Online Journal of Biological Sciences

Literature Reviews

Unraveling Sterile Inflammation in Atherosclerosis: Mechanisms, Implications and Therapeutic Strategies

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Article history
Received: 30-01-2024
Revised: 03-04-2024
Accepted: 17-04-2024

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Abstract: Atherosclerosis, a leading factor in the development of cardiovascular diseases, is a complex and multifaceted condition with inflammation emerging as a pivotal determinant of its pathogenesis. This review comprehensively investigates the role of sterile inflammation in atherosclerosis and deliberates on potential therapeutic interventions targeting this underlying mechanism. Despite advancements in the understanding of atherosclerosis, diagnosing, preventing, and choosing effective treatment methods remain challenging due to its intricate pathogenesis. In the absence of pathogenic agents, sterile inflammation signifies a promising avenue for therapeutic measures. Through the comprehensive analysis of current literature encompassing reviews and original articles obtained from the PubMed database, we aimed to unravel the intricate mechanisms triggered by sterile inflammation within the context of atherogenesis. Our exploration reveals that sterile inflammation contributes significantly to the development of atherosclerosis through diverse mechanisms beyond the conventional association with macrophages, highlighting the need for a multifaceted approach in therapeutic targeting. We emphasize the potential of sterile inflammation as a prospective target for the development of atherosclerosis treatments while acknowledging the imperative consideration of the disease's complex nature in devising effective therapeutic modalities. This review underscores the significance of tailored interventions aimed at modifying sterile inflammation to counteract the progression of atherosclerosis, affirming the importance of holistic strategies in addressing this intricate disease. In particular, our findings highlight the potential for targeted interventions to modulate specific pathways involved in sterile inflammation, offering new avenues for the development of precision treatments in atherosclerosis management.

Keywords: Atherosclerosis, Inflammation, DAMPs, CVD

Introduction

The objective of this review entitled "Sterile Inflammation as a Factor Impacting Atherogenesis" is to explore the role of sterile inflammation in atherogenesis, particularly focusing on the mechanisms by which sterile inflammation contributes to the advancement and evolution of vascular disorders such as atherosclerosis. The review aims to provide an in-depth understanding of sterile inflammation, its triggers, and consequences, as well as its impact on cardiovascular disease, with particular emphasis on the function of inflammasomes and the potential for targeting sterile inflammation in the development of therapeutic strategies for atherosclerosis.

The review delves into the concept of sterile inflammation, which refers to the activation of inflammatory processes in response to molecular Damage Associated Patterns (DAMPs) from non-infectious sources and underscores its significance in the context of atherosclerosis. The review also highlights the role of specific molecular triggers of sterile inflammation, like cholesterol crystals in the case of atherosclerosis and urate crystals in gout.

Furthermore, the review emphasizes the crucial function of inflammasomes, especially the NLRP3 inflammasome, in mediating sterile inflammation and its contribution to cardiovascular disease. The complex interplay between inflammasome activation, cytokine production, and cell death mechanisms, such as pyroptosis, is thoroughly explored.

Lastly, the review discusses potential clinical strategies targeting sterile inflammation, including the use of specific pharmacological agents such as canakinumab and methotrexate, as well as other potential therapeutic targets such as HMGB1 and the NF-κB pathway. The review underscores the need for further research to develop effective therapeutic strategies targeting the multifaceted mechanisms of sterile inflammation in atherosclerosis.

Overall, this review seeks to offer an in-depth analysis of sterile inflammation as a key factor impacting atherogenesis, shedding light on the intricate molecular pathways and potential therapeutic interventions associated with this process.

Methods

Literature search: A comprehensive search was conducted on the PubMed database to identify relevant articles and reviews related to sterile inflammation, atherosclerosis, and related topics. The search strategy included keywords such as "sterile inflammation," "atherosclerosis," "inflammasomes," and "vascular diseases."

Inclusion and exclusion criteria: Articles included in this study were those that were published in English and concentrated on the role of sterile inflammation in atherosclerosis and provided insights into mechanisms, triggers, and therapeutic strategies. Studies unrelated to the topic or lacking relevance were excluded. Initially, we selected 17 articles. Then expanded the number of sources to 59.

Data extraction: Data from selected articles were extracted and synthesized to identify key findings, mechanisms, and conclusions related to sterile inflammation in atherosclerosis. Relevant information on inflammasomes, triggers of sterile inflammation, and potential therapeutic interventions were summarized.

Data synthesis and analysis: The collected data were scrutinized to pinpoint prevalent themes, patterns, and deficiencies in the literature concerning the contribution of sterile inflammation to atherosclerosis progression. Emphasis was placed on understanding the mechanisms by which sterile inflammation influences the development and progression of atherosclerosis.

Review structure: The structure of the review was designed to offer a thorough examination of sterile inflammation in atherosclerosis, covering key aspects such as the role of inflammasomes, triggers of sterile inflammation, and potential therapeutic strategies. Information was organized to present a coherent narrative on the current understanding of sterile inflammation in atherosclerosis and its implications for targeted therapeutic interventions.

Inflammation

Inflammation serves as a critical defense mechanism protecting the body from invasive pathogens. When infections infiltrate the organism, inflammatory cells, primarily neutrophils and macrophages, are activated, initiating the process of phagocytosis to capture infectious agents (Chen *et al*., 2018). This leads to the secretion of more cytokines and chemokines, triggering the immune system and fostering tissue restoration and wound healing (Justiz Vaillant *et al*., 2022). In instances where pathogens do not breach the body during tissue injuries and ruptures, a process known as sterile inflammation ensues. This phenomenon mirrors the processes of inflammation in the presence of infectious agents. Neutrophils and macrophages are activated, followed by the secretion of cytokines and chemokines, such as Tumor Necrosis Factor (TNF) and Interleukin-1 (IL-1) (Rock *et al*., 2010).

Despite its crucial role in combating infectious agents, inflammation can have adverse effects on the body if pathogens cannot be removed. This includes the accumulation of aberrant collagen leading to fibrosis, as well as the generation of reactive oxygen species that may result in tissue injury. Additionally, the secretion of protease enzymes during inflammation can lead to tissue destruction (Landén *et al*., 2016; Wynn and Ramalingam, 2012).

Sterile inflammation is triggered by the release of Damage-Associated Molecular Patterns (DAMPs) from deteriorating or injured cells. Multiple triggers, like ischemia-reperfusion and conditions like gout, can induce sterile inflammation. Ischemia-reperfusion damage occurs when blood flow is temporarily interrupted in tissues, as seen in conditions like heart attacks or strokes (van Golen *et al*., 2013). In gout, the presence of monosodium urate crystals in the joints

initiates an inflammatory reaction, serving as a notable example of sterile inflammation (Deng *et al*., 2021). Similarly, pseudogout, caused by calcium dihydrate pyrophosphate crystals in the joints, also elicits a sterile inflammatory response. In both instances, crystals act as DAMPs, inciting an inflammatory response essential for immune activation.

Sterile inflammation plays a crucial role in the initiation and advancement of atherosclerosis. The sequence begins with the injury to endothelial cells that coat the inner lining of arteries. Influences like hypertension, tobacco use, or elevated levels of LDL cholesterol can cause this damage (Macmullan and McCarthy, 2012). Subsequent release of DAMPs from the damaged endothelial cells triggers the immune response, resulting in the attraction of inflammatory cells to the injury site. These cells produce lipids on the walls of arteries, leading to the creation of foam cells. The chronic sterile inflammation can ultimately result in the development of a fibrous cap over the plaque. However, if the inflammation becomes too intense, the fibrous cap could potentially thin and weaken, increasing the likelihood of plaque rupture, heart attack, or stroke (Roh and Sohn, 2018). Moreover, the presence of immune cell infiltration in the absence of pathogens is also a hallmark of tumor development, influencing cancer growth and progression (Libby, 2021).

Sterile inflammation mirrors microbial infection, prompting host immune cells to activate similar patterns of action as those triggered in response to particular microbial pathogens. Pattern Recognition Receptors (PRRs) like Toll-Like Receptors (TLRs), NOD-Like Receptors (NLRs), RIG-I-Like Receptors (RLRs), C-type Lectin Receptors (CLRs), and AIM2-Like Receptors (ALRs) have a vital function in recognizing microbial infections and kickstarting the immune reaction (Mogensen, 2009; Sameer and Nissar, 2021; Jacobs and Damania, 2012; Li *et al*., 2022). When wounds occur when there are no microbial pathogens present, the activation of PRRs stimulates the generation of DAMP molecules, leading to inflammation, akin to a scenario of an immune response.

This thorough understanding of sterile inflammation's multifaceted significance in the pathogenesis of diseases highlights the potential for it to be a key therapeutic target. By intervening in the sterile inflammatory processes, novel treatment strategies could be developed to combat various diseases (Walter, 2015).

Sterile Inflammation in Vascular Disease

As previously mentioned, Damage-Associated Molecular Patterns (DAMPs) provoke proinflammatory responses in atherosclerosis. Large cholesterol crystals, characteristic of advanced atherosclerosis, appear in later stages, while small crystals are present following two weeks of a diet known to promote atherosclerosis in ApoE-/-mice. The subendothelial deposition of cholesterol crystals activates the pyrin domain-3 inflammasome, a component of the NOD-like receptor group, resulting in the release of IL-1β. This highlights the initial role of DAMPs in the initiation and development of atherosclerosis (Tomas *et al*., 2019). Additionally, oxidized LDL and apoptotic cells harbor oxidation-specific epitopes, potentially causing secondary cell death as a result of unsuccessful phagocytosis. These markers may also initiate or exacerbate the inflammatory process by activating Toll-Like Receptors (TLRs). For instance, TLR4 activation on the endothelial cells stimulated by oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine triggers the release of proinflammatory cytokines and chemokines, in addition to the activation of adhesion molecules, facilitating monocyte recruitment. In this context, oxidized LDL can also be considered DAMPs, with lipopolysaccharide contamination possibly responsible for TLR activation (Leibundgut *et al*., 2013).

Furthermore, IL-1α is present in plaques at later stages, following significant apoptotic Vascular Smooth Muscle Cell (VSMC) death. This increases the risk of plaque destruction and detachment from the arterial walls. Additionally, impaired phagocytosis in mature plaques results in secondary necrosis and the subsequent liberation of IL-1α. This activation leads to adjacent viable VSMCs producing pro-inflammatory cytokine IL-6 and chemoattractant monocyte protein-1 (Kong *et al*., 2022). Conversely, in developed plaques with a low number of dead VSMCs, inflammation is sustained, activating new macrophages and maintaining the activity of old macrophages, perpetuating plaque destruction. Atherosclerosis is a primary factor contributing to graft failure after transplantation by activating CD4+ memory T-cells, triggered by DAMP IL-1α contained in necrotic endothelial cells. This adaptive response involving IL-1α suggests that DAMPs contribute to both innate and adaptive immunity through cellular memory (Checkouri *et al*., 2021). IL-1α, a crucial DAMP linked to the vascular system, when its receptor antagonist IL-1 is disabled, results in severe inflammation in arterial tissues, accompanied by the extensive movement of macrophages, neutrophils, and T cells, resulting in vessel wall degradation, clot dislodgment, infarction, and aneurysm formation (Dinarello, 2018).

In addition, there are reasons to view IL-1α being a subsequent cue emitted by cells during necrosis,

triggering sterile inflammation. In this context, IL-1α is not considered a primary DAMP, unlike monosodium urate and other DAMPs eliciting the primary immune response. Their activation leads to the secretion of IL-1α, subsequent induction of expression, and release of chemotactic cytokines. Despite being present in large quantities within cells, IL-1α is not utilized as the primary DAMP due to the complexity of the necrotic process, resulting in the release of various potential DAMPs that require specific receptors with limited expression. The release of IL-1α as a catalyst by resident immune cells during necrosis serves to activate local cells, allowing these cells to generate sufficient inflammatory signals, which they cannot accomplish independently. IL-1α may function as a versatile DAMP, either enhancing or inhibiting the response to necrosis, given the extensive presence of IL-1R1 in non-immune cells (Scarpa *et al*., 2015; Sachet *et al*., 2017).

The Role of Inflammasomes in Cardiovascular Disease

In recent literature, it has been elucidated that inflammasomes, intricate assemblies of multiple proteins, play a significant role in activating pro-inflammatory cytokines like interleukin-1β (IL-1β) and interleukin-18 (IL-18) (De Zoete *et al*., 2014). These inflammasomes are crucial for inducing pyroptotic cell death. Particularly in cardiovascular disease, inflammasomes have been identified as crucial regulators of inflammation. Cells such as monocytes, macrophages and dendritic cells produce IL-1β, a potent inflammatory cytokine, in response to infection or inflammation. Initially synthesized in an inactive form (pro-IL-1β), IL-1β is activated through cleavage by caspase-1, leading to the mature, active form. The release of these cytokines can trigger the recruitment of immune cells and intensify inflammation within the plaque (Wang *et al*., 2020).

Inflammasomes are comprised of various proteins, including a nucleotide-binding domain and leucine-rich Repeat-Containing protein (NLR), an apoptosis-associated speck-like protein containing a CARD (ASC) and a pro-caspase-1. NLRs, which are a group of intracellular pattern recognition receptors, have the ability to detect a broad array of signals, encompassing Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs) (Davis *et al*., 2011). These NLRs are equipped with a central Nucleotide-binding Oligomerization Domain (NOD) that plays a crucial role in the formation of inflammasome complexes.

The interaction between ASC and NLR and pro-caspase-1 is facilitated by the CARD domain of ASC, while the Pyrin Domain (PYD) of NLRs enables their interaction with ASC. Additionally, NLRs possess a NACHT domain responsible for NLR oligomerization and the formation of inflammasome complexes (Swanson *et al*., 2019). The initiation of inflammasome assembly is initiated by the recognition of PAMPs or DAMPs by NLRs, leading to the formation of inflammasome complexes.

Once formed, this complex recruits and activates pro-caspase-1, resulting in the cleavage and activation of inflammatory cytokines such as IL-1β and IL-18. The release of these cytokines can trigger the recruitment and activation of immune cells, thereby amplifying the inflammatory response. One significant inflammasome complex linked to atherosclerosis is the NLRP3 inflammasome, which can be triggered by various stimuli, including oxLDL and cholesterol crystals (Kelley *et al*., 2019). Upon activation, the NLRP3 inflammasome complex activates pro-caspase-1, leading to the cleavage and activation of IL-1β and IL-18.

The release of these cytokines further promotes the recruitment and activation of immune cells, like macrophages and T cells, thus bolstering the inflammatory response and aiding in the development and progression of atherosclerotic plaques (Blevins *et al*., 2022). The impact of NLRP3 on atherosclerosis and ischaemia/reperfusion injury is outlined in Fig. (1).

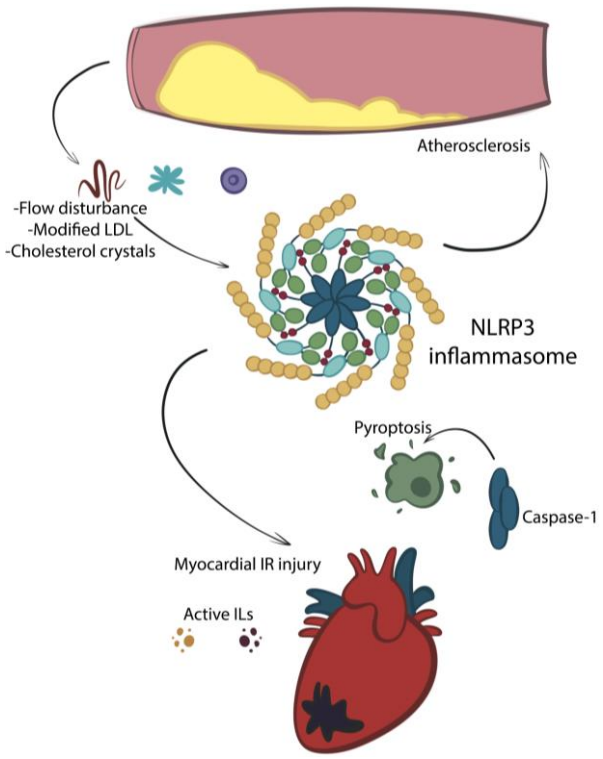

Fig. 1: The role of NLRP3 inflammasome in cardiovascular pathologies

The levels of pro-IL-1β and NLRP3 induction necessary for inflammasome function, supplied by nuclear factor-κB, are largely activated through Toll-Like Receptors (TLRs) via Lipopolysaccharide (LPS) stimulation, for example. Various proteins containing caspase-12, CARD, or PYD bind ASC or caspase-1, whereas anti-apoptotic proteins (Bcl-2 and Bcl-XL) interact with ATP bound to NACHT. This highlights that inflammasomes engage multiple, often unrelated, inhibitors, and direct ligand interaction is not the sole method of combining these signals (Ryan *et al*., 2022). Instead, a model incorporating three different pathways for the combination of signals from different inhibitors and receptors has been constructed: ROS-derived activators can bind NLRP3 directly or interact with it through an adaptor; damage to lysosomes triggers a proteolytic cascade; and potassium efflux leads to polarization changes and subsequent cellular stress. This model effectively illustrates the complex and multifactorial operation of the immune system (Zhang *et al*., 2023).

Inflammasome Activation as a Cause of Cardiovascular Disease

In response to pathogenic reactions and autoimmune diseases, inflammasome activation occurs. According to studies, In the process of atherogenesis, persistent inflammation and infiltration of monocytes result in the buildup of macrophages within the forming plaque. Phagocytosis of cholesterol crystals causes the secretion of IL-1β, acting as the primary mediator of the inflammatory cascade, from macrophages (Abderrazak *et al*., 2015). Elevated IL-1β expression triggers the release of additional proatherogenic cytokines and chemokines, in conjunction with the production of endothelial adhesion molecules and nitric oxide synthase. Accordingly, IL-1β levels often serve as an indicator of disease severity. While cholesterol in necrotic nuclei has traditionally been considered a consequence instead of being the primary factor behind lesion formation, several studies demonstrate that small crystals in Initial plaques quickly induce phagolysosomal membrane breakdown upon penetration, triggering the activation of the NLRP3 inflammasome (Rafieian-Kopaei *et al*., 2014). Additionally, research has indicated that the lack of inflammasome constituents in mice crossed with ApoE-/-mice did not alter the development of atherosclerosis. Moreover, calcification could also play a crucial role as a Damage-Associated Molecular Pattern (DAMP) in vascular disorders, as basic calcium phosphate crystals have been demonstrated to activate NLRP3 inflammasomes (Menu *et al*., 2011).

Revascularization often triggers extensive inflammation. Several hours following reperfusion injury, myocardial harm, and bleeding happen because of the generation of Reactive Oxygen Species (ROS) and potassium outflow, leading to a peak discharge of pro-inflammatory cytokines. Following this, elevated levels of ASC expression as well as macrophage and neutrophil concentrations indicate further involvement of inflammasomes and ASC-/-mice exhibited a reduced infarct area and diminished comparative infiltration of macrophages and neutrophils into the ischemic heart tissue in contrast to normal wild-type mice. Inflammation often accounts for up to half of the total lesion area in infarction (Carbone *et al*., 2020).

Inflammasome Activation and Cell Death

Inflammasomes have the ability to initiate cell death through a highly inflammatory form of programmed cell death called pyroptosis. Pyroptosis is triggered by the activation of inflammasomes, resulting in the activation of caspase-1, which then cleaves gasdermin D (GSDMD) into an active form. The active GSDMD creates pores in the cell membrane, leading to osmotic lysis and the release of NLRP3. Activation of NLRP3 induces the formation of the inflammasome complex, comprising NLRP3, ASC, and pro-caspase-1. Assembly of the inflammasome complex activates caspase-1, which cleaves pro-IL-1β and pro-IL-18 into their active forms. The release of IL-1β and IL-18 not only enhances the inflammatory response but also potentially contributes to the development and progression of cardiovascular disease (Hsu *et al*., 2021). Furthermore, the release of these cytokines can worsen the inflammatory response and play a role in the pathogenesis of various diseases, including cardiovascular disease. Cell death can also trigger inflammasomes through the release of Damage-Associated Molecular Patterns (DAMPs) and Pathogen-Associated Molecular Patterns (PAMPs). DAMPs are internal molecules released from dying cells that can activate inflammasomes, while PAMPs originate from microbial pathogens and can also activate inflammasomes (Kany *et al*., 2019). Upon release, DAMPs and PAMPs bind to Pattern Recognition Receptors (PRRs) on adjacent cells, resulting in the recruitment and activation of inflammasomes. The activated inflammasomes can then stimulate the production of pro-inflammatory cytokines (e.g., NLRP3) and the induction of pyroptosis, further fueling inflammation and cell death in the affected tissue (Amarante-Mendes *et al*., 2018).

Crystal-Based Sterile Inflammation

Crystal-induced sterile inflammation represents a form of sterile inflammation triggered by the accumulation of crystals in tissues, such as urate crystals in gout and cholesterol crystals in atherosclerosis. These

crystals serve as danger signals or DAMPs, activating the innate immune system and causing the recruitment of immune cells, production of pro-inflammatory cytokines, and induction of cell death. Urate crystals, arising from purine breakdown in the body, are characteristic of gout, a condition known for its painful and inflamed joints (Cabău *et al*., 2020). NLRP3 inflammasomes recognize urate crystals, leading to the production of IL-1β and the recruitment of neutrophils and monocytes to the site of crystal deposition. Activation of the inflammasome complex and subsequent production of IL-1β can contribute to the persistent inflammation and tissue damage seen in gout. On the other hand, cholesterol crystals, resulting from cholesterol deposition in the arterial wall, are a significant component of atherosclerotic plaques (Eleftheriadis *et al*., 2020). Recognition of cholesterol crystals by various pattern recognition receptors (e.g., toll-like receptors and NLRP3 inflammasomes) triggers the production of pro-inflammatory cytokines like IL-1β and IL-18, along with the recruitment of immune cells like macrophages and neutrophils. Activation of the inflammasome complex and subsequent production of IL-1β and IL-18 can facilitate the development and progression of atherosclerosis by inducing endothelial dysfunction, heightening oxidative stress, and promoting plaque destabilization and rupture (Karasawa and Takahashi, 2017). Recent research has highlighted the ability of High-Density Lipoprotein (HDL) to interact directly with and regulate the activity of inflammasomes, including the NLRP3 inflammasome. HDL has been shown to impede NLRP3 inflammasome activation by preventing inflammasome complex assembly and caspase-1 activation, resulting in reduced production of IL-1β and IL-18. Moreover, HDL can inhibit the generation of Reactive Oxygen Species (ROS) and the expression of adhesion molecules on endothelial cells, thereby diminishing immune cell recruitment and preventing the onset of crystal-induced inflammation (Thacker *et al*., 2016).

Potential Clinical Strategies Targeting Sterile Inflammation

Canakinumab, a monoclonal antibody, is designed to target and inhibit the activity of interleukin-1 beta (IL-1β), a pro-inflammatory cytokine crucial in mediating inflammation and implicated in the pathogenesis of various inflammatory disorders, including cardiovascular diseases like atherosclerosis. By specifically binding to IL-1β, canakinumab disrupts its interaction with its receptor, thereby dampening its pro-inflammatory effects. This inhibition of IL-1β by canakinumab helps alleviate inflammation and has shown promising results in managing multiple inflammatory conditions (Mai and Liao, 2020). Notably, canakinumab has been extensively researched in the realm of atherosclerosis, demonstrating a decrease in the incidence of major cardiovascular events, such as heart attacks and strokes, notably in individuals with a history of heart attacks and heightened inflammation levels indicated by high-sensitivity C-reactive protein (hsCRP) (Libby, 2017). Findings from the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) trial have unveiled a substantial reduction in the risk of recurrent cardiovascular events with canakinumab treatment, even among patients with well-managed cholesterol levels. This suggests that inflammation, as measured by hsCRP, may represent a crucial therapeutic target in preventing and treating cardiovascular diseases (Aday and Ridker, 2018).

Methotrexate has been used for several decades in therapy for several inflammatory ailments like rheumatoid arthritis, psoriasis, and inflammatory bowel disease. It functions as an inhibitor of dihydrofolate reductase, an enzyme essential for the synthesis of purines and pyrimidines, vital components of DNA. Even though the precise way methotrexate operates remains not entirely comprehended, its anti-inflammatory effects are believed to stem from multiple mechanisms (Yan *et al*., 2021; Bedoui *et al*., 2019). One of these mechanisms involves the inhibition of the generation of inflammatory cytokines like Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) by immune cells such as macrophages and T cells is affected. Methotrexate is also noted to lower the presence of adhesion molecules on endothelial cells, potentially reducing the attraction of immune cells to inflammation sites. Furthermore, methotrexate has been shown to increase by restraining the creation of inflammatory cytokines and decreasing the gathering of immune cells at inflammation sites, methotrexate stimulates the generation of adenosine, a potent anti-inflammatory molecule. Nevertheless, recent studies reveal that at low doses, methotrexate does not result in a decrease in IL-1β, IL-6, or CRP levels (Jang *et al*., 2021).

In the treatment of inflammatory and autoimmune diseases, blocking HMGB1 has shown potential benefits. This can be accomplished through the use of neutralizing antibodies that bind to and hinder HMGB1 activity, or through small molecules that can prevent HMGB1 release from cells or its binding to receptors on immune cells. One possible way in which HMGB1 blockade works involves curtailing the production of inflammatory cytokines like TNF-α and IL-1β (Xue *et al*., 2021). By inhibiting HMGB1, the output of these cytokines can be minimized, hence reducing inflammation. Inflammatory cytokines, such as IL-1β and TNF-α, can trigger the JAK/STAT pathway, leading to the production of pro-inflammatory proteins. By blocking

HMGB1, the activation of the JAK/STAT pathway may be deterred, resulting in lower pro-inflammatory cytokine production, which in turn can help ease inflammation and prevent tissue damage in autoimmune and inflammatory conditions. Additionally, the JAK/STAT pathway is involved in regulating T cell function (Chen *et al*., 2022). Noteworthy is its importance in the formation and function of regulatory T cells (Tregs), which exert suppressive actions and can assist in reducing inflammation. HMGB1 has been found to impede Treg function and inhibiting HMGB1 through blockade could potentially enhance Treg function by suppressing the JAK/STAT pathway (Xu *et al*., 2022).

A particular redox condition of the restored CYS106 is required for HMGB1 to bind to TLR4. Once this binding occurs, HMGB1 loses its capability to attach to MD-2 and then triggers TLR4 activation when CYS106 is substituted with alanine. This notably results in a noticeable reduction in HMGB1-mediated translocation of NF-κ and TNF-α in macrophages devoid of MD-2. P5779 inhibits the induced release of HMGB1 and TNF-α, thus providing protection against liver damage caused by ischemia/reperfusion, chemical toxicity, and sepsis (Yang *et al*., 2015). In cases of arterial injury, the expression of TLR4, HMGB1, and IL-6 is alleviated by intimal hyperplasia. Folic acid-based preparations, like P5779, act as mimetics and can inhibit the interaction between HMGB1 and MD-2. Targeting TLR4 offers a way to diminish the severity of atherosclerosis and presents a potential therapeutic target. Atorvastatin, known for inhibiting HMG-COA reductase activity like other statins, specifically hinders the TLR4/MyD88/NF-κB and RAGE pathways. These approaches effectively reduce the formation and activation of NLRP3 inflammasomes. Even in scenarios where HMGB1 binds to RAGE, deactivating or lacking TLR4 in macrophages suppresses cytokine production by inhibiting NF-κB translocation (Sun *et al*., 2018). While the inhibition of TLR4 and MD-2 has shown promise in attenuating atherosclerosis, the clinical application remains complex. TLR4 is crucial for detecting both external and internal pathogens. Interfering with the TLR4 pathway can dampen immune responses, especially since TLR4 is expressed in T cells. Hence, it is suggested that targeting MD-2 and impeding TLR4/MD-2 interactions may be more favorable than directly targeting TLR4. The IL-1-Associated Kinase-4 (IRAK-4) receptor receives signals via a TLR4/MyD88-dependent pathway, leading to the complex formation and recruitment of IRAK-1 and IRAK-2 (Li *et al*., 2020). Under TNF-6, MyD88 interacts with a receptor-assessed factor as a result of IRAK phosphorylation, TRAF6. While the overall sequence identity of IRAK-1 and IRAK-4 is just 31%, it exceeds about 90% of the way along the ATP-binding site, which is the typical location for inhibitor binding. Currently, the majority of inhibitors are selective for IRAK-4, prioritizing them over IRAK-1 inhibitors due to their efficacy being challenging to acquire. Furthermore, the initial kinase acquired, IRAK-4, triggers the emergence of several underlying signal molecules. In experiments involving mice with type II diabetes, the IRAK-4 inhibitor demonstrated a protective effect on vascular smooth muscle cells and led to a decrease in MCP-1 levels expression (Dunne *et al*., 2010). Additionally, IRAK-1 controls the activation of IL-1 and is implicated in the regulation of NLRP3 inflammation. Targeting TRAF6 in macrophages has demonstrated reduced atherosclerosis and anti-inflammatory effects. Various pro-inflammatory genes associated with atherosclerosis are regulated by the activation of the NF-κB pathway governs the activation of NLRP3 inflammasomes. Notably, inhibition of the NF-κB pathway weakens atherosclerosis. However, Mendelian primary immunodeficiency resulting from a genetic NF-κB defect can profoundly impact cell proliferation, survival, innate immunity, and inflammation (Zheng *et al*., 2022). Individuals with autosomal recessive deficiencies in IRAK-4 and MyD88 are more prone to recurrent pyogenic bacterial infections, particularly with IRAK-4 deficiency, often caused by Streptococcus pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa. While responses involving T and/or B cells or leukocyte formation remain unaffected in most cases, the absence of IRAK-4 and MyD88 hampers the TLR4 response and NF-κB-mediated cellular reactions. Several pharmaceuticals are currently available that can directly inhibit NLRP3 or indirectly modulate the NLRP3 inflammasome (by preventing ASC aggregation) (Picard *et al*., 2011). While direct interference with pyroptosis cannot be treated with medication, necrosulonamide has been found to inhibit gasdermin D. Additionally, salidroside inhibits gasdermin D and necrosulonamide and has been demonstrated to reduce atherosclerotic plaques by hindering the production of gasdermin and caspase-1 in endothelial cells. An in-depth analysis of these pharmaceuticals suggests that specifically focusing on the NLRP3 inflammasome could prove to be the most potent approach. Targeting the aseptic elements of inflammasomes may be a viable clinical treatment strategy, especially given recent research demonstrating that atherosclerosis is an age-related condition. Nonetheless, further investigation in the clinical context is required to effectively implement these approaches (Burdette *et al*., 2021; Puylaert *et al*., 2022).

Limitations

While this review provides a comprehensive understanding of the impact of non-infectious

inflammation in atherosclerosis, several limitations should be acknowledged. The majority of the evidence discussed is derived from preclinical studies and the translation of these findings to clinical applications necessitates further investigation. Additionally, the complex interplay of various factors in the pathogenesis of atherosclerosis presents a challenge in isolating the specific impact of sterile inflammation. Moreover, the rapidly evolving landscape of research in this field means that our review may not capture the most current advancements. Finally, while efforts were made to ensure a broad scope of literature inclusion, the possibility of publication bias cannot be entirely discounted. These limitations emphasize the necessity for ongoing investigation to completely understand the complexities of sterile inflammation in atherosclerosis and to facilitate the development of targeted therapeutic strategies.

Conclusion

Inflammation, particularly sterile inflammation, emerges as a vital factor in the development of atherosclerosis, reaching further than the conventional paradigm of foam cell formation. Our review of the literature underscores the intricate roles of inflammasomes, cytokines, and signaling pathways, illuminating the multifaceted nature of sterile inflammation within the context of atherosclerosis. The diverse mechanisms triggered by sterile inflammation, beyond the traditional association with macrophages, signify promising objectives for creating treatment plans intended to mitigate atherosclerosis progression.

Sterile inflammation represents a significant avenue for precision therapeutic interventions, with specific cytokines such as IL-1β and IL-6 emerging as prime targets for potential treatment modalities. The efficacy of canakinumab and methotrexate in addressing atherosclerosis further supports the potential of tailored interventions in targeting sterile inflammation. However, the complexities inherent highlight the necessity for diverse treatment strategies in addressing the development of atherosclerosis that acknowledge the intricate interplay of multiple mechanisms.

While the opportunity to focus on aseptic inflammation in the treatment of atherosclerosis appears promising, the translation of preclinical findings into clinical applications necessitates further comprehensive research. The multifactorial nature of atherosclerosis necessitates a holistic approach to therapeutic interventions, encompassing the intricate interactions between sterile inflammation, traditional risk factors, and disease progression. Continued exploration and validation of sterile inflammation as a cornerstone possess encouraging potential for enhancing the future control of atherosclerosis.

In conclusion, our synthesis of current literature underlines the paramount significance of sterile inflammation regarding atherosclerosis and its viability as a targeted treatment focal point. By appreciating the intricate web of interactions and signaling cascades, we can discern potential avenues for the development of precise and effective interventions to mitigate the progression of atherosclerosis. Consequently, our findings advocate for the continued exploration of sterile inflammation as a cornerstone by advancing tailored treatment approaches, instilling hope for the future handling of atherosclerosis.

Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the resources and platform provided by the publisher, which have enabled us to share our findings with a wider audience. We appreciate the efforts of the editorial team in reviewing and editing our work and we are thankful for the opportunity to contribute to the field of research through this publication.

Funding Information

This research was funded by the Russian Science Foundation, grant number 20-15-00337.

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Anastasia Vladimirovna Poznyak: Drafted preparation.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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