achituv, E. Brazowski, A. Yerushalmy-Feler, and S. Cohen, “Neutrophil extracellular traps in pediatric inflammatory bowel disease,” Pathology International, vol. 68, no. 9, pp. 517–523, 2018.

E. Y. Lin, H. J. Lai, Y. K. Cheng et al., “Neutrophil extracellular traps impair intestinal barrier function during experimental colitis,” Biomedicines, vol. 8, no. 8, p. 275, 2020.

T. B. Bennike, T. G. Carlsen, T. Ellingsen et al., “Neutrophil extracellular traps in ulcerative Colitis,” Inflammatory Bowel Diseases, vol. 21, no. 9, pp. 2052–2067, 2015.

A. S. Savchenko, A. Inoue, R. Ohashi et al., “Long pentraxin 3 (PTX3) expression and release by neutrophils in vitro and in ulcerative colitis,” Pathology International, vol. 61, no. 5, pp. 290–297, 2011.

M. J. Grainge, J. West, and T. R. Card, “Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study,” The Lancet, vol. 375, no. 9715, pp. 657–663, 2010.

Z. He, Y. Si, T. Jiang et al., “Phosphotyrosine exposure and neutrophil extracellular traps enhance procoagulant activity in patients with inflammatory bowel disease,” Thrombosis and Haemostasis, vol. 115, no. 4, pp. 738–751, 2016.

Z. G. Fridlender and S. M. Albeda, “Tumor-associated neutrophils: friend or foe?”, Carcinogenesis, vol. 33, no. 5, pp. 949–955, 2012.

S. Arekla, A. Arampatziglou, K. Kambas et al., “Gradient infiltration of neutrophil extracellular traps in colon cancer and evidence for their involvement in tumour growth,” PloS One, vol. 11, no. 5, article e0154484, 2016.

C. Alfaro, A. Teijeira, C. Oñate et al., “Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs),” Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, vol. 22, no. 15, pp. 3924–3936, 2016.

S. Najmeh, J. Cools-Lartigue, R. F. Rayes et al., “Neutrophil extracellular traps sequester circulating tumor cells viaβ1-integrin mediated interactions,” International Journal of Cancer, vol. 140, no. 10, pp. 2321–2330, 2017.

E. Dekker, P. J. Tanis, J. L. A. Vleugels, P. M. Kasi, and M. B. Wallace, “Colorectal cancer,” The Lancet, vol. 394, no. 10207, pp. 1467–1480, 2019.

L. R. Zarour, S. Anand, K. G. Billingsley et al., “Colorectal cancer liver metastasis: evolving paradigms and future directions,” Cellular and Molecular Gastroenterology and Hepatology, vol. 3, no. 2, pp. 163–173, 2017.

J. Riedl, A. Kaider, E.-M. Reitter et al., “Association of mean platelet volume with risk of venous thromboembolism and mortality in patients with Cancer,” Thrombosis and Haemostasis, vol. 111, no. 4, pp. 670–678, 2014.

A. Shang, C. Gu, C. Zhou et al., “Exosomal KRAS mutation promotes the formation of tumor-associated neutrophil extracellular traps and causes deterioration of colorectal cancer by inducing IL-8 expression,” Cell Communication and Signaling CCS, vol. 18, no. 1, 2020.

M. Kow anzetz, X. Wu, J. Lee et al., “Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes,” Proceedings of the National Academy of Sciences, vol. 107, no. 50, pp. 21248–21255, 2010.

M. Demers, S. I. Wong, K. Martinod et al., “ Priming of neutrophils toward NETosis promotes tumor growth,” Oncoimmunology, vol. 5, no. 5, article e1134073, 2016.

M. Demers, D. S. Krause, D. Schatzberg et al., “Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis,” Proceedings of the National Academy of Sciences, vol. 109, no. 32, pp. 13076–13081, 2012.

Z. Niu, W. Tang, T. Liu et al., “Cell-free DNA derived from cancer cells facilitates tumor malignancy through Toll-like receptor 9 signaling-triggered interleukin-8 secretion in colorectal cancer,” Acta Biochimica et Biophysica Sinica, vol. 50, no. 10, pp. 1007–1017, 2018.

A. C. Leal, D. M. Mizurini, T. Gomes et al., “Tumor-derived exosomes induce the formation of neutrophil extracellular traps: implications for the establishment of cancer-associated thrombosis,” Scientific Reports, vol. 7, no. 1, 2017.

J. Albrengues, M. A. Shields, D. Ng et al., “Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice,” Science, vol. 361, no. 6409, p. eaao4227, 2018.

T. A. Fuchs, A. Brill, D. Duerschmied et al., “Extracellular DNA traps promote thrombosis,” Proceedings of the National Academy of Sciences, vol. 107, no. 36, pp. 15880–15885, 2010.

C. Thalín, Y. Hisada, S. Lundström, N. Mackman, and H. Wallén, “Neutrophil extracellular Traps,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 39, no. 9, pp. 1724–1738, 2019.

J. Etulain, K. Martinod, S. L. Wong, S. M. Cifuni, M. Schattner, and D. D. Wagner, “P-selectin promotes neutrophil extracellular trap formation in mice,” Blood, vol. 126, no. 2, pp. 242–246, 2015.

N. Maugeri, L. Campana, M. Gavina et al., “Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps,” Journal of Thrombosis and Haemostasis, vol. 12, no. 12, pp. 2074–2088, 2014.

Y. Zhang, C. Wang, M. Yu et al., “Neutrophil extracellular traps induced by activated platelets contribute to procoagulant activity in patients with colorectal cancer,” Thrombosis Research, vol. 180, pp. 87–97, 2019.

J. Cools-Lartigue, J. Spicer, B. McDonald et al., “Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis,” The Journal of Clinical Investigation, vol. 123, no. 8, pp. 3446–3458, 2013.

S. Tohme, H. O. Yazdani, A. B. al-Khafaji et al., “Neutrophil extracellular traps promote the development and progression of liver metastases after surgical stress,” Cancer Research, vol. 76, no. 6, pp. 1367–1380, 2016.

W. Lee, S. Y. Ko, M. S. Mohamed, H. A. Kenny, E. Lengyel, and H. Naora, “Neutrophils facilitate ovarian cancer peritoneal niche formation in the omentum,” The Journal of Experimental Medicine, vol. 216, no. 1, pp. 176–194, 2019.

E. Pieterse, N. Rother, M. Garsen et al., “Neutrophil extracellular traps drive endothelial-to-mesenchymal transition,” Oxidative Medicine and Cellular Longevity, 9
Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 37, no. 7, pp. 1371–1379, 2017.

B. McDonald, J. Spicer, B. Giannais, L. Fallavollita, P. Brodt, and L. E. Ferri, “Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms,” International Journal of Cancer, vol. 125, no. 6, pp. 1298–1305, 2009.

A. Arzabazadeh, C. Chan, A. L. Nouvion et al., “Host-related carcinogenic embryonic antigen cell adhesion molecule 1 promotes metastasis of colorectal cancer,” Oncogene, vol. 32, no. 7, pp. 849–860, 2013.

R. F. Rayes, P. Vourtzoumis, M. Bou Rjeily et al., “Neutrophil extracellular trap-associated CEACAM1 as a putative therapeutic target to prevent metastatic progression of colon carcinoma,” The Journal of Immunology, vol. 204, no. 8, pp. 2285–2294, 2020.

L. Yang, Q. Liu, X. Zhang et al., “DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25,” Nature, vol. 583, no. 7814, pp. 133–138, 2020.

F. Wen, A. Shen, A. Choi, E. W. Gerner, and J. Shi, “Extracellular DNA in pancreatic cancer promotes cell invasion and metastasis,” Cancer Research, vol. 73, no. 14, pp. 4256–4266, 2013.

T. Alsaigh, M. Chang, M. Richter, R. Mazor, and E. B. Kistler, “In vivo analysis of intestinal permeability following hemorrhagic shock,” World Journal of Critical Care Medicine, vol. 4, no. 4, pp. 287–295, 2015.

B. P. Yoseph, N. J. Klingensmith, Z. Liang et al., “Mechanisms of intestinal barrier dysfunction in sepsis,” Shock, vol. 46, no. 1, pp. 52–59, 2016.

J. J. Patel, M. D. Rosenthal, K. R. Miller, and R. G. Martindale, “The gut in trauma,” Current Opinion in Critical Care, vol. 22, no. 4, pp. 339–346, 2016.

V. Peer, R. Abu Hamad, S. Berman, and S. Efrati, “Renoprotective effects of DNase I treatment in a rat model of ischemia/reperfusion-induced acute kidney injury,” American Journal of Nephrology, vol. 43, no. 3, pp. 195–205, 2016.

S. F. de Meyer, G. L. Suidan, T. A. Fuchs, M. Monestier, and D. W. Wagner, “Extracellular chromatin is an important mediator of ischemic stroke in mice,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 32, no. 8, pp. 1884–1891, 2012.

H. O. Yazdani, C. Kaltenmeier, K. Morder et al., “Exercise training decreases hepatic injury and Metastases Through changes in immune response to liver ischemia/reperfusion in mice,” Hepatology, vol. 73, no. 6, pp. 2494–2509, 2021.

L. Ge, X. Zhou, W.-J. Ji et al., “Neutrophil extracellular traps in ischemia-reperfusion injury-induced myocardial no-reflow: therapeutic potential of DNase-based reperfusion strategy,” American Journal of Physiology. Heart and Circulatory Physiology, vol. 308, no. 5, pp. H500–H509, 2015.

M. Boettcher, G. Eschenburg, S. Mietzsch et al., “Therapeutic targeting of extracellular DNA improves the outcome of intestinal ischemic reperfusion injury in neonatal rats,” Scientific Reports, vol. 7, no. 1, article 15377, 2017.

S. Ascher, E. Wilms, G. Pontarollo et al., “Gut microbiota restricts NETosis in acute mesenteric ischemia-reperfusion injury,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 40, no. 9, pp. 2279–2292, 2020.

C. Chu, C. Yang, X. Wang et al., “Early intravenous administration of tranexamic acid ameliorates intestinal barrier injury induced by neutrophil extracellular traps in a rat model of trauma/hemorrhagic shock,” Surgery, vol. 167, no. 2, pp. 340–351, 2020.

N. Hayase, K. Doi, T. Hiruma et al., “Recombinant thrombomodulin prevents acute lung injury induced by renal ischemia-reperfusion injury,” Scientific Reports, vol. 10, no. 1, 2020.

L. D. Healy, C. Puy, J. A. Fernández et al., “APC inhibits neutrophil extracellular trap formation,” The Journal of Biological Chemistry, vol. 292, no. 21, pp. 8616–8629, 2017.

Q. Deng, B. Pan, H. B. Alam et al., “Citrullinated histone H3 as a therapeutic target for endotoxic shock in mice,” Frontiers in Immunology, vol. 10, p. 2957, 2020.

Z. Shriver, I. Capila, G. Venkataraman, and R. Sasisekharan, “Heparin and heparan sulfate: analyzing structure and microheterogeneity,” in Handbook of Experimental Pharmacology, Springer, 2012.

T. Ito, J. Thachil, H. Asakura, J. H. Levy, and T. Iba, “Thrombomodulin in disseminated intravascular coagulation and other critical conditions—a multi-faceted anticoagulant protein with therapeutic potential,” Critical Care, vol. 23, no. 1, 2019.

Y. Shimomura, M. Suga, N. Kuriyama et al., “Recombinant human thrombomodulin inhibits neutrophil extracellular trap formation in vitro,” Journal of Intensive Care, vol. 4, no. 1, 2016.

B. Shrestha, T. Ito, M. Kakuuchi et al., “Recombinant thrombomodulin suppresses histone-induced neutrophil extracellular trap formation,” Frontiers in Immunology, vol. 10, p. 2535, 2019.

N. Hayase, K. Doi, T. Hiruma et al., “Recombinant thrombomodulin on neutrophil extracellular traps in murine intestinal ischemia-reperfusion,” Anesthesiology, vol. 131, no. 4, pp. 866–882, 2019.

Y. Xia, J. He, H. Zhang et al., “AAV-mediated gene transfer of DNase I in the liver of mice with colorectal cancer reduces liver metastasis and restores local innate and adaptive immune response,” Molecular Oncology, vol. 14, no. 11, pp. 2920–2935, 2020.

S. An, I. Raju, B. Surenkhuu et al., “Neutrophil extracellular traps (NETs) contribute to pathological changes of ocular graft-vs.-host disease (oGVHD) dry eye: implications for novel biomarkers and therapeutic strategies,” The Ocular Surface, vol. 17, no. 3, pp. 589–614, 2019.

K. van Avondt, M. van der Linden, P. H. Naccache, D. A. Egan, and L. Meyyaard, “Signal inhibitory receptor on leukocytes-1 limits the formation of neutrophil extracellular traps, but preserves intracellular bacterial killing,” Journal of Immunology, vol. 196, no. 9, pp. 3686–3694, 2016.