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Review

Atherosclerosis – A matter of unresolved inflammation

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ABSTRACT

Atherosclerosis is commonly looked upon as a chronic inflammatory disease of the arterial wall arising from an unbalanced lipid metabolism and a maladaptive inflammatory response. However, atherosclerosis is not merely an inflammation of the vessel wall. In fact, the cardinal signs of unstable atherosclerotic lesions are primarily characteristics of failed resolution of a chronic inflammation. In contrast to acute inflammatory events which are typically self-limiting, atherosclerosis is an unresolved inflammatory condition, lacking the switch from the pro-inflammatory to the pro-resolving phase, the latter characterized by termination of inflammatory cell recruitment, removal of inflammatory cells from the site of inflammation by apoptosis and dead cell clearance, reprogramming of macrophages toward an anti-inflammatory, regenerative phenotype, and finally egress of effector cells and tissue regeneration. Here we present an overview on mechanisms of failed resolution contributing to atheroprogression and deliver a summary of novel therapeutic strategies to restore resolution in inflamed arteries.

1. Atherosclerosis – continued inflammation and failed resolution

Atherosclerosis is a complex, progressive disorder affecting large and medium-sized arteries. Therapeutically, major concerns arise from the silent progression of this worldwide malady, often with no clinical evidence until occurrence of ischemic damage due to thrombosis or severe stenosis. Intrinsically arteriosclerotic vascular disease is an inflammatory condition characterized by aberrant lipid metabolism and a maladaptive inflammatory response. Classically, arterial inflammation is triggered by an insult to the endothelium, often at arterial branch points or at areas experiencing disturbed flow, ultimately leading to endothelial cell activation and recruitment of inflammatory cells to the vessel wall. At the site of endothelial activation structural alterations, in particular the exposure of proteoglycans, facilitate the retention of low-density lipoprotein (LDL) particles in the intima [1,2], where they are susceptible to oxidative modification by reactive oxygen species (ROS) and enzymes released from inflammatory cells. As macrophages progressively take up modified lipoproteins they give rise to foam cells. The continuous intracellular accumulation of lipids (including cholesterol, oxysterols and other fatty acids) induces endoplasmic reticulum stress triggering foam cell apoptosis [3]. However, aberrations in foam cells have been described, such as the deficiency of pro-apoptotic factors (e.g. Bax and p53), that prevent cell apoptosis contributing to atherosclerosis progression [4,5]. In advanced atherosclerosis the sources of apoptotic cells sturdily overwhelm the efferocytic program. Such defective efferocytosis allows apoptotic cells to undergo secondary necrosis, thereby feeding the necrotic core and a constant flow of pro-inflammatory mediators that override existing pro-resolution signals. This highly inflamed and necrotic core is central to the atherosclerotic plaque – vulnerable to structural disruption and an immediate precursor of acute cardiovascular clinical events.

In contrast to acute inflammatory events which are typically self-limiting, atherosclerosis is an unresolved inflammatory condition, lacking the switch from pro-inflammatory to anti-inflammatory mediators that characterizes the resolution phase. The resolution phase of inflammation embraces termination of inflammatory cell recruitment, removal of inflammatory cells from the site of inflammation by apoptosis and dead cell clearance, reprogramming of macrophages toward an anti-inflammatory, regenerative phenotype, and finally egress of effector cells and tissue regeneration [6]. Understanding the different aspects of failed resolution in atherosclerosis provides the opportunity to identify alternative therapeutic targets, theoretically with minimal side-effects. Thus, this review will concentrate on mechanisms of failed resolution in atherosclerosis, specifically on (1) continued

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leukocyte accumulation (as result of continued recruitment, proliferation, and failed egress), (2) unbalanced M1/M2 macrophage polarization, and (3) impaired efferocytosis. Finally, we will outline possible therapeutic ideas, many of which stem from preclinical studies of acute inflammatory models.

2. Leukocytes gradually accumulate in atherosclerotic lesions

Monocyte-derived cells are the most abundant leukocyte subset in the atherosclerotic plaque. The pertinent role of monocytes in atherosclerosis was clearly evidenced when depletion of these cells from the circulation was shown to drastically reduce plaque formation [7,8]. However, depletion of monocytes at later atherosclerotic stages did not have any effect on the accumulation of macrophages within the lesion and also not in plaque composition or necrotic core formation [9], underscoring the importance of continued recruitment of these cells to the initiation of the disease. Consistent with this notion, continued monocyte recruitment is a hallmark during atherosclerosis progression and regression of atherosclerotic lesions is primarily driven by halted recruitment of monocytes [10]. Besides monocytes, also the presence of neutrophils in the atherosclerotic plaque has been reported, and a causal contribution of neutrophils during various stages of atherosclerosis has been established [11,12]. Continued leukocyte accumulation in the lesion site feeds an inflammatory milieu and prevents a turnover toward resolution of inflammation.

Several processes contribute to the progressive accumulation of leukocytes in the atherosclerotic plaque, the most obvious, and *supra* mentioned, being leukocyte recruitment [7,10,13,14]. However macrophage survival [15] and proliferation [16,17] in the plaque as well as the limited ability of leukocytes to leave atherosclerotic lesions [18] represent important processes critically controlling the number of macrophages within atherosclerotic lesions (Fig. 1a).

2.1. Mechanisms of continued leukocyte recruitment

The classical cascade of leukocyte recruitment includes leukocyte rolling, activation, arrest and migration. Capture and rolling are mediated by selectins and the P-selectin glycoprotein ligand-1 (PSGL1) receptor, whereas leukocyte arrest is led by chemokine-activated integrins mainly lymphocyte function-associated antigene 1, LFA1, and very late antigene 4, VLA4,

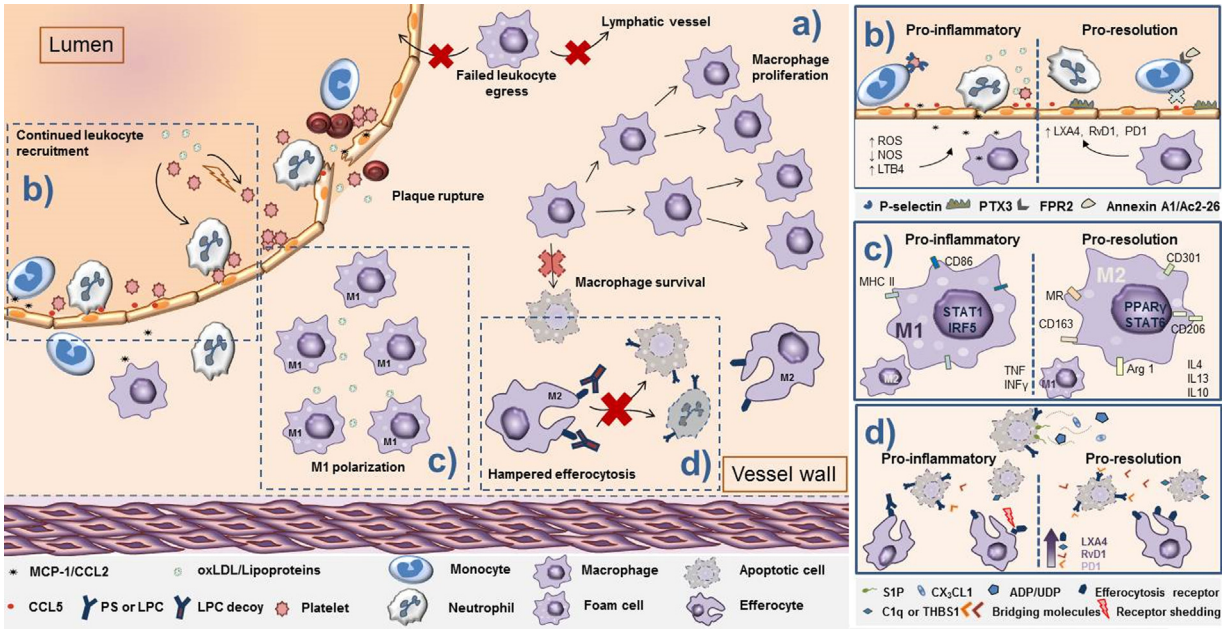

Fig. 1. Mechanisms of failed resolution in atherosclerosis. (a) Perpetuated leukocyte recruitment is one of the hallmarks of atherosclerosis, and it contributes to adjourning resolution of inflammation. In the vessel wall other mechanisms take place that aid to putting off resolution, namely: proliferation of resident macrophages, macrophage survival, failed egress of abundant monocyte-derived cells, and M1 favored polarization. Macrophages partial escape of apoptotic mechanisms is supported by aberrant gene expression, whereupon survival and tumor suppressor genes are up and down-regulated, respectively. At later stages of disease, efferocytosis (the process by which apoptotic cells are cleared) is hampered, and later on, when plaque rupture occurs, platelet aggregates are frequent and the close contact with oxLDL from the plaque is more likely to occur, aggravating the already ongoing leukocyte recruitment. (b) Under hypercholesterolemia, increased LDL and oxLDL activate platelets upon contact contributing to leukocyte recruitment via platelet chemokine (CCL5) deposition on the endothelium and the formation of aggregates (neutrophil–platelet or platelet–platelet aggregates). Platelet derived P-selectin also plays a major role, mediating the delivery of pro-inflammatory molecules to the endothelium as well as to circulating monocytes. Furthermore, the environment characterized by low availability of nitric oxide (NOS) and bountiful reactive oxygen species (ROS) favors the synthesis of leukotriene B4 (LTB4) and CCL2 secreted by endothelial cells as well as macrophages. Contrarily, pro-resolution mediators, such as lipoxin A4 (LXA4), resolving D1 (RvD1) or Protectin D1 (PD) stop the release of cytokines and leukocyte recruitment. Also pentraxin 3 (PTX3) is known as an endogenous inhibitor of engagement of neutrophils, whereas Annexin A1 acts on monocyte-derived cells via formyl peptide receptor 2 (FPR2). (c) Under inflammatory conditions, stimuli such as tumor necrosis factor (TNF) and interferon gamma (INFγ) predominate, fueling a M1 macrophage polarization. M1 macrophages express high levels of CD86 receptor as well as MHC II, and act via the transcription factors STAT1 and IRF5. Compared to M2 macrophages, M1 macrophages also possess a higher capacity to process lipids. In a pro-resolution environment, cytokines such as IL-4, IL-13 and IL-10 favor an M2 phenotype, where high PPARγ and STAT6 activity result in increased capacity of dead cell clearance. (d) Clearance of dead cells is required for many processes, including resolution of inflammation. Apoptotic cells release "find me" and "eat me" signals in order to be cleared. "Find me" signals such as sphingosine 1 phosphate (S1P), lysophosphatidylcholine 8LPC), CX3CL1 or the nucleotides adenosine or uridine-5′-triphosphate (ATP and UTP) encourage macrophages migration, whereas "eat me" signals are responsible for the engulfment process. At later stages of atherosclerosis this action is hampered. Possible contributions are attributed to lysophosphatidylcholine (LPC) decoys (oxLDL-derived LPC) that deviate efferocytic cells from their apoptotic targets by keeping the cell receptors occupied, or to low expression of bridging molecules (such as lactadherin or Gas6) as well as receptors (Mer tyrosine kinase, MERTK, or CD36 for example) directly enrolled in the phagocytic process. Alternative explanations relate to shedding of receptors directly enrolled in efferocytosis or decreased availability of bridging molecules or other similar mediators, such as complement 1q (C1q) or thrombospondin-1 (THBS1), which can bind to apoptotic cells and increase their clearance. Overall, the lesion milieu is mainly pro-inflammatory favoring an M1 polarization phenotype of macrophages and creating an imbalanced M1/M2 ratio that delays resolution of inflammation.