Inflammation and its resolution as determinants of acute coronary syndromes

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Abstract

Inflammation contributes to many of the characteristics of plaques implicated in the pathogenesis of acute coronary syndromes (ACS). Moreover, inflammatory pathways not only regulate properties of plaques that precipitate ACS but also modulate the clinical consequences of the thrombotic complications of atherosclerosis. This synthesis will provide an update on the fundamental mechanisms of inflammatory responses that govern ACS, and also highlight the ongoing balance between pro-inflammatory mechanisms and endogenous pathways that can promote the resolution of inflammation. An appreciation of the countervailing mechanisms that modulate inflammation in relation to ACS enriches our fundamental understanding of the pathophysiology of this important manifestation of atherosclerosis. In addition, these insights furnish glimpses into potential novel therapeutic interventions to forestall this ultimate complication of the disease.

Introduction

Inflammation contributes to many of the characteristics of plaques implicated in the pathogenesis of acute coronary syndromes (ACS) (Table 1).[1] Two mechanisms precipitate most ACS: a rupture of the plaque's fibrous cap and a superficial erosion of the intima. Fibrous cap fracture causes most fatal acute myocardial infarctions (MIs). The pathways by which inflammation promote fibrous cap rupture indisputably involve inflammation. Superficial erosion has a less clear relationship with inflammation. Therefore this review will focus on plaque rupture. Falk and Virmani have reviewed characteristics of plaques that cause ACS in their contribution to this series. [2] These features (Table 1) include a thin fibrous cap, a multiplicity of macrophages, a large lipid (necrotic) core, spotty calcification, and expansive remodeling. Substantial evidence supports the participation of inflammatory pathways in each of these characteristics associated with plaques that have caused ACS.

Inflammation also influences the consequences of a given plaque disruption. While the "solid state" of the atheroma itself determines the propensity to rupture, the "fluid phase" of

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blood can determine whether a plaque disruption results in a limited mural thrombus that would evade clinical detection or give rise to a sustained occlusive thrombus that could lead to an ST segment elevation MI (Figure 1)

Maintenance of homeostasis in health and limiting excessive inflammation responses in disease requires countervailing mechanisms. This review will illustrate how inflammation drives the properties of plaques and blood that predispose to ACS, how endogenous anti-inflammatory and resolution measures mitigate over exuberant inflammatory responses, and various therapeutic opportunities afforded by the inflammatory aspects of ACS.

Inflammation Drives ACS

Thin fibrous cap

Plaques that have ruptured and caused fatal acute MI generally have thin fibrous caps, measured in many studies at around 60–70 microns.[3] Recent studies with optical coherence tomography (OCT), which provides splendid near field high-resolution views of the intima, have corroborated in humans in vivo the observations of pathologists on autopsy specimens implicating thin fibrous caps in plaque disruption.[4] Inflammation decisively governs the metabolism of collagen, a major constituent of the fibrous cap that confers upon it much of its tensile strength. As recently reviewed, inflammatory signals such as the T_{H1} cytokine gamma interferon impair the ability of the smooth muscle cell (SMC), the source of most arterial interstitial collagen, to synthesize new collagen required to repair and maintain the extracellular matrix of the fibrous cap.[1] Considerable biochemical and experimental data support the role of matrix-degrading proteinases, tightly regulated by inflammatory mediators, as contributors to dissolution of interstitial collagen that thins and weakens the fibrous cap and hence renders a plaque susceptible to rupture.[5] Death of SMCs in the inflamed atheroma can deplete the plaque of the very cells that synthesize collagen required to form and maintain the fibrous cap.[6, 7]

Inflammatory cell recruitment

Observations on human atheroma specimens, in vitro studies, and animal experiments have elucidated pathways by which leukocytes from blood adhere to the endothelial cells (ECs) at sites predilected to atheroma formation, enter the intima, and undergo activation to sustain and amplify inflammation locally (Figure 2). Many recent reviews have highlighted the role of pro-inflammatory cytokines as inducers of endothelial-leukocyte adhesion molecules that capture blood leukocytes, most prominently the blood monocyte.[8] A series of chemokines that interact with cognate receptors on various classes of leukocytes cause their directed migration to penetrate into the intima. [8, 9] Recent refinements of our understanding of leukocyte accumulation in plaques include an expanded understanding of monocyte/macrophage heterogeneity.[10] In particular, in hypercholesterolemic mice, a pro-inflammatory subset of monocytes marked by high levels of expression of the surface marker Ly6c enter plaques early in development of experimental atherosclerotic lesions.[11, 12] A preformed pool of pro-inflammatory monocytes in the spleen provides many of these pro-inflammatory monocytes that populate early plaques.[13] The Ly6c high monocyte subpopulation uses CCL2/CCR2 or CXCL1/CXCR2 as a major chemokine-

receptor dyad related to their recruitment. The less inflammatory subpopulation of monocytes containing low levels of Ly6c may favor fractalkine (CX3CL1) and its receptor (CX3CR1) and CCR5 as trafficking mechanisms.[14] The Ly6c low population may arise from Ly6c high monocytes, a transition mediated by the transcription factor Nr4a1 (Nur77.) [15] Mononuclear phagocytes can proliferate in plaques.[16] The macrophage scavenger receptor A, known to bind modified lipoproteins, may contribute to mediating this process.[17] Macrophage colony stimulating factor (M-CSF/CSF-1) participates in the maturation of monocytes to macrophages, induces the expression of scavenger receptors that permit accumulation of modified lipoproteins within the phagocyte, fostering the formation of foam cells, the hallmark of the atherosclerotic lesion.[18] While less apparent morphologically, but functionally pivotal, other leukocyte populations reside in plaques including various classes of T lymphocytes, B lymphocytes, mast cells, and dendritic cells (DCs).[9, 19, 20] A recently recognized population of innate response activator B cells can produce granulocyte-macrophage colony stimulating factor (GM-CSF/CSF-2) in the spleen of hypercholesterolemic mice where it can activate dendritic cells which in turn promote T_{H1} cells capable of producing gamma-interferon that can migrate to atheroma and activate plaque macrophages.[21]

Most depictions of leukocyte recruitment to plaques portray this process as taking place at the macrovascular luminal surface overlying the lesion. While this locale likely applies to the nascent lesion, once established, atheromata develop microvasculature.[22] The plexi of plaque microvessels provide an even more abundant surface for leukocyte trafficking. Indeed, a key adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1) localizes to the micro- rather than macrovascuar endothelium in human lesions. [23] Plaque hypoxia and expression of angiogenic growth factors elaborated by inflammatory cells drive this neovascularization in plaques. [9, 22]

Large lipid pools and "necrotic" core (see also[24]

The Rosenson et al. contribution to this series discusses lipid metabolism and targets in the context of ACS.[24] Therefore, we focus here on inflammatory cell death by apoptosis or oncosis and defects in the clearance of dead cells, a process known as efferocytosis (Figure 2).

Large necrotic cores characterize plaques that cause ACS (Table 1). Plaque inflammation can promote necrotic core formation, and, in turn, plaque necrosis can worsen inflammation in advanced atheromata.[25, 26] Necrotic cores contain dead cells and their detritus, and because most of the dead cells consist of foamy macrophages, lipid debris abounds. Necrotic cores likely contribute to plaque rupture and ACS as they possess inflammatory, proteolytic, and thrombogenic properties.[27-29] Moreover, the material properties of the lipid-rich necrotic core places biomechanical stress on the overlying fibrous cap and thereby contributes to cap rupture.[30]

The dead cells that accumulate in necrotic cores likely have two origins: secondary cell death due to the combination of apoptosis and defective clearance of the apoptotic cells (efferocytosis); and primary cellular necrosis, also known as "necroptosis" or "oncosis." Macrophages have relatively long lives, but turnover of intimal macrophages occurs at all

stages of atherosclerosis. In early-stage lesions, efficient efferocytosis by neighboring phagocytes, notably macrophages, limits secondary macrophage necrosis. [31][26] As lesions progress, however, intimal cell apoptosis increases for a number of reasons, including inflammatory stimuli and factors that promote oxidative and endoplasmic reticulum (ER) stress. These stressors trigger mitochondrial pathways of apoptosis and likely work in concert with other plaque factors that activate toll-like receptors (TLRs), death receptors, and additional mitochondrial apoptotic pathways.

As plaques advance, efferocytosis begins to fail promoting secondary necrosis of the apoptotic cells and loss of efferocytosis-mediated anti-inflammatory signaling. The cytoplasmic tails of efferocytosis receptors usually mediate these anti-inflammatory pathways and result in the production of anti-inflammatory/pro-resolving IL-10 and transforming growth factor beta (TGF-β).[32] The release of damage-associated molecular patterns (DAMPs) from necrosing cells likely further amplifies plaque inflammation. DAMPs trigger inflammatory pathways in macrophages by activating innate immune receptors, including TLRs, and DAMPs likely promote plaque progression toward those prone to provoke ACS.

Many cell types can carry out efferocytosis, but dedicated phagocytes like macrophages and DCs do so most efficiently. The process involves recognition of particular features on apoptotic cells, notably cell-surface phosphatidylserine (PS), by efferocyte receptors that bind PS directly or through a bridging molecule. Although observations in mice with advanced atherosclerosis have identified macrophage efferocytosis receptors relevant to necrotic core formation, the mechanisms of defective efferocytosis in advanced atheromata are not known. In theory, the problem could reside with the efferocytes themselves (e.g., receptor or internalization defects), or with an apoptotic cell-related property that decreases their recognition by efferocytes. As discussed below, failed efferocytosis characterizes impaired resolution of the inflammatory response.

Primary necrosis refers to a pathway of cell death that is morphologically and biochemically distinct from apoptosis.[33] In contrast to apoptosis, membrane leakiness occurs early in primary necrosis, chromosome condensation does not occur. Activation of a family of kinases called receptor-interacting protein (RIP) triggers a particular programmed form of necrosis, called necroptosis. Deficiency of one of the RIP kinases, RIP3, in myeloid-derived cells in both *Ldlr-/-* and *Apoe-/-* mice leads to a decrease in necrotic macrophages in advanced atherosclerotic lesions, but no observable changes in early atherosclerosis. The trigger to necrosis in atheromata remains unknown, but RIP3-dependent cell death can occur in cultured macrophages exposed to oxidized LDL together with a caspase inhibitor.[34] Phagocytes have specific mechanisms for the clearance of necrotic cells, so the presistence of dead cells in advanced atheroma may indicate a defect in this subtype of efferocytosis.

As alluded to above, numerous studies using *Ldlr-/-* and *Apoe-/-* mice that consume an atherogenic diet support the overall concepts related to intimal cell death, efferocytosis, inflammation, and plaque necrosis as a function of lesion stage, and the underlying molecular-cellular mechanisms. Although these experiments do not recapitulate the processes of plaque rupture and thrombosis, ACS in humans associate with coronary arterial

lesional ER stress, intimal cell apoptosis, and the features associated with plaque instability (Table 1). Moreover, advanced human coronary artery lesions show signs of defective efferocytosis.[35]

Spotty calcification characterizes plaques associated with ACS

Traditional thinking has regarded calcification of atherosclerotic plaques as a passive "degenerative" process. In contrast, contemporary concepts view calcification as a dynamic active process critically dependent on inflammatory cells and signaling. Mice deficient in M-CSF, an activator of macrophages, accumulate calcium in atheromata, likely due to impaired osteoclastic activity of the mononuclear phagocytes.[36] Molecular imaging studies colocalized niduses of inflammation with early mineralization in mouse atheromata.[37] Inflammatory mediators also instruct SMCs to alter functions related to the formation of calcifying foci.

Large plates of calcium mineral may influence the stability of plaques. Recent work using computational methodology has shown that pinpoint calcification can introduce biomechanical inhomogeneities implicated in precipitation of plaque rupture.[38] Nuclear imaging studies using NaF provides a quantifiable index related to calcification in carotid and coronary artery plaque that correlates with Framingham risk. [39, 40] Imaging studies using computed tomographic (CT) approaches have associated spotty calcification with propensity of plaques to provoke ACS.[41, 42] The implication of inflammation in mechanisms related to cardiovascular calcification provides a novel link to ACS pathogenesis.

Outward remodeling

The observations of Clarkson in non-human primates and of Glagov in human atherosclerotic plaques pointed to the role of expansive remodeling or compensatory enlargement during atherogenesis.[43, 44] Imaging studies using intravascular ultrasound and computed tomography have associated expansive remodeling with risk of plaques to precipitate ACS.[45] Outward remodeling of arteries necessitates degradation of the extracellular matrix (including collagen) and of elastin, the major protein of the elastic laminae of arteries. Just as inflammatory mediators influence the expression of proteinases involved in collagenolysis in thinning of the fibrous cap, similar regulation of elastases and other matrix-degrading enzymes by inflammatory mediators can control expansive remodeling.[46] In atherosclerosis-susceptible mice, genetically produced alterations in signaling by the key inflammatory mediator interleukin-1 (IL-1) leads to a failure of expansive remodeling during atherogenesis.[47] In particular, reduced induction of the matrix metalloproteinase MMP-3 due to lack of IL-1 signaling appears to contribute causally to this defective remodeling.[47] Thus, inflammatory pathways also govern this characteristic of plaques that provoke ACS.

Thrombogenicity

As indicated above, the consequences of plaque disruption depend on the balance between pro-coagulant and anti-coagulant and pro-fibrinolytic and anti-fibrinolytic factors both in the solid state of plaque and fluid phase of blood (Figure 1).[48] Within the plaque, a subpopulation of mononuclear phagocytes and activated SMCs overexpress the potent pro-

coagulant tissue factor in response to inflammatory stimuli, notably CD40 ligand (CD154).[49-51] The normal intima expresses a number of functions that combat thrombus accumulation.[52] ECs normally express thrombomodulin and endogenous fibrinolytic mediators urokinase type and tissue type plasminogen activator. The normal intima also contains heparan sulfate proteoglycans that have anti-coagulant properties. The balance between these anti-thrombotic and pro-fibrinolytic mechanisms shifts in response to inflammatory mediators. The local production of plasminogen activator inhibitor-1 (PAI-1), a major blocker of fibrinolysis, rises in SMCs and ECs exposed to pro-inflammatory stimuli. Thus, the solid state of the plaque augments thrombogenicity and resists fibrinolysis in response to inflammatory activation.

Likewise, the fluid phase of blood promotes clot accumulation in response to systemic inflammation. The hepatocyte augments productions of acute phase reactants in the presence of systemic inflammation, notably interleukin-6 (IL-6), a pro-inflammatory cytokine strongly implicated in the pathogenesis of ACS by recent genetic studies.[53] Among the acute phase reactants released by the liver in response to IL-6, fibrinogen participates directly in clot formation. The hepatocyte also increases production of PAI-1 when exposed to IL-6. Platelets participate pivotally in the formation of arterial thrombi, and furnish an endogenous source of preformed mediators that amplify and sustain local inflammatory responses.[54] Once plaques have disrupted, neutrophils also enhance local inflammation and oxidative stress, including through the formation of neutrophil extracellular traps (NETs).[55] Hence, in response to inflammation, both the solid state of plaque and the fluid phase of blood conspire to promote thrombus accumulation by increased thrombogenicity, decreases anti-coagulant properties, and impaired fibrinolytic capacity.

Pharmacologic anti-inflammatory interventions

Statin drugs have transformed cardiovascular prevention in the last decades. Beyond their LDL-lowering effects, statins have direct anti-inflammatory actions mediated by inhibition of prenylation of small G-proteins and induction of transcription factors such as Krüppel-like factor-2 (KLF-2) that alter inflammatory pathways in a concerted manner.[56] Thus, the statins likely reduce ACS risk in part by these, and perhaps other, anti-inflammatory properties. Among anti-inflammatory strategies, colchicine has recently demonstrated promising reductions in cardiovascular events, potentially through inhibiting the inflammasome pathway in macrophages.[57, 58] Other direct anti-inflammatory interventions that do not affect LDL are currently under investigation.[58, 59]

The endogenous resolution response and its defect in advanced atherosclerosis

The acute inflammatory response in host defenses unleashes oxidative and protease-mediated tissue injury that can lead to collateral damage. The inflammatory response normally also involves a resolution phase that, after attacking the acute injury or infection, quells inflammation and restores tissue homeostasis.[60, 61] Multiple lipid-derived and protein mediators (Table 2) promote resolution by blocking inflammatory cell influx and promoting their egress; clearing pathogens, cellular debris, inflammatory cytokines, and dead cells (efferocytosis); and repairing tissue damage.[62, 63] The molecules that mediate the resolution response include specialized pro-resolving mediators (SPMs), endogenous lipids generated

actively during inflammation.[62] SPM families include lipoxins, resolvins, protectins, and maresins. Lipoxins derive from omega-6 arachidonic acid, while resolvins, protectins, and maresins arise from omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Lipoxygenase (LOX)- and cyclooxygenase (COX)-initiated mechanisms biosynthesize SPMs. SPMs each possess distinct structures that bind and activate select cell-surface receptors.

In chronic diseases, persistence of the irritative stimulus, such as intimal lipids in atherosclerosis, can mute the resolution phase, leading to a cycle of persistent tissue injury, which generates DAMPs that propagate inflammation. Impaired resolution of inflammation can contribute to many of the features of plaques associated with ACS, including persistent influx and defective egress of myeloid cells; accumulation of DAMPs; secondary necrosis of intimal cells due to defective efferocytosis, augmenting necrotic core formation; and degradation of the extracellular matrix.

Mechanisms of SPM dysregulation relevant to advanced atherosclerosis and ACS

In addition to a persistent inflammatory stimulus, such as retained subendothelial apoB lipoproteins, additional factors may hamper resolution inflammation in advanced atherosclerosis. LC-MS-MS profiling in other chronic inflammatory conditions, such as cystic fibrosis, asthma, and localized aggressive periodontitis has revealed decreased SPMs compared with healthy controls.[64-66] Moreover, lower plasma levels of a specific SPM called aspirin-triggered LXA_4 (ATL) associate with increased risk for peripheral and coronary atherosclerosis in humans, even after correcting for age, sex, and C-reactive protein levels.[67] Unraveling the mechanism(s) by which SPM concentrations decrease in chronic inflammatory diseases and whether this drop also applies to advanced atherosclerosis will require further studies. Possibilities include insufficient substrate availability (i.e., lack omega-3 fatty acids in the diet); dysregulated signaling, perhaps due to suppression of SPM receptors; impaired biosynthetic capacity, such as decreased lipoxygenase expression and/or function; and/or hyperactive catabolism of these SPMs.

Observational studies show that populations that have a high intake of foods containing omega-3 compared with omega-6 fatty acids have better cardiovascular outcomes.[68] Yet, studies evaluating omega-3 fish oil supplementation in humans have shown variable results. This discrepancy may result from differences in study endpoints (e.g., incident atherosclerotic disease versus coronary death), whether subjects entered the studies already having sufficient omega-3 fatty acid intake, a lack of uniformity and quality of the fish oils being tested, and/or to the difficulty of showing beneficial effects on subjects already receiving statins.[69] In mice, dietary omega-3 supplementation attenuates atherogenesis,[70-72] and high-dose EPA causes lesion regression.[73] Genetically-mediated increase of the n-3/n-6 ratio in mice also yields less atherosclerosis.[74] Prostaglandin E2, via the macrophage EP4 receptor, can also mitigate vascular inflammation.[75]

Overexpression of a key SPM biosynthetic enzyme, 12/15-LOX, in chowfed *Apoe-/-* mice limited atherosclerosis, providing support for a role of endogenous biosynthesis of SPMs,[76] but mice consuming a high-fat diet did not show this protection.[77] Consistent with the concept that a high-fat diet impairs resolution, diet-induced obesity associates with defective

inflammation resolution.[78] Although the mechanisms underlying this experimental observation remain speculative, the results suggest that diet may modulate ACS risk in part by effects on inflammation resolution.

The therapeutic potential of SPMs in ACS: inflammation suppression without compromise of host defense

As impaired inflammation resolution may aggravate atherosclerosis and ACS risk, new approaches to stimulate resolution have considerable interest. SPMs limit inflammation without causing immunosuppression, setting them apart from many other current anti-inflammatory strategies.[79] In this context, SPMs can enhance efferocytosis.[62] Obese atherosclerotic mice that consumed an omega-3 fish oil diet containing the SPM precursors EPA and DHA had amelioration of impaired efferocytosis.[80] Conversion of EPA and DHA to SPMs within the vasculature might contribute to this benefit. Likewise, the resolvins RvD1 and RvD2 may benefit injured atherosclerotic arteries.[81] Indeed, vascular injury in vivo augments D-series resolvin generation, and therapeutic administration of resolvins decreases intimal hyperplasia and leukocyte trafficking to injured arteries. These resolvins block migration, proliferation, monocyte adhesion, and inflammatory signaling in human primary vascular SMCs. Thus, resolution agonists merit consideration as therapeutic targets to reduce ACS risk.

Potential resolution-enhancing effects of low-dose aspirin in relation to protection against ACS

Low-dose aspirin (acetylsalicylic acid [ASA]) prevents recurrent ACS, a benefit traditionally ascribed to anti-platelet action mediated by COX inhibition.[82] Yet, aspirin has anti-inflammatory actions in humans, such as blocking leukocyte trafficking to inflamed tissues, that are difficult to attribute solely to reduced prostanoid biosynthesis.[83] Indeed, aspirin alters the active site of COX-2 in a manner that permits conversion of arachadonic acid (AA) to 15R-HETE in vascular ECs, a precursor for leukocyte production of pro-resolving 15-epilipoxins.[84] Humans taking low-dose aspirin form these lipoxins, which limit neutrophil infiltration into inflammatory sites and stimulate nitric oxide (NO) production. COX-2 acetylated by ASA can also process EPA and DHA to boost generation of other resolvins.[85, 86] Thus, non-prostanoid-related, resolution-mediating effects of low-dose aspirin may contribute to their ability to protect against ACS.

Adaptive immune modulation can also mute inflammation

Some functions of both innate and adaptive immune cells can also counterbalance pro-inflammatory pathways implicated in ACS and mitigate inflammation (Figure 1). For example, Ly6c low monocytes and alternatively activated macrophages (denoted "M2") can modulate inflammation. While B2 lymphocytes appear to aggravate atherosclerosis, B1 lymphocytes elaborate natural antibodies that can quell atherogenesis.[87] T_{H2} lymphocytes can elaborate interleukin-4 and interleukin-10 that may limit inflammation and sway macrophages towards M2 polarization. Regulatory T cells, by elaborating TGF-β, can balance the pro-inflammatory cascade unleashed by T_{H1} cells and thus modulate inflammatory responses in the atheroma.[9, 88]

Innate immune modulation in atherosclerosis and its relevance to ACS

The innate immune system represents a defense system against pathogens — a "first responder" mechanism that can mobilize rapidly against threats and without prior exposure to the provocateur. The innate immune system also responds to tissue injury to initiate response and repair processes. Innate immune activation participates centrally in the pathogenesis of atherosclerosis. Dysregulated lipid metabolism, particularly an abundance of apolipoprotein B-containing lipoproteins and their retention in the arterial wall, contributes to the development of macrophage foam cells.[89] Such aberrations and the products of modified or native lipoproteins that accumulate in atherosclerotic plaques can trigger pattern recognition receptors (PRRs) expressed by macrophages, including the NOD-like receptors (NLRs), scavenger receptors, and TLRs, thereby activating the inflammatory response. [90, 91]

NLRs and atherosclerosis

Atherosclerotic plaques contain cholesterol crystals, both in extracellular spaces and within plaque macrophages. Although previously considered a feature of advanced plaques, a recent study showed the presence of cholesterol crystals in early lesions in *Apoe*-/- mice.[92] Macrophages can engulf these crystals, resulting in the induction and activation of the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome. This intracellular complex processes IL-1β to its active form — the target of an ongoing clinical trial (CANTOS) to prevent recurrent ACS.[93] In addition to preformed cholesterol crystals, recent work indicates that loading of macrophages with cholesterol can lead to de novo formation of intracellular cholesterol crystals that trigger NLRP3 in a process mediated in part by CD36.[94]

Although not yet investigated, other crystalline or amyloid substances in atherosclerotic plaques, such as calcium phosphate crystals or serum amyloid A, may also represent DAMPS that could trigger the inflammasome and IL-1β secretion. In addition to specifically inhibiting IL-1β, blocking of inflammasome activation might offer another strategy to prevent ACS. That colchicine decreases the prevalence of ACS in gout patients, and the recent LoDoCo intervention trial in patients without gout but having CAD, affirm the potential of this approach.[57]

TLRs and atherosclerosis

The role of TLR signaling pathways in promoting atherosclerosis received support from mouse studies in which whole-body deletion of *Tlr2* or *Tlr4* or of the adaptor proteins used by these TLRs — including IL-1 receptor-associated kinase 4 (IRAK4),[79, 80] TNF receptor-associated factor 6 (TRAF6),[81] TIR-domain–containing adaptor protein inducing IFN-β (TRIF; also known as TICAM-1) and myeloid differentiation primary-response protein 88 (MYD88) — confers protection from atherosclerosis.[95-103] These findings have initiated investigations of the endogenous ligands that accumulate during hypercholesterolemia and in plaques that may trigger these microbial-sensing pathways in macrophages. Among the candidates proposed, oxidized LDL (oxLDL) species have undergone extensive study,[104] but numerous factors and pathways may contribute to the initiation and the maintenance of TLR-induced macrophage inflammation in atherosclerotic plaques. Nonetheless, oxLDL has remained firmly in the sights of investigators as a major therapeutic target, with recent

efforts directed towards a vaccine strategy to reduce ACS risk by eliminating oxLDL as it is formed — before it can stimulate TLR-related inflammatory pathways in macrophages — among other potential benefits.[105]

Macrophage Polarization in the Plaque

Histological analysis of mouse and human plaques has shown the presence of both M1 and M2 macrophages. For example, in human plaques, M1 macrophages localize to areas distinct from those in which the less inflammatory M2 macrophages ("alternatively activated" macrophages) situate.[106] Studies of M1 and M2 macrophages polarized *in vitro* by, for example, IFN-γ or IL-4, respectively, and in atherosclerotic mice have led to the simplified view that M1 macrophages promote while M2 macrophages limit plaque inflammation.[107] This construct caricatures the complex range of macrophage functions *in vivo*, as macrophages encounter microenvironments of diverse, and even opposing, signals. For example, in addition to inducing the aforementioned TLR signaling, which can lead to M1 polarization, oxidized LDL can also induce the expression of the M2 macrophage phenotypic marker arginase 1 via the activation of peroxisome proliferator activated receptor-γ (PPARγ).[108]

The full range of influences in the plaque microenvironment that promote the polarization of macrophages *in vivo* in general, and in plaques in particular, remain incompletely defined, but undoubtedly include interactions with the adaptive immune system; e.g., T helper 1 (TH1) and TH2 cells, important players in plaque inflammation as reviewed earlier, secrete potent macrophage-polarizing factors (e.g., IFN-γ and IL-4, respectively; Figure 1).[107] Although the spectrum of macrophage functions *in vivo*, particularly in humans, likely exceeds that typically described *in vitro*,[109] the M1/M2 classification still provides a useful framework, albeit oversimplified. Considerable data support its main point, that there exist subsets of macrophages with different properties, such as inflammatory and tissue repair. This concept has high relevance to the inflammatory state and its resolution in plaques of the type that cause ACS. For example, M1 macrophages secrete proatherosclerotic inflammatory cytokines (such as IL-6 and IL-12), as well as reactive oxygen and nitrogen species that would exacerbate oxidative stress in the plaque. M2 cells, on the other hand, secrete low levels of IL-6 and IL-12, but high levels of one of the endogenous anti-inflammatory cytokine, IL-10. M2 macrophages also exhibit scavenging of necrotic debris, enhanced efferocytotic activity, and reduced production of reactive nitrogen species, all considered to be pro-resolving processes as explained above.

Atherosclerotic mice display a dynamic balance between M1 and M2 macrophages in plaques. Advanced lesions have a high proportion of macrophages that express mainly markers of the M1 state. In various mouse models of regression (aortic transplantation, generic reversal of hyperlipidemia, anti-miR33 treatment, injection of apoAI, the HDL-forming protein) the balance between the macrophage polarization markers shifts toward the M2 panel of functions.[110-113] From a functional point of view, the properties of M2 macrophages align well with the macroscopic changes observed in regressing plaques — the loss of inflammatory cells and the remodeling of tissue to a morphology associated with less risk of rupture, such as a reduction in the necrotic core.[114]

That the M1/M2 state might undergo manipulation in human plaques as a preventive or therapeutic approach to ACS, as suggested by one (albeit small) clinical study in which patients who received HDL infusions prior to peripheral atherectomies showed a tendency for decreased inflammatory mediators in the plaques relative to those excised from the control group.[115] Experimental studies support this notion. Two recent studies in atherosclerotic mice in which the M2 state was induced and maintained by injection of either IL-13 (a strong polarizer in vitro, that in addition to IL-4 is secreted by TH1 lymphocytes) or helminth antigens showed reduced atherosclerosis progression and plaque inflammation.[116, 117]

In summary, the pre-clinical data in progressing and regressing plaques provide a considerable rationale for efforts to enrich plaques in M2 macrophages clinically to either prevent plaques from reaching an "ACS-prone state" or to promote rapid resolution of inflammation in the ACS setting. Based on the evolving literature, this enrichment might be accomplished, for example, by treatment with direct (small molecule) mediators of TH2-related pathways, indirect regulators of M2 polarization, such as HDL, or by plaque targeted nanoparticle-based delivery of agents, including siRNA, anti-sense RNA, and peptides.

Retention and clearance of plaque macrophages

As noted earlier, enrichment in macrophages characterizes plaques that have provoked fatal ACS (Table 1). Major kinetic processes that contribute to determining the content of plaque macrophages already reviewed include the recruitment of circulating monocytes, their local proliferation, cell death, and efferocytosis (Figure 2). An inkling that another process — that of emigration of macrophages from plaques — might also contribute is found in the 1980s studies of Ross Gerrity in atherosclerotic pigs[118] and of Russell Ross in monkeys,[119] in which electron micrographs showed images compatible with macrophage foam cells exiting between ECs into the arterial lumen. The availability of atherosclerotic mice coupled with methods to follow cell trafficking that are relatively convenient in mice led to a direct demonstration after aortic transplantation that during disease progression, emigration was quite low, but placement of plaques into a regression environment readily demonstrated macrophage exit.[111, 120] Other types of mouse experiments have also documented macrophage emigration from plaques during atherosclerosis regression, extending these initial observations.[90]

The capacity of macrophages to leave plaques appears to depend on at least two sub-pathways: one that regulates macrophage retention (chemostasis) and one that regulates macrophage locomotion (chemotaxis). For the former, recent work has shown that neuronal guidance molecules mediate much macrophage retention in plaques. Macrophages in human and mouse plaques express the best studied such guidance molecule, netrin-1, which inhibits the chemotactic responses of macrophages to a number of chemokines in vitro.[121] When *Ldlr-/-* mice received bone marrow transplants from netrin-1–deficient mice, compared to *Ldlr-/-* mice reconstituted with netrin-1–sufficient bone marrow, plaque progression slowed significantly. Cell trafficking assays showed increased macrophage emigration from the plaques in the mice lacking leukocyte netrin-1.[121] Other factors that inhibit cell movement, such as adhesion molecules (which are more highly expressed in macrophages in

progressing versus regressing plaques[122]), also likely promote the retention of macrophages, a process that would be expected to contribute to the macrophage enrichment that characterizes plaques with features associated with precipitating ACS.

The signals that guide macrophages to exit plaques, either by reverse transmigration through the endothelium to the lumen or by migrating through the media to the adventitial lymphatics, remain for the most part poorly defined. Data from studies of regression of atherosclerosis in aortic transplant studies have, however, implicated CCR7 in this process.[123] Emigrating CD68+ cells (predominately macrophages) had augmented expression of CCR7, the receptor for the chemokines CCL19 and CCL21. Furthermore, blocking this pathway led to substantial retention of these cells in the plaque.[123] The molecular basis for this augmented CCR7 appears to depend on the presence of a sterol response element (SRE) in the promoter of the mouse (and human) CCR7 gene.[124] Thus, in a regression environment, which reduces macrophage cholesterol content, CCR7 expression rises. Further experiments support the concept that the plaque content of macrophages depends on the balance between retention and chemotactic pathways. Netrin-1 (or another neuronal guidance molecule, semaphorin 3E) can block macrophage chemotaxis to CCL19 or CCL21, and in regressing plaques — coincident with CCR7 induction — netrin-1 and semaphorin 3 concentrations fall.[121, 125]

Manipulating retention or migration factors could be envisioned to have therapeutic applications to the prevention or treatment ACS by reducing the plaque content of inflammatory cells. The recent exciting demonstration that siRNA molecules that target monocyte/macrophage inflammation in vivo can be delivered in nanoparticles[126] could be adapted, for example, to similarly inhibit netrin-1 expression. Statin treatment induces CCR7 in plaque macrophages in mice and results in macrophage emigration.[124] Because the human CCR7 gene also has a SRE, the same process might occur in humans and contribute to the known reduction in peri-ACS mortality by high-dose statins.[127] In any case, the pre-clinical findings support the consideration and exploration of strategies based on retention/migration pathways to limit plaque enrichment in macrophages or to rapidly remove them as part of preventing or acutely treating, respectively, ACS.

Conclusions

Inflammation drives many aspects of the ACS both locally and systemically. We have come to appreciate increasingly the critical operation of pro- and anti-inflammatory pathways implicated in ACS pathogenesis. Thus the risk of ACS depends critically on the prevailing balance between promotion of inflammation and its resolution through the pathways discussed herein. Manipulation of this balance provides opportunities for novel therapeutic manipulation in the future. Yet, a number of unresolved issues present challenges for future research regarding the role of inflammation in ACS. The role of inflammation in superficial erosion remains controversial and underexplored. Novel therapies that target inflammation and its resolution require rigorous evaluation as an adjunct to current standard of care in preventing ACS. While the concepts of inflammation have transformed our understanding of the pathophysiology of ACS, we have yet to reap fully the potential therapeutic benefits of translation of these basic science discoveries to the prevention of ACS in patients.

Nonstandard Abbreviations and Acronyms

OCT	optical coherence tomography
DC	dendritic cell
DAMP	damage-associated molecular pattern
RIP	receptor-interacting protein
PRR	pattern recognition receptor
SPM	specialized pro-resolving mediators

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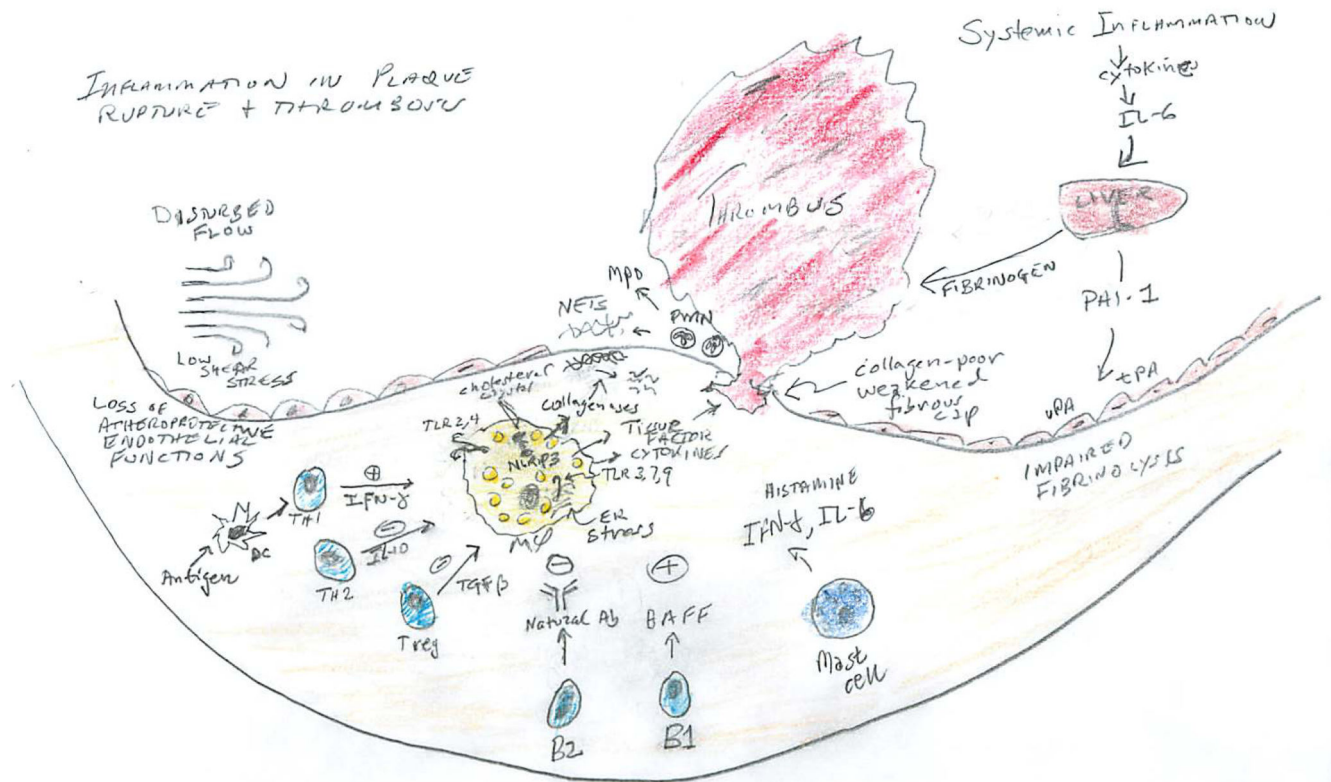
Figure 1. Inflammation in plaque rupture and thrombosis
This diagram shows a cross section the intima of part of an artery affected by atherosclerosis. Altered hydrodynamics, illustrated in the upper left, cause loss atheroprotective functions of endothelial cells — including vasodilator, anti-inflammatory, pro-fibrinolytic, and anti-coagulant properties. Antigens presented on antigen-presenting cells such as dendritic cells (DCs) can activate Th1 lymphocytes to produce interferon gamma (IFN-γ), which activates macrophages (MΦ, yellow). Other subtypes of lymphocytes (shown in blue) include Th2 lymphocytes, which can elaborate the anti-inflammatory cytokine interleukin 10 (IL-10) and regulatory T cells that secrete the anti-inflammatory cytokine transforming growth factor beta (TFG-β). On its surface, the macrophage contains Toll-like receptors (TLRs) 2 and 4, which can bind PAMPs and DAMPs (see text). The intracellular TLRs 3, 7, and 9 may also contribute to lipid accumulation and other pro-atherogenic functions of the macrophage. Macrophages can undergo stress of the endoplasmic reticulum (ER) under atherogenic conditions. Cholesterol crystals found in plaques can activate the NLRP3 inflammasome (see text) that can generate mature IL-1β from its inactive precursor. The activated macrophage secretes collagenases that can degrade the triple helical interstitial collagen that lends strength to the plaque's fibrous cap. Activated macrophages also express tissue factor, a potent pro-coagulant, and elaborate pro-inflammatory cytokines that amplify and sustain the inflammatory process in the plaque. When the plaque ruptures due to a collagen-poor, weakened fibrous cap, blood in the lumen can contact tissue factor in the lipid core, triggering thrombus formation (red). When the

thrombus forms, polymorphonuclear leukocytes (PMNs) can accumulate and elaborate myeloperoxidase (MPO), which in turn elaborates the potent pro-oxidant hypochlorous acid. Dying PMNs extrude DNA that can form neutrophil extracellular traps (NETs), which can entrap leukocytes and promote thrombosis. Other inflammatory cells modulate atherosclerosis. B2 lymphocytes secrete natural antibody that can inhibit plaque inflammation. On the other hand, B1 lymphocytes, in part via B-cell activating factor (BAFF), can promote inflammation and plaque complication. Mast cells can augment atherogenesis by releasing histamine and the cytokines IFN-γ and IL-6. The consequences of a given plaque rupture depend not only on the solid state of the intimal plaque, but also on the fluid phase of blood, as depicted in the upper right. Systemic inflammation can give rise to cytokines, culminating in the overproduction of IL-6, the trigger of the hepatic acute phase response. The acute phase reactant fibrinogen participates directly in thrombus formation. Another acute phase reactant, plasminogen activator inhibitor-1 (PAI-1), can impair fibrinolysis by inhibiting the endogenous fibrinolytic mediators, urokinase and tissue-type plasminogen activators (uPA and tPA).
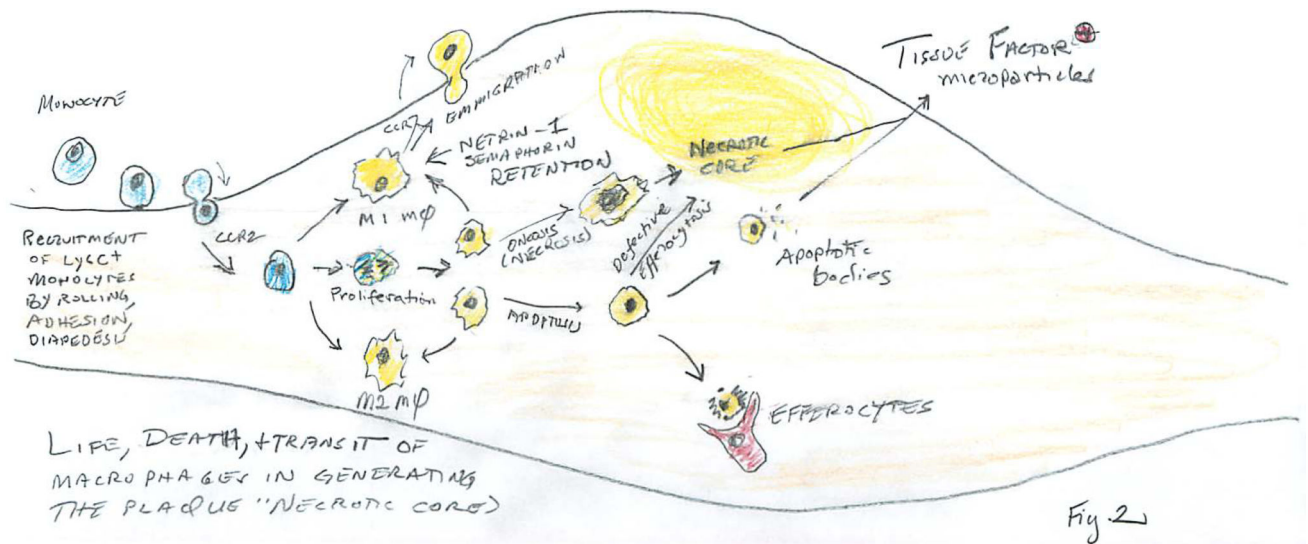
Figure 2. Life, death, and transit of macrophages in generating the plaque's "necrotic" core

Blood monocytes interact with adhesion molecules expressed by endothelial cells exposed to inflammatory mediators. They first roll and attach more firmly, and ultimately diapedese into the intima in response to chemoattractant molecules that bind to surface receptors on the leukocyte — exemplified here by chemokine receptor 2 (CCR2). The monocytes recruited to the intimal lesion can proliferate, giving rise to daughter cells, or can differentiate into macrophages with a pro-inflammatory slant (denoted M1) or those that are alternatively activated (denoted M2). Macrophages can traffic, including leaving the plaque by emigration, as shown here on the macrovascular surface. Retention factors such as netrin-1 or semaphorin-3 can promote retention accumulation of mononuclear phagocytes in the plaque. Macrophages can die by oncosis (sometimes called necrosis) or by programmed cell death (apoptosis). Advanced plaques show a defect in clearance of apoptotic cells leading to their accumulation, a process denoted "mummification" due to a defect in efferocytosis (see text). Dying cells can also release apoptotic bodies and microparticles bearing the potent pro-coagulant tissue factor that triggers thrombus formation in disrupted plaques. The detritus of dead and dying cells that accumulate due to defective efferocytosis give rise to the lipid-rich "necrotic" core of the plaque.

Table 1

Characteristics of Atherosclerotic Plaques Associated with Rupture and Thrombosis.

Thin fibrous cap[128]
Large lipid/necrotic core[128]
Many intimal inflammatory cells[128]
Few smooth muscle cells in the fibrous cap[6, 128]
Outward remodeling (compensatory enlargement)[129]
Spotty or microscopic calcification[130]

Table 2

Mediators of Inflammation Resolution Possibly Relevant to Atherosclerosis.

Characteristics of Advanced Lesions	Mediators that Reverse Processes Associated with Advanced Lesions	Refs
Persistent inflammatory cell influx	LXA$_4$, RvE1, PD1, AT-PD1, RvD1, RvD2, RvD5, MaR1, RvD3, Ac2-26, AnnexinA1	131-139
Excessive proinflammatory mediators	LXA$_4$, RvE1, PD1, RvD1, RvD2, RvD5, MaR1, RvD3, Ac2-26, AnnexinA1, IL-10	134-136, 138, 140-144
Defective efferocytosis	LXA$_4$, RvE1, PD1, RvD1, RvD2, RvD5, MaR1, RvD3, Ac2-26, IL-10	79, 137, 138, 142, 145-149
Exuberant oxidative stress	LXA$_4$, 15-epi-LXA$_4$, RvE1, PD1, RvD1, RvD2, Ac2-26	81, 150-155
Decreased collagen production/fibrous cap thinning	RvE1	156
Impaired egress	RvE1, PD1, ATLa, carbon monoxide, CCR7	123, 142, 157
Uncontrolled platelet activation	RvE1	158, 159