Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Review

The role of NETosis in enhancing of atherosclerosis

Alexander V. Blagov[1*], Alexey V. Churov[1,2], Irina A. Starodubtseva[3], Dmitry F. Beloyartsev[4], Tatiana I. Kovyanova[1,5], Tamara Pecherina[6], Vasily N. Sukhorukov[1] and Alexander Orekhov[1]

[1] Laboratory of Angiopathology, Institute of General Pathology and Pathophysiology, Moscow 125315, Russia
[2] Institute on Aging Research, Russian Gerontology Clinical Research Centre, Pirogov Russian National Research Medical University, 16, 1st Leonova Street, 129226 Moscow, Russia
[3] Department of Polyclinic Therapy, NN Burdenko Voronezh State Medical University, 10 Studencheskaya Street, 394036 Voronezh, Russia
[4] Vascular Surgery Department, A. V. Vishnevsky National Medical Research Center of Surgery, 27 Bolshaya Serpukhovskaya Street, 117997 Moscow, Russia
[5] Institute for Atherosclerosis Research, Osennyaya Street 4-1-207, 121609 Moscow, Russia
[6] Laboratory for Cardiac Fibrosis, Research Institute for Complex Issues of Cardiovascular Diseases, 650002, Kemerovo, Russia

Article Info

Article history:

Received: June 23, 2024
Accepted: September 04, 2024
Published: September 30, 2024

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Abstract

Activated neutrophils release neutrophil extracellular traps (NETs), complex structures composed of extracellular genetic material and proteins sourced from the nucleus, granules, and cytoplasm in response to pathogenic inflammatory conditions. These NETs play a crucial role in the host's innate immune defense against invasive infections. Notably, in conditions like atherosclerosis, these extracellular formations can also be elicited by inflammatory stimuli such as lipids, prothrombotic factors, platelet aggregation, or proinflammatory cytokines. NETs have been identified on the inner arterial walls in cardiovascular disease states. By promoting inflammation through NETosis-mediated cell adhesion processes and exerting cytotoxic effects leading to cellular dysfunction and tissue damage, NETs contribute to the pathogenesis of inflammatory conditions.

Keywords: NETs, NETosis, Atherosclerosis, Neutrophils

1. Introduction

Atherosclerosis is a disease characterized by the accumulation of lipids, fibrous elements and calcification in large arteries. Atherosclerosis is the underlying pathophysiology of CVD, which occurs due to lipid-induced chronic inflammatory disease of the large arteries [1]. Long-term accumulation of plasma lipoproteins, namely hyperlipidemia, causes endothelial cell dysfunction, monocyte adhesion, intimal penetration and differentiation into macrophages. In turn, macrophages ingest lipoproteins, including oxidized low-density lipoproteins and triglyceride-rich lipoprotein remnants, resulting in the formation of foam cells. Subsequently, migration, proliferation, and synthesis of extracellular matrix macromolecules in resident smooth muscle cells are involved in lesion progression. Expanding foam cells and dead cells promote the formation of cholesterol-rich necrotic nuclei, which can ultimately lead to clinical complications [2]. Atherosclerosis, a multifactorial disease involving the interaction of genetic and environmental factors, is estimated to cause 18 million deaths per year worldwide. Although the tendency to develop atherosclerosis is higher in men than women, the incidence of atherosclerosis is increasing in women as a result of dietary habits, smoking and mental stress [3].

Neutrophils are the most abundant inflammatory cells in the peripheral circulation, which play an antimicrobial role through phagocytosis and degranulation as first responders to acute inflammation [4]. Given their limited lifespan, neutrophils have long been underappreciated in chronic inflammatory diseases such as atherosclerosis, diabetes mellitus, and autoimmune disorders. Recently, the resurgence of neutrophils in chronic cardiovascular inflammation has become a concern, resulting from the discovery of a third antimicrobial mechanism, NETs, as

* Corresponding author.
E-mail address: al. blagov2014@gmail. com (A. V. Blagov).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.9.25

a significant factor in atherosclerosis and its clinical complications [5].

Despite optimal management of traditional risk factors according to current treatment guidelines, significant cardiovascular risk still exists [6]. Therefore, it is important to investigate every aspect of atherosclerosis as a potential therapeutic target and carefully study each relevant treatment effect. In regard to atherosclerosis, reducing inflammation and creating public health prevention plans for changeable cardiovascular risks will greatly reduce the burden of disease, raise the rate of disability worldwide, enhance quality of life, and eventually improve human health. This review aims to present a summary and illustrates of the most recent research on the processes underlying the production of neural extracellular matrix (NETs), the role that NETosis plays in the promotion of atherosclerosis and its sequelae, and the potential utility of NETs as enabling biomarkers and therapeutic targets in medical care.

2. General pathogenesis of atherosclerosis

2.1. The occurrence of atherosclerosis and the formation of fatty streaks

Low-density lipoproteins (LDL) are retained and undergo alteration in the intima as a result of endothelial dysfunction, which is the first step in atherosclerosis [7]. Once within the subendothelial region, the trapped LDL particles undergo oxidation, which is aided by the lack of protective antioxidants in the plasma, such as serum albumin, tocopherol, ascorbate, urate, or different apolipoproteins [8]. Because they include oxidized lipids and byproducts of their breakdown that relate to the pathogenic mechanisms of atherosclerosis, oxidized low-density lipoproteins, or oxLDLs, are important inflammatory components that play a role in the formation of atherosclerotic plaques [9].OxLDL, together with additional atherogenic factors, promotes the activation of endothelial cells (EC), which leads to the recruitment of monocytes into the intima. Once in the intima, monocytes differentiate into macrophages, which can polarize into an M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotype [10]. Nonetheless, macrophages are sensitive to alterations in the inflammatory milieu and can transform from a pro-inflammatory to an anti-inflammatory phenotype in response to novel cues [11]. An effective response is largely dependent on the adaptability of macrophages, of which M1 is prominent during disease development and M2 during regression. M1 macrophages generate NO and reactive oxygen species (ROS), as well as inflammatory cytokines and chemokines, which encourage the activation of monocytes and the spread of the inflammatory response [11].

Furthermore, increased macrophage lipid absorption promotes the inflammatory response and sets up signaling reactions that activate NF-κB pathway targets [12], all of which are necessary for foam cell formation, monocyte recruitment, and EC stimulation. Macrophages' absorption of oxLDL can be viewed as a defense mechanism since it rids the intima of cytotoxic substances. However, enhanced monocyte migration into the intima and subsequent macrophage differentiation results in a high quantity of foam cells, which in turn promote the development of atherosclerotic lesions [13]. As a result, the buildup of cholesterol is thought to identify atherosclerotic lesions [14]. Modified LDL is also actively taken up by differentiated monocytes and vascular smooth muscle cells (VSMCs), which promote the formation of foam cells [15]. Moreover, multiple inflammatory signaling pathways are triggered, leading to the formation of fatty streaks, the initial feature of atherosclerosis development. This process is associated with the substantial accumulation of lipids within cells (vascular smooth muscle cells and macrophages) and in the extracellular milieu. [15].

2.2. Development of fibrous plaques

During fibrous plaque development, atheromatous plaques undergo a transition from a fatty streak to intimal growth, a stage characterized by the presence of an acellular and lipid-rich region known as the necrotic core. The necrotic core constitutes the core of atherosclerotic plaques. Covered by a fibrous sheath, the necrotic core consists of a lipid-laden hypocellular region with reduced supporting collagen [16]. Two key causes contribute to the growth of the necrotic core in an atherosclerotic plaque: macrophage mortality and impaired efferocytosis, which eliminates apoptotic cells. These occurrences heighten plaque susceptibility by fostering an inflammatory milieu, oxidative stress, and thrombogenicity as well as the death of nearby cells such as VSMCs [17]. The necrotic core is wrapped with fibers to form a fibrous capsule, which stabilizes the plaque. Advanced atherosclerosis is characterized by a necrotic core and fibrous capsule, and atheromatous plaque regression is relatively uncommon situation in this process [18].

2.3. Plaque rupture and thrombus formation

Every step of the plaque growth process, from initiation to rupture, is influenced by the inflammatory reaction. In fact, its significance grows at this point since it adds to the fibrous capsule's fragility. Certain proinflammatory cytokines, including IFN-γ, have the ability to prevent VSMCs from producing collagen. Furthermore, as shown in vitro [19], inflammatory mediators often present in atheroma, such as TNF-α, IL-1β, and CD40 ligand (CD154), can raise matrix metalloproteinase (MMP) production in VSMCs. The data points to the possibility that the most frequent mechanism of plaque rupture, hemodynamic stresses, might cause the robust and tough fibrous cap to become unstable and brittle when inflammation is predominant [19].

When a plaque cracks or ruptures, the subendothelial space is exposed to blood, triggering the coagulation process to cover the wound. Initially, platelets adhere to the subendothelial collagen and are activated, and then more platelets collect and aggregate in this area to initiate wound healing [13]. Prothrombotic components of the lipid core are simultaneously liberated and come into touch with substances that cause plasma coagulation. To be more precise, nuclear tissue factor and plasma factor VII combine to initiate the coagulation cascade, which results in the production of thrombin, an intermediate that is required for the creation of fibrin. Together with thrombocytes, fibrin, an insoluble protein, produces networks of fibrin fibers to cover the lesion and create a solid, orderly structure. This formation is called a thrombus [20].

2.4. Clinical complications

A sequence of events that occur when a clot forms render the lesion more stable and fibrotic, hence decreasing

its susceptibility to rupture. Nonetheless, the likelihood of blood vessel obstruction rises as a result of plaque development. As a result, there is less blood supply in the coronary arteries, which can cause ischemic cardiopathy and conditions like angina or heart failure [21]. Furthermore, a myocardial infarction " nearly stroke results from a blocked heart. An embolus is a kind of blood clot that travels throughout the circulatory system when a blood clot breaks free from the artery wall. In the end, the embolus lodges in the distal arteries, where it restricts blood flow and may cause infarction, organ failure, or local ischemia [21].

3. Mechanism of occurrence and development of NETosis

The majority of myeloid leukocytes, or neutrophils, typically make up approximately 60% of all leukocytes and are crucial for innate immunity [22]. A novel anti-inflammatory mechanism has been identified: neutrophil extracellular traps (NETs). NETosis is a unique type of programmed cell death generated when neutrophils are activated, in contrast to apoptosis and necrosis [23]. With the aid of thrombocytes, proinflammatory cytokines, or other signaling molecules, endotoxins and other microbial compounds activate neutrophils, initiating the process of NETosis. Toll-like receptors (TLRs) and complement receptors are two examples of substances that bind to neutrophil receptors in order to activate neutrophils and induce NETosis. Histone citrullination and chromatin decondensation come next, which are followed by the activation of intracellular granule proteases and nicotinamide adenine dinucleotide phosphate oxidase (NADPH) [24]. The process of citrullination is facilitated by the enzyme peptidylarginine deiminase 4 (PAD4), which weakens the link between histones and DNA by converting arginine residues to citrullines and removing positive charges from core histones [24]. Subsequently, the neutrophil nuclear envelope bursts, releasing decondensed nuclear chromatin that mixes with granular and cytoplasmic elements such as myeloperoxidase, neutrophil elastase, cathelicidin (LL37), high mobility group protein B1 (HMGPB1), cathepsin G, and proteinase 3 [23]. After that, and also the neutrophil undergoes NETosis, which results in death of cell and also cell membrane becomes permeable [24, 25].

In the event that nuclear annihilation does not occur, blebbing of the nuclear envelope and the quick transfer of microvesicles that include NETs outside the cell constitute an alternate strategy for preserving the structural integrity of the granulocyte. This is a quick reaction of granulocytes drawn to infection sites and infiltrating them [24]. After DNA is released, neutrophils are still alive and still have the capacity to combat germs. NETs can be produced from mitochondrial DNA as a third pathway [25]. In this process, mitochondria move to the cell surface and displace the NETs. NETs play an important role in the antimicrobial response of neutrophils in tissues and blood vessels. The release of NETs and their degradation by DNases must be strictly regulated to prevent excessive inflammatory reactions [24].

Both overproduction and deficiencies in the purification of NETs have been found to contribute to numerous pathologies [25]. Neutrophils die in inflamed tissues by undergoing NETosis or one of various other cell death mechanisms such as apoptosis, necrosis, necroptosis, pyroptosis or autophagy [24, 26]. The interaction of these processes is necessary to combat foreign agents and is responsible for the further resolution of inflammation [25]. All mechanisms of cell death contribute to the regulation of the number of neutrophils and the production of pro- and anti-inflammatory mediators [27]. Dysregulation of neutrophil death occurs in various pathological conditions [24, 27]. It is assumed that NETosis, apoptosis and autophagy are largely dependent on the function of NADPH oxidase and ROS production [24]. Redox imbalance in the neutrophil likely accelerates the induction of the death mechanism [25]. Numerous clinical diseases result in disorders of NETosis [24, 27]. It is thought that ROS generation and NADPH oxidase activity play a major role in NETosis, apoptosis, and autophagy [24]. The induction of the death process is probably accelerated by redox deprivation in the neutrophils [25].

4. The importance of netosis in the pathogenesis of atherosclerosis

4.1. Triggering of NETosis in atherosclerosis

Recently, a more detailed model has been proposed to describe the influence of NETosis on pathological processes such as atherogenesis, thrombus formation, increased inflammatory response, and leukocyte recruitment. Reduced blood flow results in neutrophil activation, which partly explains the increased detection of NETs in atherosclerotic lesions in humans with atherosclerosis and in mice in an animal model of atherosclerosis [28]. NETosis is involved in a number of processes that lead to the development of atherogenesis and increased plaque instability. In particular, interactions between NETosis processes and pathological processes such as hyperlipidemia and oxidative stress are suggested [28].

According to one hypothesis, inflammation in atherosclerosis occurs as a result of endothelial damage and dysfunction. NETs are able to induce the release of interferon-α (IFN-α) and the initiation of TLR2 signaling in affected arteries, which leads to the development of endothelial stress [29]. Based on studies of atherosclerotic plaques in human carotid arteries, it was possible to demonstrate a higher frequency of NETs occurrence at sites of rupture than at sites of erosion [30]. Neutrophils, localized near the inflamed endothelial surface, degranulate and emit reactive oxygen species (ROS) during plaque erosion, contributing to endothelial cell damage and death. Prothrombotic factors that are exposed to the injured endothelium cause platelets to aggregate and recruit, which in turn causes neutrophil induction and the creation of NETs. Released neutrophil epithelial cells (NETs) have the ability to trigger the alternative complement cascade locally in the proximity of vascular endothelial cells. This activation is reliant on the synthesis of anaphylatoxins C3a, C5a, and the membrane assault complex. The eventual result is erosion and chronic endothelial cell activation [31]. Therefore, by contributing to the process of induced endothelial cell death dependent on complement activation, NETs play a crucial role in the advancement of acute atherothrombotic events linked to atherosclerotic lesions susceptible to erosion [30].

4.2. The influence of netosis on the development of inflammation

The inflammatory endothelium surface of the atherosclerotic artery can be crossed by neutrophil-derived proteins that are a component of the NETs scaffold, like cathep-

sin G or cathelicidin-associated antimicrobial peptide (CRAMP) [32]. Nonetheless, it appears that NET-associated histone H2a and neutrophil-derived proteins can both drive a charge-dependent mode of monocyte adherence to the vasculature. In a murine model of severe endotoxemia, this finding has been demonstrated to accelerate atherosclerosis following activation with lipopolysaccharide (LPS). By inducing the release of neutrophil-endothelial cells (NETs) in the artery lumen, a single injection of lipopolysaccharide (LPS) into a hypercholesterolemic murine model was able to expand atherosclerotic lesions. Here, monocyte attachment and subsequent accumulation are thought to be facilitated by NETs, which in turn promote inflammation and may account for the elevated risk of serious vascular complications linked to infections like pneumonia [33].

Neutrophil extracellular traps (NETs) produce myeloperoxidase (MPO), which binds to the CD206 mannose receptor on macrophages to cause reactive oxygen species (ROS) to be produced. ROS are proven inflammatory mediators, but they also contribute to cytotoxicity. Elevated levels of ROS oxidize LDL, and oxidized LDL causes increased expression of the oxidized low-density lipoprotein receptor-1 (LOX-1) in endothelial cells, leading to activation of apoptosis and increased inflammation. ROS also cause oxidation of cellular DNA, membrane lipids and proteins, which leads to disruption of cellular homeostasis, accumulation of toxic metabolites and subsequent death of endothelial cells[33].

When macrophages are activated, the oxidative spurt leads to the transformation of low-density lipoprotein (LDL) into oxidized low-density lipoprotein (ox-LDL), which facilitates the growth of foam cells and activates the NLRP3 inflammasome, which leads to the development of the production of pro-inflammatory cytokines IL-1β and IL-18 through the launch of the NF-κB pathway and the expansion of the inflammatory network including the attraction and activation of new neutrophils which form new NETs . [31]. Moreover, by activating the inflammasome and stimulating the macrophage scavenger receptor CD36 on these macrophages, NETs can cause primed macrophages to produce proinflammatory IL-1α, IL-1ß, and IL-6. Moreover, the combined effects of IL-1 and cathepsin G, which stimulate the production of ICAM-1, VCAM-1, and tissue factors by endothelial cells and are linked to the development of thrombus due to the erosion of the surface of atherosclerotic plaque, might cause damage to the endothelium in NETs.T helper 17 cells which activated by IL-1ß then produce more IL-17, another proinflammatory cytokine, as a result of this mechanism [31]. It is assumed that the production of IL-17 draws neutrophils to tissues and heightens systemic inflammation. It is hypothesized that the pro-inflammatory cytokine macrophage IL-8, which interacts with CXCR2 in human neutrophils to stimulate the production of blood of patients NETs and impact the course of atherosclerosis, is also higher in atherosclerotic lesions [34].

A pro-inflammatory M1-like phenotype was recently demonstrated to be induced in diabetic mice's atherosclerotic NETs by impacted macrophages, as demonstrated by an elevated transcriptome characteristic in their glycolytic and inflammasome mechanisms [35]. In diabetic mice, there was an increase in the number of neutrophil epithelial cells (NETs), and regardless of any related risk factors, their interaction with macrophages led to chronic inflammation generated by macrophages and poor resolution of atherosclerotic lesions. In addition to reducing lesion size and instability, combination therapy of lipid reduction and DNase I injections was also able to decrease the amount of macrophages and NETs [36]. It has also been shown that M2 macrophages, as a result of exposure to cholesterol granules, can undergo polarization into M4 macrophages, which have an increased ability to attract neutrophils and activate NETosis [37]. In conclusion, data point to the idea that lesion macrophages' proinflammatory phenotype is caused by NETS-macrophage interactions in a proatherogenic milieu, which in turn accelerates the development of atherosclerosis.

4.3. The influence of netosis on the formation and destabilization of plaques

The results of the studies demonstrated that the affected plaques contain neutrophils, which is especially noticeable in plaques containing a lipid core, infiltrated by macrophages, with SMC and a reduced collagen content [29]. In view of this, a conclusion is made about the important role that neutrophils play in the development and destabilization of atherosclerotic plaques. SMCs are able to bind to recruited neutrophils, which contributes to the hyperactivation of these immune cells. The SMC-synthesized C–C motif of chemokine ligand 7 (CCL7) enhances the formation of NETs, which subsequently causes the death of SMCs. It is believed that one of the main activators of the death of SMCs of the arterial wall is histone H4, which is part of NETs. It exhibits its cytotoxic effect through destabilization of the plasma membrane of SMCs with the formation of pores [38], [39]. SMC depletion results in a decrease in the thickness of the fibrous cap by removing the source of collagen synthesis, leading to plaque instability. Consistent with this, it was found that mice with increased neutrophil levels in diseased vessels exhibited higher rates of SMC loss, whereas neutrophil-depleted mice or mice lacking PAD4 (incapable of producing NETs) had atherosclerotic plaques with increased SMC levels [39]. This supports a direct role for neutrophils in regulating SMC viability in atherosclerosis and a direct cytotoxic capacity of NETs.

4.4. Interaction between NETs and platelets

Certain parts of NETs have prothrombotic characteristics and can activate thrombin to start the coagulation cascade. Platelet adhesion, activation, and aggregation can be facilitated by NETs, which can create a scaffold resembling fibrin [29]. Furthermore, it is believed that NETSs contribute to the development, expansion, and stability of thrombi by acting as a scaffold for the deposition of fibrin, the aggregation of red blood cells and platelets inside the thrombus, and the deposition of procoagulant and prothrombotic compounds like these: tissue factor, fibrinogen, von Willebrand factor (VWF), fibronectin, and fibrinogen [40]. Therefore, it is assumed that the fibrin scaffold in thrombi is stabilized by the histone DNA backbone of NETs. There is a complicated interplay between platelets, activated neutrophils, and NETosis. Activated platelets can also trigger NETs production through processes that involve TLR4, high mobility group box 1 (HMGB1), and P-selectin [41]. Furthermore, is was shown in animal models that tissue factor and platelet-derived VWF via platelet-de-

rived GPIbα were implicated in NET-mediated deep vein thrombosis. A positive feedback loop coordinating the prothrombotic coagulation cycle and the pro-inflammatory response, which can ultimately obstruct the arterial and result in organ damage, is at the center of the interaction between NETs, activated platelets, and coagulation factors [41]. To summarize, ample evidence supports that NETs play a critical role not only in atherogenesis and lesion progression but also in the formation of atherothrombotic events. The participation of NETosis in the pathogenesis of atherosclerosis is shown in Figure 1.

5. Potential therapy aimed at eliminating netosis in atherosclerosis

NETs are now being explored as novel targets for the control and diagnosis of atherosclerotic disorders due to their crucial involvement in atherogenesis and the advancement of atherosclerosis. Mice with the broad PAD inhibitor Cl-Amidine do not develop NETS plaques. A decrease in the amount of intimal macrophages, a decline in the enrollment of neutrophils in the artery wall, a drop in arterial IFN-α levels, and eventually a considerable reduction in the size of the atherosclerotic lesion are all caused by the elimination of NETs production [42]. Mice lacking NETs formation due to genetics or pharmacological means can generally lower proinflammatory cytokine production in the aorta area, have less inflammatory adipocyte inflammation, have more stable plaques in the aortic root, are better protected from malnutrition and inflammation, and show improved metabolic parameters such as improved glucose tolerance and insulin resistance [43].

In recent research, collagen IV-rich endothelial areas exhibiting superficial erosion were treated with the PAD4 inhibitor GSK484 using a nanoparticle method. By blocking PAD4 enzymatic activity, systemic injection of targeted nanoparticles prevents the development of NETs while maintaining endothelial integrity [44]. All things considered, PAD4 is a validated therapeutic target that could dramatically impede the advancement of NET-induced illness. In neutrophil-mediated disorders where NETs production is harmful, treatment with DNase I has also been suggested: systemic injection of DNase I has been shown to reduce lesion size, the number of impacted NETSs, and the quantity of proinflammatory cytokines in atherosclerotic plaques in ApoE/PR3/NE −/− murine model [44].

Nevertheless, in the early stages of atherosclerosis, comparable investigations in the same mouse model did not support these findings. This is supported by the observation that DNase I treatment led to reduced surface endothelium erosion, a restricted number of adhering neutrophils, and enhanced endothelial cell survival in Ldlr/PAD4-deficient mice models. Thoughts that NETs have a clot-stabilizing impact during thrombus formation have given rise to the contentious notion of systemic administration of DNase I, since it has also been demonstrated to boost tissue-type plasminogen activator (tPA)-mediated thrombolysis in human coronary artery disease [44].

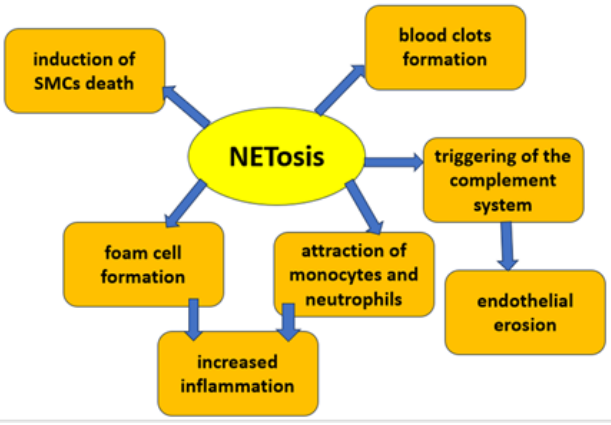

Fig. 1. The participation of NETosis in the pathogenesis of atherosclerosis.

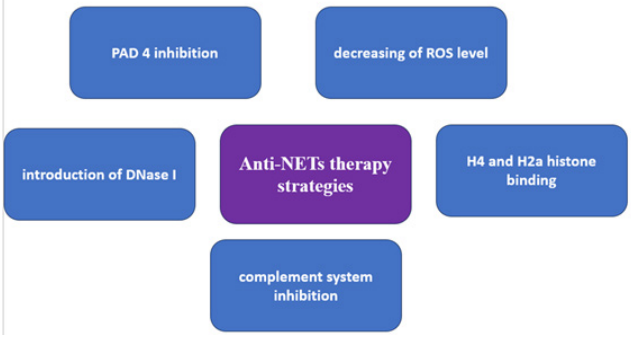

Fig. 2. Strategies for anti-NETs therapy in atherosclerosis.

It has been demonstrated that using neutralizing antibodies against histone H4 or H2a to mediate NETs improves plaque stability in mice models of atherogenesis [45]. This approach may potentially be beneficial therapeutically. Neutralization of NETS-containing proteins, complement inhibitors, ROS scavenging, low molecular weight heparin administration, phosphodiesterase 4 (PDE4) usage, and partial neutrophil removal are other well-established treatment strategies that suppress NETS production [46]. Taking everything into account, anti-NETs therapy has been suggested as a helpful treatment for atherosclerosis because of its low level of immunosuppression and minimal interference with the patient's immune system. The main strategies for anti-NETs therapy are presented in Figure 2.

6. Discussion

The activation of mobilized granulocytes and the start of the innate immune response are two aspects of the chronic inflammatory reaction which is present in vascular tissues and is a typical hallmark of disorders like atherosclerosis. Once engaged, neutrophils can interact with different cellular subpopulations inside the atherosclerotic lesion to undergo NETs release and promote inflammation. There, the creation of NETs seems to set off a chain reaction that includes proinflammatory cytokine release, endothelial tissue stimulation and damage, more neutrophil recruitment and repeated NET generation. This vicious cycle ultimately results in tissue damage, cellular malfunction, and persistent chronic inflammation. As a result, NETs are involved in the connection that exists between innate immunity, oxidative stress, endothelial dysfunction, inflammation of the arterial wall, and cardiovascular disease [47].

Lifestyle variables impact disease-associated trajectories such as inflammation, oxidative stress, and tissue and organ malfunction, and they also have a significant impact

on the chance of acquiring cardiovascular disease. Cardiovascular risk can be significantly reduced by leading a healthy lifestyle that includes modest lifestyle adjustments such as frequent physical activity, better eating and sleeping habits, and other lifestyle improvements [48].

Clearly, additional research is required to clarify the significance of histone citrullination for NETosis and to fully examine the specific processes of NETs development in vivo [49]. NETosis is frequently triggered by the production of reactive oxygen species (ROS) by different kinds of leukocytes; however, the precise mechanism of ROS-induced NETS generation and eventual endothelial dysfunction remains unknown. Throughout every stage of cardiovascular disorders, NETs have been found [50].

Nevertheless, it is still unclear if NETs have distinct functions at various phases. Furthermore, investigating whether NETs are engaged in interaction with smooth muscle cells which are another important source of foam cells in atherosclerosis—will be challenging. In order to better understand the role of NETs in venous thrombi and atherosclerotic plaques, as well as to identify, validate, and target the best molecular candidates for therapy, a deeper knowledge of NETosis—both in terms of its structural elements and context-specific functional design—will be necessary.

Potentially, the study of NETosis may be useful not only for the development of new therapeutic agents aimed at reducing the symptoms of atherosclerosis, but also for the search for new diagnostic tools that will determine the stage of the disease depending on the concentration of the selected marker. Researchers may consider NETosis-related biomolecules such as PAD4, MPO, extracellular DNA, and extracellular H4 histone as primary candidates for the role of biomarkers.

The constructed model of the development of NETosis in atherosclerosis can also be useful for studying other inflammatory diseases, regarding the influence of NETosis on the development of inflammation. For this reason, some of the proposed therapeutic targets may also be suitable for the treatment of other related inflammatory diseases. This is due to the fact that the mechanism of initiation of inflammatory pathways is similar in different chronic inflammatory diseases. However, the pathogenesis of atherosclerosis has its own characteristics, for example, the formation of atherosclerotic plaques, which is also affected by NETosis and isn't appropriate for understanding the other diseases.

7. Conclusion

Neutrophils, despite being the most abundant leukocytes in human blood and key players in acute inflammation, have historically been understudied in cardiovascular inflammatory processes and atherosclerosis. Recent investigations have highlighted the crucial roles of neutrophils and neutrophil extracellular traps (NETs) throughout the various stages of atherosclerosis and atherothrombosis, making them promising targets for cardiovascular inflammation therapy. However, advancing treatment strategies will require further research and clinical evaluation to enhance specificity in targeting and minimize side effects. Despite existing challenges, ongoing research on NETs holds the potential to uncover novel insights that could revolutionize the diagnosis and management of atherosclerosis and associated conditions.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors had equal roles in study design, work, statistical analysis and manuscript writing.

Funding

This study was supported by the Russian Science Foundation, Grant# 24-15-00123.

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