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Review

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Review

The role of neutrophil extracellular traps and proinflammatory damage-associated molecular patterns in idiopathic inflammatory myopathies

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Summary

Idiopathic inflammatory myopathies (IIMs) are a group of systemic autoimmune diseases characterized by immune-mediated muscle injury. Abnormal neutrophil extracellular traps (NETs) can be used as a biomarker of IIM disease activity, but the mechanism of NET involvement in IIMs needs to be elucidated. Important components of NETs, including high-mobility group box 1, DNA, histones, extracellular matrix, serum amyloid A, and S100A8/A9, act as damage-associated molecular patterns (DAMPs) to promote inflammation in IIMs. NETs can act on different cells to release large amounts of cytokines and activate the inflammasome, which can subsequently aggravate the inflammatory response. Based on the idea that NETs may be proinflammatory DAMPs of IIMs, we describe the role of NETs, DAMPs, and their interaction in the pathogenesis of IIMs and discuss the possible targeted treatment strategies in IIMs.

Keywords: idiopathic inflammatory myopathies, neutroph extracellular traps, damage-associated molecular patterns

Abbreviations: cIIMs: idiopathic inflammatory myopathies; DAMPs: damage-associated molecular patterns; ECM: extracellular matrix; fDNA: cell-free DNA; HMGB1: high-mobility group box 1; mtDNA: mitochondrial DNA; NETs: neutrophil extracellular traps; SAA: serum amyloid A.

Introduction

Idiopathic inflammatory myopathies (IIMs) are a group of autoimmune diseases characterized by symmetrical proximal muscle weakness and muscle enzyme elevation, which can also affect multiple organs, including the lung, heart, skin, gastrointestinal tract, and joints [1]. As a rare complex connective tissue disease, the exact pathogenesis is unknown, and clinical diagnosis and treatment are challenging [2, 3]. In recent years, studies have found that neutrophil dysregulation is a causative factor of IIMs [4, 5]. Neutrophil extracellular traps (NETs), as a disease activity biomarker [6, 7], play an important role in the pathogenesis of IIMs. However, the mechanism of how NETs participate in the occurrence and development of IIMs still needs to be explored.

Neutrophils are the most abundant white blood cells in the human body and can timely and effectively remove invading pathogens and necrotic tissues, promote damage repair, and maintain the body's homeostasis. They are the first line of defense against infection [8, 9]. Initially, neutrophils were thought to capture and kill invading pathogens through necrotizing apoptosis and phagocytosis, but it was later shown that the release of NETs is a crucial mechanism to eliminate pathogens. When the body receives a certain stimulus, neutrophils spontaneously form a network of deoxyribonucleic acid (DNA)-based proteins, consisting of histones, myeloperoxidase (MPO), neutrophil elastase, antimicrobial peptide LL-37, matrix metalloproteinase 9 (MMP9), and other granular proteins, which can eliminate invading pathogens. This process is also known as NETosis [10–13]. The ability to capture pathogens and enhance host defenses is unquestionable to NETs. Yet, excessive activation and inadequate degradation of NETs can exacerbate the inflammatory response and tissue damage and cause autoimmune diseases, such as IIMs [14–16]. NETosis facilitates the release of nuclear contents into the extracellular space, a process similar to damage-associated molecular patterns (DAMPs).

DAMPs are endogenous molecules that initiate and enhance the noninfectious inflammatory response. DAMPs are released into the extracellular environment during sterile or infectious tissue damage and interact with the pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs) and nucleotide-binding oligomerized domain-like receptor proteins 3 (NLRP3) inflammasome. By activating the innate immune cells, including neutrophils, tissue macrophages, and dendritic cells, DAMPs trigger the innate immune response and defend against external danger signals. However, they also promote a pathological inflammatory

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response, leading to the development of various autoimmune diseases [17–19]. With the deepening of relevant research, the number of DAMPs has gradually increased, mainly including high-mobility group protein B1 (HMGB1), cell-free DNA (cfDNA), histones, and mitochondrial DNA (mtDNA) [20]. Interestingly, most of the important NET components or substances closely associated with NETs are DAMPs and are involved in the pathogenesis of IIMs, suggesting that a clear understanding of NETs, DAMPs, and their interactions is critical to unravel the pathogenesis of IIMs and guide novel treatments (Figure 1).

NETs in IIMs

Abnormal formation of NETs in IIMs

Our research team previously used IIMs patients' plasma to induce NET formation and degrade NETs *in vitro* and found that NETs excessively formed and cannot be degraded completely in IIMs patients, especially in patients with interstitial lung disease (ILD). We further found that NETs could not be completely degraded by patients' plasma due to the reduction of DNase I activity [21]. Another study showed that the serum NET levels in anti-melanoma differentiation-associated gene 5 (MDA5)-positive patients with rapidly progressive interstitial lung disease (RP-ILD) was significantly higher than that of patients without RP-ILD, and the serum of anti-MDA5 antibody-positive patients could induce normal neutrophils to form NETs [22]. Recent studies have shown that NETs not only are related to the disease activity of IIMs but also can serve as biomarkers [6, 23]. These results suggest that the abnormal formation and accumulation of NETs are involved in the pathogenesis of IIMs and maybe a possible factor in the occurrence and development of ILD.

Detrimental effects of NETs in IIMs

NETs can activate the innate immune system and induce several inflammatory mediators. When NETs cause cell damage, the inflammatory reaction may be more severe, with more inflammatory mediators. In autoimmune diseases, such as IIMs, the DNA of NETs can combine with antibacterial peptide LL-37 to form a complex, which can activate dendritic cells and promote them to produce interferon-alpha (IFN-α), interleukin-6 (IL-6), and other cytokines to initiate adaptive immunity and cause an autoimmune response [24].

To assess the effect of NETs on the proliferation ability of muscle cells and the viability of myotubes in IIMs patients, cultured skeletal muscle cells stimulated with NETs isolated from IIMs patients showed significantly reduced viability of myotubes in a citrullinated histone-dependent manner [4]. These results suggest that NETs are closely related to the disease activity and muscle injury of IIMs, which also indicates that NETs may play a pathogenic role in IIMs by directly damaging muscle cells. Our research group previously found that NETs accelerated the proliferation of lung fibroblasts, as well as their differentiation into myofibroblasts in a TLR9-dependent manner. Finally, it was confirmed that NETs could induce IIM-related ILD through the TLR9-miR-7-Smad2 pathway [25]. The isolated NETs interacted with alveolar epithelial cells and endothelial cells and results indicated that the damage of NETs to cells was dose dependent, and the induced cytotoxicity could directly cause cell death. These results showed that most proteins in NETs were involved in NET-mediated cytotoxic effects on epithelial and endothelial cells, in which histones played an important role [26]. Recent studies have suggested that after the abnormally formed NETs act on epithelial and endothelial cells, the cells lose their original characteristics and acquire the morphology and characteristics of mesenchymal cells, eventually

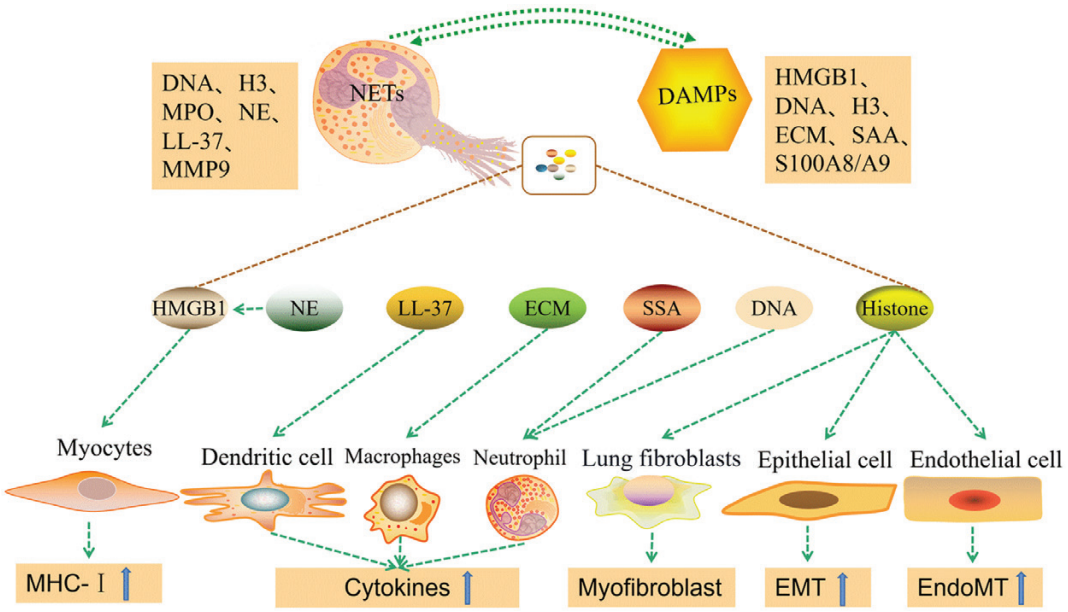

Figure 1. NETs may be proinflammatory DAMPs of IIMs. Important components of NETs, including high-mobility group box 1, DNA, histones, extracellular matrix, serum amyloid A, and S100A8/A9, act as DAMPs to promote inflammation in IIMs. NETs can act on different cells to release large amounts of cytokines and activate the inflammasome, which can subsequently aggravate the inflammatory response. Based on the idea that NETs may be proinflammatory DAMPs of IIMs, we describe the role of NETs, DAMPs, and their interaction in the pathogenesis of IIMs and discuss the possible targeted treatment strategies in IIMs.

transforming into myofibroblasts and causing extracellular matrix deposition. In other words, NETs can induce epithelial and endothelial mesenchymal transformation to accelerate the development of ILD [27, 28].

DAMPs closely related to NETs in IIMs

High-mobility group box 1

A new group of chromatin-related proteins, called "high-mobility histone" proteins, such as high-mobility group box (HMGB), were discovered in calf thymus in 1973. HMGB1, which is a highly conserved, non-histone, nuclear protein that can be released by activated or necrotic cells, plays an important role in various inflammatory reactions, as well as congenital and adaptive immunity [24]. It is also the first known DAMP. Previously, it was discovered that incubating neutrophils with HMGB1 increased the release of DNA and histone 3, the citrullination of histone 3, and the formation of NETs. Moreover, this study also showed that the lack of TLR4 would reduce the ability of neutrophils to produce NETs, indicating that the interaction between HMGB1 and TLR4 promoted the formation of NETs [29–31]. In a study of liver ischemia and reperfusion, it was found that HMGB1 and histone can also stimulate the formation of NETs through the TLR9-MyD88 signaling pathway [32]. The proteolysis of HMGB1 mediated by neutrophil elastase DNA may promote the activity of extracellular HMGB1 [33]. Several studies have shown that HMGB1 is involved in multiple autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis. Increasing studies have found that HMGB1 plays an important role in the development of IIMs [34–37].

One study found that the level of serum HMGB1 in IIMs patients, especially in IIM patients with ILD, was significantly higher than that in the healthy control group, and was also a prognostic indicator of patient survival [38]. The expression of HMGB1 was found in muscle biopsies of IIMs patients at the early stage [39], and high-dose prednisolone could downregulate the abnormal expression of HMGB1 [40]. In 1986, it was reported that IFN-I and IFN-II were found in muscle biopsies of IIMs, and proinflammatory cytokine IFN-γ (IFN-II) stimulation is related to the pathogenesis of IIMs [38, 41]. When myocytes were stimulated with IFN-γ *in vitro*, it was found to increase HMGB1 expression and promote its translocation into the myoplasm. Then HMGB1 induced reversible MHC I expression in myocytes, which is a major histopathological feature of IIMs. In addition, HMGB1 expression was increased in the myofibers of patients with IIMs in the early stages of the disease compared to MHC I [41, 42]. These results suggest that HMGB1 may be the initial step in the pathogenesis of IIMs, which can directly lead to muscle weakness. However, this does not rule out the possibility that HMGB1 overexpression may participate in the development of ILD in patients with IIMs.

cfDNA and mitochondria DNA (mtDNA)

DNA plays an important role in genetic regulation. However, when extracellular DNA from dead cells enters another cell, DNA can interact as DAMPs with PRRs, which recognize bacterial and viral DNA in cells, to trigger an immune response [43]. In addition to viral, bacterial, and host cfDNA, mtDNA is another source of extracellular DNA that has been shown to be a DAMP [44, 45]. Mitochondria can release DAMPs into the extracellular space during cell activation or death, including proteins, mtDNA, and lipids, that can induce inflammatory response and participate in the pathogenesis of various diseases [46].

Our research team found that the plasma concentration of cfDNA in the IIM patients was significantly higher than that in healthy controls, and the cfDNA was higher in patients with ILD than in those without ILD [21]. A subsequent study found that the concentration of cfDNA was more obvious in those who were anti-MDA5 antibody-positive [22]. Recent studies have also evaluated the association between single nucleotide polymorphisms in mtDNA copy number with the disease risk of IIM *in vivo*, suggesting the mtDNA copy number mediates mitochondrial dysfunction and may precede the onset of IIMs [47]. In a rat model, mtDNA was found to induce systemic inflammatory response syndrome and lung injury by activating the TLR9/NF-κB signaling pathway and inducing proinflammatory cytokine production [48]. Additionally, the antimicrobial peptide LL-37 enhances the binding of DNA to receptors and promotes the secretion of interferon and inflammatory cytokines, such as IL-12 and IL-23, by dendritic cells, thus promoting an inflammatory response [49]. While the above studies suggest that cfDNA and mtDNA are involved in the pathogenesis of IIMs, the specific role of cfDNA and mtDNA as important DAMPs in the development of various diseases still needs to be explored.

Histones

Histones, including core histones H2A, H2B, H3, H4, and junctional histone H1, are positively charged nuclear proteins. Core histones can form the basic structural unit of chromatin nucleosomes with eukaryotic DNA and play an important role in packaging of eukaryotic DNA and gene expression regulation. Histones in blood circulation are mainly derived from apoptosis, necrosis, and NETs [50, 51]. When neutrophils are stimulated, peptidylarginine deiminase 4 (PAD4) makes histone citrullination, which weakens the binding of DNA to histones, depolymerizes chromatin, and finally expels histones and other substances out of the cell [10, 52]. Once released into the blood circulation, these free histones will cause cytotoxicity and immunostimulatory effects [53, 54].

Histones released into the extracellular space can directly interact with TLR2 and TLR4, inducing the production of cytokines and tissue damage [55–57]. Recently, it has been found that endogenous histones play the role of DAMPs via TLR9 in mouse models of liver ischemia/reperfusion injury, thus causing inflammation [58]. NLRP3 knockout leads to reduce histone-induced interleukin-1 beta (IL-1β) production and neutrophil recruitment in mice, suggesting that extracellular histones trigger innate immunity through activation of the NLRP3 inflammasome [59, 60]. Finally, histones can prevent the degradation of extracellular DNA, thus promoting autoimmunity, anti-nuclear antibody formation, and autoimmunity of susceptible individuals, such as SLE, RA, and other autoimmune diseases [61–63].

Our research group found that histone 3 participated in the pathogenesis of IIMs with ILD by promoting the proliferation of lung fibroblasts and their differentiation into myofibroblasts [25]. *In vitro*, the extracellular histones are cytotoxic to endothelial cells, and the mechanism underlying damage to endothelial cells may be that elevated plasma histones can induce Ca^{2+} influx into vascular endothelial cells, leading to intracellular Ca^{2+} overload, endothelial cell death,

and vascular dysfunction, even though an influx of Ca^{2+} does not cause vasodilation [64, 65]. Based on the available studies, we can determine that histones are involved in the pathogenesis of IIMs, especially in the pathogenesis of IIMs-complicated ILD, but the exact mechanism is not fully understood.

Extracellular matrix

Extracellular matrix (ECM) is a noncellular, dynamic structure that exists in all tissues. The ECM, which consists of an interstitial matrix and basement membrane, provides tissue integrity and elasticity while continuously remodeling to maintain tissue microenvironment stability, thereby playing an important role in immune regulation [66–68]. ECM components, such as hyaluronic acid, fibrinogen, and fibronectin, interact with PRRs as DAMPs to induce the release proinflammatory mediators, such as IL-1β and tumor necrosis factor-alpha (TNF-α), and increase the expression of chemokines to promote various pathophysiological developments *in vivo* [69–71]. For example, abnormal deposition of ECM has been observed in autoimmune diseases, such as systemic sclerosis, primary Sjogren's syndrome [72–74], and fibrosis [75].

Neutrophils can provide matrix metalloproteinases (MMPs) and produce NETs to mediate ECM remodeling. The ECM can also drive disease progression by regulating the function of neutrophils [76, 77]. Both the presence of the ECM and interaction between the ECM and neutrophils are important mechanisms of inflammation. Although there are no clear studies on the role of ECM in IIMs at this stage, several studies have shown that ECM occupies an important position in the development of other autoimmune diseases. Therefore, ECM has great potential research value in the pathogenesis of IIMs.

Serum amyloid A and S100A8/A9

Serum amyloid A (SAA) is an acutely reactive protein produced mainly by hepatocytes and adipocytes, which can increase by up to 1000 times in the acute phase of inflammation compared to normal conditions [78, 79]. Increasing evidence has shown that SAA is a reliable biomarker for monitoring the disease activity of various autoimmune diseases [80], including RA [81, 82], systemic sclerosis [83], and SLE [84]. A recent study found that SAA1 activates neutrophils and macrophages isolated from the peripheral blood of asthmatic patients, and releases NETs and other related proinflammatory cytokines [85].

S100A8/A9 are two important members of the calcium-binding protein S100 family. Almost half of the intracellular proteins in neutrophils are composed of S100A8 and S100A9, which are released extracellular as DAMPs during NET formation, accelerating the production of proinflammatory cytokines [86]. At the same time, S100A8/A9 can also promote the formation of NETs by activating the TLR4/reactive oxygen species signaling pathway [87]. Recently, it was found that S100A8/A9 is highly expressed in a variety of diseases, especially autoimmune diseases, such as SLE and RA [88–90]. The levels of S100A8/A9 in the serum of IIM patients with ILD were found to be significantly higher than that of the healthy control group and closely related to IL-4, IL-6, IL-10, and other cytokines. Thus, S100A8/9 may be valuable predictors for evaluating the clinical severity of IIMs with ILD [91].

At present, studies on SAA and S100A8/A9 are mostly focused on RA. However, based on the existing studies, it is reasonable to speculate that SAA and S100A8/A9 play an important role in the pathogenesis and clinical course of IIMs. Future studies are also needed to further validate the value of SAA and S100A8/A9 in monitoring patients with IIMs.

Treatment strategy based on NETs as DAMPs in IIMs

According to the idea that NETs may be new DAMPs, PRRs, such as TLRs and NLRP3 inflammasome, may be important therapeutic targets for IIMs. Our group previously found that hydroxychloroquine can inhibit the formation of NETs via TLR9, and the TLR9 agonist CpG-ODN can reverse the inhibition of NETs formation by hydroxychloroquine [92]. Recent studies have also suggested that NETs are formed after stimulation of TLR4 by lipopolysaccharide and that inhibition of TLR4 can reduce NETs formation [93]. It has been reported that NETs formation can be reduced by gene silencing of the NLRP3 inflammasome or by the addition of the NLRP3 inflammasome inhibitor MCC950 [94]. In a murine fibrosis model, a PAD4 inhibitor or *Pad4* gene knockout reduces fibrosis and inflammation [95].

Colchicine is an effective anti-inflammatory drug with growing evidence to indicate the therapeutic value in patients with gouty arthritis, coronavirus disease 2019 (COVID-19), and coronary disease [96]. Colchicine has been shown to act by restoring cytoskeletal dynamics, thus inhibiting inflammasome activation and NETosis [97, 98]. Colchicine also has anti-fibrotic activities and various effects on endothelial function [99]. However, its effects in IIMs have not been explored. Our research group found that low-dose colchicine can alleviate the occurrence of ILD in an experimental autoimmune myositis mouse model (unpublished results), and we are conducting a clinical study to investigate the efficacy and safety of colchicine in the treatment of anti-MDA5-DM patients with ILD (Registration number: ChiCTR2200067219).

At present, the targeted therapies for IIMs mainly inhibit the formation of NETs, but for patients with formed NETs *in vivo*, promoting the elimination of DAMPs is a new potential therapeutic strategy. More valuable therapeutic methods that specifically target the NLRP3 inflammasome need to be further explored, with a particular focus on the emerging role of colchicine.

Conclusion

Research on the pathogenesis of IIMs continues to be a challenge. Only by exploring the specific mechanism can we obtain a better understanding of the disease, reduce the delay of clinical diagnosis and treatment, and improve the prognosis of IIM patients. In this review, we focused on the role of DAMPs and NETs in IIMs and the relationship between them. At this stage, it can be determined that NETs and DAMPs play a significant role in the pathogenesis and pathological mechanism underlying IIMs. NETs contain many active DAMPs molecules, such as cfDNA, mtDNA, and histones. In addition, there are other DAMPs closely related to NETs, like HMGB1, ECM, and SAA. Increasing evidence supports the assumption that NETs are a new type of DAMP that may directly or indirectly promote the development of IIMs. Future research in this field has great value, and the impact of NETs and

DAMPs on IIMs will be gradually discovered. In conclusion, we reviewed the role and possible relationship between NETs and DAMPs in IIMs and proposed a new concept suggesting NETs might be a new DAMP. Further investigation into NETs in IIMs may increase and update our understanding of this disease process.

Conflict of Interests

The authors have no conflict of interest to declare.

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Author contributions

W.M. and L.B. prepared the initial draft of this manuscript; S.Z. and J.Z. edited subsequent drafts; and P.Z. and F.L. prepared and submitted the final draft.

References

1. Cardelli C, Zanframundo G, Cometi L, Marcucci E, Biglia A, Cavagna L, et al. Idiopathic inflammatory myopathies: one year in review 2021. *Clin Exp Rheumatol* 2022, 40, 199–209. doi:10.55563/clinexprheumatol/vskjxi.
2. Miller FW, Lamb JA, Schmidt J, Nagaraju K. Risk factors and disease mechanisms in myositis. *Nat Rev Rheumatol* 2018, 14, 255–68. doi:10.1038/nrrheum.2018.48.
3. Oddis CV, Aggarwal R. Treatment in myositis. *Nat Rev Rheumatol* 2018, 14, 279–89. doi:10.1038/nrrheum.2018.42.
4. Seto N, Torres-Ruiz JJ, Carmona-Rivera C, Pinal-Fernandez I, Pak K, Purmalek MM, et al. Neutrophil dysregulation is pathogenic in idiopathic inflammatory myopathies. *JCI Insight* 2020, 5, e134189–e134189.
5. Liu L, Wang J, Zhang P, Sun W, Zhu X, Sun X, et al. Promising neutrophil-associated biomarkers in lung diseases of patients with antisynthetase syndrome and dermatomyositis. *J Immunol Res* 2022, 2022, 1886083. doi:10.1155/2022/1886083.
6. Torres-Ruiz J, Carrillo-Vazquez DA, Leal-Alanis A, Zentella-Dehesa A, Tapia-Rodriguez M, Maravillas-Montero JL, et al. Low-density granulocytes and neutrophil extracellular traps as biomarkers of disease activity in adult inflammatory myopathies. *J Clin Rheumatol* 2022, 28, e480–7. doi:10.1097/RHU.0000000000001772.
7. Wigerblad G, Kaplan MJ. Neutrophil extracellular traps in systemic autoimmune and autoinflammatory diseases. *Nat Rev Immunol* 2022, 1, 15.
8. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 2013, 13, 159–75. doi:10.1038/nri3399.
9. Liew PX, Kubes P. The neutrophil's role during health and disease. *Physiol Rev* 2019, 99, 1223–48. doi:10.1152/physrev.00012.2018.
10. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004, 303, 1532–5. doi:10.1126/science.1092385.
11. Yipp BG, Petri B, Salina D, Jenne CN, Scott BN, Zbytnuik LD, et al. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat Med* 2012, 18, 1386–93. doi:10.1038/nm.2847.
12. Yipp BG, Kubes P. NETosis: how vital is it? *Blood* 2013, 122, 2784–94. doi:10.1182/blood-2013-04-457671.
13. Manfredi AA, Ramirez GA, Rovere-Querini P, Maugeri N. The neutrophil's choice: phagocytose vs make neutrophil extracellular traps. *Front Immunol* 2018, 9, 288. doi:10.3389/fimmu.2018.00288.
14. Thieblemont N, Wright HL, Edwards SW, Witko-Sarsat V. Human neutrophils in auto-immunity. *Semin Immunol* 2016, 28, 159–73. doi:10.1016/j.smim.2016.03.004.
15. Duvvuri B, Pachman LM, Morgan G, Khojah AM, Klein-Gitelman M, Curran ML, et al. Neutrophil extracellular traps in tissue and periphery in juvenile dermatomyositis. *Arthritis Rheumatol* 2020, 72, 348–58. doi:10.1002/art.41078.
16. Opinc AH, Makowska JS. Antisynthetase syndrome - much more than just a myopathy. *Semin Arthritis Rheum* 2021, 51, 72–83. doi:10.1016/j.semarthrit.2020.09.020.
17. Denning NL, Aziz M, Gurien SD, Wang P. DAMPs and NETs in Sepsis. *Front Immunol* 2019, 10, 2536. doi:10.3389/fimmu.2019.02536.
18. Murao A, Aziz M, Wang H, Brenner M, Wang P. Release mechanisms of major DAMPs. *Apoptosis* 2021, 26, 152–62. doi:10.1007/s10495-021-01663-3.
19. Danieli MG, Antonelli E, Piga MA, Claudi I, Palmeri D, Tonacci A, et al. Alarmins in autoimmune diseases. *Autoimmun Rev* 2022, 21, 103142. doi:10.1016/j.autrev.2022.103142.
20. Roh JS, Sohn DH. Damage-associated molecular patterns in inflammatory diseases. *Immune Netw* 2018, 18, e27. doi:10.4110/in.2018.18.e27.
21. Zhang S, Shu X, Tian X, Chen F, Lu X, Wang G. Enhanced formation and impaired degradation of neutrophil extracellular traps in dermatomyositis and polymyositis: a potential contributor to interstitial lung disease complications. *Clin Exp Immunol* 2014, 177, 134–41. doi:10.1111/cei.12319.
22. Peng Y, Zhang S, Zhao Y, Liu Y, Yan B. Neutrophil extracellular traps may contribute to interstitial lung disease associated with anti-MDA5 autoantibody positive dermatomyositis. *Clin Rheumatol* 2018, 37, 107–15. doi:10.1007/s10067-017-3799-y.
23. Lu X, Peng Q, Wang G. Biomarkers of disease activity in dermatomyositis. *Curr Opin Rheumatol* 2022, 34, 289–94. doi:10.1097/BOR.0000000000000905.
24. Grayson PC, Kaplan MJ. At the Bench: Neutrophil extracellular traps (NETs) highlight novel aspects of innate immune system involvement in autoimmune diseases. *J Leukoc Biol* 2016, 99, 253–64. doi:10.1189/jlb.5BT0615-247R.
25. Zhang S, Jia X, Zhang Q, Zhang L, Yang J, Hu C, et al. Neutrophil extracellular traps activate lung fibroblast to induce polymyositis-related interstitial lung diseases via TLR9-miR-7-Smad2 pathway. *J Cell Mol Med* 2020, 24, 1658–69. doi:10.1111/jcmm.14858.
26. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS One* 2012, 7, e32366. doi:10.1371/journal.pone.0032366.
27. Pieterse E, Rother N, Garsen M, Hofstra JM, Satchell SC, Hoffmann M, et al. Neutrophil extracellular traps drive endothelial-to-mesenchymal transition. *Arterioscler Thromb Vasc Biol* 2017, 37, 1371–9. doi:10.1161/ATVBAHA.117.309002.
28. Pandolfi L, Bozzini S, Frangipane V, Percivalle E, De Luigi A, Violatto MB, et al. Neutrophil extracellular traps induce the epithelial-mesenchymal transition: implications in post-COVID-19 fibrosis. *Front Immunol* 2021, 12, 663303. doi:10.3389/fimmu.2021.663303.
29. Tadie JM, Bae HB, Jiang S, Park DW, Bell CP, Yang H, et al. HMGB1 promotes neutrophil extracellular trap formation through

interactions with Toll-like receptor 4. *Am J Physiol Lung Cell Mol Physiol* 2013, 304, L342–9. doi:10.1152/ajplung.00151.2012.
30. Ma YH, Ma TT, Wang C, Wang H, Chang DY, Chen M, et al. High-mobility group box 1 potentiates antineutrophil cytoplasmic antibody-inducing neutrophil extracellular traps formation. *Arthritis Res Ther* 2016, 18, 2. doi:10.1186/s13075-015-0903-z.
31. Shinde-Jadhav S, Mansure JJ, Rayes RF, Marcq G, Ayoub M, Skowronski R, et al. Role of neutrophil extracellular traps in radiation resistance of invasive bladder cancer. *Nat Commun* 2021, 12, 2776.
32. Huang H, Tohme S, Al-Khafaji AB, Tai S, Loughran P, Chen L, et al. Damage-associated molecular pattern-activated neutrophil extracellular trap exacerbates sterile inflammatory liver injury. *Hepatology* 2015, 62, 600–14. doi:10.1002/hep.27841.
33. Wang X, Mayorga-Flores M, Bien KG, Bailey AO, Iwahara J. DNA-mediated proteolysis by neutrophil elastase enhances binding activities of the HMGB1 protein. *J Biol Chem* 2022, 298, 102577. doi:10.1016/j.jbc.2022.102577.
34. Dong Y, Ming B, Dong L. The role of HMGB1 in rheumatic diseases. *Front Immunol* 2022, 13, 815257. doi:10.3389/fimmu.2022.815257.
35. Song X, Zhang H, Zhao Y, Lin Y, Tang Q, Zhou X, et al. HMGB1 activates myeloid dendritic cells by up-regulating mTOR pathway in systemic lupus erythematosus. *Front Med (Lausanne)* 2021, 8, 636188. doi:10.3389/fmed.2021.636188.
36. Zheng JN, Li Y, Yan YM, Yu Y, Shao WQ, Wang Q. Increased serum calpain activity is associated with HMGB1 levels in systemic sclerosis. *Arthritis Res Ther* 2020, 22, 110. doi:10.1186/s13075-020-02195-y.
37. Harris HE, Andersson U, Pisetsky DS. HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nat Rev Rheumatol* 2012, 8, 195–202. doi:10.1038/nrrheum.2011.222.
38. Shu X, Peng Q, Lu X, Wang G. HMGB1 may be a biomarker for predicting the outcome in patients with polymyositis/dermatomyositis with interstitial lung disease. *PLoS One* 2016, 11, e0161436. doi:10.1371/journal.pone.0161436.
39. Cseri K, Vincze J, Cseri J, Fodor J, Csernatony Z, Csernoch L, et al. HMGB1 expression and muscle regeneration in idiopathic inflammatory myopathies and degenerative joint diseases. *J Muscle Res Cell Motil* 2015, 36, 255–62. doi:10.1007/s10974-015-9411-7.
40. Ulfgren AK, Grundtman C, Borg K, Alexanderson H, Andersson U, Harris HE, et al. Down-regulation of the aberrant expression of the inflammation mediator high mobility group box chromosomal protein 1 in muscle tissue of patients with polymyositis and dermatomyositis treated with corticosteroids. *Arthritis Rheum* 2004, 50, 1586–94. doi:10.1002/art.20220.
41. Isenberg DA, Rowe D, Shearer M, Novick D, Beverley PC. Localization of interferons and interleukin 2 in polymyositis and muscular dystrophy. *Clin Exp Immunol* 1986, 63, 450–8.
42. Peng QL, Zhang YM, Liu YC, Liang L, Li WL, Tian XL, et al. Contribution of necroptosis to myofiber death in idiopathic inflammatory myopathies. *Arthritis Rheumatol* 2022, 74, 1048–58. doi:10.1002/art.42071.
43. Barrera MJ, Aguilera S, Castro I, Carvajal P, Jara D, Molina C, et al. Dysfunctional mitochondria as critical players in the inflammation of autoimmune diseases: potential role in Sjogren's syndrome. *Autoimmun Rev* 2021, 20, 102867. doi:10.1016/j.autrev.2021.102867.
44. Magna M, Pisetsky DS. The alarmin properties of DNA and DNA-associated nuclear proteins. *Clin Ther* 2016, 38, 1029–41. doi:10.1016/j.clinthera.2016.02.029.
45. Tumburu L, Ghosh-Choudhary S, Seifuddin FT, Barbu EA, Yang S, Ahmad MM, et al. Circulating mitochondrial DNA is a proinflammatory DAMP in sickle cell disease. *Blood* 2021, 137, 3116–26. doi:10.1182/blood.2020009063.
46. Nakahira K, Hisata S, Choi AM. The roles of mitochondrial damage-associated molecular patterns in diseases. *Antioxid Redox Signal* 2015, 23, 1329–50. doi:10.1089/ars.2015.6407.
47. Zhao Y, Peng C, Lai R, Zhang J, Zhang X, Guo Z. The SNPs of mitochondrial DNA displacement loop region and mitochondrial DNA copy number associated with risk of polymyositis and dermatomyositis. *Sci Rep* 2022, 12, 5903. doi:10.1038/s41598-022-09943-x
48. Zhang JZ, Liu Z, Liu J, Ren JX, Sun TS. Mitochondrial DNA induces inflammation and increases TLR9/NF-kappaB expression in lung tissue. *Int J Mol Med* 2014, 33, 817–24. doi:10.3892/ijmm.2014.1650
49. Takahashi T, Yamasaki K. Psoriasis and antimicrobial peptides. *Int J Mol Sci* 2020, 21, 6791.
50. Singh A, Verma S, Modak SB, Chaturvedi MM, Purohit JS. Extranuclear histones: origin, significance and perspectives. *Mol Cell Biochem* 2022, 477, 507–24. doi:10.1007/s11010-021-04300-4
51. Andres M, Garcia-Gomis D, Ponte I, Suau P, Roque A. Histone H1 post-translational modifications: update and future perspectives. *Int J Mol Sci* 2020, 21, 5941.
52. Hamam HJ, Khan MA, Palaniyar N. Histone acetylation promotes neutrophil extracellular trap formation. *Biomolecules* 2019, 9, 32. doi:10.3390/biom9010032
53. Szatmary P, Huang W, Criddle D, Tepikin A, Sutton R. Biology, role and therapeutic potential of circulating histones in acute inflammatory disorders. *J Cell Mol Med* 2018, 22, 4617–29. doi:10.1111/jcmm.13797
54. Hamam HJ, Palaniyar N. Post-translational modifications in NETosis and NETs-mediated diseases. *Biomolecules* 2019, 9, 369.
55. Allam R, Scherbaum CR, Darisipudi MN, Mulay SR, Hagele H, Lichtnekert J, et al. Histones from dying renal cells aggravate kidney injury via TLR2 and TLR4. *J Am Soc Nephrol* 2012, 23, 1375–88. doi:10.1681/ASN.2011111077
56. Tsourouktsoglou TD, Warnatsch A, Ioannou M, Hoving D, Wang Q, Papayannopoulos V. Histones, DNA, and citrullination promote neutrophil extracellular trap inflammation by regulating the localization and activation of TLR4. *Cell Rep* 2020, 31, 107602. doi:10.1016/j.celrep.2020.107602
57. Wilson AS, Randall KL, Pettitt JA, Ellyard JI, Blumenthal A, Enders A, et al. Neutrophil extracellular traps and their histones promote Th17 cell differentiation directly via TLR2. *Nat Commun* 2022, 13, 528. doi:10.1038/s41467-022-28172-4
58. Huang H, Evankovich J, Yan W, Nace G, Zhang L, Ross M, et al. Endogenous histones function as alarmins in sterile inflammatory liver injury through Toll-like receptor 9 in mice. *Hepatology* 2011, 54, 999–1008. doi:10.1002/hep.24501
59. Allam R, Darisipudi MN, Tschopp J, Anders HJ. Histones trigger sterile inflammation by activating the NLRP3 inflammasome. *Eur J Immunol* 2013, 43, 3336–42. doi:10.1002/eji.201243224
60. Beltran-Garcia J, Osca-Verdegal R, Perez-Cremades D, Novella S, Hermenegildo C, Pallardo FV, et al. Extracellular histones activate endothelial NLRP3 inflammasome and are associated with a severe sepsis phenotype. *J Inflamm Res* 2022, 15, 4217–38. doi:10.2147/JIR.S363693
61. Allam R, Kumar SV, Darisipudi MN, Anders HJ. Extracellular histones in tissue injury and inflammation. *J Mol Med (Berl)* 2014, 92, 465–72. doi:10.1007/s00109-014-1148-z
62. Okamoto Y, Devoe S, Seto N, Minarchick V, Wilson T, Rothfuss HM, et al. Association of sputum neutrophil extracellular trap subsets with IgA anti-citrullinated protein antibodies in subjects at risk for rheumatoid arthritis. *Arthritis Rheumatol* 2022, 74, 38–48. doi:10.1002/art.41948
63. Corsiero E, Bombardieri M, Carlotti E, Pratesi F, Robinson W, Migliorini P, et al. Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. *Ann Rheum Dis* 2016, 75, 1866–75. doi:10.1136/annrheumdis-2015-208356
64. Collier DM, Villalba N, Sackheim A, Bonev AD, Miller ZD, Moore JS, et al. Extracellular histones induce calcium signals in the endothelium of resistance-sized mesenteric arteries and cause loss of endothelium-dependent dilation. *Am J Physiol Heart Circ Physiol* 2019, 316, H1309–22. doi:10.1152/ajpheart.00655.2018

65. Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, et al. Extracellular histones are major mediators of death in sepsis. *Nat Med* 2009, 15, 1318–21. doi:10.1038/nm.2053
66. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science* 2009, 326, 1216–9. doi:10.1126/science.1176009.
67. Mishra YG, Manavathi B. Focal adhesion dynamics in cellular function and disease. *Cell Signal* 2021, 85, 110046. doi:10.1016/j.cellsig.2021.110046
68. Vyas M, Peigney D, Demehri S. Extracellular matrix-natural killer cell interactome: an uncharted territory in health and disease. *Curr Opin Immunol* 2022, 78, 102246. doi:10.1016/j.coi.2022.102246
69. Frevert CW, Felgenhauer J, Wygrecka M, Nastase MV, Schaefer L. Danger-associated molecular patterns derived from the extracellular matrix provide temporal control of innate immunity. *J Histochem Cytochem* 2018, 66, 213–27. doi:10.1369/0022155417740880
70. Ferreira N, Sanz CK, Raybolt A, Pereira CM, Dossantos MF. Action of hyaluronic acid as a damage-associated molecular pattern molecule and its function on the treatment of temporomandibular disorders. *Front Pain Res (Lausanne)* 2022, 3, 852249.
71. Oxford JT, Reeck JC, Hardy MJ. Extracellular matrix in development and disease. *Int J Mol Sci* 2019, 20, 205.
72. Bhattacharyya S, Midwood KS, Varga J. Tenascin-C in fibrosis in multiple organs: translational implications. *Semin Cell Dev Biol* 2022, 128, 130–6. doi:10.1016/j.semcdb.2022.03.019.
73. Kiripolsky J, Kasperek EM, Zhu C, Li QZ, Wang J, Yu G, et al. Immune-intrinsic Myd88 directs the production of antibodies with specificity for extracellular matrix components in primary Sjogren's syndrome. *Front Immunol* 2021, 12, 692216. doi:10.3389/fimmu.2021.692216
74. Sofat N, Wait R, Robertson SD, Baines DL, Baker EH. Interaction between extracellular matrix molecules and microbial pathogens: evidence for the missing link in autoimmunity with rheumatoid arthritis as a disease model. *Front Microbiol* 2014, 5, 783. doi:10.3389/fmicb.2014.00783
75. Sharma V, Letson J, Furuta S. Fibrous stroma: driver and passenger in cancer development. *Sci Signal* 2022, 15, eabg3449. doi:10.1126/scisignal.abg3449
76. Zhu Y, Huang Y, Ji Q, Fu S, Gu J, Tai N, et al. Interplay between extracellular matrix and neutrophils in diseases. *J Immunol Res* 2021, 2021, 8243378. doi:10.1155/2021/8243378
77. Galliera E, Tacchini L, Corsi RM. Matrix metalloproteinases as biomarkers of disease: updates and new insights. *Clin Chem Lab Med* 2015, 53, 349–55.
78. Ye RD, Sun L. Emerging functions of serum amyloid A in inflammation. *J Leukoc Biol* 2015, 98, 923–9. doi:10.1189/jlb.3VMR0315-080R
79. Maury CP. Comparative study of serum amyloid A protein and C-reactive protein in disease. *Clin Sci (Lond)* 1985, 68, 233–8. doi:10.1042/cs0680233
80. Soric HI, Kos I, Lamot L. Serum amyloid A in inflammatory rheumatic diseases: a compendious review of a renowned biomarker. *Front Immunol* 2020, 11, 631299.
81. Zhou J, Dai Y, Lin Y, Chen K. Association between serum amyloid A and rheumatoid arthritis: a systematic review and meta-analysis. *Semin Arthritis Rheum* 2022, 52, 151943. doi:10.1016/j.semarthrit.2021.12.011
82. Ciregia F, Nys G, Cobraiville G, Badot V, Di Romana S, Sidiras P, et al. A cross-sectional and longitudinal study to define alarmins and A-SAA variants as companion markers in early rheumatoid arthritis. *Front Immunol* 2021, 12, 638814. doi:10.3389/fimmu.2021.638814
83. Lis-Swiety A, Widuchowska M, Brzezinska-Wcislo L, Kucharz E. High acute phase protein levels correlate with pulmonary and skin involvement in patients with diffuse systemic sclerosis. *J Int Med Res* 2018, 46, 1634–9. doi:10.1177/0300060518760955
84. Zhao L, Zhang Q, Feng Z, Zhang J, He F. Serum amyloid A-to-albumin ratio as a potential biomarker to predict the activity, severity, and poor prognosis of systemic lupus erythematosus. *J Clin Lab Anal* 2022, 36, e24282. doi:10.1002/jcla.24282
85. Bich T, Quoc QL, Choi Y, Yang EM, Trinh H, Shin YS, et al. Serum amyloid A1: a biomarker for neutrophilic airway inflammation in adult asthmatic patients. *Allergy Asthma Immunol Res* 2022, 14, 40–58.
86. Sprenkeler E, Zandstra J, van Kleef ND, Goetschalckx I, Verstegen B, Aarts C, et al. S100A8/A9 is a marker for the release of neutrophil extracellular traps and induces neutrophil activation. *Cells* 2022, 11, 236.
87. Zhan X, Wu R, Kong XH, You Y, He K, Sun XY, et al. Elevated neutrophil extracellular traps by HBV-mediated S100A9-TLR4/RAGE-ROS cascade facilitate the growth and metastasis of hepatocellular carcinoma. *Cancer Commun (Lond)* 2022, 43, 225–245.
88. Inciarte-Mundo J, Frade-Sosa B, Sanmarti R. From bench to bedside: Calprotectin (S100A8/S100A9) as a biomarker in rheumatoid arthritis. *Front Immunol* 2022, 13, 1001025. doi:10.3389/fimmu.2022.1001025
89. Donohue SJ, Midgley A, Davies JC, Wright RD, Bruce I, Beresford MW, et al. Differential analysis of serum and urine S100 proteins in juvenile-onset systemic lupus erythematosus (jSLE). *Clin Immunol* 2020, 214, 108375. doi:10.1016/j.clim.2020.108375
90. Nys G, Cobraiville G, Servais AC, Malaise MG, de Seny D, Fillet M. Targeted proteomics reveals serum amyloid A variants and alarmins S100A8-S100A9 as key plasma biomarkers of rheumatoid arthritis. *Talanta* 2019, 204, 507–17. doi:10.1016/j.talanta.2019.06.044
91. Lou Y, Zheng Y, Fan B, Zhang L, Zhu F, Wang X, et al. Serum levels of interleukins and S100A8/A9 correlate with clinical severity in patients with dermatomyositis-associated interstitial lung disease. *BMC Pulm Med* 2020, 20, 196. doi:10.1186/s12890-020-01226-3
92. Zhang S, Zhang Q, Wang F, Guo X, Liu T, Zhao Y, et al. Hydroxychloroquine inhibiting neutrophil extracellular trap formation alleviates hepatic ischemia/reperfusion injury by blocking TLR9 in mice. *Clin Immunol* 2020, 216, 108461. doi:10.1016/j.clim.2020.108461
93. Liu Y, Zhang X, Chen S, Wang J, Yu S, Li Y, et al. Gut-derived lipopolysaccharide promotes alcoholic hepatosteatosis and subsequent hepatocellular carcinoma by stimulating neutrophil extracellular traps through toll-like receptor 4. *Clin Mol Hepatol* 2022, 28, 522–39. doi:10.3350/cmh.2022.0039
94. Munzer P, Negro R, Fukui S, di Meglio L, Aymonnier K, Chu L, et al. NLRP3 inflammasome assembly in neutrophils is supported by PAD4 and promotes NETosis under sterile conditions. *Front Immunol* 2021, 12, 683803. doi:10.3389/fimmu.2021.683803
95. Suzuki M, Ikari J, Anazawa R, Tanaka N, Katsumata Y, Shimada A, et al. PAD4 deficiency improves bleomycin-induced neutrophil extracellular traps and fibrosis in mouse lung. *Am J Respir Cell Mol Biol* 2020, 63, 806–18. doi:10.1165/rcmb.2019-0433OC
96. Surma S, Basiak M, Romanczyk M, Filipiak KJ, Okopien B. Colchicine - From rheumatology to the new kid on the block: coronary syndromes and COVID-19. *Cardiol J* 2023;30, 297–311.
97. Li YW, Chen SX, Yang Y, Zhang ZH, Zhou WB, Huang YN, et al. Colchicine inhibits NETs and alleviates cardiac remodeling after acute myocardial infarction. *Cardiovasc Drugs Ther* 2022. doi:10.1007/s10557-022-07326-y
98. Vaidya K, Tucker B, Kurup R, Khandkar C, Pandzic E, Barraclough J, et al. Colchicine inhibits neutrophil extracellular trap formation in patients with acute coronary syndrome after percutaneous coronary intervention. *J Am Heart Assoc* 2021, 10, e018993. doi:10.1161/JAHA.120.018993
99. Zhang FS, He QZ, Qin CH, Little PJ, Weng JP, Xu SW. Therapeutic potential of colchicine in cardiovascular medicine: a pharmacological review. *Acta Pharmacol Sin* 2022, 43, 2173–90. doi:10.1038/s41401-021-00835-w