Inflammation in atherosclerosis

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Abundant data link hypercholesterolaemia to atherogenesis. However, only recently have we appreciated that inflammatory mechanisms couple dyslipidaemia to atheroma formation. Leukocyte recruitment and expression of pro-inflammatory cytokines characterize early atherogenesis, and malfunction of inflammatory mediators mutes atheroma formation in mice. Moreover, inflammatory pathways promote thrombosis, a late and dreaded complication of atherosclerosis responsible for myocardial infarctions and most strokes. The new appreciation of the role of inflammation in atherosclerosis provides a mechanistic framework for understanding the clinical benefits of lipid-lowering therapies. Identifying the triggers for inflammation and unravelling the details of inflammatory pathways may eventually furnish new therapeutic targets.

Cardiovascular disease, currently the leading cause of death and illness in developed countries, will soon become the pre-eminent health problem worldwide[1]. Atherosclerosis — a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries — constitutes the single most important contributor to this growing burden of cardiovascular disease. Our views of the pathophysiology of this important malady have evolved substantively over the past century. The link between lipids and atherosclerosis dominated our thinking until the 1970s, based on strong experimental and clinical relationships between hypercholesterolaemia and atheroma[2]. The emerging knowledge of vascular biology led to a focus on growth factors and the proliferation of smooth muscle cells in the 1970s and 1980s. The daunting clinical problem of restenosis (narrowing of the vessel lumen) following arterial intervention, considered a problem of proliferation, reinforced this interest in vascular growth control. A fusion of these views led to the concept of the atheroma as a graveyard of acellular lipid debris enrobed by a capsule of proliferated smooth muscle cells.

Over the past decade, however, we have come to appreciate a prominent role for inflammation in atherosclerosis and its complications. Whereas most clinicians previously regarded atheroma as a bland lesion, the current notion that inflammation and immune response contribute to atherogenesis has garnered increased interest[3]. As laboratory advances in vascular biology enabled new thinking about the clinical aspects of atherosclerosis, so too have emerging clinical data instructed our laboratory work, shifting its emphasis considerably. Formerly focused on luminal narrowing due to the bulk of atheroma, our current concepts recognize the biological attributes of the atheroma as key determinants of its clinical significance. This review will weave together laboratory and clinical advances to provide an update on inflammation in atherosclerosis.

Inflammation and the initiation of the atherosclerosis

The time-tested association of cholesterol with atherosclerosis stimulated a century-long study of the mechanisms linking lipids with atheroma. From the early years of the twentieth century onward, the pathogenesis of experimental atherosclerosis induced by hypercholesterolaemia has yielded to scrutiny at ever-deeper degrees of analysis. Indeed, instigation of inflammation may well link hyperlipidaemia to atherogenesis mechanistically. Soon after initiating an atherogenic diet, light microscopy reveals attachment of blood leukocytes to the endothelial cells that line the intima, the innermost layer of arteries[4]. Under ordinary circumstances, the endothelial monolayer in contact with flowing blood resists firm adhesion of leukocytes. We now possess considerable information about the molecular mechanisms of the attachment of white blood cells to endothelium. One endothelial–leukocyte adhesion molecule has emerged as a particularly attractive candidate for the early adhesion of mononuclear leukocytes to arterial endothelium at sites of atheroma initiation (Fig. 1). Vascular cell adhesion molecule-1 (VCAM-1) binds particularly those classes of leukocytes found in nascent atheroma: the monocyte and the T lymphocyte (Fig. 2).

In addition to its leukocyte selectivity, other features of VCAM-1 make it an interesting candidate. Endothelial cells express VCAM-1 in response to cholesterol feeding selectively in areas prone to lesion formation[5]. In addition, VCAM-1 rises before leukocyte recruitment begins in both rabbit and mouse models of cholesterol-induced lesion formation[6]. Targeted deletion of VCAM-1 in mice causes embryonic lethality. However, experiments with hypomorphic variants of VCAM-1 introduced into mice rendered susceptible to atherogenesis (by inactivation of the apolipoprotein E (*apoE*) gene) show reduced lesion formation[7]. In addition to VCAM-1, P- and E-selectin also seem to contribute to leukocyte recruitment in atherosclerosis-susceptible mice[8,9].

The mechanism of VCAM-1 induction early after initiating an atherogenic diet probably depends on inflammation instigated by modified lipoprotein particles accumulating in the arterial intima in response to the hyperlipidaemia. Constituents of modified lipoprotein particles, among them certain oxidized phospholipids and short-chain aldehydes arising from lipoprotein oxidation, can induce transcriptional activation of the VCAM-1 gene mediated in part by nuclear factor-κB (NF-κB)[10]. Pro-inflammatory cytokines such as interleukin (IL)-1β or tumour-necrosis factor-α (TNF-α) induce VCAM-1 expression in endothelial cells by this pathway. Human atherosclerotic lesions contain these cytokines. Thus, pro-inflammatory cytokines may link hypercholesterolaemia to VCAM-1 expression.

Endogenous anti-inflammatory pathways and 'atheroprotection'

The mechanism of focal expression of VCAM-1 selectively in sites of lesion formation has been the subject of intense recent investigation. One novel idea to emerge from experi-

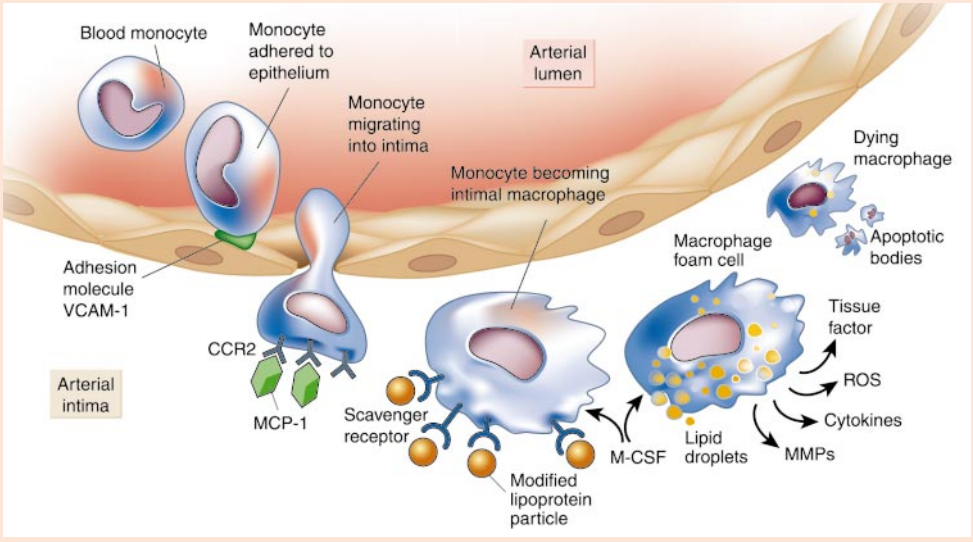

Figure 1 Mononuclear phagocytes in atherogenesis. This figure schematizes steps in the recruitment of mononuclear phagocytes to the nascent atherosclerotic plaque and some of the functions of these cells in the mature atheroma. The steps are depicted in an approximate time sequence proceeding from left to right. The normal arterial endothelium resists prolonged contact with leukocytes including the blood monocyte. When endothelial cells undergo inflammatory activation, they increase their expression of various leukocyte adhesion molecules. In the context of monocyte recruitment to the atheroma, vascular cell adhesion molecule-1 (VCAM-1) seems to have a major role. Once adherent to the activated endothelial layer, the monocyte diapedeses between intact endothelial cells to penetrate into the tunica intima, or innermost layer of the arterial wall. This directed migration requires a chemoattractant gradient. Various chemokines seem to participate in this process, particularly interaction of monocyte chemoattractant protein-1 (MCP-1) with its receptor CCR2. Once resident in the intima the monocyte acquires characteristics of the tissue macrophage. In the atheroma in particular, the macrophage expresses scavenger receptors that bind internalized lipoprotein particles modified for example by oxidation or glycation. These processes give rise to the arterial foam cell, a hallmark of the arterial lesion, so named because of the foamy appearance under the microscope, which is the result of accumulation of lipid droplets within the cytoplasm. Within the arterial intima, the macrophage serves many functions related to atherosclerosis and its complications. Notably, the foam cell secretes pro-inflammatory cytokines that amplify the local inflammatory response in the lesion, as well as reactive oxygen species. The activated mononuclear phagocyte has a key role in the thrombotic complications of atherosclerosis by producing matrix metalloproteinases (MMPs) that can degrade extracellular matrix that lends strength to the plaque's fibrous cap. When the plaque ruptures as a consequence, it permits the blood to contact another macrophage product, the potent pro-coagulant protein tissue factor. Eventually the macrophages congregate in a central core in the typical atherosclerotic plaque. Macrophages can die in this location, some by apoptosis, hence producing the so-called 'necrotic core' of the atherosclerotic lesion.

mental work — 'atheroprotection' — stands the traditional view on its head. Rather than asking what goes awry at sites of lesion formation, one can reverse the question and ask what qualities of endothelium in unaffected areas confer resistance to lesion initiation. Regions of the arterial tree protected from atherosclerosis usually experience laminar shear stress due to orderly blood flow. Sites predisposed to lesion formation include branch points of arteries, which experience disturbed rather than laminar flow.

A number of genes with potentially 'atheroprotective' properties contain shear-stress response elements in their promoter regions. Many such atheroprotective genes may modulate inflammation. For example, superoxide dismutase, expressed at higher levels in regions of laminar flow, may combat oxidative stress and hence limit VCAM-1 expression and other inflammatory pathways[11]. Likewise, nitric oxide arising from endothelial nitric oxide synthase, another shear stress-regulated gene, can inhibit VCAM gene expression through a novel pathway involving inhibition of the activation of NF-κB, the central transcriptional control point in vascular inflammation[12]. These new insights from the laboratory provide a potential explanation for the tendency of atheroma to form in characteristic sites of flow disturbance in the arterial tree despite similar exposure to fluid-phase risk factors such as hypercholesterolaemia.

Mechanisms of leukocyte chemoattraction

Morphologic studies have established that, once adherent to the endothelial cell, leukocytes enter the intima by diapedesis between endothelial cells at their junctions. This phenomenon of directed migration of leukocytes through the endothelium, known for well over a century, has in the past few years yielded to molecular analysis. Investigators have defined families of chemoattractant cytokines (chemokines) capable of recruiting leukocytes into the arterial intima. For example, monocyte chemoattractant protein-1 (MCP-1), overexpressed in human and experimental atheroma, can recruit the mononuclear phagocytes that characteristically accumulate in the nascent atheroma (Fig. 1). Recent work using compound mutant mice lacking MCP-1 or its receptor CCR2, and susceptible to atherosclerosis owing to the absence of genes encoding apoE or the low-density lipoprotein (LDL) receptor, has shown striking decreases in mononuclear phagocyte accumulation and local lipid levels[13,14]. IL-8 may have a similar role as a leukocyte chemoattractant during atherogenesis[15]. Atheroma overexpress other chemokines that may contribute to lymphocyte recruitment, including a trio of CXC chemokines induced by interferon-γ (IFN-γ)[16] (Fig. 2). Chemoattraction of mast cells found in atheroma may depend on eotaxin, a CC chemokine also overexpressed in these lesions[17] (Fig. 3).

Mechanisms of leukocyte activation in the intima

Once resident in the arterial intima, monocytes acquire the morphological characteristics of macrophages, undergoing a series of changes that lead ultimately to foam cell formation. The monocytes increase expression of scavenger receptors from modified lipoproteins such as the scavenger receptor A (SRA) and CD36, and then internalize modified lipoproteins, such that cholesteryl esters accumulate in cytoplasmic droplets (Fig. 1). These lipid-laden macrophages, known as foam cells, characterize the early atherosclerotic lesion. Macrophages within atheroma also secrete a number of growth factors and cytokines involved in lesion progression and complication (see below). In addition, macrophages replicate within the intima.

Studies performed a decade ago identified macrophage colony-stimulating factor (M-CSF) as a candidate activator of several of the steps that stimulate transition of the monocyte to the lipid-laden macrophage. M-CSF augments SRA expression, increases production of cytokines and growth factors by these cells, and also serves as a survival and co-mitogenic stimulus. Both experimental and human atherosclerotic plaques overexpress M-CSF[18,19].

Studies of mice with mutations that inactivate M-CSF, bred onto atherosclerosis-susceptible backgrounds, permitted direct testing of

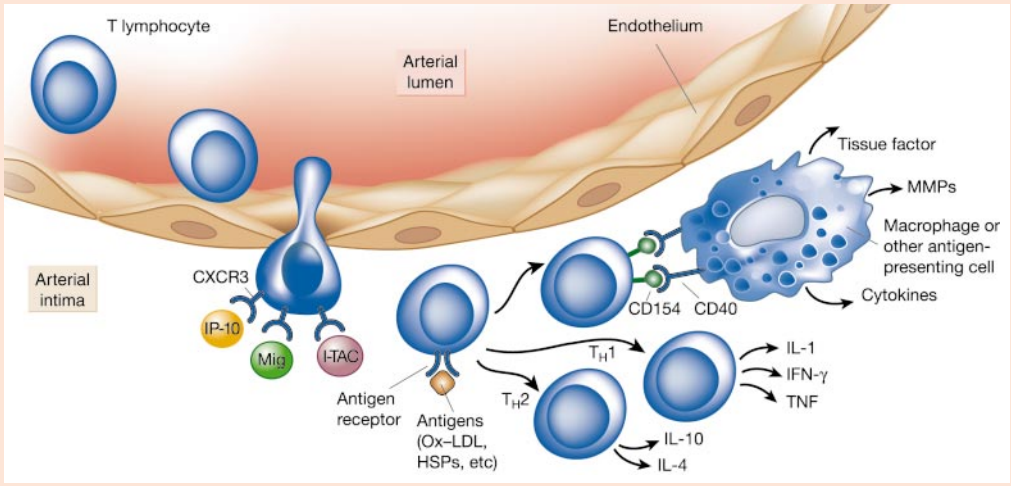

Figure 2 The roles of T lymphocytes in atherogenesis. As in the case of the mononuclear phagocyte, lymphocytes enter the intima facilitated by binding to adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1) and in response chemoattractants selective for lymphocytes. Known chemoattractants include a trio of interferon-γ (IFN-γ)-inducible chemokines of the CXC family including inducible protein-10 (IP-10), monokine induced by IFN-γ (Mig), and IFN-inducible T-cell α-chemoattractant (I-TAC). These chemokines bind to chemokine receptor CXCR3 expressed by T cells in the atherosclerotic lesion. Once resident in the arterial intima, the T cell may encounter antigens such as oxidized low-density lipoprotein (Ox-LDL) and heat-shock proteins (HSPs) of endogenous or microbial origin, among others. Upon activation by engagement of the receptor and antigen, the T cell can produce cytokines that can influence the behaviour of other cells present in the atheroma. Notably, CD154 binding to CD40 ligand, particularly on macrophages, may induce the expression of tissue factor, matrix metalloproteinases (MMPs) and pro-inflammatory cytokines. The production of these mediators provides an amplification loop resulting from crosstalk between the prototypical cell of acquired immunity (the T lymphocyte) and that of innate immunity (the mononuclear phagocyte). Within the atheroma, as in other tissues, the helper T cells can polarize into those secreting generally pro-inflammatory cytokines (known as T_H1 cells) and or those secreting predominantly anti-inflammatory cytokines (denoted T_H2 cells). In general, T_H1 cells predominate in the atheroma. But experimental data in mice suggest that with extreme levels of hypercholesterolaemia the balance may shift towards T_H2 predominance. Recent evidence indicates that in abdominal aortic aneurysms, T_H2 cytokines predominate in contrast with the situation in occlusive atherosclerotic disease.

the role of M-CSF in the formation of atheromatous lesions. Mice lacking M-CSF show retarded lesion development with markedly reduced macrophage accumulation[20,21]. This effect occurred in mice lacking both apoE and the LDL receptor and depended on gene dosage[22]. Granulocyte–macrophage colony-stimulating factor (GM-CSF) may also promote inflammation in the atheroma. GM-CSF aids the survival of a population of mononuclear phagocytes that contain myeloperoxidase, an enzyme that gives rise to the pro-oxidant hypochlorous acid, a potential source of oxidative stress and inflammation in the human plaque[23].

These examples illustrate how specific candidates identified by descriptive studies have proven causally related to inflammation during atherogenesis using genetically altered mice. From the adherence to VCAM-1, to the chemoattractant response to MCP-1, to the activation by M-CSF, we are now beginning to understand the mechanisms by which mononuclear phagocytes and inflammatory signalling pathways participate in formation of the fatty streak, the initial lesion of atherosclerosis (Fig. 1).

Inflammation in atheroma progression and complication

After formation of the fatty streak, the nascent atheroma typically evolves into a more complex lesion, which eventually leads to clinical manifestations. Although past discussions neatly separated the progression and complication phases of atherosclerosis, we now recognize the blurred barriers between these different aspects of atherogenesis.

According to the traditional notion, fatty streaks evolve into complicated atheroma through multiplication of smooth muscle cells, which accumulate in the plaque and lay down an abundant extracellular matrix. As the lesion becomes more bulky, the arterial lumen narrows until it hampers flow and leads to clinical manifestations: in the coronary circulation, unstable *angina pectoris*, or acute myocardial infarction. Growth factors elaborated by macrophages in the atherosclerotic intima supposedly stimulated the smooth muscle replication responsible for lesion growth. According to the classical view, this process occurred in an inevitable and progressive fashion gradually during time.

Plaque disruption and discontinuous progression of atheroma

Clinical observations have challenged the concept of continuous growth of atheroma, prompting a re-evaluation of the biology thought to underlie atheroma progression. Data that emerged from serial angiographic studies suggest that many coronary arterial lesions in humans develop stenoses discontinuously. In patient populations successively undergoing angiography at three different times, smooth progression of the lesions proved the exception rather than the rule[24,25].

What might explain the apparent 'bursts' in growth of atheroma in these studies in humans? Observations on the microscopic pathoanatomy of atherosclerotic plaques provided clues. Current evidence suggests that physical disruption of plaques may trigger thrombosis and thus promote sudden expansion of atheromatous lesions[26]. Three types of physical disruption may occur[27].

Superficial erosion, or microscopic areas of desquamation of endothelial cells that form the monolayer covering the intima, occurs frequently in both humans and animals with experimentally induced atherosclerosis. Such areas of limited endothelial desquamation often form the nidus of a platelet thrombus as they uncover subendothelial collagen and von Willebrand factor that promote platelet adhesion and activation[28]. Although common and most often asymptomatic, such superficial erosion may account for approximately one-quarter of fatal coronary thromboses.

Disruption of the microvessels that form in atherosclerotic plaques furnishes another scenario for sudden plaque progression[29]. Atheromata develop microvascular channels as a result of neoangiogenesis. Like those that form in the diabetic retina, the new blood vessels in the plaque may be particularly fragile and prone to micro-haemorrhage. Multiple lines of evidence support thrombosis *in situ* within plaques during human atherogenesis. Intra-plaque deposition of fibrin and fibrin-split products and haemosiderin provide evidence of intra-plaque haemorrhage. The thrombosis *in situ* leads to thrombin generation, which, in addition to cleaving fibrinogen, can potently stimulate smooth muscle migration and proliferation. Thrombin triggers platelet release of growth factors such as platelet-derived growth factor (PDGF) from their alpha

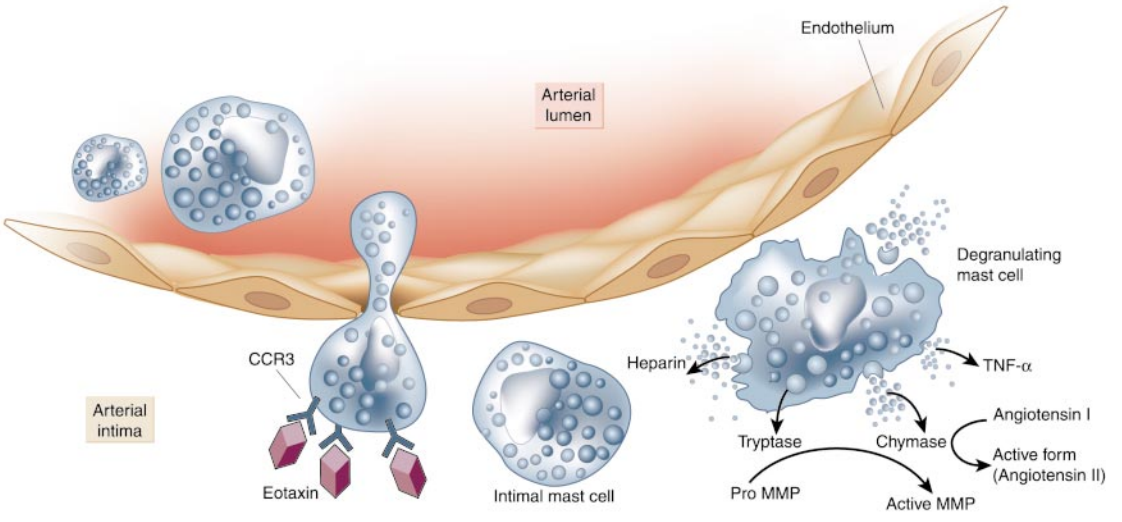

Figure 3 Recruitment and functions of mast cells in atherogenesis. The leukocytic infiltrate within atheromatous plaques includes a small but potentially important population of mast cells. Eotaxin, a chemoattractant that interacts with the chemokine receptor CCR3, may mediate the trans-endothelial migration of this specialized leukocyte. Once resident in the intima, the mast cell can undergo degranulation, releasing preformed tumour-necrosis factor-α (TNF-α), heparin with its anti-coagulant and potentially growth inhibitory effects on smooth muscle cells, and the serine proteinases tryptase and chymase. These proteinases may activate the inactive zymogen forms of matrix metalloproteinases (MMPs) to their proteolytic forms. Chymase may also generate active forms of angiotensin from their precursor, angiotensin I.

granules, further stimulating smooth muscle migration and proliferation. Activated platelets also elaborate transforming growth factor beta (TGF-β), the most potent stimulus known for interstitial collagen synthesis by smooth muscle cells. In this manner, a silent microvascular haemorrhage within the atherosclerotic intima could give rise to a growth spurt in the evolution of the plaque.

The third and most common mechanism of plaque disruption, a fracture of the plaque's fibrous cap, also involves inflammation (Fig. 4). The plaque's fibrous cap usually serves to sequester the thrombogenic lipid-rich core of the atheroma from the bloodstream, which contains circulating coagulation proteins. Fissure of the fibrous cap allows the coagulation factors contact with tissue factor, the main pro-thrombotic stimulus found in the lesion's lipid core. Although the ruptured fibrous cap causes some three-quarters of acute myocardial infarctions, like the other forms of plaque disruption, most episodes probably cause no clinical symptoms. When the prevailing fibrinolytic mechanisms outweigh the pro-coagulant pathways, a limited mural thrombus, rather than an occlusive and sustained blood clot, forms. With healing, however, resorbtion of the mural thrombus and the release of PDGF and the anti-inflammatory mediator TGF-β combine to engender a healing response that leads to fibrous tissue formation. The consequent smooth muscle accumulation and collagen accretion allow rapid evolution of a fatty lesion to one of more fibrous character (Fig. 4).

These examples illustrate the inextricable links between thrombosis and lesion progression. Usually below the clinical threshold, evolution of the lesion most often occurs silently, leading to transition from the fatty to the fibrous atherosclerotic plaque.

Inflammation causes various forms of plaque disruption

We know little of the mechanisms of superficial erosion of atherosclerotic plaques. Two processes related to inflammation may participate in endothelial desquamation. The first, endothelial cell death (perhaps by apoptosis) may result from local production of inflammatory mediators or cytolytic attack by activated killer T cells. Additionally, inflammatory mediators and oxidized lipoproteins can stimulate the expression and activation of matrix metalloproteinases (MMPs) specialized in degrading components of the sub-endothelial basement membrane[30]. Thus, inflammatory stimulation may promote the production by endothelial cells of enzymes that degrade the extracellular matrix constituents to which they adhere under normal circumstances. In this fashion, inflammation can promote loss of endothelium, the hallmark of superficial erosion.

The mechanisms of microvessel formation in atheroma probably resemble those common to other sites of angiogenesis. In addition to secreting growth factors for smooth muscle cells, inflammatory cells residing in the plaque, including macrophages, produce angiogenic mediators such as acidic and basic fibroblast growth factor and vascular endothelial growth factor (VEGF)[31,32]. Microvessels in plaques may not only serve as a site for haemorrhage *in situ* and thrombosis, but may also perform a nutritive function promoting plaque growth. Indeed, administration of inhibitors of angiogenesis retards microvessel formation and lesion evolution in atherosclerosis-prone mice[33]. The plaque microvasculature may therefore promote lesion evolution in two ways. The potential adverse effects of promoting plaque angiogenesis require consideration when contemplating strategies for promoting therapeutic angiogenesis in ischaemic hearts.

Among the forms of plaque disruption, we best understand fracture of the fibrous cap[34]. Interstitial collagen molecules confer most of the tensile strength on the fibrous cap[35], and several tightly regulated processes determine the level of collagen crucial for stability of this structure. Certain pro-inflammatory cytokines, such as IFN-γ, can inhibit collagen production by smooth muscle cells, the principle source of this extracellular matrix macromolecule in the arterial wall. Interstitial collagen fibrils usually resist proteolytic degradation, and only a limited number of interstitial collagenases can make an initial proteolytic nick in the collagen chains that make up the triple helical collagen fibril. We have found overexpression of all three human interstitial collagenases in atheromatous plaques (MMP-1, -8 and -13)[36–38].

After the limited proteolytic cleavage arising from the action of interstitial collagenases, gelatinases continue collagen catabolism. Extracts of atheroma show augmented active forms of two gelatinases (MMP-2 and MMP-9)[36]. Arteries do express the endogenous antagonists of MMPs, the tissue inhibitors of metalloproteinases (TIMPs). However, evidence for collagenolysis *in situ* indicates excess active forms of interstitial collagenases over the TIMPs in

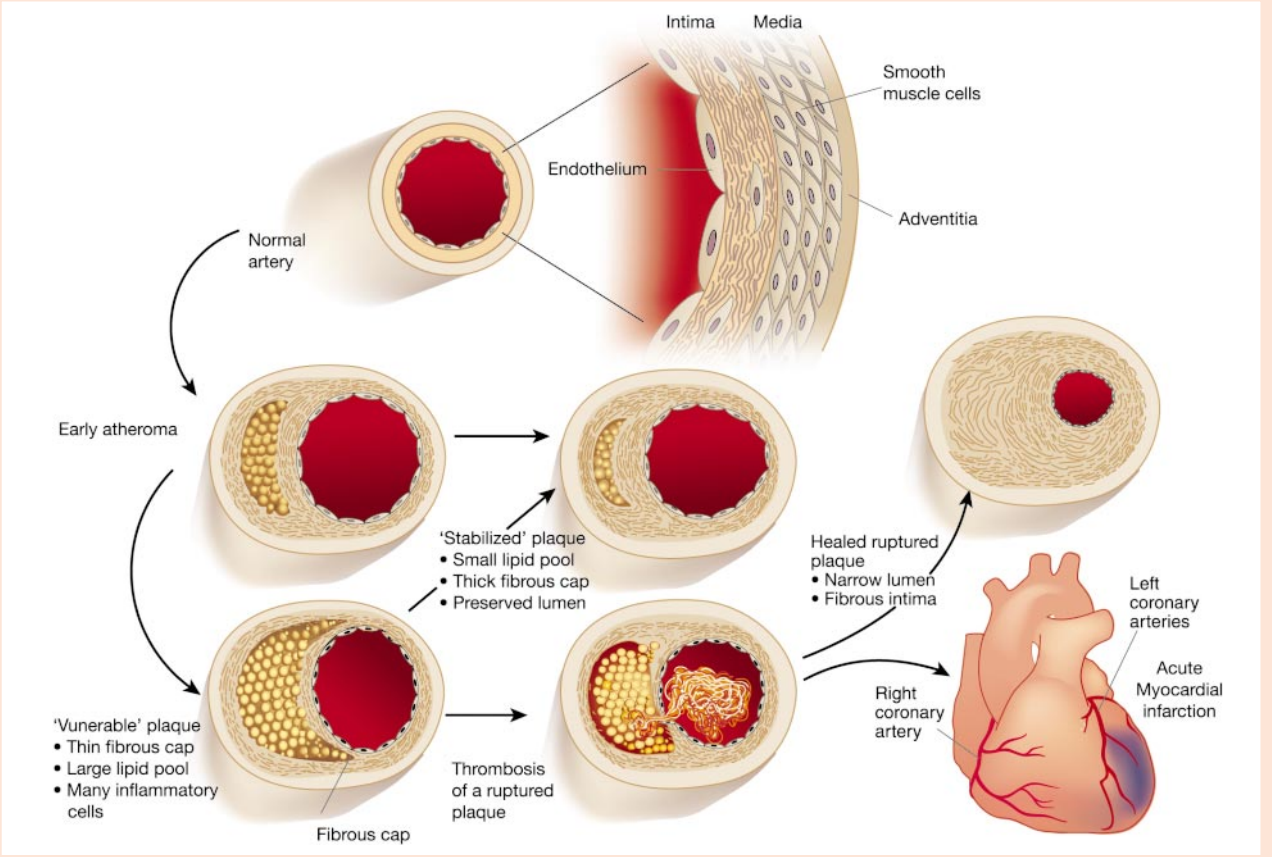

Figure 4 Schematic of the life history of an atheroma. The normal human coronary artery has a typical trilaminar structure. The endothelial cells in contact with the blood in the arterial lumen rest upon a basement membrane. The intimal layer in adult humans generally contains a smattering of smooth muscle cells scattered within the intimal extracellular matrix. The internal elastic lamina forms the barrier between the tunica intima and the underlying tunica media. The media consists of multiple layers of smooth muscle cells, much more tightly packed than in the diffusely thickened intima, and embedded in a matrix rich in elastin as well as collagen. In early atherogenesis, recruitment of inflammatory cells (Figs 1–3) and the accumulation of lipids leads to formation of a lipid-rich core, as the artery enlarges in an outward, ablumenal direction to accommodate the expansion of the intima. If inflammatory conditions prevail and risk factors such as dyslipidaemia persist, the lipid core can grow, and proteinases secreted by the activated leukocytes can degrade the extracellular matrix, while pro-inflammatory cytokines such as interferon-γ (IFN-γ) can limit the synthesis of new collagen. These changes can thin the fibrous cap and render it friable and susceptible to rupture. When the plaque ruptures, blood coming in contact with the tissue factor in the plaque coagulates. Platelets activated by thrombin generated from the coagulation cascade and by contact with the intimal compartment instigate thrombus formation. If the thrombus occludes the vessel persistently, an acute myocardial infarction can result (the dusky blue area in the anterior wall of the left ventricle, lower right). The thrombus may eventually resorb as a result of endogenous or therapeutic thrombolysis. However, a wound healing response triggered by thrombin generated during blood coagulation can stimulate smooth muscle proliferation. Platelet-derived growth factor (PDGF) released from activated platelets stimulates smooth muscle cell migration. Transforming growth factor-β (TFG-β), also released from activated platelets, stimulates interstitial collagen production. This increased migration, proliferation and extracellular matrix synthesis by smooth muscle cells thickens the fibrous cap and causes further expansion of the intima, often now in an inward direction, yielding constriction of the lumen. Stenotic lesions produced by the lumenal encroachment of the fibrosed plaque may restrict flow, particularly under situations of increased cardiac demand, leading to ischaemia, commonly provoking symptoms such as *angina pectoris*. Advanced stenotic plaques, being more fibrous, may prove less susceptible to rupture and renewed thrombosis. Lipid lowering can reduce lipid content and calm the intimal inflammatory response, yielding a more 'stable' plaque with a thick fibrous cap and a preserved lumen (centre).

human atherosclerotic plaques[37]. *In vitro* studies have shown that inflammatory mediators found in atheroma, such as IL-1β, TNF-α, and CD40 ligand (CD154), augment MMP expression in mononuclear phagocytes and endothelial and smooth muscle cells. Mast cells in the lesion may release the MMP inducer TNF-α as well as serine proteinases that can activate latent MMP proenzymes[39,40] (Fig. 3).

Converging lines of evidence point to the dynamic regulation of collagen levels in the plaque's fibrous cap. When inflammation prevails in the intima, smooth muscle cell production of new collagen required for repair and maintenance of the fibrous cap decreases. Meanwhile, collagen degradation increases due to overexpression of active MMPs. The net result, dissolution of the collagenous matrix of the fibrous cap, renders this structure weak, friable and susceptible to fracture when exposed to haemodynamic stresses. Indeed, pathologists categorize plaques as those exhibiting signs of stability, notably a thick fibrous cap, and those prone to rupture, having a thin fibrous cap and a scant collagenous skeleton on pathological examination.

Triggers for inflammation

Although the concept that inflammation occupies a central position in the pathophysiology of atherosclerosis has gained considerable currency, knowledge of the inciting factors remains remarkably sketchy. Much of the progress in understanding atherosclerosis over the past 50 years has depended on the lipid hypothesis. LDL cholesterol undoubtedly contributes importantly to atherosclerosis in many cases, and may indeed constitute a ubiquitous permissive factor for atherogenesis. However, most individuals with proven coronary artery disease in the United States have 'average' levels of cholesterol. ('Average' levels of cholesterol in developed countries probably exceed by far truly normal levels for our species as suggested by extrapolation

from data on animals and humans in agrarian societies.) Even extremely effective therapies targeting LDL cholesterol reduce coronary events by at most one-third over a five-year treatment period. Earlier or longer lipid-lowering therapy might further reduce the residual risk of atherosclerotic disease. However, addressing risk factors other than LDL cholesterol may also ameliorate atherosclerosis.

The strength of evidence supporting 'non-traditional', emerging risk factors in atherogenesis currently lags behind cholesterol, and further study is required to clarify their role. Examples of novel risk factors include lipoprotein (a), homocysteine, infectious agents such as herpesvirus and *Chlamydia pneumonia*, and oxidant stress evoked by the pressor hormone angiotensin II. The view of angiotensin II as a pro-inflammatory and pro-oxidant stimulus furnishes a satisfying link between the mechanisms at play in hypertension and its common companion, atherosclerosis.

As an epidemic of obesity sweeps the world, with insulin resistance and diabetes close behind, the so-called 'metabolic syndrome' has emerged as one of the main contributors to risk for atherosclerosis. Adipose tissue itself can give rise to cytokines that worsen insulin sensitivity, and provide a systemic pro-inflammatory stimulus. In the metabolic syndrome, LDL levels often remain in the average range, although the particles may have qualitative alterations that render them small and dense, making them particularly prone to oxidation and hence evoking inflammation. The low levels of high-density lipoprotein (HDL) that characteristically accompany the elevated triglycerides in the metabolic syndrome blunt another endogenous anti-inflammatory and hence atheroprotective mechanism[41]. HDL particles may owe their protection against atherosclerosis not only to reverse cholesterol transport, but also to provision of antioxidant enzymes such as paraoxonase and platelet-activating factor acetyl hydrolase. Persistent hyperglycaemia in diabetes can accelerate the formation of advanced glycation end-products, yet another trigger to arterial inflammation[42]. Thus, in addition to LDL, many putative non-traditional factors may aggravate atherogenesis by promoting inflammation.

Inflammation as a therapeutic target in atherosclerosis

Our new understanding of the pivotal position of inflammation in the pathogenesis of atherosclerosis raises questions and opens opportunities in prevention and therapy of this disease. A series of large, well-designed, randomized and controlled clinical trials have recently established the utility of several different pharmacological strategies for preventing recurrent myocardial infarction or death beyond the recognized roles of aspirin and β-adrenergic blocking agents. Newer drug classes shown to be effective in this regard, and listed in decreasing order of the strength of evidence, include inhibitors of hydroxymethylglutaryl coenzyme A (statins); angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers; and fibric acid derivatives (activators of the nuclear receptor/transcription factor peroxisome proliferator-activated receptor-α or PPAR-α). The success of these categories of agents in the clinic has prompted intense investigation, in the context of inflammation biology in atherosclerosis, to garner a more complete picture of the mechanism(s) of the clinical benefit observed.

For example, statins not only inhibit cholesterol synthesis, but also block the production of isoprenoid intermediates such as farnesyl- or geranylgeranyl-pyrophosphates, which are important in modifying small G proteins, among other biochemical effects. A number of laboratory studies have addressed the hypothesis that the non-lipid-lowering effects of statins may contribute to their clinical benefit. The possible 'pleiotropic' effects of this class of drugs include anti-inflammatory actions such as reduction in leukocyte adhesion, and antagonizing aspects of macrophage activation including replication, metalloproteinase production, and tissue factor procoagulant gene expression[43].

The degree to which certain clinical benefits of statins derive from such direct anti-inflammatory effects remains controversial. Many of the *in vitro* studies that demonstrate statin-induced reduction in pro-atherogenic functions of isolated cells have used concentrations of these agents not likely to be achieved in tissues clinically. In addition, pravastatin, which is relatively cell-impermeant owing to its hydrophilicity compared to most other statins, lacks such *in vitro* effects, but has proven effective in reducing cardiovascular events in multiple clinical trials. Statins certainly do stem inflammation in patients with atheroma, as gauged by the marker C-reactive protein (CRP)[44]. However, the degree of lowering of CRP correlates poorly with a patient's drop in LDL, hinting that some of the anti-inflammatory effect may not derive simply from a lipid-lowering action.

Just as reduced LDL may not account for all of the benefits of statins, recent clinical trials suggest benefits of interrupting angiotensin II signalling that are not accounted for by the degree of blood pressure lowering[45,46]. Indeed, angiotensin II's actions extend far beyond vasoconstriction. Considerable evidence now supports a role for angiotensin II as a pro-inflammatory mediator, elevating it to the category of an 'honorary' cytokine[47]. For example, this peptide can elicit VCAM-1 and MCP-1 expression by endothelial cells, and IL-6 production by smooth muscle cells.

The recent clinical success of fibric acid derivatives in certain patient populations, including those with diabetes or diabetic-like insulin-resistant states, has stimulated intense interest in the PPAR-α pathway. PPAR-α agonism increases the synthesis of apoA1, the main apoprotein of HDL, a particle that protects against lesion formation, probably owing to its role in reverse cholesterol transport (removing cholesterol from the artery wall and delivering it to peripheral tissues and the liver). Other laboratory studies have established that PPAR-α agonists also possess anti-inflammatory properties of potential relevance to atherogenesis. For example, these agents can reduce VCAM-1 and tissue factor gene expression by cells found in atheroma[48–50]. Interference with the activation of NF-κB, resulting from competition for co-activators, may explain part of this anti-inflammatory action of PPAR-α agonism[51].

These examples provide illustrations of unexpected anti-inflammatory effects of existing therapies for atherosclerosis. Uncovering inflammatory pathways has raised the possibility that future treatments may target effectors of inflammation directly to add to the benefit of current treatments. Potential targets include proximal triggers such as infectious agents, central signalling hubs in inflammation such as NF-κB, and distal effectors such as MMPs, adhesion molecules, and the like. Targeting NF-κB transcription pathways for a chronic disease such as atherosclerosis may well prove impractical given the key role of inflammation and innate immunity in normal host defences. The redundancy of distal effectors of inflammation suggests to me that narrow-spectrum inhibition may not effectively modify the disease process, while broad blockade of these mediators will impair host defences much as would interruption of NF-κB activation. I foresee targeting the proximal triggers as the most promising strategy for interrupting inflammation in atherogenesis.

Inflammatory markers as gauges of atherosclerotic risk

As noted above, many individuals develop coronary heart disease in the absence of abnormalities in the lipoprotein profile. The availability of effective therapies for preventing even a first myocardial infarction renders imperative the need to identify individuals at risk for concerted intervention before problems manifest. Based on the evidence supporting a role for inflammation in the pathogenesis of atherosclerosis, serum markers of inflammation have garnered substantial interest as markers of atherosclerotic risk; these add to the information available from traditional measures such as the lipid profile.

One of these markers, CRP, has proven remarkably robust as a marker of cardiovascular risk. Plasma CRP, an acute phase reactant produced primarily by the liver in response to inflammatory cytokines such as IL-6, prospectively identifies asymptomatic individuals at risk for coronary events. Although many candidates as novel markers of risk exist, they must meet several criteria to prove

clinically useful. The marker must have a rigorously standardized and reproducible assay, be relatively stable from day to day in a given individual, and add to estimates of risk provided by established markers such as the lipid profile as determined in prospective study. The promise of CRP in this regard has engendered clinical trials that will test its ability to guide preventive therapy in apparently well individuals. We therefore stand on the threshold of clinical application of the basic biology of inflammation in atherosclerosis that could fundamentally alter the way in which we practice preventive medicine and prove immeasurably beneficial to the public as well. □

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