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Review

Innate and adaptive immunity in atherosclerosis

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ABSTRACT

Atherosclerosis is a chronic inflammatory disorder of the large and medium-size arteries characterized by the subendothelial accumulation of cholesterol, immune cells, and extracellular matrix. At the early onset of atherogenesis, endothelial dysfunction takes place. Atherogenesis is further triggered by the accumulation of cholesterol-carrying low-density lipoproteins, which acquire properties of damage-associated molecular patterns and thereby trigger an inflammatory response. Following activation of the innate immune response, mainly governed by monocytes and macrophages, the adaptive immune response is started which further promotes atherosclerotic plaque formation. In this review, an overview is given describing the role of damage-associated molecular patterns, NLRP3 inflammasome activation, and innate and adaptive immune cells in the atherogenesis process.

1. Introduction

Atherosclerosis is a progressive chronic inflammatory disorder and emphasizes endothelial dysfunction following the response-to-injury hypothesis of atherosclerosis at the early onset of atherogenesis [1,2]. Endothelial dysfunction, characterized by a reduction in nitric oxide bioavailability in the absence of any structural changes of the vessel wall [3,4], can be caused by elevated and modified low-density lipoproteins (LDL), free radicals caused by cigarette smoking, hypertension, diabetes mellitus, age, and combination of these or other factors. Atherogenesis is further triggered by the retention of cholesterol-carrying LDL, which particularly upon oxidative modification acquire properties of damage-associated molecular patterns (DAMPs) and thereby trigger an inflammatory response. This response includes the entry of monocytes into the intima, their differentiation into macrophages and subsequently into foam cells, followed by an adaptive immune response with T helper (Th) 1 cells, but also Th2, $CD8^+$ T cells, Th17, natural killer T (NKT) cells, T regulatory (Treg) cells and B cells.

Essentially, dysregulated lipid metabolism and inflammatory processes jointly contribute to the formation of the atherosclerotic plaque in the arterial wall [5].

This review briefly describes the role of DAMPs and the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome in atherosclerosis and gives an overview of how innate and adaptive immune cells contribute to atherogenesis.

2. Role of DAMPs in atherogenesis

2.1. DAMPS

Recent advances in the field of innate immunity have revealed a complex role of innate immune signaling pathways in atherosclerosis. Among them, the so-called *senescence associated secretory phenotype* (SASP) pathway [6,7] has been linked to "*sterile inflammation*" or

Abbreviations: AAASP, age-associated arterial secretory phenotype; AIM, Absent in Melanoma; ALRs, AIM 2-like receptors; AGER/RAGE, advanced glycation endproduct-specific receptor; Aldo/MR, aldosterone/mineral corticoid receptor; Ang II, angiotensin II; apo, apolipoprotein; ATLOs, artery tertiary lymphoid organs; BAFF, B cell activating factor; CCR, chemokine receptor; DAMPs, damage-associated molecular patterns; ECs, endothelial cells; ET-1, endothelin-1; ET_A, endothelin-1 receptor A; G-CSF, granulocyte colony-stimulating factor; HDL, high-density lipoproteins; IFN-γ, interferon-γ; Ly, lymphocyte antigen; MCP-1, monocyte chemo-attractant protein-1; MMPs, matrix metalloproteinase; MPO, myeloperoxidase; NETs, neutrophil extracellular traps; NOD, nucleotide-binding and oligomerization domain; NK, natural killer; NKT, natural killer T cells; NLRs, NOD-like receptors; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PRRs, pattern recognition receptors; ROS, reactive oxygen species; RIG, retinoic acid-inducible gene; RLRs, RIG-I-like receptors; SASP, senescence associated secretory phenotype; Th, T helper; TNF, tumor necrosis factor; TLRs, Toll-like receptors; VSMCs, vascular smooth muscle cells

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"*inflamm-aging*" that occurs during aging-associated tissue damage and chronic inflammation, in the absence of detectable pathogens [8]. SASP is represented by a myriad of factors collectively called DAMPs [6,9]. According to the modern "danger theory" recently introduced by Polly Matzinger [10], DAMPs initiate the primary function of the immune system in detecting and protecting the host against danger. Thus, the immune response is not induced by foreign or stranger microbes (non-self), but by alarm signals generated by injured or damaged cells and tissues, collectively referred to as DAMPs or alarmins [9]. Well-known DAMPs are heat shock proteins, breakdown products of the extracellular matrix, oxidized lipoproteins, ATP, cholesterol crystals, and S100 proteins. DAMPs induce sterile inflammation through their binding to innate immune receptors, collectively defined as pattern recognition receptors (PRRs), including advanced glycation end-product-specific receptors (AGER/RAGE), Toll-like receptors (TLRs), C-type lectin receptors (CLR), nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), retinoic acid–inducible gene (RIG)-I-like receptors (RLRs), and Absent in Melanoma (AIM) 2-like receptors (ALRs) [11]. The evocation of sterile inflammation causes tissue inflammatory/immune cell infiltration, which acts as vicious cycle to further induce tissue and cell damages, and therefore DAMP release [12,13]. For example the alarmins S100A8 and S100A9, which form the heterodimer S100A8/S100A9, and are known to be involved in several inflammatory diseases [14,15] further regulate the NLRP3 inflammasome [16]. Both S100A8 and S100A9 are expressed in the human arterial wall [17] and deletion of these proteins partly protects apolipoprotein (Apo)$E^{-/-}$ mice from atherosclerosis [18]. Increased S100A8/A9 plasma levels are known to predict cardiovascular events [19,20].

According to findings from Lakatta s' [21] and Šabovic's group [22], additional mediators of DAMPs are renin/Angiotensin (Ang) II, aldosterone/mineral corticoid receptor (Aldo/MR) and the endothelin-1 (ET-1)/endothelin-1 receptor A (ET_A). They act as inflammatory drivers as well as hypertensive agents, thus linking high blood pressure with the genesis of atherosclerotic lesions. Recently, it has been evidenced that Ang II signaling cascades promote aortic atherosclerosis by inducing expression and/or activity of several pro-inflammatory transcription factors, including the Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [23,24]. Its activation determines the production and release of a myriad of factors, including inflammatory mediators, mitotic and trophic factors, proteoglycans and metalloproteinase (MMP)s (especially MMP-2 and -9), and vaso-active molecules. Overall, they constitute the so-called *age-associated arterial secretory phenotype (AAASP)* which create a microenvironment driving the phenotype shift of both endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). Thus, ECs and VSCMs become secretory, migratory, proliferative and senescent, resulting in changes in aortic wall such as intimal-medial thickening, fibrosis, calcification, aneurysms and endothelial dysfunction, associated with reduction in endothelial-dependent vasodilatation and increased stiffness. Among AAASP components, the main contributors to aorta aging and remodeling are MMP-9 and -2, which degrade collagen, elastin, and other extracellular matrix molecules, resulting in increased DAMPs [25,26]. Their relevance in vascular remodeling has been confirmed by MMP inhibition studies from Lakatta's group, who used chronic administration of MMP inhibitor PD166739 in 16-month-old rats for 8 months. They showed that MMP inhibition delays age-associated aortic proinflammatory signaling and preserves intact elastin and collagen fibers within the aortic wall [27]. Thus, MMP activation and consequent DAMPs induction play a central role in age-related pro-inflammatory aorta remodeling and stiffening, which are typical pathological entities of aortic diseases such as atherosclerosis and aneurysms (see Balistreri et al. [28] for comprehensive review on aneurysmal aortic disease).

2.2. DAMPS-mediated NLRP3 inflammasome activation

Inflammasomes are multi-protein complexes in the cytoplasm that intensify inflammation in response to DAMPs and pathogen-associated molecular patterns via caspase 1-mediated proteolytic cleavage of pro-IL-1ß and pro-IL18 to mature IL-1ß and IL-18, respectively. The most well-known inflammasome is the NLRP3 inflammasome [29], which consists of NLRP3, the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) and caspase-1 [30]. NLRP3 activation involves 1) an NF-kB-mediated increase in NLRP3 and pro-IL-1ß mRNA expression (NLRP3 priming) following a TLR-, tumor necrosis factor (TNF) receptor (*R*)-, IL-1R-, or NOD2-depending pathway [31] and 2) formation of the NLRP3 inflammasome complex for full activation [30]. A wide range of stimuli converges on the NLRP3 inflammasome complex for full activation. Besides ATP, particularly the DAMPs cholesterol crystals and oxidized LDL are known to activate the NLRP3 inflammasome in the context of atherogenesis [32], whereas also reactive oxygen species (ROS), potassium efflux and lysosomal damage are established NLRP3 inflammasome activators. NLRP3 components are expressed on EC [33], VSMC [34], and primarily on phagocytic antigen-presenting cells such as macrophages and dendritic cells. Their increased expression is associated with vascular inflammation [35]. In patients with coronary atherosclerosis, high protein expression levels of NLRP3 in the aorta correlate with the severity of coronary artery stenosis [36]. The NLRP3 inflammasome and its product IL-1ß contribute to atherogenesis at different stages: IL-1ß induces the expression of 1) the pro-inflammatory cytokines IL-6 and TNF-α [37,38]; 2) IL-8, which attracts neutrophils; 3) the adhesion molecule, vascular cell adhesion molecule-1 on endothelial cells [39], facilitating the adhesion and subsequent infiltration of circulating monocytes; and 4) monocyte chemo-attractant protein-1 (MCP-1) attracting circulating monocytes [40]. Besides pro-inflammatory effects, IL-1ß induces the proliferation and migration of VSMCs [41–43], raises the susceptibility of macrophages to lipid deposition, boosting foam cell formation [44], and increases the expression of MMPs [45,46] leading to collagen degradation and promoting plaque instability (Fig. 1).

3. Role of innate immune cells in atherogenesis

3.1. Neutrophils

Neutrophils are the first responders following tissue damage or infection and release pro-inflammatory mediators to neutralize the danger [47]. If the mission is successful, the inflammation is resolved. However, in case of a persistent inflammatory trigger, the neutrophils continue to accumulate and secrete neutrophil granules filled with pro-inflammatory proteins like myeloperoxidase (MPO), lactoferrin, proteinase-3, MMP-9, defensin, azurocidin, cysteine-rich secretory protein 3 (CRISP3), gelatinase and ficolin 1, inflicting tissue damage and chronic inflammation [48]. Moreover, neutrophils have the ability to perform NETosis, which represents a process of expelling of cytosolic and nuclear material that traps extracellular microbes in neutrophil extracellular traps (NETs), which are web-like structures containing DNA, histones, neutrophil elastase and MPO [48]. Initially, neutrophils have rarely been detected in atherosclerotic lesions probably due to their limited lifespan, phenotypic plasticity [49] and rapid clearance by macrophages, resulting in underestimation of their role. However, gradually substantial evidence has emerged showing hyperlipidemia-associated neutrophilia and neutrophils-mediated atherogenesis [50–52] and plaque destabilization [53]. Also a direct correlation between neutrophil blood count and the severity of the coronary damage in patients with coronary artery disease has recently been demonstrated [54].