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Inflammatory factors driving atherosclerotic plaque progression new insights

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ABSTRACT

Coronary atherosclerosis is a chronic inflammatory disease that can lead to varying degrees of blood flow obstruction and a common pathophysiological basis of cardiovascular disease. Inflammatory factors run through the whole process of atherosclerotic lesions. Macrophages, T cells, and neutrophils play important roles in the process of atherosclerotic inflammation. Considering the evolutionary characteristics, atherosclerosis can be divided into different stages as early atherosclerotic plaque, plaque formation stage, and plaque rupture stage. In this paper, the changes in inflammatory cells at different stages of lesions and their related mechanisms are discussed, which can provide new insights from a clinical to bench perspective for atherosclerosis me chanism.

Key words: atherosclerosis, inflammation, by stage

INTRODUCTION

It is currently believed that atherosclerosis (AS) is a chronic inflammatory disease characterized by vigorous immune activity. Inflammation plays an important role in the occurrence and development of atherosclerotic lesions, and is the most common and important type of atherosclerotic vascular disease.[1] Coronary atherosclerotic heart disease is a heart disease caused by stenosis or obstruction of vascular lumen caused by coronary artery vessels after AS, resulting in myocardial ischemia, hypoxia, or necrosis. Cardiovascular disease (CVD) has become a major health burden worldwide, and AS is the main cause. Ischemic heart disease (IHD) after AS is the most common cause of cardiac death worldwide.[2,3] Therefore, the study of the pathogenesis of AS has important practical significance for the scientific research and clinical treatment of AS.

As for the pathogenesis of AS, various theories have been expounded from different perspectives, including lipid infiltration theory, thrombosis theory, smooth muscle cell cloning theory, and so on.[4,5] In recent years, most scholars have supported the "endothelial injury response theory," believing that various major risk factors of this disease will eventually damage the intima of arteries and cause local inflammatory response of vascular endothelium, while AS lesions are the inflammation–fibroproliferative reaction caused by intimal injury of arteries.[6]

In recent years, many scholars have devoted themselves to the relevant studies of inflammatory cells on AS. Macrophages are capable of engulfing lipids and forming foam cells. And they are considered the main cells that play a key role in the process of AS.[7,8] Some studies have shown that the type and number of T cells showed significant changes during the early stage, indicating that the immune response occurred before plaque instability and was accompanied by plaque instability.[9] In recent years, the role of polymorphonuclear neutrophils (PMNs) in the evolution of atherosclerotic plaque has drawn people's eyes. Studies have shown that the increase of PMNs is positively correlated with the

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risk of CVD.[10] AS is a dynamic process, and inflammatory cells in the lesion site are in a constantly changing state.[11] The modified American Heart Association classification proposed by Virmani[12] defined AS pathological lesions as nine stages. But such a complex classification is difficult to apply in clinical practice and scientific research.

In combination with clinical disease scenario, AS with some uncertain characteristics of stable and vulnerable, and the vulnerable state can be divided into early plaque and progressive plaque. However, various limitations existed in previous literatures; those researches mostly focused on a certain inflammatory cell access mechanism or individually discussed certain types of inflammatory cells in the process of AS changes. And they lack of inflammatory factor to drive the comprehensive research of the progress of AS plaques. In this article, AS staging model is put forward as three phases, which are the prophase of AS plaque, the plaque formation and progress stage, and the plaque rupture stage. This paper will discuss the changes of inflammation in these three periods.

PROPHASE OF AS PLAQUE: VASCULAR ENDOTHELIAL INJURY TRIGGERS INFLAMMATORY CELL RECRUITMENT

Prophase of plaque refers to the period from vascular endothelial dysfunction (ED) to AS plaque formation. ED is considered to be the "promoter" that induces AS.[13] Abnormal expression of adhesion molecules and chemokines results in the recruitment of inflammatory cells to the lesion site, which is an important manifestation of this stage. The excessive deposition of low-density lipoprotein (LDL) in arteries can cause vascular endothelial injury. LDL through the vascular endothelial cells (ECs) and then it is oxidized into oxidized low-density lipoprotein (ox-LDL). It promotes the release of cytokines and attracts monocytes to recruit to the lesion site and then triggers the recruitment of PMNs. After recruitment, the neutrophils release granulocytes and differentiated monocytes into the macrophages with the assistance of T cells.

Macrophage recruitment

Dysfunction of vascular endothelium is the initial step of AS. At this stage, the vasomotor function is out of balance and vascular endothelium permeability is increased, accompanied by local inflammatory reactions.[14] Physiologically, LDL in plasma binds to cholesterol and transports it to tissue cells without causing a buildup of LDL. LDL, which can form AS, is usually desialylated in the blood and contains fewer antioxidants. It can stay under the ECs of the arterial wall longer and is more sensitive to oxidation.[15] The LDL under ECs is easily oxidized to ox-LDL in an anoxic environment.[16] Under the influence of factors such as hypertension, hyperglycemia, hyperlipidemia, and smoking, more reactive oxygen species (ROS) are produced and the endogenous antioxidant capacity is exceeded, resulting in oxidative stress reaction, which further promotes the oxidation of LDL.[17] Meanwhile, the damaged ECs release damage-associated molecular patterns (DAMPs) and monocyte chemoattractant protein-1 (MCP-1).[18,19] Adenosine triphosphate (ATP), a critical compound released from death of damaged ECs, activates the NOD^{-}, LRR^{-}, and pyrin domain-containing protein 3 (NLRP3) inflammasome in local macrophages, which subsequently activate the pro-inflammatory protease caspase-1 and then increase the secretion of interleukin (IL)-1. This ultimately leads to EC expression of the intercellular adhesion molecule (ICAM), which is beneficial to the adhesion of macrophages.[20,21] MCP-1 is an important chemokine of macrophages. Through the specific binding between protein molecules, it guides the migration of monocytes across the endothelium and aggregates to the lesion site, and meanwhile induces its differentiation into macrophages.[22] Macrophages are divided into MI type and M2 type. Lymphocytes play an important role in the occurrence of AS. During the inflammation process, IL-12 and iIL-18 released by pathological cells can induce the expression of helper T cell (Th) 1-specific transcription factor (T-BET) in naive $CD4^{+}$ T cells; meanwhile, they induce the differentiation of Th1 cells and the production of interferon (IFN).[23] Factors such as IFN-γ released by Th1 can induce monocytes to differentiate into M1-type macrophages.[24] M1-type macrophages secrete high levels of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-1α, IL-1β, IL-6, and so on.[25] TNF-α is the main cause of the increase of ROS release and can imprint ox-LDL instead of the accumulation.[26] Studies using microcomputed tomography and histological evaluation have shown that increased IL-1α levels play a key role in the development of early AS.[27] IL-6 can stimulate T and B cells to secrete inflammatory mediators and promote the maturation of B cells, thereby enhancing the effects of IL-1β and TNF-α. In inflammation, IL-6 has a chemotactic effect on inflammatory cells such as mononuclear macrophages.[28] B cells are activated after vascular EC injury. Activated B cells can express peptide antigens via major histocompatibility complex II (MHC II) molecules and bind to T cell antigen receptor (TCR) on the surface of $CD4^{+}$ cells. TCR cannot directly activate T cells after binding with antigen, but must rely on its neighboring CD3 molecules to transmit activation information into the cell. At the same time, $CD4^{+}$ cells are differentiated into Th1 cells. The pro-inflammatory effect of Th1 cells can promote the formation of AS plaques. At the same time,

B cells can also release pro-inflammatory factors such as TNF-α, which further promotes the formation of AS.[29] Moreover, inflammatory factors, such as TNF-α, can promote the production of reduced nicotinamide adenine dinucleotide phosphate (NAPDH) and lead to the increase of ROS, which may further lead to the damage of ECs.[30] M2-type macrophages are induced and differentiated by Th2 cytokines IL-4, IL-13, and so on. They have strong phagocytosis ability, which can remove debris and dead cells, promote tissue repair and wound healing, and finally inhibit AS.[31,32] Sirtuin 2 (SIRT2) was found to slow the progression of atherosclerotic plaques in $LDLr^{-/-}$ mice by inhibiting the polarization of macrophages to the M1-type phenotype.[33]

The recruitment of PMNs promotes the recruitment of macrophages

PMN is an important inflammatory cell. When the degree of local lesion exceeds the scavenging capacity of macrophages, macrophages will further expand the intensity of inflammatory response by releasing chemokines, thus activating PMNs and initiating the recruitment process of PMNs. PMN recruitment is a multilevel cascade reaction jointly completed under the regulation of adhesion molecules and chemokines. Mainly four types of adhesion molecules are included, that is, selectin, integrin, immunoglobulin superfamily, and mucin-like glycoproteins. The activated PMNs express L-selectin, which interacts with E-selectin and P-selectin expressed by ECs to slow it down and roll it on the surface of ECs.[34] Integrins are transmembrane molecules of cells that consist mainly of β2 and α subunits, of which LFA1 (αLβ2: CD11a/CD18) and Mac1 (αMβ2: CD11b/CD18) are the main expression molecules of PMNs.[34-36] The activated PMNs can express integrin β2 and bind to the intracellular adhesion molecules of immunoglobulin superfamily on the surface of ECs, making the PMNs adhere and aggregate to the surface of the inflammatory site.[37,38] Meanwhile, ICAM further enhances the adhesion of PMNs to vascular ECs. Cells attracted to chemokines migrate toward the source of chemokines along the signal of increased chemokine concentration. IL-8 released by macrophages is the main chemokine of PMNs, so it is also known as chemokine (C-X-C motif) ligand 8 (CXCL-8), which can promote the transmembrane movement of PMN and enable it to recruit to the inflammatory site.[39] White blood cells involved in the inflammatory response release leukotriene B4 (LB4), a chemokine that can promote the activation and recruitment of PMN, and it can also stimulate the production of ROS as well as the release of particle enzymes in PMN.[40,41] The expression of ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) is promoted by the lysis of azure granules and protease-3 (PR3) shortly after PMN recruitment, which contribute to the adhesion of macrophages.[42] Studies have shown that the effect of PMN recruitment on mononuclear macrophage recruitment is crucial in the early formation of AS.[43] At the same time, PMN-released proteinase 3 (PR-3) can amplify mononuclear cell recruitment by stimulating ECs to express MCP-1.[44] In addition, PMN also releases cathepsin G and antimicrobial peptides (human: IL-37; mouse: CRAMP), which directly attract the adhesion of monocytes.[45] Studies have also shown that the number of monocytes and macrophages in arterial plaques is significantly reduced after PMN reduction (Figure 1).[46]

Besides, of course, several other factors can contribute to the formation of atherosclerotic plaque. For example, normal vascular shear stress (SS) can protect vascular ECs. When infected with a pathogen, such as sepsis, the pathogen can disrupt the fibrous junctions between ECs and the polysaccharides–protein complex, causing SS to decline. This is followed by an increase in local vascular pro-inflammatory cytokine expression, an increase in leukocyte adhesion and permeability, and a decrease in nitric oxide (NO) production. At the same time, the immune response after pathogen infection triggers a "cytokine storm" of pro-inflammatory cytokines such AS TNF-α, IL-1β, IL-6, IL-12, and IL-17, which in turn trigger the release of EC adhesion molecules and chemokines, followed by the recruitment of neutrophils and monocytes, further aggravating the risk of AS.[47] The inflammation caused by aging plays an important role in the development of CVD. The characteristic of inflammatory aging is that with the increase of age, the level of pro-inflammatory factors such as TNF-α and IL-6 in the circulatory system gradually increases, while the level of anti-inflammatory factors such as IL-10 significantly decreases, leading to the gradual accumulation of inflammation.[48] Through the above process, the disease progresses continuously and ox-LDL accumulates subcutaneously in the blood vessels. After recruitment, a large number of macrophages phagocytose ox-LDL and then form foam cells, which then enter the progressive stage of atherosclerotic plaque formation. At this point into the atherosclerotic plaque formation of the advanced stage.

PLAQUE FORMATION AND DEVELOPMENT STAGE: CONTINUOUS ACCUMULATION OF FOAM CELLS

Foam cells refer to monocytes or tissue cells where phagocytosis of a large amount of lipids occurs, and their cytoplasm contains many lipid droplets, which are characteristic pathological cells in AS plaques.[49]

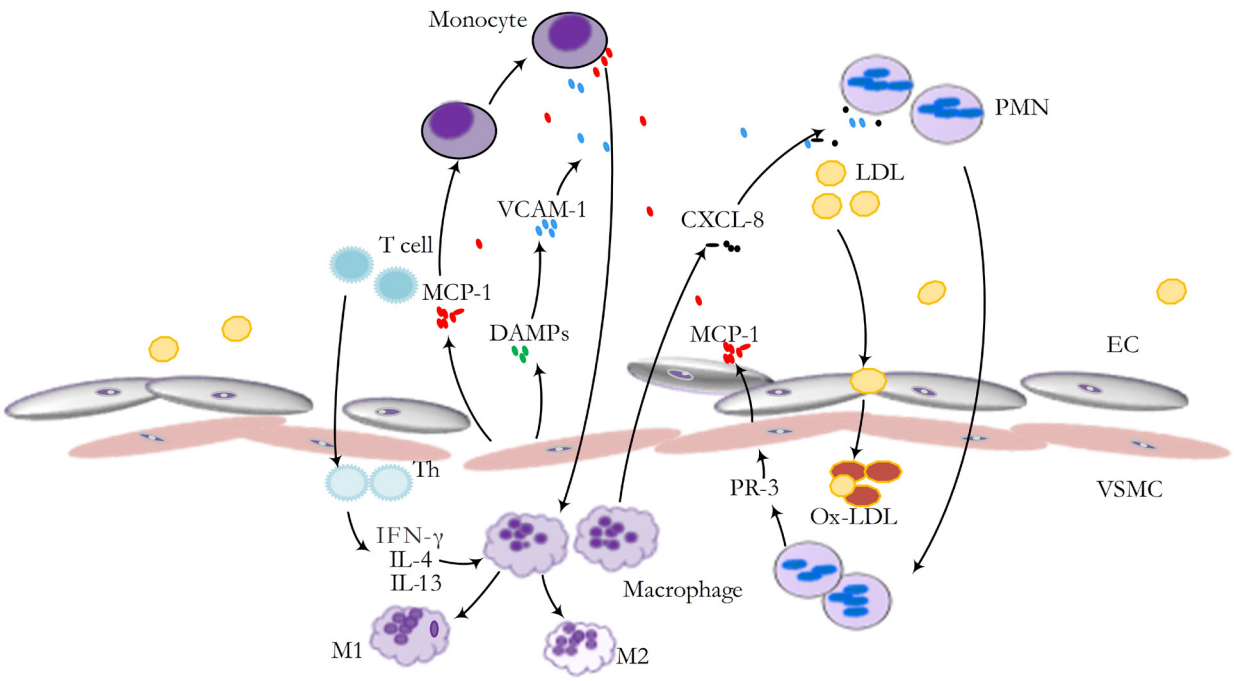

Figure 1: After vascular endothelial function is impaired, vascular permeability changes. LDL in the blood passes through ECs, then stimulates ECs to release DAMPs, MCP-1, and other factors to promote the recruitment and differentiation of monocytes and macrophages. Meanwhile, with the help of T helper cells, macrophages further differentiate into Ml-type and M2-type macrophages. The macrophages then release CXCL-8 to drive the recruitment of PMNs. Activated PMNs also can release the recruitment of MCP-1 primary macrophages. M1-type macrophages can release TNF-α, IL-6, and other inflammatory factors, which further aggravate the inflammatory response of the lesion site. LDL: low-density lipoprotein; ECs: endothelial cells; DAMP: damage-associated molecular patterns; MCP-1: monocyte chemoattractant protein-1; CXCL: chemokine (C-X-C motif) ligand 8; PMNs: polymorphonuclear neutrophils; VSMC: vascular smooth muscle cell; TNF-α: tumor necrosis factor alpha; IFN-γ: interferon-γ; IL-1α: interleukin-1α; IL-1β: interleukin-1β; IL-4: interleukin-4; IL-6: interleukin-6; IL-12: interleukin-12; IL-13: interleukin-13; IL-18: interleukin-18; PR-3: proteinase 3; ox-LDL: oxidized low-density lipoprotein; Th: T helper cell; M1: M1-type macrophages; M2: M2-type macrophages; VCAM-1: vascular cell adhesion molecule-1.

In the evolution of AS lesions, ox-LDL is a key molecule that, after binding to the receptor, can stimulate macrophages to absorb a large amount of ox-LDL and thus reduce cholesterol transport, leading to a large amount of lipid cholesterol deposition in the cytoplasm to form foam cells.[50] At the same time, a large number of inflammatory factors are produced to initiate a series of inflammatory responses, and thus promote the development of AS. According to the different activation conditions of macrophages, they can be divided into classical activated M1 type and selective activated M2 type.[51,52] Some scholars made pathological observation on human AS plaque and found that both M1- and M2-type macrophages in the plaque increased significantly during the AS process.[53] In order to clarify the relationship between macrophages and foam cells of different subtypes and the relationship between macrophages and plaque, the researchers observed the plaque conditions of patients with symptomatic and asymptomatic acute stroke and found that M1 macrophages were abundant in the vulnerable plaques of patients with symptoms.[54] It has been found that M1-type macrophages play a crucial role in the formation and development of AS plaques. A large number of scavenger receptors (SRs) are expressed on the surface of M1-type macrophages, which can mediate the phagocytosis of denatured lipoprotein, namely, ox-LDL, to form foam cells and promote the development of AS.[55] Normally, type M2 macrophages have anti-inflammatory effect. They include several different subtypes: type M2a macrophages can inhibit a variety of inflammatory factors (such as INF-γ, IL-1), the release of M2b and M2c macrophages can by adjusting the factors of chronic inflammation related to suppress M1 macrophages produce, thus inhibiting pathological changes to the further development of the AS.[56] At the same time, M2-type macrophages can express SR family members CD36 and SR-A1, and other receptors,[57] which can promote lipid intake and lead to cholesterol accumulation, and then activate liver X receptor (LXR), resulting in the large expression of ATP-binding transporters (ABCA1 and ABCG1),[58] thus increasing cholesterol transport. MCP-1 released by damaged ECs in AS lesions not only has chemotaxis effect on monocytes, but also has the function of inducing monocytes to differentiate into M1-type macrophages.

Through the above process, ox-LDL production in large quantities leads to the continuous accumulation of lipids in macrophages, which leads to the breaking of the balance

between macrophages and lipoproteins, and finally, foam cells are formed.[59] It has also been found in animal experiments that the use of simvastatin nanoparticles to inhibit the proliferation of macrophages in progressive atherosclerotic plaques can rapidly reduce plaque inflammation in ApoE$^{-/-}$ mice.[60] This well verified the important role of macrophages in the development of AS.

In the inflammatory process of AS, activated PMNs can release a variety of chemokines and granulosa proteins, creating a favorable environment for the formation of foam cells, among which the main ones are azurophilic granule and α defensins. The former can directly activate M1-type macrophages.[61] The latter can induce the production of nitro-amino acids in macrophages to directly increase the oxidation of LDL and the expression of ox-LDL receptors CD36 and CD68, thus promoting the formation of foam cells.[62] In addition, the myeloperoxidase (MPO) stored in the pellet can also interact with the mannose receptor of macrophages to cause the release of ROS and pro-inflammatory factors. ROS can directly oxidize LDL, while MPO can also generate free radicals and oxidize LDL to ox-LDL. They further promote the development of intra-lesion inflammation and generation of foam cells.[63-65] With the continuous development of inflammation, foam cells gradually accumulate to form lipid core and the surface is covered by the fibrous cap composed of cell matrix and ECs, thus forming atherosclerotic plaque.[9] Studies have shown that neutrophil microvesicles accelerate the formation of atherosclerotic plaques by delivering Mir-155 and then activating NF-kB pathway to promote the expression of inflammatory genes and macrophage differentiation.[66] Liang *et al.* showed in their study that after the interaction between PMN and platelet activation, myeloid-related protein (MRP)-8/MRP-14/toll-like receptor(TLR)-4/NF-kB signaling pathway in promoting the development of AS played an important role in the process.[67] Nam found that increased PMN/lymphocyte ratio was predictive of intracranial AS in healthy people. Human neutrophil polypeptide 1 can bind to the apolipoprotein of LDL and help remove LDL particles from the liver, thereby reducing cardiovascular risk.[68,69]

In recent years, the role of neutrophil extracellular traps (NETs) in promoting the development of AS has attracted more and more attention, while its mechanism of action is still unclear. NETs are produced primarily through a process called NETosi or NETotic cell death[70,71] or through a non-decomposition pathway called an "important pathway."[72,73] Studies have found that NETs may be associated with CVDs, rheumatoid arthritis, and other diseases, especially AS lesions in human and animal models. So, it is inferred that NETs may participate in various mechanisms of AS.[74,75] NETs can induce oxidative stress and oxidize high-density lipoprotein (HDL) particles to reduce their ability to facilitate cholesterol outflow, while NETs can also induce ED and apoptosis.[76] Josefs *et al.*[77] observed the transcription maps of NET+ and NET− region plaque macrophages in LDLr$^{-/-}$ mice and found that NET could upregulate the inflammatory body and glycolytic pathway and increase the content of macrophages, thereby inhibiting the regression of AS and increasing the risk of CVD. Zhang *et al.*[78] found in their study that the exome Mir-146A secreted by ox-LDL–treated macrophages promotes the release of ROS and NETs by targeting superoxide dismutase 2, thus promoting the development of AS. An *et al.*[79] found that IL-8 interacts with receptor chemokine receptor 2 (CXCR2) on PMNs to increase the generation of NETs by influencing extracellular regulated protein kinases (ERK) and mitogen-activated protein kinase (MAPK) signals, thus promoting the development of AS, and preliminarily revealed the interaction mechanism of PMN–macrophages in AS development. These results suggest that NET is a novel therapeutic target for AS and other CVDs (Figure 2).

With the continuous interaction of various inflammatory factors in the chronic inflammatory response, the lipid core of atherosclerotic plaque gradually becomes larger and the enlarged plaque bulges toward the lumen, leading to local blood flow obstruction. In the environment with the aggravation of ischemia and hypoxia, the oxidative stress reaction is gradually further activated *in vivo*, resulting in the imbalance of oxidative system and antioxidant system, which leads to excessive production of highly active oxidation molecules such as ROS and reactive nitrogen free radicals (RNS), and finally, increased production of ox-LDL and the inflammatory response in the plaques.[80] At the same time, the activated inflammatory cells release matrix metalloproteinases (MMPs) and fibrinogen proteinases and other substances to destroy the tissue structure of the fibrous cap, leading to the formation of vulnerable plaques, which then enter the plaque rupture stage. A recent study shows that intestinal flora has a great influence on the occurrence of AS. Brandsma transplanted fecal microflora from CASP1$^{-/-}$ mice into the intestines of LDLr$^{-/-}$ mice and used the fecal microflora of LDLr$^{-/-}$ mice as the control. After 13 weeks of high cholesterol diet, LDLr$^{-/-}$(CASP1$^{-/-}$) mice significantly increased the plaque size by 29% compared with LDLr$^{-/-}$(LDLr$^{-/-}$) mice, along with increased numbers of circulating monocytes and neutrophils and elevated plasma levels of pro-inflammatory cytokines. These results suggest that the introduction of pro-inflammatory CASP1$^{-/-}$ microorganism in LDLr$^{-/-}$ mice can enhance systemic inflammation and accelerate the development of AS.[81] It is known that systemic lupus erythematosus (SLE) can be related to the arteries of the

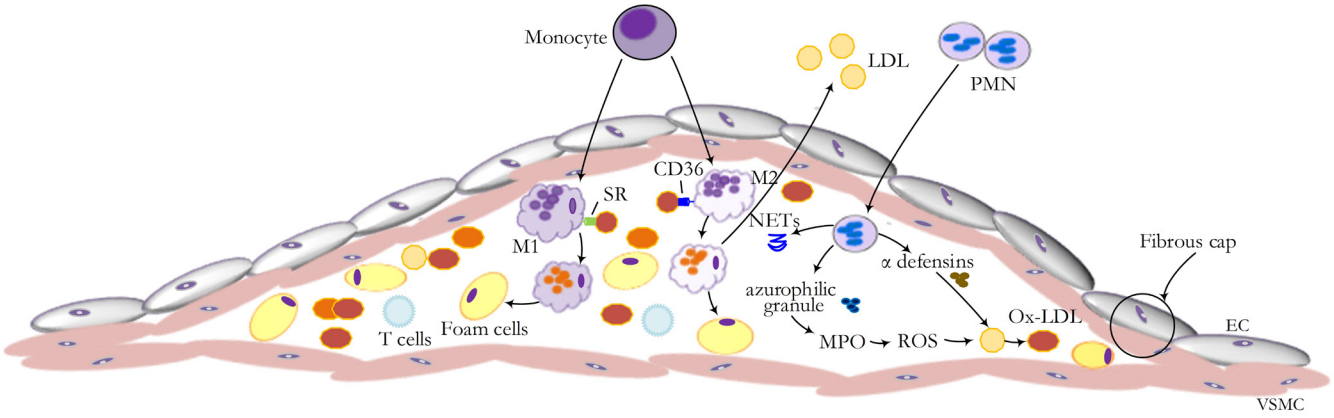

Figure 2: Atherosclerotic plaque progresses under the stimulation of various inflammatory factors. During the plaque formation and development stage, SR on the surface of MI-type macrophages recognize ox-LDL and engulf it to form foam cells. Normally, receptors such as CD36 expressed by M2-type macrophages phagocytose ox-LDL and then promote lipid output. At the same time, α defensins and azurophilic granule released by neutrophils can further promote the oxidation of LDL to produce more ox-LDL. Excessive production of ox-LDL leads to lipid accumulation and the formation of more foam cells. EC: endothelial cell; VSMC: vascular smooth muscle cell; M1: M1-type macrophages; M2: M2-type macrophages; LDL: low-density lipoprotein; ox-LDL: oxidized low-density lipoprotein; SR: scavenger receptor; MPO: myeloperoxidase; ROS: reactive oxygen species; PMNs: polymorphonuclear neutrophils; NETs: neutrophil extracellular traps.

body and AS is one of its common complications.[82] IFN-γ plays a central role in the pathogenesis of SLE and can promote the progression of AS. IFN-γ activates monocytes/macrophages and dendritic cells, leading to the persistent existence of pathogenic Th1, thereby promoting the proliferation of smooth muscle cells, accelerating the accumulation of inflammatory cells and foam cells, and promoting the continuous progression of AS.[83] Studies have found that the increase of intestinal permeability during the aging process leads to higher circulating bacteria content.[49] However, some microorganisms can produce a metabolite, trimethylamine nitrogen oxide (TMAO), which can enhance polarization of M1 macrophages through the activation of NLRP33 inflammasome.[84,85] Skye *et al.*[86] found that in germ-free animals transplanted with synthetic microbial colonies capable of producing TMAO, the accumulation of foam cells and the plaque area are both increased, allowing AS plaques to progress continuously.

PLAQUE RUPTURE STAGE: INFLAMMATORY CELLS AND VULNERABLE PLAQUES

Studies have shown that plaque rupture with thrombosis is a common pathological form of severe heart disease, while vulnerable plaque is its early manifestation.[87] Vulnerable plaque refers to vulnerable plaques prone to thrombosis and likely to develop rapidly into criminal plaques, with pathological features such as thin fibrous caps, large necrotic lipid cores, abundant inflammatory cells, and a small amount of smooth muscle cells.[88] It can be inferred that the stability of AS plaque is negatively correlated with the number of inflammatory cells and the size of necrotic lipid core, while it is positively correlated with the thickness of fibrous cap. Epidemiological studies have shown that about 66.67% of acute coronary syndromes (ACS) occur in coronary artery critical lesions, among which vulnerable plaque is an independent predictor of plaque rupture and thrombosis.[89]

At the late stage of AS, ischemia and hypoxia are further aggravated due to the gradually enlarged plaque of foam cells, which makes the inflammatory response continue to be activated.[90] The increase of inflammatory load in the plaque is the key factor causing instability of the plaque. Plaque rupture and plaque erosion often coexist with inflammation. Studies have found that inflammation in the plaque is always upregulated in the clinically vulnerable state.[91] Pathological studies found that when the plaque ruptures, a large number of macrophages, thinning fibrous caps, and proliferating new blood vessels could be observed in the lesion site of the criminal; at the same time, the number of T cells also reaches a peak.[92,93] This suggests that T cells and macrophages play an important role in the rupture process of late-stage AS plaques.

AS plaque fiber cap is formed by a large number of collagen fibers, smooth muscle cell (SMC), a few elastic fibers, and proteoglycan.[94] When activated, T cells, macrophages, and other inflammatory cells can secrete cytokines that inhibit the formation of fibrous caps and proteases that can destroy fibrinogen.[95] In vulnerable plaques, IFN-γ secreted by Th1 almost completely inhibits collagen fiber synthesis and affects collagen tissue maintenance as well as its repairment, thereby reducing collagen content in the fibrous cap and reducing plaque stability qualitatively.[96] Also, the transforming

growth factor-β (TGF-β) secreted by regulatory T cells (Tregs) has an obvious fibrogenic effect on smooth muscle cells and fibroblasts. At the same time, TGF-β can inhibit the activity of Th-1 cells and macrophages, thus weakening the inflammatory response in plaques.[97] Th-17 cells can participate in wound healing and has strong fiber activity.[98] As Th-17 cells secrete specific cytokines, IL-17A can promote the expression of procollagen. Therefore, during the process of AS, Th-17 cells can promote the formation of collagen fibers and enhance the ability of plaque to withstand the impact of blood flow on its surface, thus increasing the stability of plaque.[99]

Collagen in the fibrous cap is mainly derived from extracellular matrix. T cells degrade most of the proteins in the extracellular matrix by inducing macrophages to release MMPs, resulting in fibrin cap thinning, rupture, and even thrombosis.[100] Activated T cells specifically express CD40L and bind to CD40 of the macrophage system to promote the secretion of cytokines and MMPs.[101] MMPs are a group of destructive proteins containing zinc ions. They mainly include interstitial collagenase (MMP-1), gel enzymes A and B (MMP-2 and MMP-9), matrix degrading enzymes (MMP-3 and MMP-7), and human metalloproteinases (MMP-12). First, MMP-1 destroys the structure of collagen, then MMP-2 and MMP-9 continue to break down the collagen fragments, and then MMP-3 and MMP-7 break down the matrix and degrade elastin by activating other MMPs into active enzymes. Meanwhile, MMP-3 and MMP-7 can also break down the main component of the extracellular matrix, the polysaccharide core protein. And almost all MMP-12 that activate MMPs, this not only can be decomposed elastin, but also will be able to degrade the extracellular matrix components such as collagen type IV layer of protein, protein fiber connection, plate, and so on.[102,103,104] Studies have found that inhibiting the activity of MMP-8 or MMP-13 can shrink the plaque area and reduce the number of macrophages, thus increasing the plaque stability.[105,106] PMN, as an important source of MMPs, is significantly related to the rupture of atherosclerotic plaques. Experiments have found that the rupture of plaques is positively correlated with the number of PMNs.[107] Therefore, PMN is also an important therapeutic target in the stage of plaque rupture at the end of AS.

The apoptosis of macrophages runs through the whole course of AS, which can be caused by excessive inflammatory reaction, oxidized lipid and cholesterol, especially in the stage of unstable rupture of atherosclerotic plaque. Apoptosis of macrophages is the main reason for the formation of necrotic lipid core in plaques.[108] In the initial stage of the development of AS lesions, apoptosis has protective significance. It can clear cells with cholesterol overload and reduce the diversity of plaque cell structure. However, this beneficial effect depends on whether apoptotic macrophages can be effectively cleared from the lesion site.[109] Efferocytosis is a process in which phagocytes remove programmed dead apoptotic cells, and it is an important way to clear dead cells from plaques.[110] At the initial stage of the development of AS lesions, apoptosis is of protective significance, which can reduce the diversity of plaque cell structure and remove cells with cholesterol overload. However, this beneficial effect depends on whether apoptotic macrophages can be effectively removed from the lesion site through cytology.[111] Efferocytosis at the site of the lesion begins with the cell surface receptor c-mer proto-oncogene tyrosine kinase. However, in the late stage of AS, the activity of c-mer proto-oncogene tyrosine kinase is impaired, leading to reduced clearance of apoptotic macrophages, and then apoptotic debris begin to accumulate and lead to secondary necrosis.[112] After efferocytosis is impaired, apoptotic cells accumulate in large quantities and then the necrotic core grows gradually, leading to an increased risk of plaque rupture.[113] In the process of AS lesion formation, endoplasmic reticulum stress, cell apoptosis, and necrosis occur in macrophages to adapt to the continuous accumulation of lipids with the continuous uptake of LDL by macrophages.[114] Endoplasmic reticulum stress stimulates the production of the proapoptotic molecule C/enhancer-binding protein (EBP) homologous protein (CHOP), specific for endoplasmic reticulum stress.[115] Studies have shown that the endoplasmic reticulum stress CHOP pathway can mediate the apoptosis of macrophages, thus increasing the instability of AS plaques.[116] Meanwhile, ox-LDL can initiate the apoptosis of macrophages and EC by upregulating CHOP pathway.[117,118] It has been found that the genetic deletion of CHOP in mice can prevent macrophage apoptosis and plaque necrosis in advanced lesions (Figure 3).[119] Sterpetti *et al.*'s study also confirmed the influence of hemodynamics on AS.[120] When they transplanted mice veins into a simulated arterial system, they observed that the vessels became stiff and pale. The release of IL-6 and TNF-α increased 6 h after transplantation, and the secretion of IL-1 and IL-2 began to increase 24 h after transplantation.[120] These pro-inflammatory factors can accelerate the formation of AS. When the vein was inserted back into the venous system, the experimenter observed the blood vessel return to a soft, elastic appearance and change in color from pale to pink. More importantly, the continuous blood vessel wall is easy to be washed by rapid blood flow, which is easy to cause the rupture of AS plaque.[120]

A new study has found that activated PMNs can promote thrombosis by releasing NETs. NETs are strands of DNA excreted by activated or dead neutrophils that,

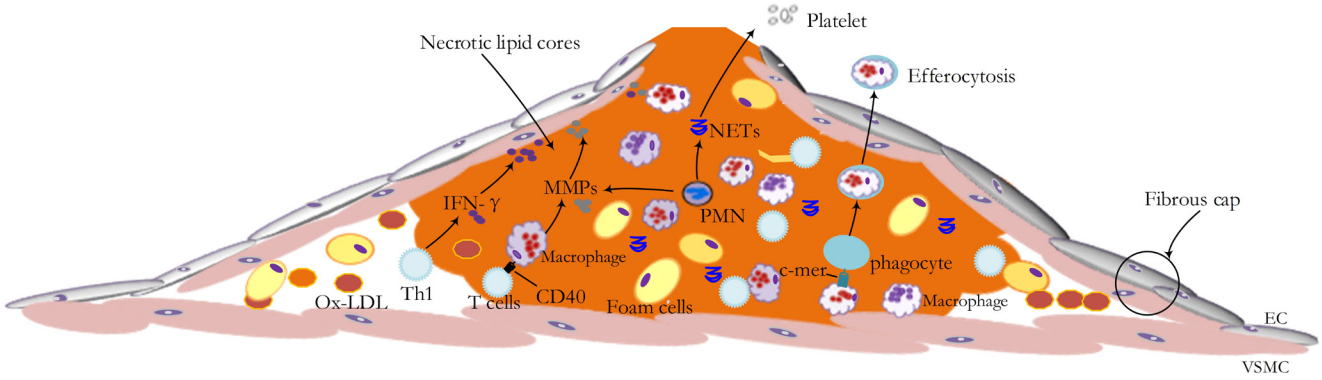

Figure 3: The increase of inflammatory load in plaque is the key factor leading to plaque instability. During the rupture period, the plaque contains a large number of T cells and macrophages. Expression of CD40 in T cells stimulates macrophages to release MMPs to destroy the matrix protease contained in the plaque fibrous cap. IFN-γ released by Thl cells inhibits fibrin synthesis in the fibrous cap. The damage of c-mer proto-oncogene tyrosine kinase on the surface of phagocytes results in the destruction of cellular burial effect and the timely removal of necrotic cells. PMNs can release MMPs and NETs, while breaking down the fibrous cap and causing platelets to accumulate, increasing the risk of thrombosis. EC: endothelial cell; VSMC: vascular smooth muscle cell; MMPs: matrix metalloproteinases; IFN-γ: interferon-γ; Th1: T helper cell 1; PMNs: polymorphonuclear neutrophils; NETs: neutrophil extracellular traps.

decorated with a variety of protein mediators, act as solid reactants. They are present in the blood and on the intimal surface of the diseased artery. NETs have been linked to inflammation, thrombosis, oxidative stress, and CVD.[121] NETs are released to form fibrin-like bases that promote platelet activation, adhesion, and aggregation. NETs also promote the production of prethrombotic molecules such as von Willebrand factor and fibrinogen, which contribute greatly to the formation of blood clots during the rupture of atherosclerotic plaques.[74] Therefore, inhibition of PMNs and NETs may play a role in preventing thrombosis and reducing cardiovascular adverse events.

ANTI-INFLAMMATORY TREATMENT OF AS

The anti-inflammatory drugs have not yet been clinically used in the treatment of patients with AS. Current clinical resistance to AS treatment mainly concentrated in the lower lipid levels, and control inflammation, but several studies have shown that the occurrence of inflammation in AS plays a very important role in the development, so the anti-inflammatory treatment for AS cannot be ignored.[122-124] It has been found that the deficiency of nerve injury-inducing protein (NINJ1), a novel MMP-9 substrate, promotes the progression of AS. NINJ1-deficient macrophages promote pro-inflammatory gene expression by activating MAPK and inhibiting phosphoinositide 3-kinase (PI3K)/Akt signaling pathway. Treatment with soluble NINJ1 simulator peptides $Ninj1_{1–56}$ (ML56) and $Ninj1_{26–37}$ (PN12) reduced the expression of pro-inflammatory genes in activated macrophages in humans and mice, thereby reducing monocyte transmembrane migration and the occurrence of AS.[125] Kim *et al.*[126] encapsulated the anti-inflammatory cytokine IL-10 into a cyclic arginine–glycine–aspartic acid tripeptide (cRGD)-coupled pluronic based nanocellular carrier (NC) for targeted delivery to atherosclerotic plaques. NC releases bioactive IL-10 and reduces the production of pro-inflammatory cytokine IL-1β in the lesion site of AS model mice, which significantly reduces the plaque area. Pseudorlaric acid D (PLAD) is the main active ingredient of Pseudolarix kaempferi Gorde. Chen *et al.*[127] established a vertical motor AS model in ApoE−/− mice fed with a high-fat diet and then gavaged them with 5 mg/kg PLAD for 4 weeks. The results showed that PLAD inhibited the inflammatory response by downregulating the level of monocytes and inhibiting the formation of NETs, thereby reducing the progression of AS plaques in mice. Meanwhile, PLAD also significantly reduced the expression of pro-inflammatory factors IL-1β and IL-18, suggesting that PLAD is a potential drug for the anti-inflammatory treatment of AS.[127] It was found that an octimibate derivative, OXA17, enhanced the expression of ATP-binding cassette-transporter A1 (ABCA1) through LXRα activation to stabilize plaques. ABCA1 is located at 9q31.1 on chromosome 9. It promotes the conversion of cholesterol and phospholipids to HDL. Tangier disease (TD) caused by ABCA1 gene mutation is a severe HDL deficiency syndrome, which can lead to cholesterol deposition in macrophages, increasing the risk of CVD. LDLr−/− mice treated with OXA17 had increased HDL levels and decreased aortic plaques. This suggests that plaque stability can be improved by reducing macrophage accumulation and reducing the size of the necrotic core.[128] To test the role of anti-inflammatory therapy in AS, Ridker *et al.*[129] conducted a randomized, double-blind, controlled trial. To 1061 patients with coronary heart disease, they gave different doses of canazumab, a therapeutic monoclonal

antibody against IL-1β, every 3 months. The results showed that 150 mg of canakinumab administered every 3 months resulted in a significantly lower recurrence rate of cardiovascular events than placebo, without a reduction in blood lipid levels.[129] Peach kernel-safflower is a traditional Chinese medicine commonly used in the treatment of CVDs. The study found that in mice on a high-fat diet with $ApoE^{-/-}$, the peach kernel-safflower treatment group had lower body weight, triglycerides, cholesterol, IL-6, TNF-α, smaller plaques, fewer lymphatic vessels, and fewer T cells in lymph nodes, but higher IL-10 levels. In lipopolysaccharide (LPS)-stimulated mouse mononuclear macrophage leukemia cells (RAW264.7 cells), the levels of IL-6 and TNF-α were lower than those of LPS group, while the level of IL-10 was higher than that of LPS group.[130] Meanwhile, NF-κB p65 was transferred from the cell and nucleus to the cytoplasm. It is suggested that peach kernel-safflower has a good application prospect in the treatment of AS plaque by anti-inflammatory approach.[130]

SUMMARY

AS is a chronic disease caused by non-single factor. In this process, multiple inflammatory factors interact to promote its development. Based on the relevant literature in the past 10 years, it was found that the movement, differentiation, phagocytosis, and apoptosis of mononuclear macrophages played a leading role in the process of AS, while PMN and T lymphocytes played an important inducing and promoting role in the development of AS. At the very beginning of the disease, after vascular endothelial injury, monocytes were activated and then the inflammatory cascade was initiated. PMNs and T lymphocytes were then induced to release cytokines and various proteins to assist macrophages to complete the recruitment of the diseased sites. In the stage of AS plaque formation and progression, M1-type macrophages are differentiated under the inducement of Th and other factors. PMNs and NETs can promote the production of ox-LDL in a variety of ways, which breaks the lipid metabolism balance at the plaque site, causing the macrophages to engulf excess AS and form foam cells, thus generating lipid streaks and atherosclerotic plaques. Continuing with the inflammatory response, apoptosis of macrophages and ECs prompts atherosclerotic plaque formation of necrotic core and at the same time, T cell secretion of interferon and MMPs of PMN releases substances that can destroy an atherosclerotic plaque fiber cap structure, which greatly increases the risk of rupture of vulnerable plaques.

So far, it has been clear that inflammatory factors play an important role in the whole process of AS, while many studies only focus on a single pathway or the mechanism of action of a single inflammatory cell, which brings limitations to the scientific research and clinical treatment of inflammation-related AS. In this paper, the mechanism of inflammatory changes in plaques at different stages has been discussed through the proposed AS stage three lesion model system, which broke the limitations of previous studies. Based on the above discussion, we believe that the development of AS is caused by the interaction and continuous action of various inflammatory cells. Therefore, researchers and clinicians should comprehensively grasp and dynamically analyze the changes of inflammation in the process of AS to obtain the most satisfactory results.

Conflict of Interest

The authors declare that there is no conflict of interest.

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