Inflammation contributes to many of the characteristics of plaques implicated in the pathogenesis of acute coronary syndromes (ACS; Table 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 infarction. The pathways by which inflammation promotes 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 the characteristics of plaques that cause ACS in their contribution to this series. 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. Although the solid state of the atheroma itself determines the propensity to rupture, the fluid phase of blood can determine whether a plaque disruption results in a limited mural thrombus that would evade clinical detection.
Nonstandard Abbreviations and Acronyms

| ACS         | acute coronary syndromes |
| CSF        | colony stimulating factor |
| DAMP       | damage-associated molecular pattern |
| EC         | endothelial cell |
| EPA        | eicosapentaenoic acid |
| LDL        | low-density lipoprotein |
| SMC        | smooth muscle cell |
| SPM        | specialized proresolving mediator |
| Tₘᵦ       | T helper 1 |
| TLR        | Toll-like receptor |

Inflammation Drives ACS

Thin Fibrous Cap

Plaques that have ruptured and caused fatal acute myocardial infarction generally have thin fibrous caps, measured in many studies at ≈60 to 70 μm. Recent studies with optical coherence tomography, 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. Inflammation decisively governs the metabolism of collagen, a major constituent of the fibrous cap that confers on it much of its tensile strength. As recently reviewed, inflammatory signals such as the T helper 1 (Tₘᵦ) cytokine γ-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. Considerable biochemical and experimental data support the role of matrix-degrading proteinases, tightly regulated by inflammatory mediators, as contributors to the dissolution of interstitial collagen that thins and weakens the fibrous cap and hence renders a plaque susceptible to rupture. Death of SMCs in the inflamed atheroma can deplete the plaque of the same cells that synthesize collagen required to form and maintain the fibrous cap.

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 predirected 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 proinflammatory cytokines as inducers of endothelial-leukocyte adhesion molecules that capture blood leukocytes, most prominently the blood monocyte. A series of chemokines that interact with cognate receptors on various classes of leukocytes causes their directed migration to penetrate into the intima. Recent refinements of our understanding of leukocyte accumulation in plaques include an expanded understanding of monocyte/macrophage heterogeneity. In particular, in hypercholesterolemic mice, a proinflammatory subset of monocytes marked by high levels of expression of the surface marker Ly6c enter plaques early in development of experimental atherosclerotic lesions. A preformed pool of proinflammatory monocytes in the spleen provides many of these Ly6c high leukocytes that populate early plaques. The Ly6c high monocyte subpopulation uses chemokine (C-C motif) ligand 2 (CCL2)/chemokine receptor 2 (CCR2) or chemokine (C-X-C motif) ligand 1 (CXCL1)/chemokine (C-X-C motif) receptor 2 (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. The Ly6c low population may arise from Ly6c high monocytes, a transition mediated by the transcription factor Nr4a1 (Nur77). Mononuclear phagocytes can proliferate in plaques. The macrophage scavenger receptor A, known to bind modified lipoproteins, may contribute to mediating this process. Macrophage colony stimulating factor (CSF) 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. Although 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. A recently recognized population of innate response activator B cells can produce granulocyte-macrophage CSF in the spleen of hypercholesterolemic mice where it can activate dendritic cells, which in turn promote Tₘᵦ cells capable of producing γ-interferon that can migrate to atheroma and activate plaque macrophages.

Most depictions of leukocyte recruitment to plaques portray this process as taking place at the macrovascular luminal surface overlying the lesion. Although this locale likely applies to the nascent lesion, once established, atheromata develop a microvasculature. The plexi of plaque microvessels provide an even more abundant surface for leukocyte trafficking. Indeed, a key adhesion molecule, vascular cell adhesion
molecule-1, localizes to the micro- rather than macrovascular 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
The Rosenson et al contribution to this series discusses lipid metabolism and targets in the context of ACS. 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).

Figure 1. Inflammation in plaque rupture and thrombosis. This diagram shows a cross-section of the intima of part of an artery affected by atherosclerosis. Altered hydrodynamics, illustrated in the top left, cause loss of atheroprotective functions of endothelial cells—including vasodilator, anti-inflammatory, profibrinolytic, and anticoagulant properties. Antigens presented on antigen-presenting cells such as dendritic cells (DCs) can activate T<sub>H</sub><sub>1</sub> lymphocytes to produce interferon-γ (IFN-γ), which activates macrophages (MΦ, yellow). Other subtypes of lymphocytes (shown in blue) include T<sub>H</sub><sub>2</sub> 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-β (TGF-β). On its surface, the macrophage contains Toll-like receptors (TLRs) 2 and 4, which can bind pathogen-associated molecular patterns and damage-associated molecular patterns (see text). The intracellular TLRs 3, 7, and 9 may also contribute to lipid accumulation and other proatherogenic functions of the macrophage. Macrophages can undergo stress of the endoplasmic reticulum (ER) under atherogenic conditions. Cholesterol crystals found in plaques can activate the NOD-, LRR- and pyrin domain-containing 3 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 procoagulant, and elaborate proinflammatory cytokines that amplify and sustain the inflammatory process in the plaque. When the plaque ruptures because of 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 propagate thrombosis. Other inflammatory cells modulate atherosclerosis. B1 lymphocytes secrete natural antibody that can inhibit plaque inflammation. On the contrary, B2 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 top 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 (not shown) 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). (Illustration credit: Ben Smith.)

...other 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 characterise 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 2 origins: secondary cell death because of the combination of apoptosis and defective clearance of the apoptotic cells (efferocytosis), and primary cellular necrosis, also known as necrosis 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.\textsuperscript{26,31} As lesions progress, however, intimal cell apoptosis increases for several reasons, including inflammatory stimuli and factors that promote oxidative and endoplasmic reticulum 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/proresolving interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) 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.\textsuperscript{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 kinases (RIPs) in myeloid-derived cells in both LDLr\textsuperscript{−/−} and Apoe\textsuperscript{−/−} mice leads to a decrease in necrotic macrophages in advanced atherosclerotic lesions, but no observable changes in early atherosclerosis. The trigger to necrosis in atheroma remains unknown, but RIP3-dependent cell death can occur in cultured macrophages exposed to oxidized low-density lipoprotein (LDL) together with a caspase inhibitor.\textsuperscript{34} Phagocytes have specific mechanisms for the clearance of necrotic cells, so the presence of dead cells in advanced atheroma may indicate a defect in this subtype of efferocytosis.

As alluded to above, numerous studies using LDLr\textsuperscript{−/−} and Apoe\textsuperscript{−/−} 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 lesions. Advanced human coronary artery lesions show signs of defective efferocytosis.\textsuperscript{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 macrophage CSF, an activator of macrophages, accumulate calcium in atheromata, likely because of impaired osteoclastic activity of the mononuclear phagocytes. Molecular imaging studies colocalized niduses of inflammation with early mineralization in mouse atheromata. 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. Nuclear imaging studies using NaF provide a quantifiable index related to calcification in carotid and coronary artery plaque that correlates with Framingham risk. Imaging investigations using computed tomographic approaches have associated spotty calcification with propensity of plaques to provoke ACS. The implication of inflammation in mechanisms related to cardiovascular calcification provides a novel link to ACS pathogenesis.

Outward Remodeling

The observations of Clarkson in nonhuman primates and of Glagov in human atherosclerotic plaques pointed to the role of expansive remodeling or compensatory enlargement during atherogenesis. Imaging studies using intravascular ultrasound and computed tomography have associated expansive remodeling with risk of plaques to precipitate ACS. 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. In atherosclerosis-susceptible mice, genetically produced alterations in signaling by the key inflammatory mediator IL-1 leads to a failure of expansive remodeling during atherogenesis. In particular, reduced induction of the matrix metalloproteinase-3 because of lack of IL-1 signaling seems to contribute causally to this defective remodeling. 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 procoagulant and anticoagulant and profibrinolytic and antifibrinolytic factors both in the solid state of plaque and fluid phase of blood (Figure 1). Within the plaque, a subpopulation of mononuclear phagocytes and activated SMCs overexpress the potent procoagulant tissue factor in response to inflammatory stimuli, notably CD40 ligand (CD154). The normal intima expresses several functions that combat thrombus accumulation. ECs normally express thrombomodulin and endogenous fibrinolytic mediators urokinase- and tissue-type plasminogen activator. The normal intima also contains heparan sulfate proteoglycans that have anticoagulant properties. The balance between these antithrombotic and profibrinolytic mechanisms shifts in response to inflammatory mediators. The local production of plasminogen activator inhibitor-1, a major blocker of fibrinolysis, rises in SMCs and ECs exposed to proinflammatory 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 IL-6, a proinflammatory cytokine strongly implicated in the pathogenesis of ACS by recent genetic studies. 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 plasminogen activator inhibitor-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. Once plaques have disrupted, neutrophils also enhance local inflammation and oxidative stress, including through the formation of neutrophil extracellular traps. 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, decreased anticoagulant properties, and impaired fibrinolytic capacity.

Pharmacological Anti-Inflammatory Interventions

Statin drugs have transformed cardiovascular prevention in past 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 that alter inflammatory pathways in a concerted manner. 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 inflammosome pathway in macrophages. Other direct anti-inflammatory interventions that do not affect LDL are currently under investigation.

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, following the acute injury or infection, quells inflammation and restores tissue homeostasis. 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. The molecules that mediate the resolution response include specialized proresolving mediators (SPMs), endogenous lipids generated actively during
Table 2. Mediators of Inflammation Resolution Possibly Relevant to Atherosclerosis

<table>
<thead>
<tr>
<th>Characteristics of Advanced Lesions</th>
<th>Mediators that Reverse Processes Associated With Advanced Lesions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent inflammatory cell influx</td>
<td>LXA₄, RvE1, PDI, AT-PD1, RvD1, RvD1, RvD2, RvD5, MaR1, RvD3, Ac2-26, AnnexinA1</td>
<td>131–139</td>
</tr>
<tr>
<td>Excessive proinflammatory mediators</td>
<td>LXA₄, RvE1, PDI, RvD1, RvD2, RvD5, MaR1, RvD3, Ac2-26, AnnexinA1, IL-10</td>
<td>134–136, 138, 140–144</td>
</tr>
<tr>
<td>Defective efferocytosis</td>
<td>LXA₄, RvE1, PDI, RvD1, RvD2, RvD5, MaR1, RvD3, Ac2-26, IL-10</td>
<td>79, 137, 138, 142, 145–149</td>
</tr>
<tr>
<td>Exuberant oxidative stress</td>
<td>LXA₄, 15-epi-LXA₄, RvE1, PDI, RvD1, RvD2, Ac2-26</td>
<td>81, 150–155</td>
</tr>
<tr>
<td>Decreased collagen production/fibrous cap thinning</td>
<td>RvE1</td>
<td>156</td>
</tr>
<tr>
<td>Impaired egress</td>
<td>RvE1, PDI, ATLa, carbon monoxide, CCR7</td>
<td>123, 142, 157</td>
</tr>
<tr>
<td>Uncontrolled platelet activation</td>
<td>RvE1</td>
<td>158, 159</td>
</tr>
</tbody>
</table>

Ac2-26 indicates annexin peptide; AT-PD1, aspirin-triggered protectin D1; ATLa, aspirin-triggered lipoxin analog; CCR7, chemokine receptor 7; IL-10, interleukin-10; LXA₄, lipoxin A₄; MaR1, maresin 1; PDI, protectin D1; RvD, resolvin D; and RvE1, resolvin E1.

inflammation. SPM families include lipoxins, resolvins, protectins, and maresins. Lipoxins derive from omega-6 arachidonic acid, whereas resolvins, protectins, and maresins arise from omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid. Lipoxynogenase- and cyclooxygenase-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 because of 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 apolipoprotein B lipoproteins, additional factors may hamper resolution inflammation in advanced atherosclerosis. Liquid chromatography–mass spectrometry profiling in other chronic inflammatory conditions such as cystic fibrosis, asthma, and localized aggressive periodontitis has revealed decreased SPMs compared with healthy controls. Moreover, lower plasma levels of a specific SPM called aspirin-triggered lipoxin A4 associate with increased risk for peripheral and coronary atherosclerosis in humans, even after correcting for age, sex, and C-reactive protein levels. Unraveling the mechanism(s) by which SPM concentrations decrease in chronic inflammatory diseases and whether this drop also applies to advanced atherosclerosis will require additional studies. Possibilities include insufficient substrate availability (ie, lack of omega-3 fatty acids in the diet); dysregulated signaling, perhaps because of suppression of SPM receptors; impaired biosynthetic capacity, such as decreased lipoxynogenase expression or function; 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. Yet, studies evaluating omega-3 fish oil supplementation in humans have shown variable results. This discrepancy may result from differences in study end points (eg, 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, or to the difficulty of showing beneficial effects on subjects already receiving statins. In mice, dietary omega-3 supplementation attenuates atherosclerosis, and high-dose EPA causes lesion regression. Genetically mediated increase of the n-3/n-6 ratio in mice also yields less atherosclerosis. Prostaglandin E2, via the macrophage EP4 receptor, can also mitigate vascular inflammation.

Overexpression of a key SPM biosynthetic enzyme, 12/15-lipoxygenase, in chow-fed Apoe⁻/⁻ mice limited atherosclerosis, providing support for a role of endogenous biosynthesis of SPMs, but mice consuming a high-fat diet did not show this protection. Consistent with the concept that a high-fat diet impairs resolution, diet-induced obesity associates with defective inflammation resolution. 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.

Therapeutic Potential of SPMs in ACS: Inflammation Suppression Without Compromise of Host Defense

Because 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. In this context, SPMs can enhance efferocytosis. Obese atherosclerotic mice that consumed an omega-3 fish oil diet containing the SPM precursors EPA and docosahexaenoic acid had amelioration of impaired efferocytosis. Conversion of EPA and docosahexaenoic acid to SPMs within the vasculature might contribute to this benefit. Likewise, the resolvins RvD1 and RvD2 may benefit injured atherosclerotic arteries. 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) prevents recurrent ACS, a benefit traditionally ascribed to antiplatelet action
mediated by cyclooxygenase inhibition. 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. Indeed, aspirin alters the active site of COX-2 in a manner that permits conversion of arachidonic acid to 15R-HETE (15R-hydroxyeicosatetraenoic acid) in vascular ECs, a precursor for leukocyte production of proresolving 15-epi-lipoxins. 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 acetylsalicylic acid can also process EPA and docosahexaenoic acid to boost the generation of other resolvins. Thus, non-prostanoid-related, resolution-mediated effects of low-dose aspirin may contribute to their ability to protect against ACS.

Adaptive Immune Modulation Can Also Mute Inflammation (Figures 1 and 2)

Some functions of both innate and adaptive immune cells can also counterbalance proinflammatory pathways implicated in ACS and mitigate inflammation (Figure 1). For example, Ly6c low monocytes and alternatively activated macrophages (denoted M2) can modulate inflammation. Although B2 lymphocytes seem to aggravate atherosclerosis, B1 lymphocytes elaborate natural antibodies that can quell atherogenesis. T helper 2 lymphocytes can elaborate IL-4 and IL-10 that may limit inflammation and sway macrophages toward M2 polarization. Regulatory T cells, by elaborating transforming growth factor-β, can balance the proinflammatory cascade unleashed by T helper cells and thus modulate inflammatory responses in the atheroma.

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 (Figures 1 and 2). 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. Such aberrations and the products of modified or native lipoproteins that accumulate in atherosclerotic plaques can trigger pattern recognition receptors expressed by macrophages, including the NOD-like receptors, scavenger receptors, and TLRs, thereby activating the inflammatory response.

NOD-Like Receptors 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. Macrophages can engulf these crystals, resulting in the induction and activation of the 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 (Canakinumab Anti-inflammatory Thrombosis Outcomes Study [CANTOS]) to prevent recurrent ACS. 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 NOD-, LRR- and pyrin domain-containing 3 in a process mediated in part by CD36.

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 Low-Dose Colchicine (LoDoCo) intervention trial in patients without gout but having coronary artery disease, affirm the potential of this approach.

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 4,79,80 tumor necrosis factor receptor–associated factor 6,81 Toll/interleukin-1 receptor-domain–containing adaptor protein inducing interferon-β (also known as TICAM-1), and myeloid differentiation primary-response protein 88—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 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, oxidized LDL has remained firmly in the sights of investigators as a major therapeutic target, with recent efforts directed toward a vaccine strategy to reduce ACS risk by eliminating oxidized LDL as it is formed—before it can stimulate TLR-related inflammatory pathways in macrophages—among other potential benefits.

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. Studies of M1 and M2 macrophages polarized in vitro by, for example, interferon-γ or IL-4, respectively, and in atherosclerotic mice have led to the simplified view that M1 macrophages promote whereas M2 macrophages limit plaque inflammation. This construct caricatures the complex range of macrophage functions in vivo, because 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
The full range of influences in the intimal microenvironment that promote the polarization of macrophages in vivo in general, and in plaques in particular, remains incompletely defined, but undoubtedly includes interactions with the adaptive immune system; for example, T regulatory (Treg) and T helper 2 cells, important players in plaque inflammation as reviewed earlier, secrete potent macrophage-polarizing factors (e.g., interferon-γ and IL-4, respectively; Figure 1). Although the spectrum of macrophage functions in vivo, particularly in humans, likely exceeds that typically described in vitro, 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 contrary, secrete low levels of IL-6 and IL-12, but high levels of one of the endogenous anti-inflammatory cytokines, IL-10. M2 macrophages also exhibit scavenging of necrotic debris, enhanced efferocytotic activity, and reduced production of reactive nitrogen species, all considered to be proresolving 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 apolipoprotein AI, the high-density lipoprotein–forming protein), the balance between the macrophage polarization markers shifts toward the M2 panel of functions. 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.

That the M1/M2 state might undergo manipulation in human plaques as a preventive or therapeutic approach to ACS, suggested by 1 (albeit small) clinical study in which patients who received high-density lipoprotein infusions before peripheral atherectomies showed a tendency for decreased inflammatory mediators in the plaques relative to those excised from the control group. 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, which in addition to IL-4 is secreted by T regulatory lymphocytes) or helminth antigens showed reduced atherosclerosis progression and plaque inflammation.

In summary, the preclinical 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 T regulatory–related pathways, indirect regulators of M2 polarization, such as high-density lipoprotein, or by plaque targeted nanoparticle–based delivery of agents, including small interfering RNA, antisense 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—emigration of macrophages from plaques—might also contribute emerged from the 1980s’ studies of Ross Gerrity in atherosclerotic pigs and of Russell Ross in monkeys, 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 low, but placement of plaques into a regression environment readily demonstrated macrophage exit. Other types of mouse experiments have also documented macrophage emigration from plaques during atherosclerosis regression, extending these initial observations.

The capacity of macrophages to leave plaques seems to depend on at least 2 subpathways: 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 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 several chemokines in vitro. When Ldlr−/− mice received bone marrow transplants from netrin-1–deficient mice, compared with 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.

Other factors that inhibit cell movement, such as adhesion molecules (which are more highly expressed in macrophages in progressing versus regressing plaques), 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. 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.\textsuperscript{123} The molecular basis for this augmented CCR7 seems to depend on the presence of a sterol response element in the promoter of the mouse (and human) CCR7 gene.\textsuperscript{124} Thus, in a regression environment, which reduces macrophage cholesterol content, CCR7 expression rises. More 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.\textsuperscript{121,125}

Manipulating retention or migration factors could be envisioned to have therapeutic applications to the prevention or treatment of ACS by reducing the plaque content of inflammatory cells. The recent exciting demonstration that small interfering RNA molecules that target monocyte/macrophage inflammation in vivo can be delivered in nanoparticles\textsuperscript{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.\textsuperscript{124} Because the human CCR7 gene also has a sterol response element, the same process might occur in humans and contribute to the known reduction in peri-ACS mortality by high-dose statins.\textsuperscript{122} In any case, the preclinical 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, several 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. Although the concepts of inflammation have transformed our understanding of the pathophysiology of ACS, we are yet to reap fully the potential therapeutic benefits of translation of these basic science discoveries to the prevention of ACS in patients.

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None.

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