REVIEW ARTICLE

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Atherosclerotic Plaque Healing

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Atherosclerotic plaques typically develop over a period of years or decades. In contrast, the thrombotic complications of atherosclerotic disease occur suddenly, often without warning.[1] The notion that acute coronary syndromes develop from the rupture or superficial erosion of an atherosclerotic plaque is an oversimplification of a process involving plaque activity, blood thrombogenicity, and healing.[2,3] Pathological studies have shown that many (if not most) atherosclerotic plaques destabilize without resulting in a clinical syndrome.[4,5] The occurrence of an acute coronary syndrome probably depends on the disruption of a balance between instability ("activation") and healing ("passivation") of an atherosclerotic plaque. During the past 30 years, research efforts have mostly been focused on the mechanisms of plaque instability.[2,3] Yet the risk of acute myocardial infarction or sudden death from coronary causes remains difficult to predict,[6] suggesting that other pathogenic mechanisms should also be investigated. Recently, the notion that plaque healing may play a key role in the natural history of atherosclerotic disease has been gaining attention, in part because of the development of new imaging techniques, allowing in vivo study of the morphologic features of atherosclerotic plaque.[7,8] This review examines the mechanisms of atherosclerotic plaque healing, their role in the progression of atherosclerotic disease and in the development of acute coronary syndromes, and the clinical and potential therapeutic implications of the healing process.

Mechanisms of Plaque Rupture, Erosion, and Healing

Atherosclerotic plaque consists of extracellular lipid particles, foam cells, and debris that have accumulated in the intima of the arterial wall and formed a lipid or necrotic core. The core is surrounded by a layer of collagen-rich matrix and smooth-muscle cells covered by endothelial cells, known as the fibrous cap (Fig. 1A). Inflammatory cells (mainly T cells and macrophages) infiltrate the lesion and are involved in plaque progression and thrombosis, leading to an acute coronary syndrome.[1-3] The two most frequent causes of thrombosis are plaque rupture and superficial erosion. Plaque rupture occurs when the fibrous cap covering the necrotic core fissures, exposing the highly thrombogenic core to flowing blood. The thin-cap fibroatheroma, a plaque with a large necrotic core covered by a thin (<65-μm) fibrous cap infiltrated by activated macrophages, is considered the prototype of the rupture-prone plaque.[1-3,9] Plaque erosion is caused by endothelial damage or denudation and overlying thrombosis in the absence of frank cap rupture. Plaques subject to erosion tend to be proteoglycan-rich and lipid-poor and usually lack prominent inflammatory infiltrates.[2,3,9] When plaque rupture or erosion occurs in a prothrombotic milieu, subocclusive or occlusive thrombosis results, causing a symptomatic acute coronary event; otherwise, if thrombosis-resisting factors prevail, thrombus formation is contained, and plaque healing occurs.[1,10]

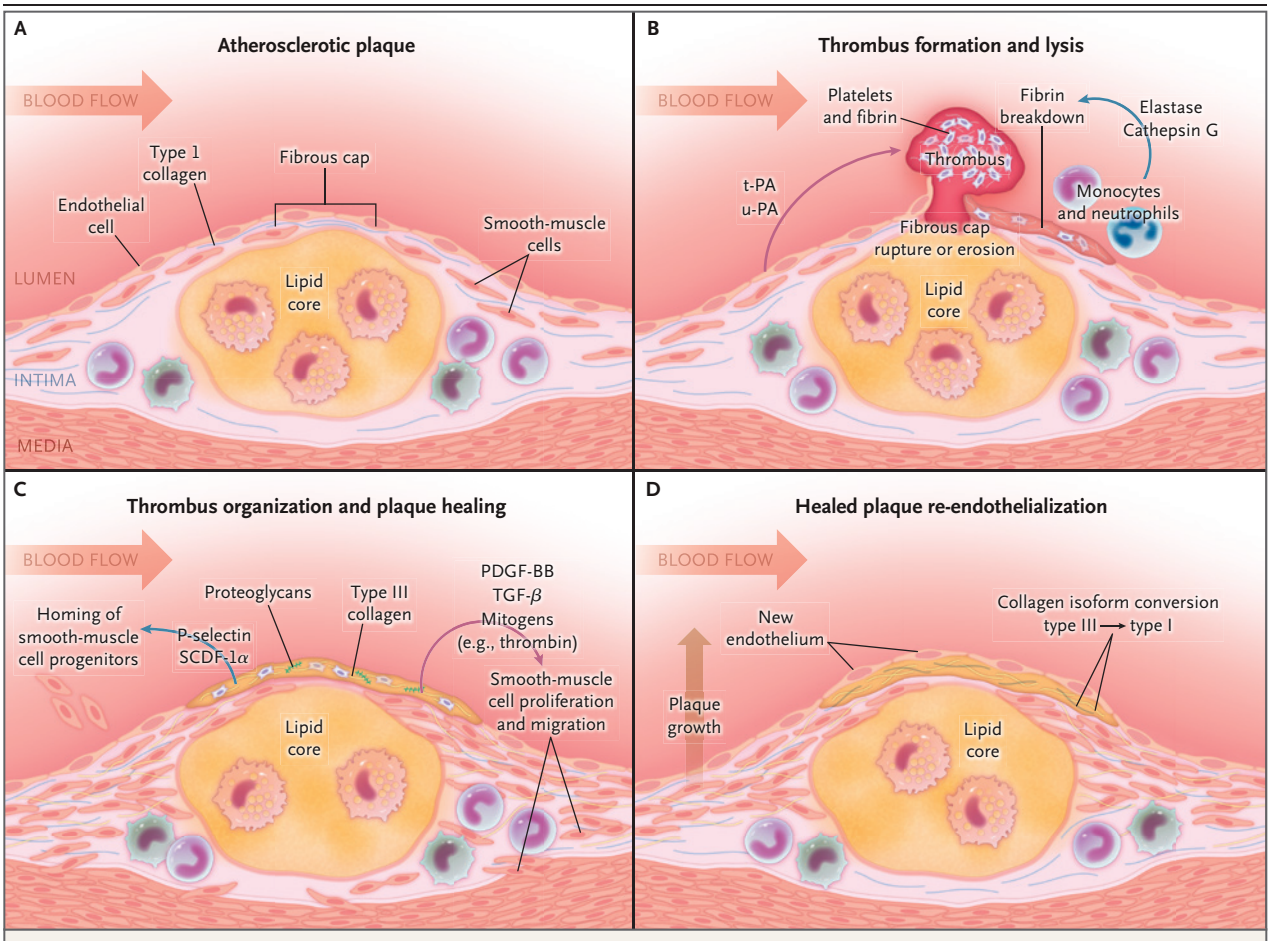

Figure 1. Mechanisms of Atherosclerotic Plaque Healing.

Shown are the phases of rupture and healing of an atherosclerotic plaque. The cross-section of a plaque in Panel A shows a central core containing lipids and necrotic debris (yellow) and macrophage foam cells (pink cells), surrounded by a fibrous cap composed of collagen-rich matrix and smooth-muscle cells. Inflammatory cells, including monocytes (lavender) and macrophages (aqua), infiltrate the intima. Rupture of the fibrous cap, shown in Panel B, leads to exposure of the highly thrombogenic necrotic core, causing platelet activation and aggregation and thrombus formation. In the case of plaque erosion, thrombus formation takes place in the presence of endothelial denudation, which is more characteristic of plaques without a necrotic core. In a patient with an efficient endogenous fibrinolytic system, rupture or erosion of the fibrous cap triggers a cascade of enzymatic processes such as the release of tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) from endothelial cells and the release of elastase and cathepsin G from neutrophils and monocytes, facilitating fibrin breakdown and lysis of thrombus. In Panel C, the release of growth factors such as platelet-derived growth factor BB (PDGF-BB) and transforming growth factor β (TGF-β) and the increased expression of P-selectin and stromal cell–derived factor 1α (SCDF-1α) stimulate a proliferative response of local plaque smooth-muscle cells and homing of smooth-muscle cells from the underlying media, as well as bone marrow–derived smooth-muscle cell progenitors from circulating blood. The proliferating smooth-muscle cells, in turn, synthesize proteoglycans and type III collagen, which form a provisional extracellular matrix. As shown in Panel D, when plaque healing is complete, type I collagen gradually replaces type III collagen, and re-endothelialization occurs. This process prevents the rapid development of occlusive thrombus but can cause the slow progression of a nonocclusive, lipid-rich plaque to a stenotic, more fibrous lesion.

Atherosclerotic plaque healing is a dynamic process that takes place after disruption of the plaque and prevents the formation of a thrombus, promotes plaque repair, and restores the integrity of the vessel. This process, which involves circulating blood cells, soluble mediators, local plaque cells, and extracellular matrix, has three main, overlapping phases: thrombus lysis, granulation-tissue formation, and vessel re-endothelialization. Atherosclerotic plaque healing differs from plaque stabilization; healing follows plaque disruption and takes place in a limited

time period, whereas stabilization is a progressive transformation of a lipid-rich plaque into a more fibrotic and calcific plaque.

The mechanisms of atherosclerotic plaque healing have been studied in humans and in experimental models of mechanical injury to the vessel wall (Fig. 1).[11-14] After plaque rupture or erosion, the exposure of thrombogenic plaque components (e.g., the necrotic core, tissue factor, and collagen) provides a potent stimulus for platelet activation and aggregation and for thrombus formation[2,15] and simultaneously triggers endogenous fibrinolysis to physiologically preserve vessel patency[10] (Fig. 1B). Data obtained from both clinical studies and animal models show that prevention of lasting occlusive thrombus formation requires an intact and functional endogenous fibrinolytic system, including the release of tissue plasminogen activator[16-18] and urokinase plasminogen activator[19] from endothelial cells, the release of elastase and cathepsin G from neutrophils and monocytes trapped in the thrombus (which directly break down fibrin),[17,20] and the dispersing effect of laminar blood flow.[21] The structural properties of fibrin fibers are another important determinant of susceptibility to endogenous thrombolysis, with thick fibers appearing to be more susceptible to lysis than compact, thin fibers.[22]

Plaque disruption also activates a proliferative response in the smooth-muscle cells of the local plaque, as well as migration of smooth-muscle cells from the underlying media to the intima (Fig. 1C). Factors promoting this smooth-muscle cell response include the release of growth factors (e.g., platelet-derived growth factor BB and transforming growth factor β [TGF-β]) and mitogens (e.g., thrombin) by platelets.[23] In addition, the release of TGF-β stimulates extracellular matrix production by smooth-muscle cells.[13,14] Experimental studies in mice have shown that activated platelets adhering to and aggregating over an injured arterial wall can also stimulate the homing of bone marrow–derived smooth-muscle cell progenitors from circulating blood through the expression of P-selectin and stromal cell–derived factor 1α.[12,24,25] At the site of plaque healing, proliferating smooth-muscle cells synthesize a provisional extracellular matrix (granulation tissue), which is rich in proteoglycans and type III collagen[4,26] (Fig. 1C). When plaque healing is complete, type I collagen gradually replaces type III collagen, and complete re-endothelialization of the plaque surface with creation of a neointima eventually occurs[27] (Fig. 1D). After the healing cycle has been completed, atherosclerotic plaque may undergo further cycles of destabilization and subsequent healing, with accumulation of new granulation tissue.

PREVALENCE AND PHENOTYPE OF PLAQUE HEALING

The reported prevalence of healed plaques varies greatly (ranging from <5% to >80%), depending on the method of detection, the vascular bed assessed, and the clinical presentation.[4,5,7,8,28-31] The introduction of new imaging techniques, such as optical coherence tomography (OCT), intravascular ultrasonography, and cardiovascular magnetic resonance imaging (MRI), has enabled the assessment of atherosclerotic plaque healing in vivo.[7,8,28,30] Studies using these techniques suggest that the prevalence of healed plaques is considerably higher among patients with chronic manifestations of atherosclerotic disease than among those in whom clinical stability is frequently interrupted by acute events, in both coronary[7,8,29-31] and noncoronary[28] vascular beds. Pathological studies reveal similar findings. In histologic studies of nonculprit lesions in patients who died suddenly from ischemic heart disease, more than half the lesions were clinically silent, healed coronary plaques.[4,5] Observations from noncoronary vascular beds are consistent with these findings. Serial carotid MRI in patients with a transient ischemic attack showed healing of plaque rupture or erosion in almost 90% of patients who had no recurrence of ischemic symptoms at 12 months of follow-up, whereas this tendency toward healing was less clear in patients with subsequent cerebrovascular events.[28]

Why do some ruptured or eroded plaques fail to heal, resulting in vessel occlusion, whereas other plaques heal, entering a quiescent phase? Knowing the phenotypic features of a healed plaque may help in understanding the mechanisms that promote plaque healing. On histopathological evaluation with the use of picrosirius red staining, healed plaque ruptures are typically identified as newly synthesized type III collagen (which appears green under polarized light) over a disrupted fibrous cap containing

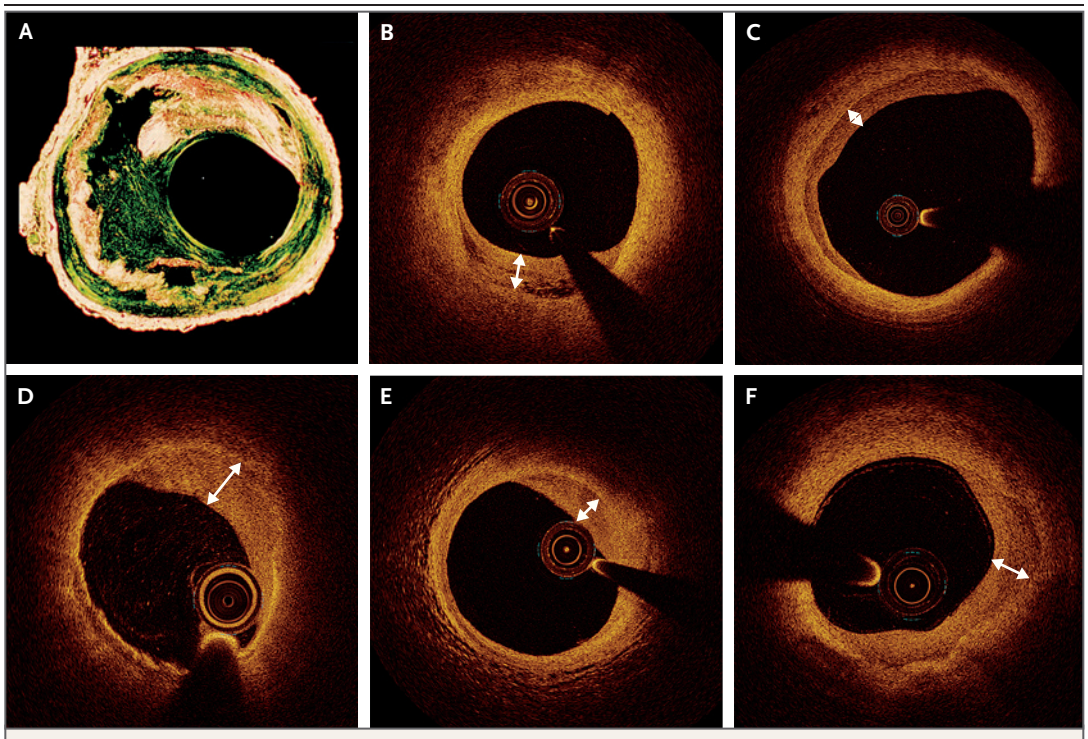

Figure 2. Histologic and Optical Coherence Tomographic (OCT) Images of Healed Coronary Plaques.

In Panel A, a histologic cross-section of human coronary atherosclerotic plaque stained with picrosirius red and viewed under polarized light shows a healed plaque rupture; the majority of plaque collagen (type I collagen) is yellow–white, and the new, loosely arranged collagen repairing the disrupted plaque (type III collagen) is green. Panels B through F show representative in vivo OCT images of human healed coronary plaques with heterogeneous, signal-rich layers of healing tissue (white double-headed arrows) characterized by a different optical-signal intensity from the underlying plaque, giving the lesion a characteristic layered, "onion-like" appearance. (Panel A is reprinted from Mann and Davies[5] with the permission of the publisher.)

mostly type I collagen (which appears yellow to white). Such lesions may have multiple layers of lipid and necrotic core, indicative of previous episodes of thrombosis and healing (Fig. 2A).[4,29] In contrast, healed plaque erosions appear as distinct layers of dense collagen interspersed with smooth-muscle cells and proteoglycans, without evidence of preexisting disruption of the fibrous cap.[4,29] On OCT imaging, healed plaques appear as heterogeneous signal-rich layers with optical-signal intensity that is different from the underlying plaque, creating a characteristic onion-like appearance, without interruption of the old fibrous cap or with interruption that is suggestive of a previous rupture[7,8,29] (Fig. 2B through 2F). In a histopathological study by Shimokado et al., half of the analyzed multilayered lesions were healed plaque ruptures, and half healed plaque erosions. These lesions had an underlying lipid core in about two thirds of cases and consisted of fibrous plaque in the remaining third.[29] The morphologic features of healed carotid plaques at autopsy are similar to those of healed coronary plaques, with multiple layers of fibrous tissue and a lipid-rich necrotic core.[32]

Coronary thrombi overlying ruptured or eroded plaques may also be assessed for evidence of healing.[33] In a pathological study involving 111 patients who died suddenly from coronary causes, almost half the thrombi overlying plaque ruptures had no evidence of healing, and the remaining half showed various phases of healing. In contrast, more than 85% of thrombi overlying plaque erosions showed late stages of healing, characterized by inflammatory-cell degradation, infiltration of smooth-muscle cells, proteoglycan deposition, and platelet and fibrin layering.[33] In a study by Geary et al.,[34] who analyzed healing thrombi that developed after experimental angioplasty of the left iliac artery in

nonhuman primates, thrombus was replaced by smooth-muscle cells expressing hyaluronan and an associated protein, versican, which are known to be specifically involved in the pathogenesis of erosion.[15,35] Taken together, these observations suggest that plaque erosion may be more likely to heal than plaque rupture. The ingrowth of microvessels is a common feature during thrombus organization and is observed more frequently in healed plaques than in nonhealed plaques.[8] Neoangiogenesis appears to be a crucial step in thrombus organization (and generally in tissue repair), as the new microvessels provide oxygen and nutrients to the healing tissue.[36]

INFLAMMATION AND PLAQUE HEALING

Although the mechanisms through which inflammation can precipitate or exacerbate the thrombotic complications of atherosclerosis have been extensively investigated, the link between inflammation and plaque healing remains incompletely elucidated.[1,37] A study of human vascular smooth-muscle cells showed that exposure to interferon-γ, secreted by activated type 1 helper T (Th1) cells, inhibits the ability of smooth-muscle cells to produce the interstitial collagen needed to repair the fibrous cap and maintain its integrity, even when the smooth-muscle cells are maximally stimulated by TGF-β (Fig. 3).[23] In addition, cross-talk between Th1 cells and macrophages (through the interaction between the T-cell–derived cytokine CD40 ligand and the macrophage receptor CD40) boosts the production of interstitial collagenases, including matrix metalloproteinases 1, 8, and 13, which promote interstitial collagen breakdown, weakening the fibrous cap.[38,39] These phagocytes, known as M1 macrophages, are part of Th1 responses and are involved in proinflammatory activities.[40]

In contrast, so-called alternative M2 macrophages are triggered by type 2 helper T (Th2) cytokines, including interleukin-4 and interleukin-13, and secrete antiinflammatory cytokines, including interleukin-10, which counterbalance the proinflammatory activity of M1 macrophages and promote tissue repair (Fig. 3).[40,41] M2 macrophages have been categorized into three subtypes: M2a, M2b, and M2c.[41] M2a macrophages, traditionally recognized as wound-healing macrophages, express high levels of scavenger, mannose, and galactose receptors and produce profibrotic factors, such as fibronectin, insulin-like growth factor 1, and TGF-β, which may contribute to plaque healing.[40-42] Studies suggest a potential role for M2 macrophages in plaque stability and atherosclerosis regression in mouse models, as well as in humans.[43,44]

It has been shown that macrophages are not terminally differentiated in atherosclerotic disease but can switch from one phenotype to another in response to environmental cues.[41,45] The resolution of inflammation in atherosclerosis is mediated by specialized, proresolving mediators, such as resolvins, lipoxins, maresins, and protectins. These mediators modulate the phenotypic conversion of M1 proinflammatory macrophages into alternative M2 macrophages, promote apoptosis and efferocytosis of inflammatory cells, and may play a role in the resolution of inflammation during plaque healing.[46] Mounting evidence suggests that CD31, an immunoglobulin-like membrane receptor expressed by leukocytes, platelets, and endothelial cells, might play a part in atherosclerotic plaque healing by regulating platelet activity and modulating T-cell activation and macrophage phenotypic conversion.[47,48] Other studies suggest that smooth-muscle cells are also not terminally differentiated during the natural history of atherosclerosis and can undergo a phenotypic conversion to a macrophage-like state in response to changes in the microenvironment. Whether smooth-muscle cells can switch to an alternative M2 phenotype and whether some dedifferentiated intimal smooth-muscle cells can redifferentiate to repair the fibrous cap during the healing process are questions that remain largely unexplored.[49] OCT imaging data suggest a higher prevalence of macrophage infiltration in healed plaques than in nonhealed plaques,[8] but in vivo molecular insights into the macrophage phenotype in healed plaques are lacking.

In the late phases of the healing process, M2 macrophages not only prompt the production of extracellular matrix but also may promote plaque calcification by stimulating osteoblastic differentiation and maturation of vascular smooth-muscle cells (Fig. 3).[50,51] Calcium formation dur-

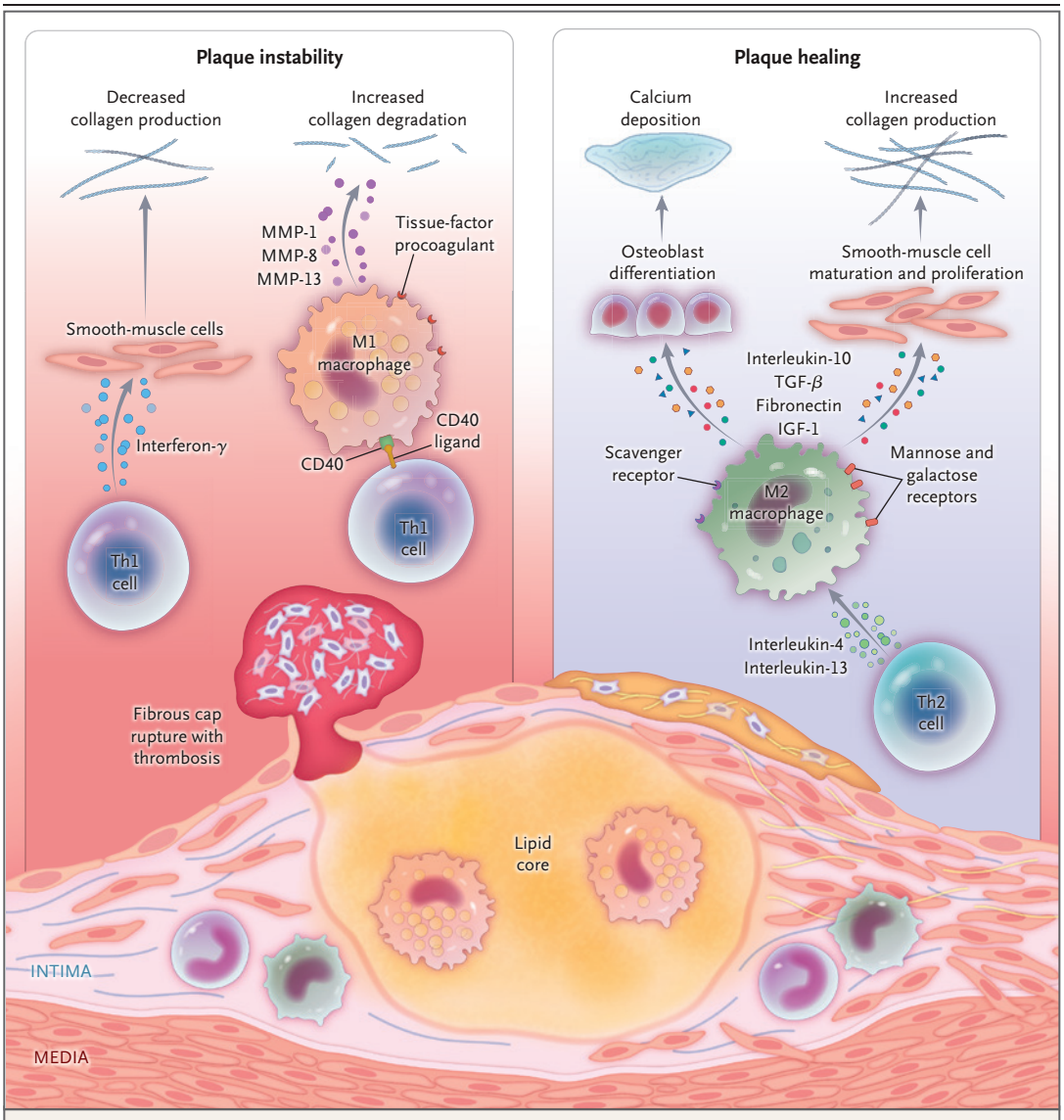

Figure 3. Inflammatory Pathways Involved in Plaque Instability and Plaque Healing.

A cross-section of a plaque (bottom) shows two phases of the natural history of atherosclerosis (i.e., plaque rupture with thrombosis on the left, and plaque healing on the right). The panel on the left depicts the key inflammatory pathways involved in plaque instability. Activated T cells (type 1 helper T [Th1] cells) produce interferon-γ, which inhibits the synthesis of interstitial collagen by smooth-muscle cells. Th1 cells can also activate M1 macrophages through a CD40 ligand–CD40 macrophage receptor interaction, stimulating an overproduction of interstitial collagenases, including matrix metalloproteinases 1, 8, and 13 (MMP-1, -8, and -13), which in turn promote collagen breakdown. The CD40 ligand–CD40 interaction also induces an overexpression of tissue factor by M1 macrophages. The decreased synthesis and increased breakdown of interstitial collagen render the fibrous cap more susceptible to rupture, and the enhanced thrombotic potential due to inflammatory activation triggers thrombus formation over the disrupted plaque. The panel on the right shows the key antiinflammatory pathways involved in plaque healing. Alternative M2 macrophages, mainly triggered by type 2 helper T (Th2) cytokines interleukin-4 and interleukin-13, produce antiinflammatory cytokines, including interleukin-10, and secrete profibrotic factors, including fibronectin, insulin-like growth factor 1 (IGF-1), and TGF-β, which prompt the production of interstitial collagen and extracellular matrix by smooth-muscle cells. In the late phases of the healing process, M2 macrophages also induce osteoblast differentiation and maturation of vascular smooth-muscle cells, promoting plaque calcification.

ing plaque healing is therefore not merely a passive degenerative process but rather is the result of dysregulated deposition and impaired clearance. Pathological studies have shown that maximum calcification is present in healed plaque rupture and in fibrocalcific plaques and that the calcification area steadily enlarges with progressive luminal narrowing. Healed plaques frequently contain diffuse sheets of calcification, which provide mechanical support for the healed plaque.[52]

ROLE OF PLAQUE HEALING IN CLINICAL EVENTS

Much of the work addressing the mechanisms of acute coronary syndromes during the past three decades has focused on the pathogenesis of plaque instability.[1-3] However, recent intracoronary imaging studies suggest that acute coronary syndromes may require a "double hit" of plaque disruption and impaired healing[7] (Fig. 4). The acute destabilization of an atherosclerotic plaque by either rupture or erosion (the first hit) leads to thrombosis and an acute coronary syndrome in patients with an impaired healing capacity (the second hit). In contrast, in patients with an effective healing system, the first hit is contained, the unstable plaque is "pacified," and the healing process promotes the development of a more fibrous, stable plaque.

Repeated cycles of thrombosis and healing lead to progressive encroachment on the arterial lumen, with silent, stepwise stenotic progression possibly leading to high-grade coronary occlusion in the absence of acute coronary events. In a seminal pathological study by Mann and Davies,[5] only approximately 16% of coronary lesions with 0 to 20% diameter stenosis (the percentage of cross-sectional diameter lost to stenosis) and approximately 19% of coronary lesions with 21 to 50% diameter stenosis had healed disruption, whereas approximately 73% of lesions with more than 50% diameter stenosis had a healed disruption pattern. In vivo, healed coronary plaques detected on OCT imaging have been consistently associated with a significantly smaller luminal area,[7,8,30] and the prevalence of healed plaques steadily increases with a greater degree of stenosis.[30]

The clinical significance of plaque healing is still a matter of debate.[7,8,30] In a study from our group, impaired healing was associated with recurrent acute coronary syndromes, whereas the presence of healed plaques was a predictor of long-term clinical stability.[7] Whether plaque healing, through the silent progression of stenosis, may translate into more frequent revascularization procedures warrants investigation. Recent data suggest that patients with OCT evidence of healed plaques in nonculprit coronary segments may have higher rates of revascularization in the absence of acute events than patients without evidence of healed plaques.[53] Taken together, these observations suggest that plaque healing may protect patients with atherosclerotic disease from the occurrence of acute coronary syndromes, instead leading to a chronic coronary syndrome (Fig. 4).

THERAPEUTIC IMPLICATIONS AND FUTURE PERSPECTIVES

Both established and experimental therapeutic interventions may influence plaque healing and thereby reduce the risk of acute coronary syndromes (Table 1). In rabbits with atherosclerosis induced through balloon injury of the thoracic aorta and an atherogenic diet, subsequent dietary lipid lowering reduced the proteolytic activity of matrix metalloproteinases and increased interstitial collagen within the fibrous cap of the plaque,[54] reduced oxidative stress and endothelial-cell activation,[55] and reduced tissue factor expression and activity,[56] rendering atheroma less susceptible to disruption and thrombosis.

Lipid-lowering medication also has beneficial effects on plaque healing. Statin therapy has been shown to increase the number of intimal smooth-muscle cells and the expression of type I procollagen and to reduce the proliferation and activation of macrophages, as well as tissue factor expression, in atherosclerotic lesions in Watanabe heritable hyperlipidemic rabbits.[57,58] In a recent study by Schuster et al., treatment of APOE*3Leiden.CETP mice with neutralizing monoclonal antibodies against proprotein convertase subtilisin–kexin type 9 (PCSK9) resulted in a reduction in macrophage infiltration within aortic plaques and an increase in the numbers of endothelial progenitor cells and circulating angiogenic cells.[59] Lipid lowering by conditional inactivation of the gene encoding the microsomal triglyceride transfer protein (MTTP) has

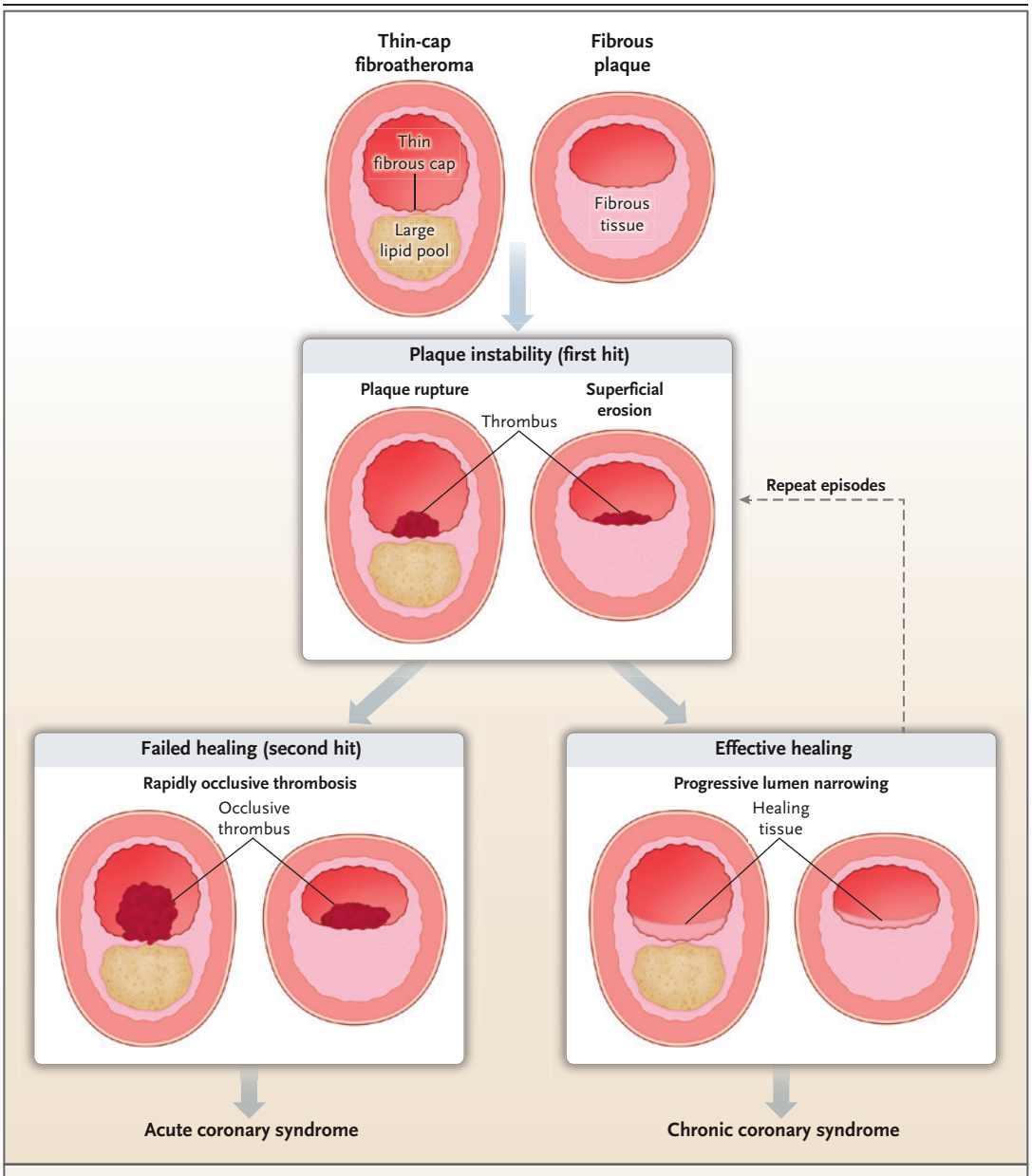

Figure 4. Role of Plaque Healing in the Natural History of Ischemic Heart Disease.

In the "double hit" theory of atherosclerosis, the first hit is the acute disruption of an atherosclerotic plaque (rupture or erosion), and the second hit is an impaired healing capacity. In patients with a double hit of plaque disruption and failed healing, occlusive or subocclusive thrombosis and an acute coronary syndrome will develop. In contrast, the first hit is contained in patients with an effective healing system, and the healing process promotes evolution to a more fibrous, stable plaque, often accompanied by constrictive remodeling. Repeated cycles of thrombosis and healing lead to progressive luminal loss with stepwise stenotic progression in the absence of acute events.

been associated with an increase in collagen deposition, as well as a reduction in cholesterol and CD68+ macrophage content within atherosclerotic plaques in low-density lipoprotein receptor–deficient mice.[60] In vivo OCT imaging studies suggest that coronary plaques undergo an increase in fibrous-cap thickness and a reduction in lipid content and macrophage infiltration after the initiation of statin therapy[61,62] or other lipid-lowering therapies.[63,64] A similar response

Table 1. Therapeutic Interventions That Might Enhance Atherosclerotic Plaque Healing.*

Therapy	Potential Favorable Effects on Plaque Healing
Lipid-lowering diet (e.g., low-cholesterol, low-fat diet)	Reduces expression and proteolytic activity of interstitial collagenase (MMP-1) Increases interstitial collagen content within fibrous cap Reduces oxidative stress Reduces endothelial-cell activation Reduces tissue factor expression and activity (reduces thrombotic potential)
Lipid-lowering drugs (e.g., statins, PCSK9 inhibitors)	Reduce expression and proteolytic activity of interstitial collagenase (MMP-1) Increase smooth-muscle cells and interstitial collagen within fibrous cap Reduce proliferation and activation of macrophages Reduce oxidative stress Reduce endothelial-cell activation Up-regulate endothelial progenitor cells and circulating angiogenic cells Reduce tissue factor expression and activity (reduce thrombotic potential) Reduce plasminogen activator inhibitor 1 levels (increase fibrinolytic potential)
Antiinflammatory drugs (e.g., interleukin-1β antagonists, colchicine)	Reduce expression of adhesion molecules and leukocyte recruitment on endothelial cells Reduce tissue factor expression (reduce thrombotic potential) Reduce fibrinogen and plasminogen activator inhibitor 1 levels (reduce thrombotic potential, increase fibrinolytic potential)
CD31-targeting therapies (e.g., agonist peptides, antibodies, recombinant proteins)	Polarize macrophages toward an alternative M2 phenotype
Epigenetic therapies targeting macrophage polarization (e.g., DNA methylation, histone modifications, miRNAs, lnc-RNAs)	Polarize macrophages toward an alternative M2 phenotype
PI3Kc–CXCL10 axis inhibitors	Increase re-endothelialization after vascular injury

* CXCL10 denotes C-X-C motif chemokine ligand 10, lncRNAs long noncoding RNAs, miRNAs microRNAs, MMP-1 matrix metalloproteinase 1, PCSK9 proprotein convertase subtilisin–kexin type 9, and PI3Kc phosphatidylinositol 3-kinase, catalytic domain.

to lipid-lowering therapy has been observed in carotid plaques.[65,66]

Intensive antiplatelet therapy with newer $P2Y_{12}$ inhibitors has proved effective in stabilizing plaque erosion, with a progressive reduction in the thrombotic burden at 30 days and complete healing at 1 year.[67,68] Whether patients with impaired healing capacity may benefit from prolonged antiplatelet regimens is currently unknown and may warrant investigation in larger-scale, randomized studies.

A number of studies are evaluating the effect of antiinflammatory therapies, including the interleukin-1β antagonist canakinumab and low-dose colchicine, in reducing the risk of a recurrent acute coronary syndrome.[69,70] Although the potential role of these antiinflammatory agents in plaque healing has not been directly assessed, their positive effects on clinical outcomes might be due not only to the prevention of both atherosclerotic progression and its acute thrombotic complications but also to an improvement in plaque healing capacity. Interleukin-1β stimulates the expression of tissue factor and adhesion molecules recruiting leukocytes on endothelial cells, including intercellular adhesion molecule 1 and vascular-cell adhesion molecule 1.[71] Moreover, smooth-muscle cell stimulation by interleukin-1β strongly induces the production of interleukin-6, which in turn elicits an acute-phase response culminating in the synthesis of acute-phase reactants by hepatocytes, including fibrinogen and plasminogen activator inhibitor.[72] Blockade of interleukin-1β may therefore indirectly favor plaque healing by inhibiting leukocyte adhesion, reducing thrombosis, and promoting fibrinolysis. Similarly, a recent study suggests that low-dose colchicine favorably modifies the morphologic features of coronary plaques, producing a more stable, fibrous phenotype.[73]

What about new therapeutic targets? Modulation of macrophage polarization (the process of preferential differentiation into the M1 or M2

phenotype) through CD31-targeting molecules or epigenetic therapies may provide additional opportunities for therapeutic intervention in the near future. A drug-suitable CD31 agonist peptide was recently shown to promote macrophage polarization toward a reparative M2 phenotype in experimental models of arterial injury.[47,74] Neele et al. found that the histone demethylase JMJD3 reprograms macrophages to the M2 phenotype.[75] Similarly, systemic deletion or myeloid-specific deletion of histone deacetylases 9 and 3 has been shown to promote M2 macrophage polarization.[76,77] Inhibition of microRNAs (miRNAs) such as miRNA-33 was found to polarize macrophages toward an M2 phenotype[78]; miRNA-146a and miRNA-27a also had an atheroprotective effect mediated by an alternative M2 macrophage activation.[79,80] In a study assessing the detailed expression patterns of long noncoding RNAs (lncRNAs) in macrophage polarization, Huang et al. found that knockdown of the lncRNA TCONS_00019715 can switch isolated human monocyte-derived macrophages from a proinflammatory M1 phenotype to an alternative M2 phenotype.[81]

An elegant study using an in vivo model of endovascular injury in mice recently revealed that a phosphatidylinositol 3-kinase c (PI3Kc)–dependent T-cell response stimulates production of the interferon-inducible C-X-C motif chemokine ligand 10 (CXCL10) by smooth-muscle cells, which in turn inhibits endothelial healing. In this study, both ubiquitous genetic inactivation of PI3Kc and hematopoietic cell–specific PI3Kc deletion, as well as CXCL10 neutralization, improved reendothelialization of the injured carotid artery in mice.[82] These novel findings might pave the way for new therapeutic strategies to promote endothelial healing, which may be particularly effective when a superficial erosion occurs in the absence of a frank rupture of the fibrous cap. An anti-CXCL10 antibody and a highly specific PI3Kc inhibitor have been developed and are being evaluated in clinical trials for the treatment of noncardiovascular diseases.[83,84]

CONCLUSIONS

We have learned a great deal about mechanisms governing plaque instability, and this knowledge has driven the traditional approaches to therapy for atherosclerosis. The notion that plaque healing contributes to the natural history of atherosclerotic disease, rooted in keen pathological observations in the past century, has been undergoing a research renaissance in recent years, primarily owing to the availability of new invasive imaging tools that permit the study of atherosclerosis in vivo. We now understand that healed plaques are not merely innocent bystanders. In particular, the evolution of coronary artery disease appears to be more stable in patients with healed plaques than in patients with unhealed plaques. It is unclear why some patients have a good capacity for healing and others do not. A better insight into the pathophysiology of plaque healing might allow the implementation of strategies to transform poor healing into good healing, thus further reducing the residual burden of cardiovascular disease.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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