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Inflammatory Mechanisms in Atherosclerosis

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Abstract

Atherosclerosis is a disease of chronic inflammation, characterized by a dysfunctional interplay between the immune apparatus and lipids. Immune cells, as well as nonimmune cells, drive plaque inflammation through a complex crosstalk of inflammatory mediators. The cells are activated by risk factor-induced triggers, which are present in the circulation and in the vessel wall, such as shear stress, oxidized lipoproteins and oxidative stress. Without relief from risk factors, the activation of inflammatory processes persists, resulting in a chronic nonresolving inflammation. Inflammation is associated with severity of disease, and complex lesions, which are prone to rupture and cause acute events, are characterized by extensive inflammation. Thus, inflammation is an active driver of atherosclerotic plaque development and a risk factor for atherosclerotic events. It is therefore of utmost importance to understand the mechanisms behind these inflammatory processes and to be able to develop new diagnostics and treatment modalities for atherosclerotic disorders. This chapter provides a brief overview of the most important inflammatory players and processes during atherosclerotic plaque development and of possible therapeutic targets to combat atherosclerotic disease.

Keywords: inflammation, monocytes, macrophages, T cells, cholesterol

1. Introduction

Atherosclerosis is a complex disease of the artery wall. It is the major cause of cardiovascular disease (CVD), which is the most common cause of death in the world, killing 17.5 million people each year [1]. Although previously thought of as a disorder of age and cholesterol, it is now commonly appreciated that atherosclerosis results from a complex interplay between inflammation and lipids. As early as in the nineteenth century, Rudolf Virchow described inflammation as an active driver of plaque development; however, the importance of these findings was not appreciated until over a century later. During this time, modern immunology

evolved extensively and paved the way for in-depth understanding of how the immune system works. Identification of adhesion markers on endothelial cells and thus the ability of leukocytes to migrate into atheromas gave plausibility of inflammation as a contributor in atherogenesis [2, 3]. Furthermore, findings showing that monocytes [4], and later on that vascular cells [5, 6], secrete inflammatory mediators were important evidence supporting this. These discoveries were followed by clinical proof in the 1990s. Immune activation in atherosclerotic plaques was identified [7], and myocardial infarction was recognized as a potent trigger of CRP release [8]. Since then, an extensive number of animal as well as clinical studies have established inflammation as a major driver of atherosclerotic disease. Regardless of this acceptance, our understanding of atherogenic inflammation is far from complete.

2. Inflammatory mediators in atherosclerosis

The evidence for atherosclerosis as an inflammatory disease is solid. The importance of immune activation in atherosclerosis is demonstrated in several animal models, where removal of central inflammatory mediators or cell types have been shown to extensively reduce plaque development [9–11]. Further, both communicable and noncommunicable inflammatory conditions increase the risk of cardiovascular disease (CVD), and CRP is an independent risk factor of cardiovascular events, both in healthy individuals and in patients with established disease [12–14]. Moreover, immune cells are present within all atherosclerotic plaques, from early fatty streaks to complex atheromas. Lesional inflammation increases during the course of plaque development and is most prominent in vulnerable plaques with large necrotic cores. Immune cells, but also smooth muscle cells, platelets and endothelial cells are drivers of plaque inflammation. Further, there are numerous different inflammatory triggers contributing to the great complexity of atherosclerotic plaque inflammation.

2.1. Immune cells in atherosclerotic inflammation

2.1.1. *Macrophages: linking lipid metabolism and inflammation in atherogenesis*

Macrophages are involved in all stages of plaque development, and are the most important immune cell in atherogenesis. Monocytes originate from a common stem cell in the bone marrow and migrate to various tissues where they develop into tissue-specific macrophages. Although circulating monocytes are a heterogeneous population, it is not known if distinct monocyte subtypes develop into specific macrophage subtypes in humans. Monocytes from the circulation are recruited to the intimal layer of an artery by chemokines (e.g., CCL2) and neuronal guidance molecules (e.g., ephrin-B2). Inside the plaque, the monocytes differentiate to macrophages and engulf modified lipids through scavenger receptors such as SR-A1 and CD36. These lipid-filled macrophages, called foam cells, have altered phenotype and immune function. The efferocytotic capacity (ability to clear apoptotic cells) is one of the functions affected, and as extensive cholesterol accumulation is also lethal to the cells, a necrotic core consisting of cell debris and lipids forms inside the lesion. Plaques with large necrotic cores are associated with an unstable plaque phenotype and are prone to rupture. Monocyte infiltration and foam cell formation are key elements in plaque development and provide the main link between lipid metabolism and

chronic inflammation. There is, however, not only the quantity but also the phenotype of the macrophages that is important to the fate of the plaque [15]. The terms M1 and M2 describe the “classical” activated macrophage induced by T helper cell (Th) 1 cytokine interferon (IFN) γ and the “alternatively” activated macrophage induced by Th2 cytokines IL-13 and IL-4, respectively. In short, the M1 macrophages produce pro-inflammatory cytokines and chemokines, cause tissue injury and promote atherosclerotic plaque development. M2 macrophages are often divided into “wound healing” and “regulatory” macrophages, the latter induced by immune complexes and IL-10, and produce anti-inflammatory cytokines and increase plaque stability [16]. Their pro- and antiatherogenic role is supported by studies showing that plaques enriched in M2 macrophages are associated with a stable or regressive phenotype and vice versa. Growth factors, lipids and cytokines produced by vascular cells and immune cells in the plaque affect the macrophage polarization state. Due to the complexity of inflammatory stimuli present in the plaque, the terms “M1” and “M2” and “classical” and “alternative” are overly simplified, and it is more likely that there exists a range of overlapping phenotypes in the atherosclerotic lesions [15, 17–19].

2.1.2. Dendritic cells are professional antigen presenting cells in the plaque

Another cell of the innate immune system, with great importance for atherosclerotic plaque inflammation, is the dendritic cell (DC). Increased number of DCs is present in atherosclerotic plaques of both humans and mice, and also, as described later, in tertiary lymphoid organs in the adventitia. However, the circulating number of DCs has, by the majority of studies performed, been reported to be reduced in atherosclerosis, which could reflect hampered production from the bone marrow as well as increased recruitment to the plaque [20–22]. As macrophages, the DCs engulf lipids and become foam cells, thereby contributing to plaque development. On the contrary, it has also been suggested that DCs can control cholesterol homeostasis and counteract hypercholesterolemia. It is, however, their role as antigen presenting cells (APCs) that is most described in plaque inflammation [22]. Antigen presentation to T cells occurs both inside the plaque and in the lymphatic tissue, and it is shown that DCs can leave the atherosclerotic lesion upon signals from the chemokines CCL19 and CCL21 [23]. The different subgroups of DCs activate pro- and anti-inflammatory functions in T cells. Difficulties in finding DC-specific markers, as well as the broad spectrum of different DC cell subtypes, have complicated the study of DCs in atherogenesis. There is, however, without doubt that DCs are important players in atherosclerotic disease [22, 24].

2.1.3. Other innate immune cells in atherogenesis

Neutrophils, mast cells and innate lymphoid cells, such as natural killer (NK) cells, are also important contributors to inflammation in atherogenesis, and their role is increasingly appreciated. The description of these cell types is beyond the scope of this chapter, but has been reviewed elsewhere [25–28].

2.1.4. T-cell diversity in atherogenesis

CD4⁺ Th cells are the most abundant of the adaptive immune cells in the plaque and are therefore the most studied. In the plaque, they are activated by epitopes of native as well as oxidative

LDL presented by antigen-presenting cells (i.e., DCs). Activated T cells can affect atherosclerosis in two ways: through effector functions in the arterial wall and by activating B cells in lymphoid organs to produce circulating antibodies [29]. For CD4⁺ Th cells, several subsets have been identified. Most is known about the role of Th1 and Th2 in atherosclerosis; however, in recent years, it has become evident that Th17 and Tregs also are important players in atherogenesis. Polarization of Th cells is determined by the cytokine environment, and the proinflammatory Th1 cells are the most abundant T cell in the plaques. Th1 is characterized by secretion of IFN- γ , and Th2 typically secretes IL-4, IL-5 and IL-13. Th17 secretes IL-17 and IL-22, and Tregs secrete IL-10 and transforming growth factor (TGF)- β . In short, the Th1 cells are proatherogenic, while Tregs are atheroprotective. The impact of Th2, Th17 and natural killer T cells (NKTs) on atherosclerosis has shown more conflicting results, but are all present in the plaque. CD8⁺ cytotoxic T cells are also present in atherosclerotic lesions, although less frequent than CD4⁺ effector cells. Their activation and importance in atherosclerosis is not completely understood, but they can exert proatherogenic effects through IFN- γ production and macrophage activation or through their cytotoxic activity. Recently, the CD8⁺ regulatory T cell was described, with possible atheroprotective effects, through modulatory effects on T cell–B cell interaction [30, 31].

2.1.5. B cells and atherogenic antibodies

B cells are divided into two subtypes: B1 and B2 cells, and both of these are involved in atherogenesis. B1 cells are involved in innate humoral immune response, divide upon self-renewal in the periphery and produce antibodies with low specificity. In contrast, B2 cells are conventional B cells, which differentiate to plasma cells upon antigen presentation by T cells and DCs in lymph nodes, producing antibodies with high affinity and thereby contribute in adaptive immunity [30, 32]. Several animal models with B cell depletion resulting in aggravation of atherosclerosis have suggested a protective role for B cells in atherogenesis [33, 34]. Specific depletion of B2 cells has, however, been shown to reduce the development of atherosclerosis, suggesting subset specificity with regard to B cell atherogeneity [35, 36]. In contrast to B2 cells which are mainly proatherogenic, producing IgG antibodies and activating T cells, B1 cells produce IgM antibodies, which can bind and thereby block the uptake of oxLDL by macrophages, exerting atheroprotective effects [37]. Most of these studies are performed in animal models, and thus, the importance for B cells in human atherosclerosis is unclear. In contrast to macrophages and T cells, B cells are only found in some atherosclerotic plaques, and are more abundant in so-called tertiary lymph organs, in the adventitial layer of the artery.

2.2. Tertiary lymphoid organs: extended plaque inflammation

During chronic inflammatory conditions, lymph-node-like structures, termed tertiary lymphoid organs (TLOs), can develop. Immune cells in the adventitial layer of atherosclerotic arteries were discovered decades ago [38], but the importance of these TLOs for atherosclerotic plaque inflammation is still unknown. Advanced plaques are, however, associated with increased adventitial inflammation in both humans [39, 40] and Apoe^{-/-} mice [41], suggesting that such extended plaque inflammation is important in the disease process. They likely evolve as a response to arterial wall inflammation in early lesion development. Medial SMCs are suggested as drivers of TLO development and are upon inflammatory stimuli shown to attract immune cells into

adventitia by production of the lymphorganogenic chemokines CXCL13 and CCL21 [42, 43]. The TLOs have a different composition of immune cells than the macrophage-rich plaques and is mostly composed of dendritic cells, T cells and a high number and diversity of B cells [43, 44]. This supports the role of TLOs as sites for T-cell training [45] and activation of local humoral immune responses [37]. A recent paper suggests that TLOs participate in atheroprotection [45], however, as the plaque itself, the TLOs can harbor both pro- and anti-inflammatory mediators, and thus, the net effect of adventitial inflammation is still elusive [46].

2.3. Inflammatory mechanisms of nonimmune cells in atherosclerosis

Plaque development does not occur randomly, but typically at curvatures and branching points in the arteries. At these sights, the shear stress activates the endothelial cells lining the arterial wall, leading to structural, molecular and functional alterations in the cells. Atheroprone flow activates the Nf-K β pathway and TLR2 expression in endothelial cells as well as a spectrum of other conduits leading to increased endothelial proliferation and inflammation. The activated endothelial cells adhere leukocytes and stimulate neighboring cells, e.g. vascular smooth muscle cells (VSMCs) [47–49]. Upon atherogenic stimuli, that is, from the activated endothelium, VSMCs undergo so-called phenotype switching. They progress from quiescent contractile to proliferative and migratory cells. These cells possess atheroprotective functions, as they produce extracellular matrix and proteoglycans, which protects the plaque from rupture. They do, however, also accumulate lipids and become macrophage-like foam cells, contributing to plaque development [50, 51]. Further, they express adhesion molecules such as VCAM-1 and ICAM-1 and thereby contribute to retention of monocytes and macrophages in the lesions [52, 53]. Thus, VSMCs can have both protective and destructive effects, depending on the stage of plaque development, and the stimuli present. Inflammatory monocytes can further stimulate VSMCs to secrete pro-atherogenic matrix metalloproteinases (MMPs), which increase the risk of plaque rupture through thinning of the fibrous cap [54]. Further, VSMCs produce a variety of cytokines, activating immune cells, endothelial cells and other VSMCs in the lesion [51]. The inflammatory, atheroprone contribution of VSMCs is however probably under-communicated, as lack of cell-specific markers complicates their identification. Macrophages can express “classical” smooth muscle cell markers (i.e., α -actin and SM22 α), and vice versa (i.e., CD68 and Mac2), and this is determined by the presence of lipids and inflammatory stimuli in the plaque [55, 56]. The local inflammatory micro milieu will therefore decide the inflammatory contribution of smooth muscle cells to atherogenesis by regulating the transition of VSMC into inflammatory cells. Thus, there is a need for better markers to more correctly determine the role of VSMCs in atherosclerotic inflammation.

Also nonimmune cells in the circulation can contribute to the inflammatory milieu during atherogenesis. In addition to their most known roles as blood clotting cells, platelets also possess a great inflammatory potential. In a bidirectional manner, platelets interact with both leukocytes and endothelial cells to communicate inflammation. They express a variety of inflammatory mediators and receptors and contribute to atherosclerotic inflammation throughout disease development, from development of fatty streaks to thrombus formation. For an extensive review of the role of platelets in atherogenesis, see [57].

3. Inflammasome activation: a central inflammatory driver of atherosclerosis

Inflammasomes are intracellular immune sensors, which are tightly controlled. They assemble upon stimuli from tissue damage, infection or metabolic disturbances, and their activation results in production of the pro-inflammatory cytokines interleukin (IL)-1 β and IL-18. There are several different inflammasomes, but the *NOD-like receptor containing a pyrin domain 3* (NLRP3) inflammasome is the most described and is an important constituent of innate immune apparatus. The NLRP3 inflammasome is a multimeric protein complex, which upon activation assembles and attracts caspase-1 molecules, which then is activated by self-cleavage. Active caspase-1 cleaves pro-IL-1 β and pro-IL-18 to active cytokines ready for secretion [58, 59]. IL-1 β is a prototypical proatherogenic cytokine, and NLRP3 is thus an important contributor to atherosclerotic inflammation. Cholesterol crystals, which deposit in atherosclerotic lesions, can activate the inflammasome both in vitro and in vivo [60, 61]. Further, the nonlipid danger signal ATP stimulates foam cell formation and cell migration through inflammasome activation [62]. Thus, the inflammasome promotes atherogenesis through inflammatory, as well as noninflammatory pathways, induced by lipid- as well as nonlipid stimuli. The NLRP3 inflammasome is present and activate in human atherosclerotic plaque [61]. Further, LDL receptor (LDLR)-deficient mice which received bone marrow from NLRP3-deficient mice show attenuated atherosclerosis, and silencing of NLRP3 in ApoE-deficient mice stabilizes atherosclerotic plaques, pointing to an important role in atherosclerotic disease development [60, 63].

4. Danger signals in atherosclerosis

Inflammation is a part of the body's response to harm, either from microbes, such as virus and bacteria, from burns or toxins, or from injury. The main function is to eliminate the insult, remove damaged tissue and restore tissue homeostasis. In atherosclerosis, the signals of harm, termed *triggers*, are numerous. In contrast to infectious disease, the most typical triggers in atherogenesis are however sterile. These are termed damage-associated molecular patterns (DAMPs) and are host-derived danger signals released upon tissue damage, metabolic disturbances, or environmental stress. The risk factors of CVD include hyperlipidemia, smoking, hypertension and hyperglycemia, and all these factors cause DAMPs. There is, however, also evidence supporting a role for pathogens in atherosclerosis. These are termed pathogen-associated molecular patterns (PAMPs). Bacterial and viral microbes are found in atherosclerotic plaques and are associated with disease risk [64, 65]. In addition to pathogens, gut microbiota is a potential source of PAMPs, also linked to atherogenesis [66]. The causal relationship between the endogenous DAMPs and atherosclerosis is stronger than for PAMPs. Microbes do not seem to be required for atherogenesis, as germ-free mice are not protected against disease [67]. The DAMPs comprehend the necessary evil of atherogenesis, namely lipids. As mentioned, the interaction between lipids and immune activation is the hallmark of atherosclerotic disease. Nonmodified fatty acids can activate immune responses, and while saturated fats are shown to stimulate inflammation, polyunsaturated fats are repressors [68]. It is, however, the modified lipids that are the typical triggers during atherogenesis. In hyperlipidemia, LDL undergoes oxidation, forming oxidation-specific

epitopes (OSEs), an important class of DAMPs in atherosclerosis [65, 69]. Cholesterol saturation inside the plaques leads to the formation of cholesterol crystals, which are important activators of the NLRP3 inflammasome (see Section 3) [60]. Other crystal structures can also serve as DAMPs, such as monosodium urate (MSU) crystals, which are composed of crystalized uric acid that contributes to the increased risk of atherosclerosis in patients with gout [70]. Moreover, lipids, nucleic acids and proteins can be modified in the presence of sugars, forming advanced glycation end products (AGEs), which activate immune responses through specialized receptors. These DAMPs are especially prevalent in diabetic subjects, promoting atherosclerosis through vascular dysfunction and increased inflammation [71, 72].

Necrotic cores of complex lesions are huge sources of inflammatory stimuli. In contrast to apoptosis, which is silent, necrosis and pyroptosis activate innate immune responses through the release of DAMPs such as heat shock proteins, nucleic acids, uric acid and ATP [65, 73, 74]. Further, as immune cells accumulate and the plaque develops, the demand for oxygen exceeds the availability, leading to hypoxic conditions. Hypoxia can activate the NLRP3 inflammasome and stimulate the polarization of M1 macrophages, causing increased inflammation in the plaques [75, 76]. Further, as mentioned, mechanical stress in the artery wall can also be a trigger of inflammation by stimulating endothelial activation, with subsequent activation of immune cells and VSMC in the artery wall.

The presence of risk factors provides continuous production of triggers, resulting in defective rescue mechanisms and persistent immune stimulation. Without relieve of these stimuli, a nonresolving inflammation develops, which is a hallmark of atherogenesis.

5. Defective resolution in atherosclerosis

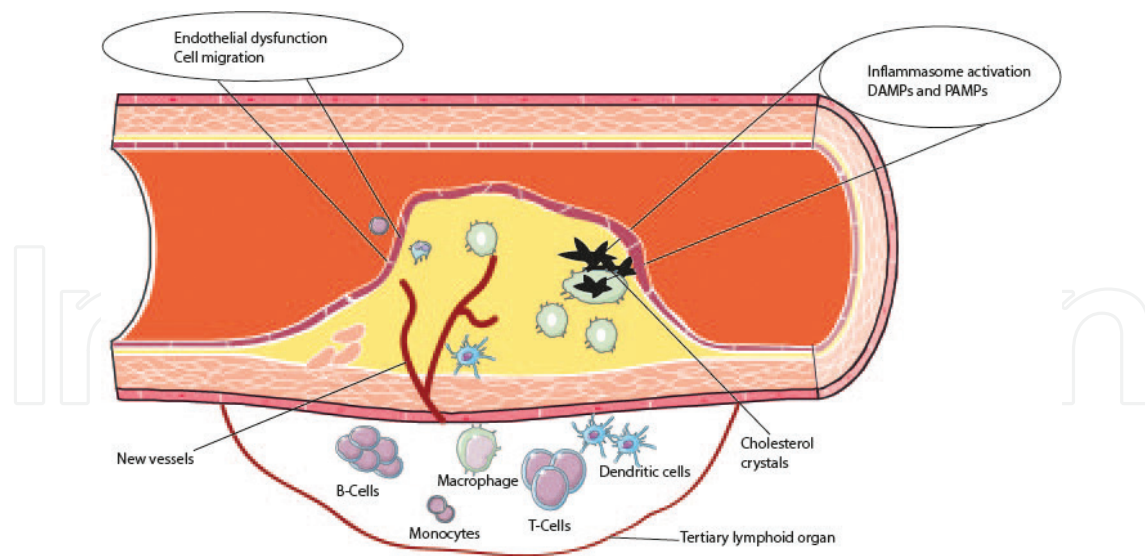
Inflammation is a beneficial process; however, it becomes detrimental if the response is too strong or too long. Cessation of inflammation was previously thought of as a passive process; however, it is now known that resolution of inflammation is a highly active process, involving a complex network of mediators. For inflammation to stay homeostatic, these mechanisms need to be intact. Atherosclerosis is characterized by a chronic nonresolving low-grade inflammation. Thus, a defective resolution of inflammation is an important contributor to atherosclerotic development and sustainability. Resolution is driven by endogenous specialized lipid-derived mediators (SPMs), which are synthesized from fatty acids, as well as proteins such as IL-10, M1 macrophages and the nucleotides inosine and adenosine. These stimulate tissue repair and regeneration and can therefore be distinguished from the classical anti-inflammatory signals, which are merely antagonists of pro-inflammatory signals. In a normal immune response, the production of SPMs is initiated by the production of the pro-inflammatory prostaglandins. Defective resolution in atherosclerosis can be summarized in three processes: (1) sustained inflammation, (2) increased infiltration/reduced egress of immune cells and (3) defective efferocytosis. In early atherosclerotic plaques, the efferocytotic capacity of macrophages (ability to clear apoptotic cells) is sufficient. Thus, inflammatory cells are cleared from the lesion, and this process elicits the release of anti-inflammatory mediators that counteract the plaque development. In advanced plaques, the efferocytotic

capacity is however, as mentioned, compromised, leading to reduced clearance of dead cells, secondary necrosis and stimulation of the pro-inflammatory environment and growth of the necrotic core. SPMs are shown to counteract these processes and stabilize plaques [73, 77]. However, in advanced atherosclerotic lesions, the ratio of SPMs to pro-inflammatory mediators is decreased, and the administration of SPMs counteracts atherosclerotic disease development. These findings provide a mechanistic explanation for the defective resolution observed in atherosclerotic disease [78, 79]. To further map the production, regulation and function of pro-resolving mediators in atherosclerosis will be of great importance to increase our understanding of how the inflammation in atherosclerosis becomes nonresolving.

6. Inflammation: a therapeutic target in atherosclerotic disease

Despite the great success of modern treatment modalities, atherosclerosis is still the leading cause of mortality and morbidity worldwide. For example, high-dose statin treatment and other standard measures only prevent a fraction of recurrent events in survivors of MI. This residual burden of events presents a pressing unmet medical need, and novel perspective on atherogenesis is needed to treat those who are not met by the current treatment regimes. An interesting new therapeutics is the enzyme proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors. PCSK9 binds to the hepatic low-density lipoprotein (LDL) receptor, thereby inhibiting recycling of this receptor, resulting in attenuated removal of LDL cholesterol (LDL-C) from the circulation. The importance of PCSK9 for LDL-C homeostasis is illustrated in individuals with loss- and gain-of function mutations in this enzyme leading to hypo- or hypercholesterolemia, respectively, with dramatic effects on the incidence of atherosclerotic disease. Recent studies have shown that anti-PCSK9 therapies markedly reduce LDL-C levels, leading to lower incidence of adverse cardiovascular disease (CVD) outcomes in high-risk patients with hyperlipidemia [80]. In patients with LDL levels that remain above the treatment target, despite statin treatment (residual LDL risk); adding a PCSK9 inhibitor should be considered. Recent network meta-analysis demonstrates that PCSK9 inhibitors significantly reduce LDL cholesterol, on top of medium to high statin therapy [81]. Of direct relevance for inflammation, a very recent study, the CANTOS trial, suggests that those with residual inflammatory risk could benefit from interleukin-1 β inhibition by Canakinumab. This anti-inflammatory treatment resulted in reduced cardiovascular risk, independent of lipid-lowering effects [82]. Another exciting approach to target inflammation in atherosclerosis is the pro-resolving mediators SPMs. In contrast to anti-inflammatory agents, these ligands will not compromise host defense, one of the most important challenges of immunosuppressive therapeutics. Many experimental studies have shown therapeutic potential for SPMs; however, more knowledge is needed to pinpoint how these mediators act, before these findings can be translated into clinical use [83].

In sum, atherosclerosis is characterized by low-grade chronic inflammation in the arteries, in tight interplay with lipids. Targeting both pathways, depending on individual risk analysis, might be the future of prevention and treatment of cardiovascular disease. However, there is still a need for tools to identify people at risk, especially for personalized treatment. There is also a need to evolve more precise targets for treatment.



Atherosclerotic plaque inflammation

Inflammation is involved in all stages of plaque development. Endothelial dysfunction allows entry of lipoproteins and migration of inflammatory cells into the intimal layer of the artery. Inside the plaque the cells are activated by PAMPs and DAMPs. Cholesterol crystals are important DAMPs which can activate the NLRP3 inflammasome and stimulate release of inflammatory cytokines. Further, accumulation of large amounts of lipids in the immune cells can lead to extensive cell death, and a necrotic core develops due to dysfunctional clearance of these cells. The necrotic core maintains the nonresolving inflammatory milieu in the lesion and is a typical feature of advanced plaques. Moreover, tertiary lymphoid organs can form in the adventitial layer of the vessel wall and feed the plaque with inflammatory cells and mediators, and can further contribute to plaque inflammation. The authors wish to acknowledge Sverre Holm for making the illustration and SERVIER Medical Art (www.servier.fr) for use of their medical art kits.

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