RESEARCH PAPER

THE ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS IN ATHEROSCLEROSIS: INSIGHTS FOR THERAPEUTIC APPROACHES

Anastasia Vladimirovna Poznyak [1], Mariam Bagheri Ekta [2], Daria Dmitryevna Borodko [3], Alexander Nikolaevich Orekhov [1]

[1] Institute for Atherosclerosis Research, 4-1-207, Osennyaya Street, 121609 Moscow, Russia;
[2] Laboratory of Cellular and Molecular Pathology of Cardiovascular System, Research Institute of Human Morphology, Petrovsky Russian National Center of Surgery, 2, Abrikosovsky Lane, 119991 Moscow, Russia;
[3] Laboratory of molecular genetic modeling of inflamaging, Institute of General Pathology and Pathophysiology, 8, Baltiiskaya Street, 125315 Moscow, Russia;

*Correspondence: tehhy_85@mail.ru (AVP)

Author's contribution: The authors confirm their contribution to the paper draft manuscript: AVP; review and editing: N.A.O., A.L.R., A.E.K., I.G.L., N.V.E., V.N.S., A.N.O. All authors reviewed the results and approved the final version of the manuscript.
Funding: This work was financially supported by Russian Science Foundation (grant number 25-15-00483)
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable.
Acknowledgments: Not applicable.
Conflicts of Interest: The authors declare no conflicts of interest.

Abstract
Cardiovascular disease (CVD), a leading cause of global mortality, encompasses a range of conditions affecting the heart and blood vessels, including but not limited to atherosclerosis, hypertension, heart failure, and arrhythmias Neutrophils, key players in innate immunity, have gained attention for their role in atherosclerosis and atherothrombosis through the formation of Neutrophil Extracellular Traps (NETs). These web-like structures, composed of chromatin, histones, and granular proteins, actively participate in the pathophysiology of atherosclerosis by promoting inflammation, vascular dysfunction, plaque destabilization, and thrombogenesis
Studies reveal that NETs contribute to endothelial dysfunction, foam cell formation, and immune cell recruitment within atherosclerotic lesions, exacerbating disease progression. Neutrophil-derived myeloperoxidase and pro-inflammatory mediators, such as IL-1β, propagate atherosclerosis by activating immune responses and hastening plaque development. Targeting NET formation pathways, notably PAD-4 inhibition, shows promise in reducing plaque size and improving vascular outcomes in experimental models.
Beyond atherosclerosis, NETs play diverse roles in immune-mediated inflammatory conditions like systemic lupus erythematosus and rheumatoid arthritis, underscoring their wider implications in disease pathogenesis. While NETosis inhibition strategies, such as DNase I, MF, and JAK inhibitors, demonstrate potential therapeutic benefits, further research is essential to unravel the intricate mechanisms of NETs in atherosclerosis and explore novel treatment avenues.
Understanding the complex interplay between neutrophils, NETs, and atherosclerosis offers opportunities for targeted interventions to mitigate disease progression and reduce cardiovascular risk. NETs emerge as a promising therapeutic target in atherosclerosis management, holding the potential to revolutionize treatment strategies and improve clinical outcomes in cardiovascular diseases and beyond.
How to cite this article: Anastasia Vladimirovna Poznyak 1 Mariam Bagheri Ekta 2 Daria Dmitryevna Borodko 3 Alexander Nikolaevich Orekhov 1. THE ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS IN ATHEROSCLEROSIS: INSIGHTS FOR THERAPEUTIC APPROACHES. Int J Drug Deliv Technol. 2026;16(82s):828-846. DOI: 10.25258/ijddt.16.82s.93.

Introduction

Cardiovascular disease (CVD) accounts for a significant portion of global mortality and morbidity, particularly in Western countries. The primary causes of CVD include coronary heart disease (CHD), which is responsible for 42.6% of deaths; stroke at 17.0%; high blood pressure at 10.5%; heart failure at 9.4%; diseases of the arteries at 2.9%; and other CVD-related conditions at 17.6%. These conditions are closely linked to atherosclerosis, a chronic inflammatory and lipid-driven disorder that adversely affects the vascular intima, leading to increased thickness through plaque formation and development [1,2].

The development of atherosclerosis is primarily triggered by endothelial damage, the accumulation of low-density lipoproteins beneath the endothelial layer, and the infiltration of various inflammatory cells, such as macrophages, neutrophils, dendritic cells, and T-lymphocytes. Recent research has highlighted a substantial presence of neutrophils within the atherosclerotic plaques of coronary arteries, which contributes to plaque erosion, rupture, and intraplaque hemorrhage [3,4]. While the role of

these cells in chronic inflammation is well understood, ongoing investigations aim to further elucidate the significance of neutrophils and their structures known as neutrophil extracellular traps (NETs) as potential biomarkers and therapeutic targets.

NETs are web-like structures formed during the activation of neutrophils and comprise decondensed chromatin, histones, and various cellular components including nuclear and cytoplasmic proteins, cytoskeletal elements, proteases, and granzymes. They play a critical role in the innate immune response against infections caused by bacteria, viruses, and fungi. Moreover, NETs have been implicated in the pathogenesis of atherosclerosis and atherothrombosis, highlighting their potential relevance in these conditions [5,6]. In Figure 1, we schematically depicted the mechanism of NETs formation.

The pathways leading to NET formation have generated considerable debate since their discovery, with various triggers being documented. However, linking the primary events of NETosis through specific signaling molecules to define distinct intracellular cascades remains a challenge. Furthermore, the absolute necessity of PAD-4 for NET formation is still contested. While some reports indicate that the knockout or inhibition of PAD-4 disrupts NET formation in both murine and human models [7], other studies have observed NETs forming in response to similar stimuli independently of PAD-4 activity. These conflicting findings highlight the variability in methodologies and interpretations across different studies. Nevertheless, citrullinated histone H3 (citH3) is widely recognized as a specific marker of NET formation. The distinction between “vital” and “suicidal” NETosis also raises questions about terminology, as the formation of NETs does not necessarily lead to immediate cell death. Despite the ongoing discussions, it is increasingly acknowledged that NETs significantly influence various diseases, including atherosclerosis and thrombosis [8,9].

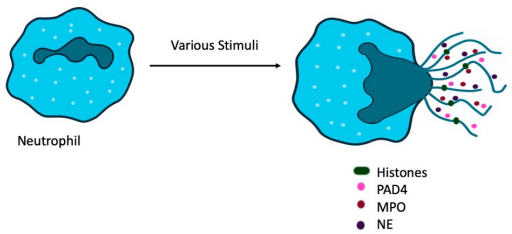

Figure 1. Formation of NETs in response to various stimuli (pathogens, cytokines,etc).

Neutrophil infiltration plays a critical role in plaque erosion, as demonstrated by an optical coherence tomography study that differentiated between plaque rupture and erosion in patients with acute coronary syndrome. Among 25 patients analyzed, seven displayed erosions, and levels of systemic MPO were significantly elevated compared to those with ruptured plaques. Analysis of human endarterectomy samples indicated a positive correlation between the presence of NETs and endothelial cell apoptosis, a characteristic feature of eroded plaques. Nevertheless, the burden of NETs did not differ significantly between the two lesion types, emphasizing the potent effector functions of neutrophils in atherosclerosis [10,11].

Methods

his review article employed a comprehensive and systematic approach to investigate the role of Neutrophil Extracellular Traps (NETs) in atherosclerosis. The methodology involved the following steps:

Literature Search: A thorough search of the scientific literature was conducted using major databases, including PubMed, Scopus, and Web of Science. The search terms included "NETs," "atherosclerosis," "inflammation," "immune response," and "cardiovascular disease." The search was limited to articles published in English between 2000 and 2024.

Inclusion and Exclusion Criteria: Articles were included if they discussed the role of NETs in atherosclerosis, inflammation, or immune response. Articles were excluded if they were review articles, case reports, or studies that did not investigate the relationship between NETs and atherosclerosis.

Study Selection: A total of 150 articles were identified through the literature search. After applying the inclusion and exclusion criteria, 50 articles were selected for in-depth analysis.

Data Extraction: Relevant data were extracted from the selected articles, including study design, sample size, population characteristics, and findings related to NETs and atherosclerosis.

Data Synthesis: The extracted data were synthesized to identify patterns and relationships between NETs and atherosclerosis. The synthesis involved the use of narrative summaries, tables, and figures to present the findings.

Critical Appraisal: The selected articles were critically appraised to evaluate the quality of the evidence and the strength of the associations between NETs and atherosclerosis.

The pathophysiology of atherosclerosis and atherothrombosis

Atherosclerosis is increasingly recognized as an inflammatory disease, with inflammation being an integral part of its pathophysiology rather than merely a secondary phenomenon. Recent studies have shown that both immune responses and infections play significant roles in the initiation and progression of atherosclerosis and atherothrombosis. Key indicators of atherosclerosis include lipid accumulation within the arterial walls at specific locations, proliferation of smooth muscle cells, increased extracellular matrix production, and recruitment of inflammatory cells, all of which contribute to the formation of atherosclerotic plaques [12,13].

The initial event in atherogenesis is endothelial cell injury, which sets off a series of processes leading to

the development of atherosclerotic lesions. Following this injury, there is an activation of the endothelium and a disruption of its normal functions, triggered by various factors such as infections, abnormal lipid levels, decreased nitric oxide availability, and disturbed blood flow [14,15]. A reduction in nitric oxide, which is essential for maintaining healthy vascular function and keeping the endothelium in a quiescent state, creates an imbalance between vasodilators and vasoconstrictors. This imbalance, along with the heightened activity of nitric oxide synthase that generates reactive oxygen species (ROS), can activate endothelial cells; importantly, this activation can be reversed if the stimulus is quickly removed [16,17]. However, prolonged exposure to these triggers leads to a chronic state of endothelial activation, characterized by the expression of adhesion molecules, pro-coagulant factors, and pro-inflammatory mediators. Over time, the cycle of endothelial injury and repair perpetuates, promoting the proliferation of vascular smooth muscle cells and the accumulation of lipids and inflammatory cells, ultimately resulting in the formation of atheromatous plaques composed of multiple layers of foam cells and lipid deposits [18,19].

While early-stage lesions at less susceptible sites can potentially be reversed, these plaques often progress rapidly to more advanced forms at areas of higher susceptibility. Advanced plaques typically contain a confluent core of extracellular lipids surrounded by fibromuscular layers. Repeated episodes of plaque surface disruption, hematoma formation, and thrombosis may lead to luminal narrowing. Additionally, advanced plaques can undergo calcification and display fibrous changes [20,21].

Atherothrombosis arises as a complication of atherosclerosis and is a significant contributor to life-threatening vascular events. The mechanisms underlying atherothrombosis can be understood through two primary processes. The first involves the rupture of the fibrous cap of a plaque, particularly in what are termed "vulnerable plaques." These plaques have thin fibrous caps with low collagen and smooth muscle content, but high levels of lipids and inflammatory cells. The second mechanism is the superficial erosion of the plaque, which is characterized by a higher presence of extracellular matrix components, smooth muscle cells, and NETs, but fewer lipids and inflammatory cells [22,23].

While lipid-lowering therapies are known to improve the conditions of vulnerable plaques and mitigate the first mechanism, the erosion process remains a significant factor contributing to the high risk of complications associated with atherosclerosis. The prominent roles of inflammation and immune responses in both the initiation and progression of atherosclerosis underscore the importance of various inflammatory cells, mediators, and immune pathways involved in its pathophysiology [24].

Toll-like receptor 4 (TLR4) signaling in vascular smooth muscle cells has been implicated in the inflammatory process and lipid accumulation through the activation of the NF-kB pathway and the regulation of ATP-binding cassette sub-family G member 1 (ABCG1), which in turn drives the progression of atheromatous plaques. Similarly, the JAK-STAT signaling pathway participates in the modulation of atherogenesis and plaque development. Pro-inflammatory cytokines such as IFN-γ, TNF-α, and interleukins like IL-1β and IL-6 act as crucial mediators in the complex interactions among the cellular components involved in atherosclerotic inflammation [25,26].

Recent studies have identified a variety of immune cells within atherosclerotic lesions, including T and B lymphocytes, natural killer cells, dendritic cells, macrophages, monocytes, and neutrophils. The presence of microbial antigens and genetic material within these plaques has been documented. This suggests that inflammation related to infections may accelerate atherogenesis and increase the severity of atherosclerotic lesions, supporting the notion that infections play a significant role in atherosclerosis pathophysiology [27,28].

Gram-negative bacterial endotoxins have been shown to trigger inflammatory responses, which can be explained by an "echo effect." These bacterial components and pathogen-associated molecular patterns (PAMPs) circulate in the bloodstream and activate immune responses in atherosclerotic lesions by interacting with TLRs present on leukocytes and vascular endothelial cells. Chronic infections can lead to persistent inflammation and immune activation, which may contribute to the development of atherosclerosis and its associated complications [29]. Furthermore, acute infectious states can create a hypercoagulable environment that may exacerbate the development of atherothrombosis. Initially identified as a defense mechanism against bacterial infections, NETs may also play a crucial role in connecting the dots between infections, atherosclerosis, and thrombosis [30,31].

Neutrophils and Neutrophil Extracellular Traps

Neutrophil granulocytes account for approximately 60% of all leukocytes in the human body and play a pivotal role in innate immunity. Characterized by their short lifespan, neutrophils are swiftly released from the bone marrow into the bloodstream, where they serve as the first line of defense against inflammation and tissue injury [32,33].

The abundance and distinctive capabilities of neutrophils enable them to combat pathogens effectively through mechanisms such as phagocytosis, degranulation, and cytokine release.

These cells contain various types of granules filled with hydrolytic enzymes, including MPO and serine proteases. Upon engulfing pathogens, neutrophils can merge their granules with phagolysosomes, resulting in the degradation of the engulfed material. Additionally, they can release their granular contents into the extracellular environment [34,35].

A notable mechanism associated with neutrophils emerged only about 15 years ago, although preliminary evidence for it was documented back in 1996. Research indicated that phorbol myristate acetate (PMA) can rapidly induce a unique form of cell death in neutrophils, which differs from traditional apoptosis or necrosis. This process involves distinct morphological changes, including signs of nuclear decondensation [36]. It has been found that not just PMA, but also lipopolysaccharide (LPS) and interleukin (IL)-8 can trigger the formation of extracellular structures comprised of fragile fibers made of decondensed DNA intertwined with histones and granule proteins. These NETs can effectively capture both Gram-positive and Gram-negative bacteria, and the presence of neutrophil elastase (NE) or MPO facilitates the degradation of vital bacterial virulence factors [37].

Live-cell imaging studies have revealed that NET formation is an active process, distinct from both apoptosis and necrosis. Upon activation, isolated neutrophils undergo dramatic morphological changes: they initially spread out and form intracellular vacuoles, followed by the loss of their lobulated nuclear appearance as they expand within the cytoplasm [38,39]. At this stage, despite these changes, neutrophils remain viable, as indicated by the retention of the vital dye calcein blue and a negative Annexin V test, indicating that they are not undergoing apoptotic death. This viability can persist until the eventual rupture of the plasma membrane occurs. Unlike apoptosis, the chromatin in these neutrophils becomes decondensed, and the fragmentation of internal membranes allows for the intermixing of nuclear, granular, and cytoplasmic components, which is not characteristic of necrotic cell death [40].

The generation of ROS is essential for the formation of NETs. Inhibition of ROS production has been shown to diminish NET formation, and neutrophils isolated from patients with chronic granulomatous disease—who possess mutations affecting phagocyte NADPH oxidase—do not produce NETs in response to PMA. However, introducing hydrogen peroxide to the system restored their ability to form NETs. This indicates that NETosis is dependent on the assembly and activation of NADPH oxidase and can be initiated through protein kinase C signaling, specifically via the raf-MEK-ERK pathway [41,42].

ROS serve as substrates for MPO, and the presence of both ROS and MPO acts as a crucial stimulus for activating NE. Following its translocation into the nucleus, NE targets core histones, facilitating chromatin decondensation in conjunction with histone citrullination mediated by PAD-4 [43,44]. Subsequently, the breakdown of intracellular membranes allows the association of granular proteases and antimicrobial agents with chromatin. The disruption of the outer cell membrane ultimately leads to the expulsion of the cellular meshwork, resulting in the formation of NETs. Many of the proteins associated with NETs are susceptible to degradation by neutrophil proteases in vitro, which likely reduces their potential to act as autoantigens in vivo [39].

In addition to mentioned stimuli such as MPO, serine proteases, LPS, PAD-4, and ROS, recent research indicates that various factors also play significant roles in triggering NET formation. Factors such as (12-) tetradecanoic acid phorbol ester (-13-) acetate, interferon, activated platelets, endothelial cells, interleukin-8, and tumor necrosis factor-alpha have been shown to enhance neutrophil activation, leading to NETosis. These stimuli can amplify the inflammatory response within atherosclerotic lesions, contributing to disease progression and representing important targets for future therapeutic interventions [45,46].

Recently, there has been a growing interest in the potential for extracellular trap (ET) formation beyond neutrophils. Among granulocytes, mast cells and eosinophils have shown capabilities for ET formation, and it has been discovered that monocytes can also release ETs containing myeloperoxidase and citH3. Additionally, evidence suggests that macrophages can expel their intracellular contents as part of ET formation. However, the significance of these non-neutrophil-associated ETs remains to be fully elucidated [47,48].

The Role of NETs in Thrombosis

Recent research has increasingly highlighted the significant involvement of NETs in both arterial and venous thrombosis. Platelets are crucial for hemostasis and thrombus formation, and studies suggest that interactions between platelets, neutrophils, and NETs are complex and pivotal [49]. NETs serve as a structural framework that facilitates the adhesion, activation, and aggregation of platelets and red blood cells. They can enhance gene expression related to coagulation factors and promote clustering of prothrombotic substances, such as von Willebrand factor (vWF) [50]. Notably, clot formation can occur even without fibrin, solely due to the NET structure. While NET-associated histones are known to trigger platelet aggregation, recent findings indicate that cathepsin G, a neutrophil protease found within NETs, is key to activating platelets [51].

Platelet activation involves specific receptors and kinases, while NETs produced by septic patients and those experiencing myocardial infarction contain

tissue factor (TF), which initiates the coagulation cascade and activates additional platelets [52].

Importantly, NETs influence the production and stability of thrombospondin-1 (TSP-1), which is essential for hemostasis. Neutrophil proteases, including neutrophil elastase and cathepsin G, convert TSP-1 to a more active form, enhancing its hemostatic properties. Meanwhile, NETs protect TSP-1 from complete degradation under high protease conditions [53].

Moreover, activated platelets appear to induce NETosis, indicating that the interaction between platelets and neutrophils extends beyond thrombosis to inflammation. Research has demonstrated that stimulated platelets can activate neutrophils to produce NETs, enhancing bacterial entrapment in the microvasculature of the liver and lungs [54].

Prominent platelet agonists, such as collagen and thrombin, lead to NET formation in a dosage-dependent manner. Mediation occurs through mechanisms like the adhesion molecule P-selectin, but evidence suggests that its role in humans is still debated. Other factors, including glycoprotein Ib and the release of vWF, also contribute to NETosis. The signaling pathways involved appear to include ERK and phosphatidylinositol 3-kinase, without a reliance on ROS production [55].

Interestingly, PMPs and inorganic polyphosphate have been identified as novel mediators of NETosis. Overall, there exists a feedback loop where activated platelets stimulate neutrophils to release NETs, which further activate platelets [56].

Arterial and Venous Thrombosis

NETs are involved in both arterial and venous thrombosis. In arterial cases, studies have shown that NETs are present in the culprit artery of myocardial infarction patients, where they coexist with activated platelets and neutrophils. NETs are found in fresh and lytic thrombi but are absent from fully organized thrombi. Recent findings indicate that various leukocyte-derived extracellular traps, including those from macrophages and mast cells, play a role in coronary atherothrombosis [57].

In the context of venous thrombosis, robust evidence supports the connection of NETs with thrombus formation in animal studies and clinical observations. The interaction between vWF, TF, and platelets contributes to NET involvement. Synergistic signaling through specific receptors in neutrophils is associated with promoting deep vein thrombosis [58].

In cancer patients, neutrophils exhibit a heightened tendency for NETosis, contributing to increased thrombin and fibrin production, while targeting neutrophils or disrupting NET structures mitigates this procoagulant activity [59].

In summary, the evidence firmly establishes the essential role of NETs in various facets of thrombosis, prominently linked to the interactions among neutrophils, NETs, and platelets.

NETs in Atherosclerosis Development

The role of neutrophils in atherosclerosis was identified indirectly when elevated levels of MPO were shown to independently predict the risk of coronary artery disease, regardless of traditional risk factors. A substantial number of circulating neutrophils, a major source of MPO, were linked to both the development and severity of atherosclerotic lesions, as well as to chronic stable angina. Investigations of human atherosclerotic lesions have revealed the presence of MPO and neutrophils producing the pro-inflammatory cytokineIL-8 [60]. The extent of neutrophil infiltration correlated with a pro-inflammatory environment and lesions prone to rupture. Additionally, surrogate markers for NETs, such as double-stranded DNA and chromatin, were independently associated with the severity of coronary atherosclerosis and the occurrence of adverse cardiovascular events [61,62].

Mechanistic Pathways of Atherosclerosis

The exploration of the mechanistic pathways of atherosclerosis frequently employs genetically modified mice, particularly those lacking apolipoprotein E (ApoE) or the low-density lipoprotein receptor. These mice are subjected to a high-fat diet to induce atherosclerotic lesions, revealing numerous factors contributing to lesion formation and maintenance, particularly underscoring the role of neutrophils in plaque progression [63,64].

In experiments, MPO-positive neutrophils were predominantly located in the fibrous caps of atherosclerotic plaques, especially in regions exhibiting marked inflammatory activity, often outnumbering macrophages in these areas. The depletion of neutrophils was shown to diminish lesion development; however, these protective effects were most evident when neutrophil depletion occurred during the initial weeks of lesion formation, suggesting that neutrophil activity is crucial in the early stages of plaque growth [65,66].

In ApoE knockout mice, neutrophils were found at the luminal surfaces of carotid atherosclerotic lesions and were observed releasing DNA, indicative of NET formation. This was further supported by the visualization of NETs in human endarterectomy specimens. The presence of NETs in atherosclerotic lesions of mice was associated with elevated levels of pro-inflammatory markers, including IL-1α, IL-1β, and IL-6. NETs were shown to induce lytic cell death in smooth muscle cells within these lesions via histones, leading to reduced plaque stability [67].

Recent classifications of atherosclerotic lesions have identified two distinct phenotypes: rupture-prone and erosion-prone. Evidence suggests that the underlying pathomechanisms differ significantly between these two types. Typically, rupture-prone lesions have high macrophage content, large lipid pools, and low levels of interstitial collagen, characterized by a thin fibrous cap. The disruption of these fibrous caps accounts for approximately two-thirds of coronary events [68]. Conversely, eroded plaques generally possess a thick or intact fibrous cap, often with a discontinuous endothelial layer.

Pre-NET Stage: Initiation of NETosis

NETs are formed in the arterial environment through various antigenic stimuli, including bacteria, viruses,

and inflammatory factors. Key pathways leading to NET production include PAD4-dependent and cell cycle-dependent pathways. A significant contributing factor to NET formation is the generation of ROS, primarily influenced by oxidized low-density lipoprotein (oxLDL) and cholesterol [69]. These components induce ROS production in monocytes and activated macrophages, promoting inflammation and atherosclerosis development. Notably, cellular cross-talk plays a critical role in neutrophil activation, particularly interactions with platelets which can synergistically enhance NET formation. This interaction is especially relevant in the context of thrombosis [70].

Diverse Stimuli for NET Induction

We have highlighted several key stimuli that can trigger NET formation [71,72]:

- Cytokines: Pro-inflammatory cytokines (e.g., IL-8 and IL-1β) have been shown to significantly promote NETosis. IL-8 facilitates neutrophil recruitment and activates pathways leading to NET formation.
- PAMPs: Agents like LPS initiate inflammatory responses that can lead to NET release. These external stimuli activate intracellular signaling cascades that include the NF-κB and cGAS-STING pathways.
- Cellular Cross-talk: The interaction between platelets and neutrophils is a key trigger for NET formation. Platelets release factors that can activate neutrophils, a process that is accentuated during thrombosis, thereby promoting NETosis in response to vascular injury.

Role of Cytokines in NET Formation

Cytokines such as IL-8 and IL-1β are crucial in regulating NET production by neutrophils, with IL-8 specifically facilitating neutrophil recruitment to inflammatory sites. The activation of these cytokines can lead to vascular damage, foam cell formation, and accelerated atherosclerosis. Furthermore, IL-18 activates the NF-κB signaling pathway, promoting additional inflammatory responses that exacerbate endothelial damage [73].

NF-κB and cGAS-STING Pathways

The NF-κB signaling pathway is essential for regulating inflammatory responses and promoting NET formation. It activates genes associated with inflammation and NET production, establishing a reciprocal relationship between NET release and NF-κB activity. Various atherosclerosis-related factors, such as cytokines and infections, can stimulate NF-κB, facilitating NETosis and further contributing to vascular inflammation [74,75].

The cGAS-STING signaling pathway is activated in response to abnormal intracellular DNA accumulation, such as mitochondrial DNA (mtDNA), which can enhance NET formation and inflammatory responses. This pathway induces ROS activation and promotes NETosis, further embedding the relationship between inflammation, NETs, and atherosclerosis [76,77].

NET Release Phase: Mechanisms of NETosis

NETosis is categorized into suicidal and non-suicidal pathways, both of which can be influenced by various activating signals. We have clarified the role of specific pathways and their interplay during NETosis:

Suicidal NETosis: Triggered by potent agonists such as phorbol-12-myristate-13-acetate (PMA), this pathway involves extensive chromatin decondensation and release of NET contents. Key pathways such as NADPH oxidase activation, mitochondrial dysfunction, and the action of PAD4 are necessary [78].

Non-Suicidal NETosis: This pathway operates in response to less potent stimuli and maintains the viability of neutrophils while still resulting in NET release. Interactions with platelets or cytokines can enhance or modulate this process, highlighting the complexity of NET formation under varying pathological conditions [79].

Key Processes in NETosis

- Mitochondrial Activation: The mitochondrial NADPH oxidase pathway generates ROS, playing a critical role in triggering NETosis.
- Cytoskeletal Disruption: The disintegration of the cytoskeleton and nuclear membrane is vital for the irreversible release of NETs.
- Chromatin Decondensation: Enzymes such as PAD4 and NE facilitate chromatin extension, leading to NET release.
- Release of NET Contents: After cell membrane rupture, NETs, which comprise nuclear and granular materials, are released to trap pathogens [80,81].

Post-NET Release Phase: Impact on Atherosclerosis

The relationship between NETs and atherosclerosis is synergistic; NETosis exacerbates atherosclerotic processes through various mechanisms [82].

Interactions with Leukocytes and Platelets

NETs significantly influence leukocyte behavior. MPO within NETs binds to receptors on macrophages, promoting inflammatory cytokine release and foam cell formation, leading to fatty streaks in arteries. Additionally, NET components can activate neutrophils and sustain inflammatory cascades [83].

NETs Activate Platelets and Drive Coagulation

NETs play a crucial role in thrombus formation by providing a negatively charged matrix that not only attracts platelets but also facilitates their activation. This dual functionality underscores their importance, serving both in the immune response against pathogens and as significant contributors to thrombogenesis [84].

NETs create a structural framework that enhances platelet adhesion and aggregation due to the presence of negatively charged DNA and other components, which allow for efficient binding of circulating platelets and promote thrombus formation. Additionally, NETs express various coagulative factors, including histones that can activate platelets through pathways mediated by TLRs. Specifically, histones H3 and H4 have been shown to initiate pro-

coagulant signaling in platelets, further advancing thrombotic processes [85].

Moreover, NETs have the capacity to ensnare circulating platelets, effectively trapping them within their fibrous network. This entrapment not only restricts the free movement of platelets but also brings them into close proximity with pro-coagulant factors, thus facilitating localized thrombus formation. The relationship between NETs, platelets, and coagulation factors is intricate and dynamic [86]. Although intact NETs might not induce coagulation in vitro, their disruption can significantly enhance thrombin generation by increasing the accessibility of histones and other proteins that play a role in coagulation activation. Additionally, platelet-derived polyphosphate, which has the capability to activate the intrinsic pathway of coagulation, supports the notion that NETs help create a microenvironment conducive to thrombus formation [87].

Pro-inflammatory and Procoagulant Effects

NETs harbor proteins that can induce oxidative stress, inflammation, and coagulation pathways. Histones and proteases from NETs activate TLR pathways and other receptors on endothelial cells and immune cells, amplifying inflammatory responses and further promoting atherosclerosis [88].

Role of mtDNA and non-coding RNAs in Atherosclerosis

mtDNA within NETs serves as a danger signal that activates inflammatory responses. Additionally, non-coding microRNAs associated with NETs modulate inflammatory mediator production in macrophages, linking immune responses to NETosis and atherosclerosis [89].

Inflammasome Engagement

Inflammasomes, particularly NLRP3, engage with NETs and modulate inflammation and lipid metabolism within atherosclerotic plaques. This interaction fosters a feedback loop that exacerbates both NETosis and atherosclerosis [90].

Mechanisms of NETs in Atherosclerosis

The mechanisms underlying atherosclerosis (AS) pathogenesis remain an area of ongoing investigation. However, it is well established that NETs play a significant role in AS progression. In studies using ApoE−/− mice, treatment with Deoxyribonuclease-I (DNaseI)—which degrades NETs—resulted in a reduction of plaque formation, indicating that NETs contribute to the development of atherosclerotic plaques. It has been shown that the DNA found in NETs, along with granule proteins released from neutrophils, can activate dendritic cells within the vascular wall, initiating a robust type I interferon (IFN-1) response that facilitates AS formation [91,92,93].

NETs recruit monocytes and lymphocytes through histone-mediated and charge-dependent adhesion mechanisms and inhibit autophagosome formation in macrophages. Additionally, NETs activate the NLRP3 inflammasome in macrophages, leading to the release of the pro-inflammatory cytokine IL-1β. This, in turn, activates T-helper 17 (Th17) cells, further enhancing the recruitment of immune cells to the arterial wall [94,95]. Moreover, NETs stimulate macrophages and promote the oxidation of low-density lipoprotein (LDL) into oxidized LDL (ox-LDL), which contributes to foam cell formation and propels the progression of atherosclerosis. The release of NADPH oxidase-dependent NETs and macrophage-extruded extracellular traps (METs) has been linked to the formation of atherosclerotic plaques. Evidence suggests that NETs are integral to the lesions associated with AS, while METs are prominent in advanced plaques. This relationship may be attributed to NET-induced macrophage recruitment and the stimulation of arterial macrophages to produce inflammatory mediators [93,96,97].

Furthermore, proteins associated with NETs can harm smooth muscle cells and accelerate plaque rupture. Research also indicated that inhibiting PAD-4, an enzyme critical for NET generation, leads to a decrease in plaque size in ApoE−/− AS mice and improves outcomes in carotid thrombosis. In ApoE−/− mice on a high-fat diet, NETs generated in the carotid arteries can induce macrophages to produce IL-1β, activating the Th17 response and promoting immune cell infiltration into atherosclerotic lesions [96,98]. It has been found that NE and proteinase 3 (PR3) play key roles in regulating NETosis; markedly reduced NET generation occurs in ApoE/NE/PR3 triple-deficient mice. After eight weeks on high-fat diet, these mice exhibited significantly smaller plaques compared to wild-type counterparts on the same diet, with reduced plasma levels of IL-1α, IL-1β, and IL-6. This suggests that NETs drive the release of inflammatory mediators [99,100,93].

NETs also contribute to endothelial cell (EC) dysfunction and apoptosis, along with triggering the production of autoantibodies against double-stranded DNA. Moreover, NETs promote the accumulation of pre-thrombotic molecules, such as von vWF and fibrinogen, intensifying the risk of thrombosis. Given the established connections between NETs and atherosclerosis, along with related cardiovascular diseases, components of NETs may serve as promising biomarkers for diagnosing and prognosing AS and associated cardiovascular risks [101,102,93].

Reactive Oxygen Species and NETs

ROS play a complex, dual role in the development of atherosclerosis, acting both as detrimental agents and as crucial mediators of cellular signaling. Atherosclerosis is a key contributor to various cardiovascular diseases, including stroke and myocardial infarction, characterized by inflammation, lipid accumulation, and plaque formation within arterial walls [103]. The influence of ROS can be likened to a double-edged sword. On one side, excessive production of ROS leads to oxidative stress, an imbalance between oxidants and antioxidants that can have harmful effects on the body. This oxidative stress contributes to endothelial dysfunction and inflammation, which escalate the progression of atherosclerotic lesions [104,105]. Sustained elevated ROS levels impair endothelial

function, causing an increase in the expression of adhesion molecules and chemokines that attract circulating monocytes. As these monocytes penetrate the endothelium and differentiate into macrophages, they give rise to foam cells, a hallmark of atherosclerosis [106].

In addition, high ROS levels are linked to the proliferation and migration of smooth muscle cells (SMCs), which can form neointimal tissue. While uncontrolled SMC proliferation may lead to plaque instability, regulated production of basal ROS is essential for SMC contractile phenotype and homeostasis. Furthermore, ROS activate immune cells, including macrophages, which secrete pro-inflammatory cytokines and exacerbate the inflammatory environment within atherosclerotic plaques. This immune response leads to the recruitment of more inflammatory cells to the site of injury [107,108].

Interestingly, ROS are not solely harmful; they are also vital for normal physiological signaling. Low, controlled levels of ROS are important for redox signaling pathways that facilitate healthy cell function and maintain vascular homeostasis. For instance, ROS are involved in activating transcription factors such as Nrf2, which regulates the expression of antioxidant genes that can mitigate oxidative stress [109].

The primary sources of ROS in atherosclerosis are NADPH oxidases, particularly Nox1 and Nox4. These enzymes produce superoxide and other ROS that modulate various cellular functions. Nox4 has demonstrated protective effects in specific contexts; for example, endothelial-specific overexpression of Nox4 has resulted in smaller lesions in mouse models, while its inhibition can worsen disease progression [110,111]. Mitochondria also contribute to ROS generation through the electron transport chain; mitochondrial oxidative stress is implicated in macrophage activation and cytokine production, further contributing to the inflammatory landscape of atherosclerotic plaques [112].

The cellular response to ROS varies significantly among different cell types. Endothelial cells exposed to elevated ROS may experience senescence and loss of function, while SMCs may proliferate. This differential response is influenced by the context of oxidative stress, the local microenvironment, and the molecular signaling pathways activated by ROS [113]. Additionally, high levels of ROS can trigger endoplasmic reticulum stress pathways, contributing to cellular dysfunction in atherosclerosis. Excess H_2O_2 initiates unfolded protein response pathways that can lead to apoptosis if unresolved [114].

In summary, NETs and their components have the potential to act as important clinical markers for AS and represent new therapeutic targets. Continued research on NETs across various stages of atherosclerosis will enhance our understanding of the disease's development and support the creation of novel treatment strategies [115]. Although evidence is accumulating regarding the involvement of NETs in AS pathogenesis, many aspects remain unclear. Specific mechanisms through which NETs exert their harmful effects include the activation of dendritic cells and macrophages, immune cell recruitment, induction of EC dysfunction and apoptosis, autoantibody production, and the promotion of thrombogenesis [116]. The elements of NETs show substantial promise as diagnostic and prognostic biomarkers in AS and may play a crucial role in mitigating the morbidity and mortality associated with this condition [117,118]. In Table 1, we provided the summary of NETs involvement in atherosclerosis.

Table 1: Mechanisms of Neutrophil Extracellular Traps (NETs) in Atherosclerosis

Mechanism	Description/Impact	Implications for Therapy
Plaque Development	NETs promote atherosclerotic plaques in ApoE−/− mice	Targeting NETs may reduce plaque size and enhance outcomes
Immune Activation	NETs stimulate dendritic cells, leading to a robust IFN-1 response	Further investigation on targeting immune pathways involved
Monocyte Recruitment	NETs recruit monocytes and macrophages via histone-mediated adhesion	Potential therapeutic targets to modulate immune cell recruitment
Inflammatory Cytokine Release	NETs trigger NLRP3 inflammasome activation, increasing IL-1β levels	Inhibition of NLRP3 may reduce inflammation and lesion progression
Foam Cell Formation	Promote oxidation of LDL to ox-LDL, enhancing foam cell development	Strategies to inhibit LDL oxidation could be beneficial

Mechanism	Description/Impact	Implications for Therapy
Smooth Muscle Cell Damage	NETs damage smooth muscle cells, accelerating plaque rupture	Protecting smooth muscle cells might stabilize plaques
Thrombogenesis Promotion	Induce accumulation of pre-thrombotic molecules, enhancing thrombosis	Development of anticoagulant therapies targeting NET components
Diagnostic Biomarkers	NETs and their components may serve as biomarkers for AS severity	Research into NET-associated biomarkers for cardiovascular risk prediction

NETs as a possible therapeutic target

Alongside therapies that target traditional atherosclerosis risk factors such as hypercholesterolemia and hypertension, there is a growing interest in addressing the inflammatory processes involved in atherosclerosis as a means to alleviate its harmful consequences. Statins, for example, not only reduce lipid levels but also exhibit anti-inflammatory properties [119,120]. The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) produced noteworthy results in the prevention of atherosclerosis-related events. This study demonstrated that Canakinumab, a monoclonal antibody that targets IL-1β, significantly lowered the risk of myocardial infarction (MI), stroke, or cardiovascular death—the primary outcome measure—by 15%.

Additionally, preventive measures against infections—such as influenza, which can exacerbate atherosclerosis and associated complications—have shown promise in reducing the incidence of atherosclerotic events [121,122]. While NETs serve as potent defenders against bacterial infections, focusing on such critical immune components requires careful consideration of potential risks. However, further investigation into specific initiators and mediators involved in the pathogenesis of atherosclerosis and atherothrombosis, as well as their life-threatening complications, is essential.

Research has indicated that DNase I can significantly reduce thrombus formation and improve outcomes in MI. Moreover, inhibiting the activation of MMP-2 has been associated with exacerbated endothelial dysfunction and NET-related vascular injury in systemic lupus erythematosus (SLE) [123,124]. Treatment with metformin has been shown to decrease PMA-induced NET formation in SLE patients. Vitamin D (1,25(OH)2D3) has also been found to reduce NET formation and subsequent endothelial injury in this condition. Tofacitinib, a Janus kinase inhibitor, has been reported to modulate NET formation while improving vascular complications associated with murine lupus. Additionally, celastrol, a triterpenoid compound, has been noted for its ability to inhibit NETosis induced by inflammatory stimuli in both rheumatoid arthritis (RA) and SLE. Tocilizumab has demonstrated effectiveness in reducing NETosis, lowering oxidative stress, and enhancing endothelial function in RA patients [125]. In Table 2, we summarized the potential mechanisms and current key features of therapeutic strategies targeting NETs.

Table 2. Therapeutic Strategies Targeting NETs in Atherosclerosis

Therapeutic Approach	Mechanism/Outcome	Key Studies/Findings
Statins	Reduce inflammation and stabilize atherosclerotic plaques.	Linked to lower cardiovascular event rates and improved plaque stability.
Canakinumab	Targets IL-1β to reduce inflammation associated with AS.	Decreased risk of MI, stroke, and death by 15% (CANTOS).
Influenza Vaccination	Mitigates inflammatory responses associated with infections that exacerbate AS.	Positive outcomes in reducing atherosclerotic events during flu seasons.
DNase I	Reduces thrombi formation; improves MI outcomes.	Enhances MI fate.

Therapeutic Approach	Mechanism/Outcome	Key Studies/Findings
Metformin	Inhibits NET formation and reduces systemic inflammation, improving endothelial function.	Shown to reduce complications associated with AS in diabetes.
Vitamin D ($1,25(OH)_2 D_3$)	Enhances endothelial function and reduces inflammatory responses linked to NETs in AS.	Protects endothelial function in SLE.
Tofacitinib	Inhibits inflammatory pathways that contribute to NET formation, promoting vascular health.	Enhances vascular health in murine lupus.
Celastrol	Reduces NETosis and associated inflammatory responses, alleviating AS progression.	Reduces NET formation in RA and SLE.
Tocilizumab	Inhibits pro-inflammatory cytokines, resulting in decreased NET formation and oxidative stress.	Provides benefits observed in RA and associated atherosclerosis.

Future perspectives of NETs

Lately, it has been revealed that NETs not only play a key past in the inflammation of atherosclerotic ECs but also in other immune or noninfectious inflammatory diseases, such as, SLE, RA, antiphospholipid syndrome. Moreover, NETs also play a part in SARS-CoV-2 lung disease. Therefore, it is important to study the mechanisms of NETs/NETosis in diseases to facilitate their treatment [126,127]. Acetylsalicylic acid, cyclosporin A, and chlor-amidine can inhibit NETosis in the regulation of anti-inflammatory and immune responses. Acetylsalicylic acid inhibits NETosis by blocking the NF-κB pathway and weakens adverse reactions caused by the overactivation of neutrophils. NETs are also a promising target antitumor therapy. NETs can provoke tumor transformation and speed up tumor growth and metastasis in the process of carcinogenesis, that is why it is possible to treat tumors by degrading NETs. The NE inhibitor, PAD-4 inhibitor, and DNase I are possible antitumor agents for NETs targeted drugs [128,129].

Moreover, NETs are connected with stent thrombosis and can support the overall stability of thrombus, which implies that targeting NETs is a significant direction in antithrombotic treatments. It has been proved that DNase may boost thrombolysis in arteries, which gives support for the use of targeted NETs as novel thrombolytic strategy. Also, heparin decreases the stability of NETs by removing the histones in NETs and neutralizes the negative impact of histones, which improves the influence of antithrombotic therapy [130,131]. Metformin (MF) is one of the most popular hypoglycemic agents with potential to cute various diseases, Metformin is able to regulate the function of macrophages in AS, for instance, by inhibiting macrophage inflammation, foam cell formation, etc. Moreover, it has been proved that MF can inhibit the formation of NETs by enhancing the function of macrophages. According to research, MF is able to inhibit the activation of the NLRP3 inflammasome in chronic diseases, while promoting the activation of the NLRP3 inflammasome in acute diseases [132]. MF also diminishes the degree to which neutrophils participate in NETosis by boosting T cell differentiation into memory and regulatory T cells, Moreover, MF inhibits pro-inflammatory phenotype of immune cells and shows positive results in animal models of autoimmune diseases and clinical trials. According to these studies, MF can be an essential therapeutic drug for diseases associated with NETs [133].

NETs are currently a popular research subject, and have been discovered to play various parts in different diseases. Despite the limited research on the mechanisms of NETs in AS and the pathways implicated, NETs are a promising target for the treatment of AS [134,135].

Conclusion

The intricate interplay between neutrophils, Neutrophil Extracellular Traps (NETs), and atherosclerosis underscores the multifaceted role of innate immune responses in cardiovascular disease pathogenesis. Neutrophils, as frontline defenders of the immune system, contribute significantly to the inflammatory milieu within atherosclerotic lesions, exacerbating vascular dysfunction and plaque instability. While NETs can promote the formation of atherosclerotic plaques and blood clots, they also serve to kill pathogenic microorganisms, helping to prevent further spread of infection. The discovery of NETs as potent effectors of immune-mediated processes in atherosclerosis highlights their potential

as both diagnostic biomarkers and therapeutic targets for mitigating disease progression.

Research efforts focused on elucidating the mechanisms by which NETs modulate atherosclerosis have revealed novel insights into the pathophysiology of the disease, shedding light on the intricate crosstalk between inflammation, immune responses, and cardiovascular complications. Targeting specific pathways involved in NET formation, such as PAD-4 inhibition or DNase I treatment, shows promise in attenuating plaque development and improving vascular outcomes in experimental models.

Moreover, the broader implications of NETs in various immune-mediated inflammatory disorders and infectious diseases emphasize the versatile nature of these structures in modulating immune responses and disease pathogenesis. Recognizing that NETs can both aid in combating infections and contribute to atherosclerosis provides a double-edged perspective that warrants further investigation. By unraveling the complex interactions between neutrophils, NETs, and atherosclerosis, novel therapeutic strategies can be devised to target key players in disease progression and reduce the burden of cardiovascular morbidity and mortality.

Moving forward, continued research into the precise mechanisms governing NET formation and activity in atherosclerosis is crucial for advancing personalized treatment approaches and enhancing clinical outcomes in cardiovascular diseases. The evolving understanding of NETs as critical mediators of inflammation and thrombosis not only offers new perspectives on disease pathophysiology but also paves the way for innovative therapeutic interventions tailored to target specific aspects of disease progression.

In conclusion, the investigation of neutrophils and NETs in the context of atherosclerosis represents a burgeoning field with significant implications for cardiovascular health. Harnessing the potential of NETs as diagnostic tools and therapeutic targets holds promise for revolutionizing the management of atherosclerosis and related cardiovascular disorders, ultimately striving towards improved patient care, outcomes, and quality of life for individuals at risk of cardiovascular events.

List of Abbreviations

Abbreviation	Full Term
ApoE	Apolipoprotein E
AS	Atherosclerosis
cGAS-STING	GMP-AMP synthase - Stimulator of Interferon Genes
citH3	citrullinated histone H3
CVD	Cardiovascular Disease
DNase I	Deoxyribonuclease I
EC	Endothelial Cell
ET	Extracellular Trap
IFN-1	Type I Interferon
IL	Interleukin
JAK	Janus kinase

Abbreviation	Full Term
LDL	low-density lipoprotein
LPS	Lipopolysaccharide
MET	macrophage-extruded extracellular trap
MF	Metformin
MI	Myocardial infarction
MPO	Myeloperoxidase
mtDNA	mitochondrial DNA
NADPH	Nicotinamide adenine dinucleotide phosphate
NE	Neutrophil Elastase
NET	Neutrophil Extracellular Trap

Abbreviation	Full Term
NF-κB	Nuclear Factor κB
NLRP3	NOD-Like Receptor Protein 3
ox-LDL	oxidized low-density lipoprotein
PAD-4	Peptidyl Arginine Deiminase 4
PAMP	pathogen-associated molecular patterns
PMA	Phorbol Myristate Acetate
PR3	Proteinase 3
RA	rheumatoid arthritis
ROS	Reactive Oxygen Species
SLE	Systemic Lupus Erythematosus

Abbreviation	Full Term
SMC	Smooth muscle cells
TF	tissue factor
Th17	T-helper 17 (cells)
TSP-1	Thrombospondin-1
vWF	von Willebrand Factor

Author's contribution: The authors confirm their contribution to the paper draft manuscript: AVP; review and editing: N.A.O., A.L.R., A.E.K., I.G.L., N.V.E., V.N.S., A.N.O. All authors reviewed the results and approved the final version of the manuscript.

Funding: This work was financially supported by Russian Science Foundation (grant number 24-15-00123)

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. [1] Einarson, T. R.; Acs, A.; Ludwig, C.; Panton, U. H. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review. *Cardiovasc. Diabetol.* **2018**, *17*(1), 83. https://doi.org/10.1186/s12933-018-0728-6.

2. [2] Di Cesare, M.; Perel, P.; Taylor, S.; Kabudula, C.; Bixby, H.; Gaziano, T. A.; McGhie, D. V.; Mwangi, J.; Pervan, B.; Narula, J.; Pineiro, D.; Pinto, F. J. The heart of the world. *Glob. Heart* **2024**, *19*(1), 11. https://doi.org/10.5334/gh.1288.

3. [3] Mostafa, M. N.; Osama, M. The implications of neutrophil extracellular traps in the pathophysiology of atherosclerosis and atherothrombosis. *Exp. Biol. Med.* **2020**, *245*(15), 1376–1384. https://doi.org/10.1177/1535370220945989.

4. [4] Mehu, M.; Narasimhulu, C. A.; Singla, D. K. Inflammatory cells in atherosclerosis. *Antioxidants* **2022**, *11*(2), 233. https://doi.org/10.3390/antiox11020233.

5. [5] Huang, S. U.; O'Sullivan, K. M. The expanding role of extracellular traps in inflammation and autoimmunity: the new players in casting dark webs. *Int. J. Mol. Sci.* **2022**, *23*(7), 3793. https://doi.org/10.3390/ijms23073793.

6. [6] Wigerblad, G.; Kaplan, M. J. Neutrophil extracellular traps in systemic autoimmune and autoinflammatory diseases. *Nat. Rev. Immunol.* **2023**, *23*, 274–288. https://doi.org/10.1038/s41577-022-00787-0.

7. [7] Li, T.; Zhang, Z.; Li, X.; Dong, G.; Zhang, M.; Xu, Z.; Yang, J. Neutrophil extracellular traps: signaling properties and disease relevance. *Mediators Inflamm.* **2020**, *2020*, 9254087. https://doi.org/10.1155/2020/9254087.

8. [8] Tilley, D. O.; Abuabed, U.; Zimny-Arndt, U.; Schmid, M.; Florian, S.; Jungblut, P. R.; Brinkmann, V.; Herzig, A.; Zychlinsky, A. Histone H3 clipping is a novel signature of human neutrophil extracellular traps. *eLife* **2022**, *11*, e68283. https://doi.org/10.7554/eLife.68283.

9. [9] Liu, X.; Arfman, T.; Wichapong, K.; Reutelingsperger, C. P. M.; Voorberg, J.; Nicolaes, G. A. F. PAD4 takes charge during neutrophil activation: impact of PAD4-mediated NET formation on immune-mediated disease. *J. Thromb. Haemost.* **2021**, *19*(7), 1607–1617. https://doi.org/10.1111/jth.15313.

10. [10] Luo, X.; Lv, Y.; Bai, X.; Qi, J.; Weng, X.; Liu, S.; Bao, X.; Jia, H.; Yu, B. Plaque erosion: a distinctive pathological mechanism of acute coronary syndrome. *Front. Cardiovasc. Med.* **2021**, *8*, 711453. https://doi.org/10.3389/fcvm.2021.711453.

11. [11] Theofilis, P.; Vlachakis, P. K.; Papanikolaou, A.; Karakasis, P.; Oikonomou, E.; Tsioufis, K.; Tousoulis, D. Coronary plaque erosion: epidemiology, diagnosis, and treatment. *Int. J. Mol. Sci.* **2024**, *25*(11), 5786. https://doi.org/10.3390/ijms25115786.

12. [12] Lusta, K. A.; Poznyak, A. V.; Sukhorukov, V. N.; Eremin, I. I.; Nadelyaeva, I. I.; Orekhov, A. N. Hypotheses on atherogenesis triggering: does the infectious nature of atherosclerosis development have a substruction? *Cells* **2023**, *12*(5), 707. https://doi.org/10.3390/cells12050707.

13. [13] Gusev, E.; Sarapultsev, A. Atherosclerosis and inflammation: insights from the

theory of general pathological processes. *Int. J. Mol. Sci.* **2023**, *24*(9), 7910. https://doi.org/10.3390/ijms24097910.

14. [14] Gimbrone, M. A., Jr.; García-Cardeña, G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ. Res.* **2016**, *118*(4), 620–636. https://doi.org/10.1161/CIRCRESAHA.115.306301.

15. [15] Malekmohammad, K.; Bezsonov, E. E.; Rafieian-Kopaei, M. Role of lipid accumulation and inflammation in atherosclerosis: focus on molecular and cellular mechanisms. *Front. Cardiovasc. Med.* **2021**, *8*, 707529. https://doi.org/10.3389/fcvm.2021.707529.

16. [16] Cyr, A. R.; Huckaby, L. V.; Shiva, S. S.; Zuckerbraun, B. S. Nitric oxide and endothelial dysfunction. *Crit. Care Clin.* **2020**, *36*(2), 307–321. https://doi.org/10.1016/j.ccc.2019.12.009.

17. [17] Cahill, P. A.; Redmond, E. M. Vascular endothelium—gatekeeper of vessel health. *Atherosclerosis* **2016**, *248*, 97–109. https://doi.org/10.1016/j.atherosclerosis.2016.03.007.

18. [18] Rajashekhar, G.; Willuweit, A.; Patterson, C. E.; Sun, P.; Hilbig, A.; Breier, G.; Helisch, A.; Clauss, M. Continuous endothelial cell activation increases angiogenesis: evidence for the direct role of endothelium linking angiogenesis and inflammation. *J. Vasc. Res.* **2006**, *43*(2), 193–204. https://doi.org/10.1159/000090949.

19. [19] Immanuel, J.; Yun, S. Vascular inflammatory diseases and endothelial phenotypes. *Cells* **2023**, *12*(12), 1640. https://doi.org/10.3390/cells12121640.

20. [20] Chiorescu, R. M.; Mocan, M.; Inceu, A. I.; Buda, A. P.; Blendea, D.; Vlaicu, S. I. Vulnerable atherosclerotic plaque: is there a molecular signature? *Int. J. Mol. Sci.* **2022**, *23*(21), 13638. https://doi.org/10.3390/ijms232113638.

21. [21] Miceli, G.; Basso, M. G.; Pintus, C.; Pennacchio, A. R.; Cocciola, E.; Cuffaro, M.; Profita, M.; Rizzo, G.; Tuttolomondo, A. Molecular pathways of vulnerable carotid plaques at risk of ischemic stroke: a narrative review. *Int. J. Mol. Sci.* **2024**, *25*(8), 4351. https://doi.org/10.3390/ijms25084351.

22. [22] Loftus, I. Mechanisms of plaque rupture. In *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists*; Fitridge, R.; Thompson, M., Eds.; University of Adelaide Press: Adelaide, AU, 2011; Chapter 4. Available online: https://www.ncbi.nlm.nih.gov/books/NBK534259/.

23. [23] Badimon, L.; Padró, T.; Vilahur, G. Atherosclerosis, platelets, and thrombosis in acute ischaemic heart disease. *Eur. Heart J. Acute Cardiovasc. Care* **2012**, *1*(1), 60–74. https://doi.org/10.1177/2048872612441582.

24. [24] Libby, P.; Pasterkamp, G.; Crea, F.; Jang, I. K. Reassessing the mechanisms of acute coronary syndromes. *Circ. Res.***2019**, *124*(1), 150–160. https://doi.org/10.1161/CIRCRESAHA.118.311098.

25. [25] Meng, T.; Li, X.; Li, C.; Liu, J.; Chang, H.; Jiang, N.; Li, J.; Zhou, Y.; Liu, Z. Natural products of traditional Chinese medicine treat atherosclerosis by regulating inflammatory and oxidative stress pathways. *Front. Pharmacol.* **2022**, *13*, 997598. https://doi.org/10.3389/fphar.2022.997598.

26. [26] Zhu, J.; Wang, Y.; Zhang, J.; Zhang, Y.; Liu, X. Immunomodulatory effects of neutrophil extracellular traps on atherosclerosis. *Front. Immunol.* **2021**, *12*, 672052. https://doi.org/10.3389/fimmu.2021.672052.

27. [27] Franck, G.; Mawson, T. L.; Sausen, G.; Salinas, M.; Masson, G. S.; Cole, A.; Beltrami-Moreira, M.; Chatzizisis, Y. S.; Quillard, T.; Tesmenitsky, Y.; Shvartz, E.; Sukhova, G. K.; Libby, P. Flow perturbation mediates neutrophil recruitment and potentiates endothelial injury via neutrophil extracellular traps in atherosclerosis. *Circulation* **2018**, *138*(18), 1819–1833. https://doi.org/10.1161/CIRCULATIONAHA.117.030843.

28. [28] Li, Y.; Zhang, H.; Li, Y.; Li, Y.; Wang, Y. Neutrophil extracellular traps: a potential therapeutic target in atherosclerosis. *Front. Immunol.* **2022**, *13*, 944249. https://doi.org/10.3389/fimmu.2022.944249.

29. [29] Döring, Y.; Soehnlein, O.; Weber, C. Neutrophil extracellular traps in atherosclerosis and atherothrombosis. *Circ. Res.* **2017**, *120*(4), 736–743. https://doi.org/10.1161/CIRCRESAHA.116.309692.

30. [30] Folco, E. J.; Mawson, T. L.; Vromman, A.; Bernardes-Souza, B.; Franck, G.; Persson, O.; Nakamura, M.; Newton, G.; Luscinskas, F. W.; Libby, P. Neutrophil extracellular traps induce endothelial cell activation and tissue factor production through interleukin-1α and cathepsin G. *Arterioscler. Thromb. Vasc. Biol.* **2018**, *38*(8), 1901–1912. https://doi.org/10.1161/ATVBAHA.118.311150.

31. [31] Knight, J. S.; Luo, W.; O'Dell, A. A.; Yalavarthi, S.; Zhao, W.; Subramanian, V.; Guo, C.; Grenn, R. C.; Thompson, P. R.; Eitzman, D. T.; Kaplan, M. J. Peptidylarginine deiminase inhibition disrupts NET formation and protects against thrombosis in mice. *J. Clin. Invest.* **2014**, *124*(10), 4690–4701. https://doi.org/10.1172/JCI76175.

32. [32] Wang, H.; Wang, C.; Zhao, M.-H.; Chen, M. Neutrophil extracellular traps can activate alternative complement pathways. *Clin. Exp. Immunol.* **2015**, *181*(3), 518–527. https://doi.org/10.1111/cei.12654.

33. [33] Wong, S. L.; Demers, M.; Martinod, K.; Gallant, M.; Wang, Y.; Goldfine, A. B.; Kahn, C. R.;

Wagner, D. D. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat. Med.* **2015**, *21*(7), 815–819. https://doi.org/10.1038/nm.3887.

34. [34] Silvestre-Roig, C.; Braster, Q.; Wichapong, K.; Lee, E. Y.; Teulon, J. M.; Berrebeh, N.; Winter, J.; Hidalgo, A.; Soehnlein, O.; Weber, C. Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death. *Nature***2019**, *569*(7755), 236–240. https://doi.org/10.1038/s41586-019-1167-6.

35. [35] Zernecke, A.; Soehnlein, O. Pathways of leukocyte recruitment during atherosclerosis. *Nat. Rev. Cardiol.* **2018**, *15*(12), 779–796. https://doi.org/10.1038/s41569-018-0093-1.

36. [36] van Avondt, K.; Hartl, D. Mechanisms and disease relevance of neutrophil extracellular trap formation. *Eur. J. Clin. Invest.* **2018**, *48*(suppl. 2), e12919. https://doi.org/10.1111/eci.12919.

37. [37] He, S.; Wang, L.; Miao, L.; Wang, T.; Du, F.; Zhao, L.; Wang, X. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-α. *Cell* **2009**, *137*(6), 1100–1111. https://doi.org/10.1016/j.cell.2009.05.021.

38. [38] Wang, Y.; Li, M.; Stadler, S.; Correll, S.; Li, P.; Wang, D.; Hayama, R.; Leonelli, L.; Han, H.; Grigoryev, S. A.; Allis, C. D.; Coonrod, S. A. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J. Cell Biol.* **2009**, *184*(2), 205–213. https://doi.org/10.1083/jcb.200806072.

39. [39] Metzler, K. D.; Fuchs, T. A.; Nauseef, W. M.; Reumaux, D.; Roesler, J.; Schulze, I.; Wahn, V.; Papayannopoulos, V.; Zychlinsky, A. Myeloperoxidase is required for neutrophil extracellular trap formation: implications for innate immunity. *Blood* **2011**, *117*(3), 953–959. https://doi.org/10.1182/blood-2010-06-290171.

40. [40] Fuchs, T. A.; Abed, U.; Goosmann, C.; Hurwitz, R.; Schulze, I.; Wahn, V.; Weinrauch, Y.; Brinkmann, V.; Zychlinsky, A. Novel cell death program leads to neutrophil extracellular traps. *J. Cell Biol.* **2007**, *176*(2), 231–241. https://doi.org/10.1083/jcb.200606027.

41. [41] Neeli, I.; Khan, S. N.; Radic, M. Histone deimination as a response to inflammatory stimuli in neutrophils. *J. Immunol.* **2008**, *180*(3), 1895–1902. https://doi.org/10.4049/jimmunol.180.3.1895.

42. [42] Leshner, M.; Wang, S.; Lewis, C.; Zheng, H.; Chen, X. A.; Santy, L.; Wang, Y. PAD4-mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding in neutrophils. *J. Biol. Chem.* **2012**, *287*(42), 35126–35138. https://doi.org/10.1074/jbc.M112.375097.

43. [43] Thiam, H. R.; Wong, S. L.; Wagner, D. D.; Waterman, C. M. Cellular mechanisms of NETosis. *Annu. Rev. Cell Dev. Biol.* **2020**, *36*, 191–218. https://doi.org/10.1146/annurev-cellbio-020520-111016.

44. [44] Douda, D. N.; Khan, M. A.; Grasemann, H.; Palaniyar, N. SK3 channel and mitochondrial ROS mediate NADPH oxidase–independent NETosis induced by calcium influx. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*(9), 2817–2822. https://doi.org/10.1073/pnas.1414055112.

45. [45] Vorobjeva, N. V.; Pinegin, B. V. Neutrophil extracellular traps: mechanisms of formation and role in health and disease. *Biochemistry (Moscow)* **2014**, *79*(12), 1286–1296. https://doi.org/10.1134/S0006297914120047.

46. [46] de Bont, C. M.; Boelens, W. C.; Pruijn, G. J. M. NETosis, complement, and coagulation: a triangular relationship. *Cell. Mol. Immunol.* **2019**, *16*(1), 19–27. https://doi.org/10.1038/s41423-018-0024-0.

47. [47] van der Linden, M.; Meyaard, L. Fine-tuning neutrophil activation: strategies and consequences. *Immunol. Lett.***2016**, *178*, 3–9. https://doi.org/10.1016/j.imlet.2016.05.008.

48. [48] Warnatsch, A.; Ioannou, M.; Wang, Q.; Papayannopoulos, V. Inflammation: Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science* **2015**, *349*(6245), 316–320. https://doi.org/10.1126/science.aaa8064.

49. [49] Nahrendorf, M.; Swirski, F. K. Innate immune cells in ischaemic heart disease: does myocardial infarction beget myocardial infarction? *Eur. Heart J.* **2016**, *37*(11), 868–872. https://doi.org/10.1093/eurheartj/ehv653.

50. [50] Soehnlein, O.; Libby, P. Targeting inflammation in atherosclerosis—from experimental insights to the clinic. *Nat. Rev. Drug Discov.* **2021**, *20*(8), 589–610. https://doi.org/10.1038/s41573-021-00198-1.

51. [51] Libby, P.; Hansson, G. K. Inflammation and immunity in diseases of the arterial tree: players and layers. *Circ. Res.***2015**, *116*(2), 307–311. https://doi.org/10.1161/CIRCRESAHA.116.301313.

52. [52] Hansson, G. K.; Hermansson, A. The immune system in atherosclerosis. *Nat. Immunol.* **2011**, *12*(3), 204–212. https://doi.org/10.1038/ni.2001.

53. [53] Gisterå, A.; Hansson, G. K. The immunology of atherosclerosis. *Nat. Rev. Nephrol.* **2017**, *13*(6), 368–380. https://doi.org/10.1038/nrneph.2017.51.

54. [54] Swirski, F. K.; Nahrendorf, M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science* **2013**, *339*(6116), 161–166. https://doi.org/10.1126/science.1230719.

55. [55] Jonasson, L.; Holm, J.; Skalli, O.; Bondjers, G.; Hansson, G. K. Regional accumulations of T cells, macrophages, and smooth muscle cells in

the human atherosclerotic plaque. *Arteriosclerosis* **1986**, *6*(2), 131–138. https://doi.org/10.1161/01.atv.6.2.131.

56. [56] Robbins, C. S.; Hilgendorf, I.; Weber, G. F.; Theurl, I.; Iwamoto, Y.; Figueiredo, J. L.; Gorbatov, R.; Sukhova, G. K.; Gerhardt, L. M.; Smyth, D.; et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat. Med.* **2013**, *19*(9), 1166–1172. https://doi.org/10.1038/nm.3258.

57. [57] Moore, K. J.; Sheedy, F. J.; Fisher, E. A. Macrophages in atherosclerosis: a dynamic balance. *Nat. Rev. Immunol.***2013**, *13*(10), 709–721. https://doi.org/10.1038/nri3520.

58. [58] Tabas, I.; Bornfeldt, K. E. Macrophage phenotype and function in different stages of atherosclerosis. *Circ. Res.* **2016**, *118*(4), 653–667. https://doi.org/10.1161/CIRCRESAHA.115.306256.

59. [59] Williams, J. W.; Giannarelli, C.; Rahman, A.; Randolph, G. J.; Kovacic, J. C. Macrophage biology, classification, and phenotype in cardiovascular disease: JACC macrophage in CVD series (Part 1). *J. Am. Coll. Cardiol.* **2018**, *72*(18), 2166–2180. https://doi.org/10.1016/j.jacc.2018.08.2149.

60. [60] Barrett, T. J. Macrophages in atherosclerosis regression. *Arterioscler. Thromb. Vasc. Biol.* **2020**, *40*(1), 20–33. https://doi.org/10.1161/ATVBAHA.119.312802.

61. [61] Stöger, J. L.; Gijbels, M. J.; van der Velden, S.; Manca, M.; van der Loos, C. M.; Biessen, E. A.; Daemen, M. J.; Lutgens, E.; de Winther, M. P. Distribution of macrophage polarization markers in human atherosclerosis. *Atherosclerosis***2012**, *225*(2), 461–468. https://doi.org/10.1016/j.atherosclerosis.2012.09.013.

62. [62] Rahman, K.; Vengrenyuk, Y.; Ramsey, S. A.; Vila, N. R.; Girgis, N. M.; Liu, J.; Gusarova, V.; Gromada, J.; Weinstock, A.; Moore, K. J.; Fisher, E. A. Inflammatory Ly6Chi monocytes and their conversion to M2 macrophages drive atherosclerosis regression. *J. Clin. Invest.* **2017**, *127*(8), 2904–2915. https://doi.org/10.1172/JCI75005.

63. [63] Hilgendorf, I.; Swirski, F. K. Making a difference: monocyte heterogeneity in cardiovascular disease. *Curr. Atheroscler. Rep.* **2012**, *14*(5), 450–459. https://doi.org/10.1007/s11883-012-0273-8.

64. [64] Shankman, L. S.; Gomez, D.; Cherepanova, O. A.; Salmon, M.; Alencar, G. F.; Haskins, R. M.; Swiatlowska, P.; Newman, A. A.; Greene, E. S.; Straub, A. C.; et al. KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. *Nat. Med.* **2015**, *21*(6), 628–637. https://doi.org/10.1038/nm.3866.

65. [65] Feil, S.; Fehrenbacher, B.; Lukowski, R.; Essmann, F.; Schulze-Osthoff, K.; Schaller, M.; Feil, R. Transdifferentiation of vascular smooth muscle cells to macrophage-like cells during atherogenesis. *Circ. Res.* **2014**, *115*(7), 662–667. https://doi.org/10.1161/CIRCRESAHA.115.304634.

66. [66] Bennett, M. R.; Sinha, S.; Owens, G. K. Vascular smooth muscle cells in atherosclerosis. *Circ. Res.* **2016**, *118*(4), 692–702. https://doi.org/10.1161/CIRCRESAHA.115.306361.

67. [67] Chistiakov, D. A.; Orekhov, A. N.; Bobryshev, Y. V. Vascular smooth muscle cell in atherosclerosis. *Acta Physiol.***2015**, *214*(1), 33–50. https://doi.org/10.1111/apha.12466.

68. [68] Bennett, M. R.; Clarke, M. C. Regulation of vascular cell apoptosis in atherosclerosis and its role in plaque stability. *Cardiovasc. Res.* **2005**, *67*(4), 594–604. https://doi.org/10.1016/j.cardiores.2005.06.002.

69. [69] Clarke, M. C.; Figg, N.; Maguire, J. J.; Davenport, A. P.; Goddard, M.; Littlewood, T. D.; Bennett, M. R. Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. *Nat. Med.* **2006**, *12*(9), 1075–1080. https://doi.org/10.1038/nm1459.

70. [70] Yurdagul, A., Jr.; Doran, A. C.; Cai, B.; Fredman, G.; Tabas, I. Mechanisms and consequences of defective efferocytosis in atherosclerosis. *Front. Cardiovasc. Med.* **2017**, *4*, 86. https://doi.org/10.3389/fcvm.2017.00086.

71. [71] Kojima, Y.; Downing, K.; Kundu, R.; Miller, C.; Dewey, F.; Lancero, H.; Raaz, U.; Perisic, L.; Hedin, U.; Schadt, E.; et al. Cyclin-dependent kinase inhibitor 2B regulates efferocytosis and atherosclerosis. *J. Clin. Invest.* **2014**, *124*(3), 1083–1097. https://doi.org/10.1172/JCI70391.

72. [72] Tabas, I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat. Rev. Immunol.* **2010**, *10*(1), 36–46. https://doi.org/10.1038/nri2675.

73. [73] Viola, J.; Soehnlein, O. Atherosclerosis—A matter of unresolved inflammation. *Semin. Immunol.* **2015**, *27*(3), 184–193. https://doi.org/10.1016/j.smim.2015.03.013.

74. [74] Lahoute, C.; Herbin, O.; Mallat, Z.; Tedgui, A. Adaptive immunity in atherosclerosis: mechanisms and future therapeutic targets. *Nat. Rev. Cardiol.* **2011**, *8*(6), 348–358. https://doi.org/10.1038/nrcardio.2011.62.

75. [75] Kyaw, T.; Winther, M. P. J. de; Toh, B. H. The role of adaptive immunity in atherosclerosis. *Clin. Transl. Immunol.***2014**, *3*(10), e33. https://doi.org/10.1038/cti.2014.29.

76. [76] Ketelhuth, D. F.; Hansson, G. K. Adaptive response of T and B cells in atherosclerosis. *Circ. Res.* **2016**, *118*(4), 668–678. https://doi.org/10.1161/CIRCRESAHA.115.306427.

77. [77] Fernandez, D. M.; Rahman, A. H.; Fernandez, N. F.; Chudnovskiy, A.; Amir, E. D.; Amadori, L.; Khan, N. S.; Wong, C. K.; Shamailova,

R.; Hill, C. A.; et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat. Med.* **2019**, *25*(10), 1576–1588. https://doi.org/10.1038/s41591-019-0590-4.

78. [78] Lahoute, C.; Herbin, O.; Mallat, Z.; Tedgui, A. Adaptive immunity in atherosclerosis: mechanisms and future therapeutic targets. *Nat. Rev. Cardiol.* **2011**, *8*(6), 348–358. https://doi.org/10.1038/nrcardio.2011.62.

79. [79] Sage, A. P.; Tsiantoulas, D.; Binder, C. J.; Mallat, Z. The role of B cells in atherosclerosis. *Nat. Rev. Cardiol.* **2019**, *16*(3), 180–196. https://doi.org/10.1038/s41569-018-0106-9.

80. [80] Kyaw, T.; Tay, C.; Khan, A.; Dumouchel, V.; Cao, A.; To, K.; Kehry, M.; Dunn, R.; Agrotis, A.; Tipping, P.; Bobik, A.; Toh, B. H. B cell depletion therapy attenuates atherosclerotic plaque development in ApoE-deficient mice. *J. Am. Heart Assoc.* **2012**, *1*(6), e000031. https://doi.org/10.1161/JAHA.111.000031.

81. [81] Kyaw, T.; Tipping, P.; Bobik, A.; Toh, B. H. Protective role of natural IgM-producing B cells in atherosclerosis. *Clin. Exp. Immunol.* **2012**, *167*(1), 109–118. https://doi.org/10.1111/j.1365-2249.2011.04490.x.

82. [82] Sage, A. P.; Mallat, Z. Multiple potential roles for B cells in atherosclerosis. *Ann. Med.* **2014**, *46*(5), 297–303. https://doi.org/10.3109/07853890.2014.907065.

83. [83] Whitman, S. C.; Ravisankar, P.; Daugherty, A. IFN-γ deficiency exerts gender-specific effects on atherogenesis in LDL receptor–deficient mice. *J. Interferon Cytokine Res.* **2002**, *22*(6), 661–670. https://doi.org/10.1089/10799900260100168.

84. [84] Buono, C.; Binder, C. J.; Stavrakis, G.; Witztum, J. L.; Glimcher, L. H.; Lichtman, A. H. T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*(5), 1596–1601. https://doi.org/10.1073/pnas.0409015102.

85. [85] Mallat, Z.; Gojova, A.; Brun, V.; Esposito, B.; Fournier, N.; Cottrez, F.; Tedgui, A.; Groux, H. Induction of a regulatory T cell type 1 response reduces the development of atherosclerosis in apolipoprotein E–knockout mice. *Circulation* **2003**, *108*(10), 1232–1237. https://doi.org/10.1161/01.CIR.0000089081.61317.F6.

86. [86] Mor, A.; Luboshits, G.; Planer, D.; Keren, G.; George, J. Altered status of CD4+CD25+ regulatory T cells in patients with acute coronary syndromes. *Eur. Heart J.* **2006**, *27*(21), 2530–2537. https://doi.org/10.1093/eurheartj/ehl203.

87. [87] Chen, T.; Wang, M.; Yang, H.; Gao, Y.; Hu, L.; Chen, W.; Zhang, S.; Zhang, C. Regulatory T cells and atherosclerosis. *Clin. Immunol.* **2022**, *238*, 109032. https://doi.org/10.1016/j.clim.2022.109032.

88. [88] Lichtman, A. H.; Binder, C. J.; Tsimikas, S.; Witztum, J. L. Adaptive immunity in atherogenesis: new insights and therapeutic approaches. *J. Clin. Invest.* **2013**, *123*(1), 27–36. https://doi.org/10.1172/JCI63108.

89. [89] Döring, Y.; Drechsler, M.; Soehnlein, O.; Weber, C. Neutrophils in atherosclerosis: from mice to man. *Arterioscler. Thromb. Vasc. Biol.* **2015**, *35*(2), 288–295. https://doi.org/10.1161/ATVBAHA.114.303564.

90. [90] Drechsler, M.; Megens, R. T. A.; van Zandvoort, M.; Weber, C.; Soehnlein, O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation* **2010**, *122*(18), 1837–1845. https://doi.org/10.1161/CIRCULATIONAHA.110.961714.

91. [91] Rotzius, P.; Thams, S.; Soehnlein, O.; Kenne, E.; Tseng, C.-N.; Björkström, N. K.; Malmberg, K.-J.; Lindbom, L.; Eriksson, E. E. Distinct infiltration of neutrophils in lesion shoulders in ApoE−/− mice. *Am. J. Pathol.* **2010**, *177*(1), 493–500. https://doi.org/10.2353/ajpath.2010.090480.

92. [92] Ionita, M. G.; van den Borne, P.; Catanzariti, L. M.; Moll, F. L.; de Vries, J. P.; Pasterkamp, G.; de Kleijn, D. P. V. High neutrophil numbers in human carotid atherosclerotic plaques are associated with rupture-prone lesions. *Atherosclerosis* **2010**, *211*(1), 67–75. https://doi.org/10.1016/j.atherosclerosis.2010.01.033.

93. [93] Quillard, T.; Franck, G.; Mawson, T.; Folco, E.; Libby, P. Mechanisms of erosion of atherosclerotic plaques. *Curr. Opin. Lipidol.* **2017**, *28*(5), 434–441. https://doi.org/10.1097/MOL.0000000000000440.

94. [94] Borissoff, J. I.; Joosen, I. A.; Versteylen, M. O.; Brill, A.; Fuchs, T. A.; Savchenko, A. S.; Gallant, M.; Martinod, K.; Ten Cate, H.; Hofstra, L.; et al. Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. *Arterioscler. Thromb. Vasc. Biol.* **2013**, *33*(8), 2032–2040. https://doi.org/10.1161/ATVBAHA.113.301627.

95. [95] Schönrich, G.; Raftery, M. J. Neutrophil extracellular traps go viral. *Front. Immunol.* **2016**, *7*, 366. https://doi.org/10.3389/fimmu.2016.00366.

96. [96] Leppkes, M.; Maueröder, C.; Hirth, S.; Nowecki, S.; Günther, C.; Billmeier, U.; Paulus, S.; Biermann, M.; Munoz, L. E.; Hoffmann, M.; et al. Externalized decondensed chromatin as an endogenous danger signal in inflammatory disease. *Nat. Commun.* **2016**, *7*, 10593. https://doi.org/10.1038/ncomms10593.

97. [97] Westman, J.; Papareddy, P.; Dahlgren, M. W.; Chakrakodi, B.; Norrby-Teglund, A.; Smeds, E.; Mörgelin, M.; Rydell, G. E.; Malmström, E.; Björck, L.; et al. Extracellular histones induce chemokine production through TLR2 and TLR4

activation in human monocytes. *PLoS Pathog.* **2015**, *11*(5), e1005319. https://doi.org/10.1371/journal.ppat.1005319.

98. [98] Papayannopoulos, V. Neutrophil extracellular traps in immunity and disease. *Nat. Rev. Immunol.* **2018**, *18*(2), 134–147. https://doi.org/10.1038/nri.2017.105.

99. [99] Mutua, V.; Gershwin, L. J. A review of neutrophil extracellular traps (NETs) in disease: potential anti-NETs therapeutics. *Clin. Rev. Allergy Immunol.* **2021**, *61*(2), 194–211. https://doi.org/10.1007/s12016-020-08804-7.

100. [100] Brinkmann, V.; Reichard, U.; Goosmann, C.; Fauler, B.; Uhlemann, Y.; Weiss, D. S.; Weinrauch, Y.; Zychlinsky, A. Neutrophil extracellular traps kill bacteria. *Science* **2004**, *303*(5663), 1532–1535. https://doi.org/10.1126/science.1092385.

101. [101] Fadini, G. P.; Menegazzo, L.; Rigato, M.; Scattolini, V.; Poncina, N.; Bruttocao, A.; Ciciliot, S.; Mammano, F.; Ciampa, A.; Albiero, M.; Avogaro, A. NETosis delays diabetic wound healing in mice and humans. *Diabetes* **2016**, *65*(4), 1061–1071. https://doi.org/10.2337/db15-0863.

102. [102] Jiménez-Alcázar, M.; Rangaswamy, C.; Panda, R.; Bitterling, J.; Simsek, Y. J.; Long, A. T.; Bilyy, R.; Krenn, V.; Renné, C.; Renné, T.; Kluge, S.; Panzer, U.; Mizuta, R.; Mannherz, H. G.; Napirei, M.; Fuchs, T. A. Host DNases prevent vascular occlusion by neutrophil extracellular traps. *Science* **2017**, *358*(6367), 1202–1206. https://doi.org/10.1126/science.aam8897.

103. [103] Bonaventura, A.; Vecchié, A.; Abbate, A.; Montecucco, F. Neutrophil extracellular traps and cardiovascular diseases: an update. *Cells* **2020**, *9*(1), 231. https://doi.org/10.3390/cells9010231.

104. [104] Warnatsch, A.; Ioannou, M.; Wang, Q.; Papayannopoulos, V. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science* **2015**, *349*(6245), 316–320. https://doi.org/10.1126/science.aaa8064.

105. [105] Delgado-Rizo, V.; Martínez-Guzmán, M. A.; Iñiguez-Gutierrez, L.; García-Orozco, A.; Alvarado-Navarro, A.; Fafutis-Morris, M. E. Neutrophil extracellular traps and its implications in inflammation: an overview. *Front. Immunol.***2017**, *8*, 81. https://doi.org/10.3389/fimmu.2017.00081.

106. [106] Gupta, S.; Chan, D. W.; Zaal, K. J. M.; Kaplan, M. J. NETosis: process and therapeutic potential. *Trends Pharmacol. Sci.* **2022**, *43*(7), 553–568. https://doi.org/10.1016/j.tips.2022.04.004.

107. [107] Pertiwi, K. R.; van der Wal, A. C.; Pabittei, D. R.; Mackaaij, C.; van Leeuwen, M. B.; Li, X.; de Boer, O. J. Neutrophil extracellular traps participate in all different types of thrombotic and hemorrhagic complications of coronary atherosclerosis. *Thromb. Haemost.* **2018**, *118*(6), 1078–1087. https://doi.org/10.1055/s-0038-1641584.

108. [108] Franck, G.; Mawson, T.; Folco, E. J.; Molinaro, R.; Ruvkun, V.; Engelbertsen, D.; Liu, X.; Tesmenitsky, Y.; Shvartz, E.; Sukhova, G. K.; Croce, K. J.; Libby, P. Roles of PAD4 and NETosis in experimental atherosclerosis and arterial injury: implications for superficial erosion. *Circ. Res.* **2018**, *123*(1), 33–42. https://doi.org/10.1161/CIRCRESAHA.118.312494.

109. [109] Stakos, D. A.; Kambas, K.; Konstantinidis, T.; Mitroulis, I.; Apostolidou, E.; Arelaki, S.; Tsironidou, V.; Giatromanolaki, A.; Skendros, P.; Konstantinides, S.; Ritis, K. Expression of functional tissue factor by neutrophil extracellular traps in culprit artery of acute myocardial infarction. *Eur. Heart J.* **2015**, *36*(22), 1405–1414. https://doi.org/10.1093/eurheartj/ehv007.

110. [110] Savchenko, A. S.; Borissoff, J. I.; Martinod, K.; De Meyer, S. F.; Gallant, M.; Erpenbeck, L.; Brill, A.; Wang, Y.; Wagner, D. D. VWF-mediated leukocyte recruitment with NETs formation promotes deep vein thrombosis in mice. *Blood***2014**, *123*(26), 4113–4123. https://doi.org/10.1182/blood-2013-07-518654.

111. [111] Wong, S. L.; Wagner, D. D. Peptidylarginine deiminase 4: a nuclear protein with cytoplasmic activity. *Curr. Opin. Hematol.* **2018**, *25*(1), 42–49. https://doi.org/10.1097/MOH.0000000000000390.

112. [112] Knight, J. S.; Zhao, W.; Luo, W.; Subramanian, V.; O'Dell, A. A.; Yalavarthi, S.; Hodgin, J. B.; Eitzman, D. T.; Thompson, P. R.; Kaplan, M. J. Peptidylarginine deiminase inhibition reduces vascular damage and modulates innate immune responses in murine lupus. *Arthritis Rheumatol.* **2015**, *67*(2), 507–517. https://doi.org/10.1002/art.38957.

113. [113] Liu, C.; Zhang, W.; Wang, J.; Si, J.; Li, L.; Zhao, Y.; Wang, W.; Lu, C.; Yang, S.; Wu, J. Inhibition of PAD4 improves inflammation and atherosclerosis progression in ApoE−/− mice through reducing NETs. *Atherosclerosis* **2018**, *273*, 1–9. https://doi.org/10.1016/j.atherosclerosis.2018.04.018.

114. [114] Döring, Y.; Libby, P.; Soehnlein, O. Neutrophil extracellular traps participate in cardiovascular diseases: recent experimental and clinical insights. *Circ. Res.* **2020**, *126*(9), 1228–1241. https://doi.org/10.1161/CIRCRESAHA.120.315931.

115. [115] Sollberger, G.; Tilley, D. O.; Zychlinsky, A. Neutrophil extracellular traps: the biology of chromatin externalization. *Dev. Cell* **2018**, *44*(5), 542–553. https://doi.org/10.1016/j.devcel.2018.01.019.

116. [116] Kenny, E. F.; Herzig, A.; Krüger, R.; Muth, A.; Mondal, S.; Thompson, P. R.; Brinkmann, V.; von Bernuth, H.; Zychlinsky, A. Diverse stimuli engage different neutrophil extracellular trap

pathways. *eLife* **2017**, *6*, e24437. https://doi.org/10.7554/eLife.24437.

117. [117] Munoz, L. E.; Bilyy, R.; Biermann, M. H.; Kienhöfer, D.; Maueröder, C.; Hahn, J.; Brauner, J. M.; Weidner, D.; Chen, J.; Scharin-Mehlmann, M.; et al. Nanoparticles size-dependently initiate self-limiting NET formation controlled by phagocytosis. *Front. Immunol.* **2016**, *7*, 557. https://doi.org/10.3389/fimmu.2016.00557.

118. [118] Cooper, P. R.; Palmer, L. J.; Chapple, I. L. C. Neutrophil extracellular traps as a new paradigm in innate immunity: friend or foe? *Periodontol. 2000* **2013**, *63*(1), 165–197. https://doi.org/10.1111/prd.12025.

119. [119] Jorch, S. K.; Kubes, P. An emerging role for neutrophil extracellular traps in noninfectious disease. *Nat. Med.* **2017**, *23*(3), 279–287. https://doi.org/10.1038/nm.4294.

120. [120] Brinkmann, V. Neutrophil extracellular traps in the second decade. *J. Innate Immun.* **2018**, *10*(5-6), 414–421. https://doi.org/10.1159/000489829.

121. [121] Benítez, S.; Villegas, V.; Bancells, C.; Jorba, O.; González-Sastre, F.; Ordóñez-Llanos, J.; Sánchez-Quesada, J. L. Impaired binding affinity of electronegative low-density lipoprotein to the LDL receptor is related to non-esterified fatty acids and lysophosphatidylcholine content. *Biochemistry* **2004**, *43*(50), 15863–15872. https://doi.org/10.1021/bi0481667.

122. [122] Uribe, K. B.; Méndez, M. T.; Gonzalez-Porras, M. A.; Fernández, M. C.; Arévalo, J. R.; Álvarez, I. B.; Osorio, A. S. Oxidized LDL triggers NETosis through TLR2 and complement activation in human neutrophils. *Free Radic. Biol. Med.* **2020**, *152*, 178–188. https://doi.org/10.1016/j.freeradbiomed.2020.03.010.

123. [123] Abi-Mansour, J.; Keck, K.; Ding, J.; Liu, W.; Fredman, G.; Tabas, I.; Wang, N. Mechanisms of defective efferocytosis in atherosclerosis. *Front. Immunol.* **2024**, *15*, 1423420. https://doi.org/10.3389/fimmu.2024.1423420.

124. [124] Pertiwi, K. R.; de Boer, O. J.; Mackaaij, C.; Pabittei, D. R.; de Winter, R. J.; Li, X.; van der Wal, A. C. Extracellular traps derived from macrophages, mast cells, eosinophils, and neutrophils are generated in atherosclerotic plaques. *Front. Immunol.* **2019**, *10*, 2138. https://doi.org/10.3389/fimmu.2019.02138.

125. [125] Kimball, A. S.; Obi, A. T.; Diaz, J. A.; Henke, P. K. The emerging role of NETs in venous thrombosis and immunothrombosis. *Front. Immunol.* **2016**, *7*, 236. https://doi.org/10.3389/fimmu.2016.00236.

126. [126] Jiménez-Alcázar, M.; Napirei, M.; Fuchs, T. A. Tissue factor-bearing extracellular vesicles and NETs in thrombosis. *Front. Immunol.* **2017**, *8*, 1278. https://doi.org/10.3389/fimmu.2017.01278.

127. [127] Martinod, K.; Wagner, D. D. Thrombosis: tangled up in NETs. *Blood* **2014**, *123*(18), 2768–2776. https://doi.org/10.1182/blood-2013-10-463646.

128. [128] Laridan, E.; Denorme, F.; Desender, L.; François, O.; Andersson, T.; Deckmyn, H.; Vanhoorelbeke, K.; De Meyer, S. F. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann. Neurol.* **2017**, *82*(2), 223–232. https://doi.org/10.1002/ana.24993.

129. [129] Vallés, J.; Lago, A.; Santos, M. T.; Latorre, A. M.; Tembl, J. I.; Salom, J. B.; Nieves, C.; Moscardó, A. Neutrophil extracellular traps are increased in patients with acute ischemic stroke: prognostic significance. *Thromb. Haemost.* **2017**, *117*(10), 1919–1929. https://doi.org/10.1160/TH17-02-0130.

130. [130] Döring, Y.; Soehnlein, O.; Weber, C. Neutrophil extracellular traps in atherosclerosis and thrombosis. *Circ. Res.***2017**, *120*(4), 736–743. https://doi.org/10.1161/CIRCRESAHA.116.309692.

131. [131] Laridan, E.; Martinod, K.; De Meyer, S. F. Neutrophil extracellular traps in arterial and venous thrombosis. *Semin. Thromb. Hemost.* **2019**, *45*(1), 86–93. https://doi.org/10.1055/s-0038-1677040.

132. [132] Pertiwi, K. R.; van der Wal, A. C.; Li, X.; Mackaaij, C.; Pabittei, D. R.; de Boer, O. J. Extracellular traps derived from macrophages, mast cells, eosinophils, and neutrophils are generated in atherosclerotic plaques. *Front. Immunol.***2019**, *10*, 2138. https://doi.org/10.3389/fimmu.2019.02138.

133. [133] Silvestre-Roig, C.; Hidalgo, A.; Soehnlein, O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood* **2016**, *127*(18), 2173–2181. https://doi.org/10.1182/blood-2015-12-636829.

134. [134] Kolaczkowska, E.; Kubes, P. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.***2013**, *13*(3), 159–175. https://doi.org/10.1038/nri3399.

135. [135] Brinkmann, V.; Zychlinsky, A. Neutrophil extracellular traps: is immunity the second function of chromatin? *J. Cell Biol.* **2012**, *198*(5), 773–783. https://doi.org/10.1083/jcb.201203170.