

## Review

# Atherosclerotic plaque rupture – pathologic basis of plaque stability and instability

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## 1. Introduction

Atherosclerosis continues to be one of the main subjects in pathology research. The intriguing complexity of its pathogenesis as well as the importance of its clinical sequelae provide a rationale for this [1]. A large number of diseases with totally different clinical presentations are basically atherosclerosis related, and among these, myocardial infarction, stroke, abdominal aneurysms and lower limb ischemia determine to a large extent the morbidity and mortality in Western style populations. But, despite this broad spectrum of clinical disease, most of the acute manifestations of atherosclerosis share a common pathogenetic feature: rupture of an atherosclerotic plaque [2–4]. Plaque disruptions may vary greatly in extent from tiny fissures or erosions of the plaque surface to deep intimal tears which extend into the soft lipid core of lesions; in all these instances, at least some degree of thrombus formation occurs [5,6]. The abdominal aorta is the arterial site most prominently involved in the process of plaque formation, and also of plaque complications. In this large diameter vessel the process of plaque disruption and thrombosis is not ended by luminal occlusion, and may lead to extensive surface ulcerations comprising large areas of the aortic wall, as can be observed in many autopsy cases at older age. Apart from the undisputable role of atherosclerosis in abdominal aneurysm formation [7], mural thrombosis leads to a surprisingly low rate of clinically significant complications in these patients, although cholesterol emboli can be regularly found in their kidneys and skin at autopsy. Still, it is presently unclear what impact the various biologically active mediators

released from eroded aortic surfaces may have on the human body.

In contrast, in small diameter vessels such as coronary arteries, occlusive thrombosis is a frequent and often fatal complication of plaque rupture, and even smaller not occluding thrombi may lead to clinical symptoms [5,6]. In coronary arteries, therefore, plaque disruption has been studied most extensively, and a number of correlations have emerged between the morphology of the culprit plaques, the degree of thrombus formation and types of ensuing ischemic coronary syndromes of patients [5,6,8]. These observations have led to a concept of unstable atherosclerotic plaques: plaques with an unstable morphology giving rise to the onset of unstable coronary artery disease.

Many research efforts have been devoted to the identification of such unstable plaques; this article tends to emphasize the central role of intrinsic plaque features in the process of plaque rupture and thrombosis.

## 2. Stable and unstable plaques

Atherosclerotic plaque formation results from complex cellular interactions in the intima of arteries, which take place between resident cells of the vessel wall (smooth muscle cells and endothelial cells) and cells of the immune system (leukocytes). Local flow disturbances and lipids as a driving force appear to be obligatory in this process [1]. Once an atherosclerotic plaque has formed, it shows the highly characteristic architecture of a fibrous cap encasing a central core of extracellular lipids and debris: the ‘atheroma’. Fibrous tissue provides the structural integrity

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of a plaque. On the other hand, the atheroma is soft, weak and highly thrombogenic. It is rich in extracellular lipids and practically devoid of living cells, but is bordered by a rim of lipid-laden macrophages. Unlimited phagocytosis of oxidized LDL by macrophages through scavenger receptors with a high ligand specificity for ox-LDL results in the formation of foam cells, which is another hallmark of atherosclerosis [9]. Foam cell death plays an important role in the formation and growth of the atheroma, together with extracellular binding of lipids to collagen fibers and proteoglycans [10,11].

Less well known are the quantitative differences in these structural components: histopathologic examinations of a large series of plaques have revealed substantial variations in the thickness of fibrous caps, in the size of atheromas, in the extent of dystrophic calcification and, as has been shown more recently, in the relative amounts of major cell types: and inflammatory cells [12,13]. This notion is of importance, since only specific types of lesions in this spectrum of morphologic variants appear to be associated with acute manifestations of atherosclerotic disease.

### *2.1. Ratios of fibrous tissue and lipids in plaques*

Any combination of cap thickness and atheroma size may occur. But, the extremes at both ends of the spectrum appear to have a totally different clinical outcome. Essentially clinically stable are fibrous plaques, composed of solid fibrous or fibrocellular tissue, and only small amounts of extracellular lipid or no lipid at all. In coronary arteries most of these lesions remain clinically silent, or on the long term, may lead to stable angina pectoris [5]. On the other hand, typically vulnerable plaques are characterized by large lipid pools and have a thin or virtually absent fibrous cap. At autopsy, lipid-rich plaques are frequently found underlying coronary thrombosis [3,14,15]. Atherectomy specimens obtained from patients with unstable coronary artery disease frequently contain more fragments of extracellular debris than those of patients with stable angina [16–18]. Lipid plaques, therefore, are considered ‘rupture prone’. Plaques derived from the aorta also show a clear relationship between the size of the lipid core and rupture events. In these plaques, easily accessible for quantitative investigations, a critical threshold for vulnerability to rupture of more than 50 volume percent of extracellular lipids was established by Davies et al. [12].

But, certainly not all the plaques in patients with stable coronary artery disease fulfil these criteria for stability. Hangartner et al. have provided a description of the histology of 448 plaques in coronary arteries of 54 men with stable angina. Sixty percent of these plaques were fibrous, but 40% had a pool of extracellular lipids. In only 15% of these patients, all the plaques causing >50% stenosis were fibrous, while in 13% of patients virtually all plaques had a lipid core. In fact, most patients had mixtures of plaque types in varying proportions [19]. On

the other hand, lipids pools appear to be not the only determinant of plaque instability. In a study on 20 thrombosed coronary arteries in our laboratory, the classical lipid-rich morphology was found indeed in 50% of the underlying ruptured plaques, but the other 50% had either a substantial fibrous cap (25% or more of the entire plaque thickness) or were almost completely fibrous in composition, albeit all with surface erosions [20].

Differences in histological composition inside the plaque and its relation to the geometry of the arterial wall have implications for the biomechanical properties of plaques. Studies using computer modeling of plaques have identified circumferential tensile stress on the fibrous cap as the most important intrinsic mechanical stress factor involved in plaque rupture [15,21]. Most plaques rupture at sites of high calculated circumferential stress, which is often at the periphery of eccentric plaques. These studies also showed the importance of the thickness of a fibrous cap (thickness in millimetres being inversely related to the peak stress in the cap), and the stenosis rate (circumferential stresses in the plaques gradually decreased when stenosis severity increased) [21]; it gives at least one explanation for the fact that many plaques rupture at a stenosis rate of less than 50%.

Intrinsic mechanical forces clearly contribute to the process of plaque rupture, but of equal importance is the tissue composition of the fibrous cap. Lendon et al. [22] tested the mechanical strength of human fibrous cap tissue and observed significantly reduced maximum stress at fracture when fibrous caps are infiltrated with macrophages. Richardson et al. [15] showed that regions with high circumferential stress correlated with sites of rupture. However, the actual sites of rupture were also influenced by variations in the mechanical strength of the fibrous cap due to accumulations of lipid-laden macrophages. It shows why lipid plaques with attenuated fibrous caps are more vulnerable to rupture, but moreover emphasizes the importance of cellular infiltrations in the cap, particularly the presence of inflammatory cells (Fig. 1).

### *2.2. Inflammatory activity in the plaque*

The continued recruitment of T-cells and macrophages at sites of ‘dysfunctional endothelium’ appears to be a constant feature of lesion initiation and progression as it accentuates the chronic inflammatory nature of atherosclerosis [23]. The recruitment appears to be specific for macrophages, T lymphocytes and mast cells [24–27], and the arterial endothelium covering the plaque surface is considered as the principal site of entrance for these cells [1]. However, recently the neovascularisation at the base of the atheroma and in the shoulder parts of advanced plaques attracted renewed attention in this respect. The same shoulder parts of eccentric lesions represent the vulnerable sites of plaques where most ruptures take place. Several adhesion molecules are expressed on the endothelium of

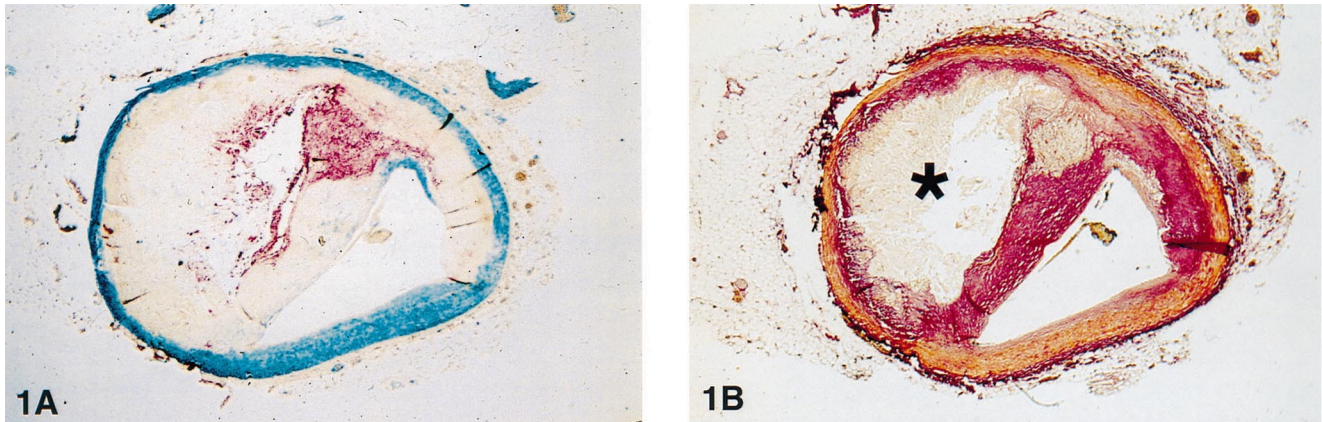


Fig. 1. (A) Example of an intact (non-ruptured) eccentric lipid-rich plaque in a coronary artery. There is focal accumulation of macrophages (red), creating a vulnerable site in the periphery of the fibrous cap. Smooth muscle cells (blue) are in the media and focally in the fibrous cap where they cover the site of macrophage accumulation. (Anti-CD68/anti- $\alpha$ -actin immunodouble stain). (B) Adjacent section stained with Picro Sirius Red (collagen red, media yellow) shows a decrease in collagen density where the macrophages have accumulated. Asterisk is in the lipid core of the plaque.

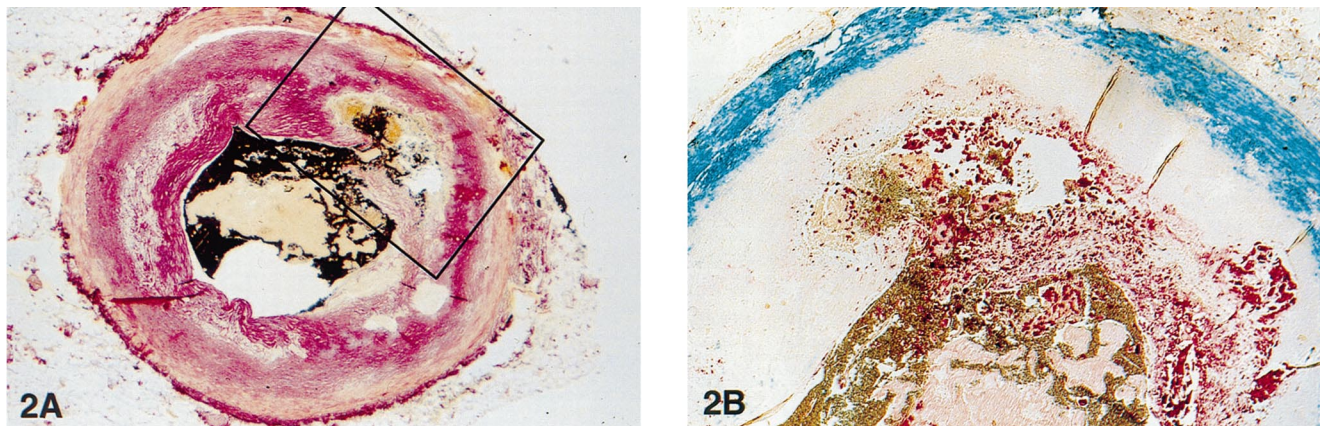


Fig. 2. (A) Coronary plaque of a 67 year old male, containing an eccentric mildly stenosed plaque with complete disruption of the fibrous cap (boxed area), mural thrombus and hemorrhage into the lipid core. (Picro Sirius red stain: collagen red). (B) Detail of the boxed area in (A). An adjacent tissue section shows accumulations of macrophages (red cells) at the rupture site. Smooth muscle cells in the media stain blue. (Anti-CD68/anti- $\alpha$ -actin immunodouble stain).

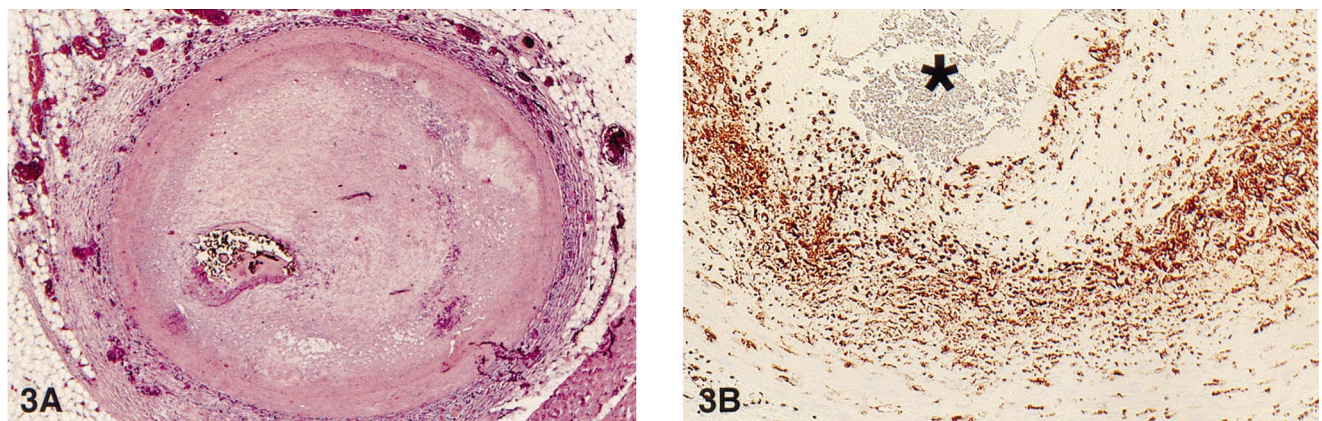


Fig. 3. (A) High grade stenosing lesion with occlusive thrombosis of a 32 year old male who died instantly of acute myocardial infarction. The plaque is largely fibrocellular/fibrosclerotic and contains only small deeply located atheromas (hematoxylin–eosin stain). (B) shows a detail of the erosion underneath the thrombus. There is abundant anti HLA-DR reactivity on plaque cells indicating active inflammation. Asterisk is in the thrombosed lumen. (anti-HLA-DR immunostain).

capillary vessels in intimal plaques, and their counter structures (ligands) are found on T cells and macrophages in the vicinity of these vessels [28,29]. Microvessels create an alternative and probably more easily accessible pathway for leukocytes to enter the so called ‘rupture prone’ sites of advanced plaques.

Fully developed plaques contain highly variable amounts of inflammatory cells, but largest concentrations can be found in lipid-rich lesions where they occupy the attenuated cap, the shoulder parts of the lesions or both [12,13]. Moreover, inflammation appears to be associated also with the initiation of plaque rupture, a notion derived from several clinicopathological investigations using autopsy materials and atherectomy specimens.

### 2.2.1. *Acute myocardial infarction*

In a series of 20 acute myocardial infarction related thrombosed coronary arteries of patients who died acute or within 2 days after the onset of the infarct, we noticed abundant infiltration of activated T cells and macrophages at the immediate site of erosion or rupture in 19 cases. This was in contrast to the overall morphology of the ruptured lesions, which was heterogeneous both with respect to plaque architecture (lipid or fibrous) and presence or absence inflammation [20]. Moreover, densities of and interstitial collagen were either low in the entire cap, or decreased at rupture sites compared with the adjacent plaque tissue. Disruptions in these cases were either deep ruptures (60%) (see also Fig. 2) or erosions of the plaque surface (40%, of which one showed hardly no inflammation) (see also Fig. 3). Kovanen et al. extended these observations by identifying in addition neutral proteases releasing mast cells as participants in the inflammatory process, providing another indication for active inflammation at rupture sites [30]. And more recently, active plaque inflammation associated with plaque rupture could be demonstrated also in carotid artery plaques obtained from stroke patients [31]. However, in an autopsy study primarily focused on plaque erosion as the underlying cause of coronary thrombosis, this type of disruption was also identified in a proteoglycan-rich and smooth muscle-rich type of plaque. These lesions, which occur more often in younger individuals and in women, have less often or smaller foci of inflammation [32].

### 2.2.2. *Unstable angina*

Pathologic analysis of coronary atherectomy specimens allowed the further investigation of the relationship between plaque inflammation and acute plaque events, also in patients with less severe coronary artery disease. In these retrospective comparative studies several histopathological parameters of plaque inflammation have been analyzed and quantified in tissue specimens of culprit lesions and correlated with the clinical status of the patient

(either chronic stable angina or one of the various forms of unstable angina). When compared with lesions underlying chronic stable angina, the lesions of patients with unstable coronary syndromes contain significantly larger amounts of inflammatory cells [17,18,33], including activated inflammatory cells, as indicated by the expression of HLA-DR molecules on cells [18]. Other findings of interest concern the various inflammatory products released by cells in unstable plaques: increased numbers of macrophages producing the proteolytic enzyme gelatinase B (MMP9) [34,35], the inflammatory cytokine TNF- and tryptase-producing mast cells [28], vasoactive substances such as angiotensin I [36] and endothelin [37], larger amounts of the thrombosis initiator Tissue Factor [38,39], and increased numbers of Interleukin-2 receptors on T cells (as marker for acute T cell activation in unstable lesions) [40]. Interesting clinicopathological correlations, demonstrating that inflammation can be seen as a marker for plaque instability.

### 2.2.3. *Stable angina*

It is important to note that the culprit plaques of clinically stable patients do not always appear stable histologically. We investigated coronary atherectomy specimens of 58 patients with clinically well defined coronary artery diseases, of which 28 had chronic stable angina of more than 2 months duration without progression. These were compared with atherectomy tissues of patients with either ‘stabilized’ unstable angina or the more severe type of ‘acute onset’ unstable angina. This study revealed on the average larger tissue areas infiltrated with macrophages and larger amounts of lymphocytes in patients with unstable angina, and larger tissue areas occupied by smooth muscle cells in patients with stable angina. But, despite these differences an overlap in the extent of inflammation was noticed between the groups of stable and unstable patients; at least a number of stable patients had considerable amounts of inflammatory cells in their culprit lesions. This is illustrated in Fig. 4. Moreover, this study and several other atherectomy investigations documented fragments of thrombus in substantial numbers (up to 20%) of apparently stable plaques [16–18,33,41,42]. Therefore, clinical stability does not always indicate biologic stability in terms of (absence of) inflammation and thrombus formation. Ongoing inflammation or a rapid progression of growth due to thrombus organization could imply a progression to unstable syndromes [18,42]. It might explain the angiographically detected rapid progression of stenosis and the onset of acute events in patients with stable angina who were placed on a waiting list for non-urgent coronary angioplasty [43,44].

An overall impression emerging from these investigations is that the biological (inflammatory) state of lesions must be considered of prime importance in determining the clinical outcome of patients with coronary atherosclerosis.

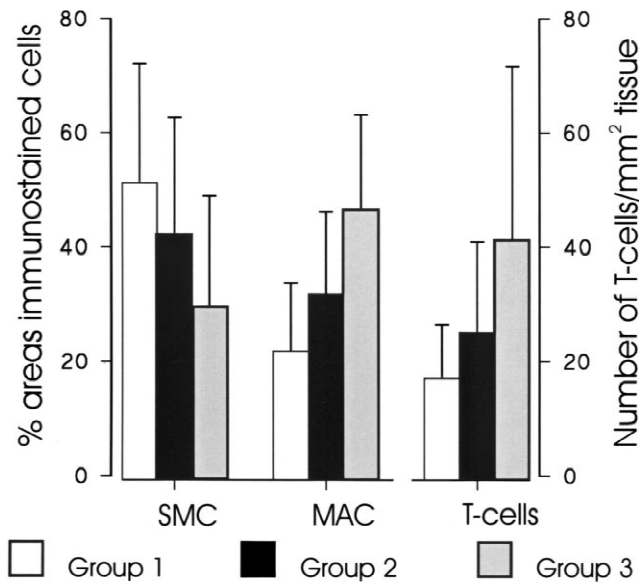


Fig. 4. Correlations between the cellular composition of atherectomy specimens taken from coronary culprit lesions and the corresponding clinical symptoms of 58 patients with ischemic coronary syndromes. Percentages of tissue areas occupied by smooth muscle cells (SMC) and macrophages (MAC) are quantified planometrically and the number of T cells are counted per mm<sup>2</sup> in immunostained sections. The atherectomy specimens are grouped according to the type of coronary syndrome. Group 1: chronic stable angina; Group 2: 'stabilized' unstable angina (Braunwald class I, II); Group 3: 'refractory' unstable angina (Braunwald class III).

### 3. Instability and stability: a balance between inflammation and repair

A principal feature of inflammation is the accumulation of leukocytes; in longstanding chronic inflammatory processes these leukocytes are macrophages, lymphocytes and mast cells. Basically the leukocytes have a protective function and serve in host defense by eliminating injurious agents, but their secretory products may also augment injury by damaging surrounding tissue components. During inflammation another series of events is initiated that must lead to the healing of injured tissue, either by regeneration or by scarring (fibrosis). In this respect inflammation is closely related to a process of repair. Over the past years several studies have pointed out that both inflammation and repair are key events in the natural course of atherosclerosis [1,23,45,46], and there is a great deal of evidence that inflammatory cells and their secretory products have profound effects on the integrity of the connective tissue meshwork of plaques [47].

#### 3.1. Smooth muscle cells: repair cells of the plaque

Smooth muscle cells produce by far most of the extracellular matrix components of a plaque, including collagens, elastin and various types of proteoglycans [48].

Transforming growth factor beta (TGF- $\beta$ ) is one of the most potent stimulators of connective tissue production by smooth muscle cells [49]. Large amounts of this growth factor are detected in restenosis lesions after PTCA [50], and it also participates in the repair process after natural plaque disruption. Thrombin generated during episodes of local thrombosis is another stimulator of smooth muscle cell growth. TGF- $\beta$  and other growth factors, including platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), play an important role in wound healing and the reparative stage of many chronic inflammatory diseases. In the atherosclerotic plaque these growth factors are produced by 'injured' endothelial cells and macrophages or released from thrombus [1,51]. Smooth muscle proliferation and matrix synthesis implies a mechanism of slowly progressive growth of plaques; it serves to encapsulate the soft atheroma and organizes episodes of thrombus formation, either spontaneously or artificially induced. The result is a reparative and stabilizing effect on the plaque structure [46,47,51].

#### 3.2. Lipids and inflammatory cells

Several mediators produced by activated T-lymphocytes and macrophages in plaques promote destabilizing effects. The T-cell cytokine IFN- $\gamma$  appears to play an important role in this process, by inhibiting the proliferation of smooth muscle cells, as well as decreasing their synthesis of collagen fibrils [47].

Apoptosis, an intrinsically programmed mode of cell death, can be activated by inflammatory mediators, and is recognized as a mechanism of foam cell death in plaques. Cell death leads to the spill of lipids and, hence, the enlargement of the soft lipid core [52]. Apoptosis of cells has been observed in atherosclerotic plaques and in restenosis lesions after PTCA [53,54]. In early lesions and in restenosis lesions, smooth muscle cell apoptosis could have beneficial effects and promote regression, but in the fibrous cap of advanced lesions it introduces another potential of plaque destabilization through the loss of repair cells.

In addition, during inflammation an even more powerful pathway of plaque desintegration is initiated by the extracellular matrix degrading metalloproteinases interstitial collagenase (MMP1), stromelysin (MMP3) and the gelatinases MMP2 and MMP9 [55,56]. Once activated by plasmin or mast cell products, they initiate a cascade of proteolytic activities with a very broad substrate specificity, including all the extracellular matrix components of the fibrous cap [57]. An observation of particular interest is that synthesis as well as lytic activity of these enzymes is most abundant in the lipid laden macrophages and in the extracellular space around lipid cores of plaques [56]. Studies on experimental atheromas have endorsed these observations: isolated lipid laden macrophages ob-

tained from the aortic wall of cholesterol fed rabbits spontaneously synthesize and release metalloproteinases, whereas alveolar macrophages derived from the same animals and under the same circumstances do not [58]. These observations provide a link between lipids and inflammation, and furthermore could give at least one explanation why the lytic effects of inflammation are most prominent in lipid-rich plaques (Fig. 5).

The preference of inflammatory cells for lipid plaques as alluded to earlier is not coincidental, and presently there are several arguments in support of an intriguing relationship between lipids and inflammation. First, during activation of the scavenging pathway for phagocytosis of oxidized lipoproteins, macrophages exert a number of secretory functions with detrimental effect on the plaque tissue [9,59–61]. In addition, specific T cell mediated immune responses appear to be involved in atherogenesis, and there is increasing evidence that a direct link may exist between accumulation of cholesterol in the vessel wall and activation of T cells, possibly by autoimmune responses to modified lipoproteins [59,62–64]. In human plaques, clusters of lymphocytes are regularly observed in close proximity of ceroid pigments [40], which are considered as an end product of lipid oxidation [61].

In conclusion, it appears that during the ongoing process of lesion formation, and also in mature clinically relevant plaques, two major tissue remodeling forces may be operative. Smooth muscle cells increase the structural strength by producing the connective tissue matrix of a plaque. On the other hand, lipid associated inflammation introduces tissue degrading effects. Whether a plaque tends to stability or instability will depend on which mechanism dominates the course of plaque formation in a given period of time. This view is illustrated in Fig. 6. Accordingly, in extreme cases that occur at both ends of the spectrum, plaques may arise with a totally different cellular composition. This is illustrated in Figs. 7 and 8, which show examples of a plaque composed of a large lipid core with an extremely attenuated cap and infiltrated by large amounts of macrophages (extremely vulnerable, Fig. 7), and a fibrous lesion of which the cellular component consists almost solely of smooth muscle cells (typically stable, Fig. 8).

### 3.3. Pathologic evidence derived from coronary atherectomy studies

Studies on atherectomy specimens of patients with different clinical ischemic syndromes have provided some circumstantial evidence for this concept. An interesting relationship was seen between the amounts of inflammatory cells in the lesions and the *severity* of various unstable ischemic syndromes [18,33,40]. A gradual increase in macrophage and T cell content can be noticed from the lesions of patients with chronic stable angina to the severest types of unstable angina. In contrast, a gradual

decline in the average tissue areas occupied is noticed in the same series of patients from stable angina to the severest types of unstable angina [18] (Fig. 4). These observations nicely reflect the balance between inflammation and repair mechanisms in lesions of patients with different types of coronary syndromes.

## 4. The acute ischemic event: a multifactorial process

Despite the pivotal role of lipids, inflammation and repair in determining the vulnerability of a plaque to rupture, the onset of an acute ischemic event depends on a complex interplay of variables in and outside the plaque. Certainly, external factors including systemic thrombotic factors and ‘rupture triggers’ such as vasospasms or elevated blood pressure will play a role in the ultimate thrombotic occlusion of the vessel or reduction of flow between a critical threshold [6,65,66]. These external factors will not be discussed, but still some other plaque features require attention.

### 4.1. The spectrum of plaque disruption and thrombus formation

Most plaques that develop during a lifetime remain unnoticed, but plaque disruption and thrombus formation are not uncommon features. Any form of endothelial denudation leads to activation of the coagulation system due to exposition of highly thrombogenic plaque constituents (lipids, tissue factor, collagens) to the blood stream. Microscopic foci of endothelial loss associated with platelet thrombi are present on the surface of in many advanced plaques [67]. They have no clinical implications at least on the short term, but may stimulate plaque growth through thrombin and PDGF related stimulation of smooth muscle growth and matrix synthesis. Larger, but apparently clinically silent ruptures have been observed also at autopsy in coronary arteries of 9% of persons who died of non-cardiac disease, increasing to 22% in those with diabetes or hypertension. Most of these are intra plaque hemorrhages, due to entrance of blood into the lipid core of the lesion and followed by healing of the rupture [68]. In another study which included 47 patients who died of myocardial infarction, a total of 103 ruptured plaques were identified and only 40 of these ruptures could be associated with the infarct related coronary thrombosis [69]. Again, organization of these mural thrombi or intra plaque hemorrhages may lead to a phase of rapid plaque growth through a repair process of smooth muscle cells growth and connective tissue deposition. Large mural thrombi due to large surface erosions or superficial fissures in the fibrous cap have found in many of the lesions underlying unstable angina. Deep intimal tears which extend into the highly thrombogenic lipid core of lesions, and sometimes showing extrusion of parts of the atheroma are often associated

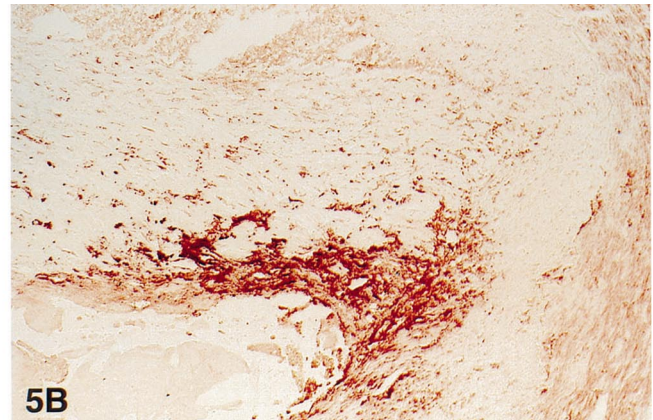
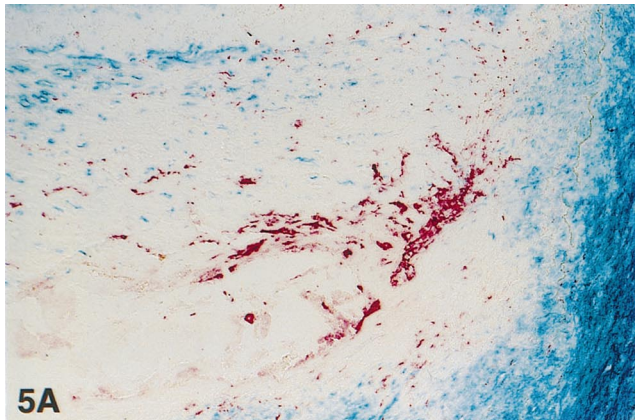


Fig. 5. (A) Detail of a lipid-rich plaque, showing part of the lipid core, bordered by foam cell macrophages (red cells), and smooth muscle cells (blue cells) in the fibrous cap (anti-CD68/anti- $\alpha$ -actin immunostain). (B) Adjacent section shows abundant stromelysin-1 (MMP3) staining of foam cell macrophages around the lipid core, and to a lesser extent some smooth muscle cells in the fibrous cap (anti-MMP3 immunostain).

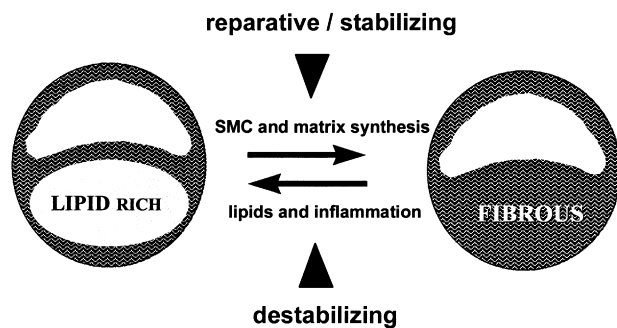


Fig. 6. Schematic view of the two major tissue remodeling forces in atherosclerotic plaques. On the left side is a lipid-rich plaque. On the right side is a fibrous plaque. Arrow to the left indicates the destabilizing effects of lipids and inflammation, thus creating a vulnerable lipid-rich plaque. Arrow to the right indicates the reparative effects of smooth muscle cells, leading to the formation of a stable fibrous plaque.

with massive thrombus formation. They are found in most cases of acute transmural myocardial infarction. However, in young patients and females, plaque erosions are a more common cause of coronary thrombosis underlying myocardial infarction [32].

#### 4.2. Plaque volume and stenosis rate

Much attention has been devoted to the rate of luminal stenosis and plaque volume of lesions that underlie coronary thrombosis. Indeed, many plaques that underlie coronary thrombosis are high grade stenotic lesions. However, data from both pathologic and angiographic studies on large series of patients indicate that most lesions that underlie coronary complications such as unstable angina or acute myocardial infarction, are only mildly to moderately

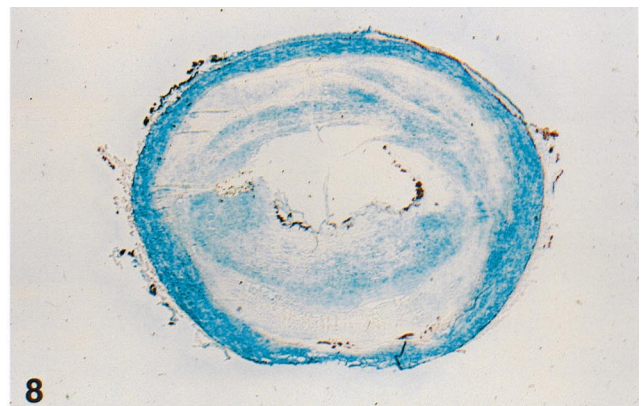
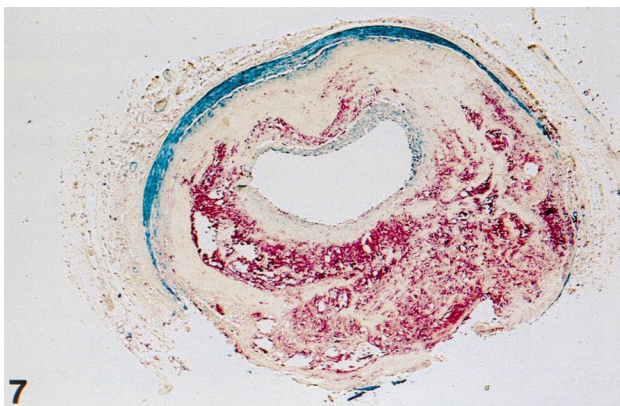


Fig. 7. Cross-section of an atherosclerotic plaque, which is heavily infiltrated with macrophages (red) and contains only scarce smooth muscle cells (blue) in the fibrous cap (anti-CD68/anti- $\alpha$ -actin immunodouble stain).

Fig. 8. This atherosclerotic plaque contains almost solely smooth muscle cells (blue) and is practically devoid of macrophages. Although basically expressions of the same disease, the plaques in Fig. 7 and Fig. 8 show a completely different cellular composition. In both sections the same immunodouble staining is applied (anti-CD68/anti- $\alpha$ -actin immunodouble stain).

stenotic [70,71]. Local arterial dilation is a well-known and important mechanism of compensatory enlargement of the vessel at sites where plaques grow [72]. For this reason, large plaques may angiographically be visualized as only mildly stenotic. However, Pasterkamp et al. extended this view by using entire (atherosclerotic) arteries to investigate atherosclerosis associated vascular wall remodeling. They also observed dilation at sites of atherosclerotic plaques, but in other instances (plaques) shrinkage of the vessel wall leading to lumen narrowing could be objectivated [73,74]. Recent investigations by this group give more insight in this paradoxical situation. An interesting relationship between the type of remodelling of the vessel wall and the tissue composition of the local plaque was found: lipid-rich plaques with many inflammatory cells were often associated with local arterial dilation. On the other hand, fibrous plaques coincided more often with local shrinkage of the vessel wall [75]. Fibrosis related contraction (a well known phenomenon in wound healing) could explain why many highly stenotic lesions are fibrous. Moreover, the association between inflamed lipid lesions and local vessel dilation provides another clue why many mildly stenotic lesions do rupture. Since inflammation occurs also at the base of the atheroma of lipid plaques, dilation may result from destruction and attenuation of the media underlying plaques [76,77].

## 5. Classical risk factors and acute plaque complications

Although the major risk factors for coronary heart disease (age, gender, hypercholesterolemia, hypertension, smoking, diabetes) clearly correlate with the extent of plaque formation in coronary arteries [78], little is known about whether, and if so, how they influence the composition and vulnerability of plaques. However, recently important data came up from investigations on large series of human plaques of patients with well documented coronary risk factors. Burke et al. compared morphologic plaque features with the profile of risk factors of corresponding patients [79]. Vulnerable plaques were defined as lesions with a fibrous cap of less than 65  $\mu\text{m}$  and infiltrated with  $>25$  macrophages per high power field. Patients with low serum levels of HDL and high LDL had more vulnerable plaques according to the criteria above. Moreover, 69% of deep plaque ruptures reaching into a lipid core were found in man, whereas in a previous study of the same group [32], 69% of superficial erosions was reported in women (in plaques composed of smooth muscle cells and matrix proteins rather than lipids and macrophages), indicating sex-related differences in the type of rupture, which could have been the result of differences in plaque composition. Smoking did not influence the composition of the plaques with respect to features of vulnerability, but appeared to be highly throm-

bogenic. However, in the PDAY investigations smokers had more lipids in their plaques, particularly oxidized lipoproteins, than non-smokers [80]. Additional support for the influence of risk factors on plaque disruption stems from autopsies on diabetic patients with coronary thrombosis, which revealed erosion of the plaque surface as the most common type of plaque disruption (66% of diabetic infarct patients) [8].

Hemodynamic factors such as local disturbances in flow velocity and alterations in shear stress form another risk factor for plaque initiation and growth [81]. It can be anticipated from the extensive experimental work in this field that local differences in arterial flow or shear stress over a bulging plaque surface may induce local variations in endothelial function (expression of adhesion molecules, production of growth factors) within one and the same plaque, with local differences in leukocyte recruitment and platelet adherence as a consequence [82–84]. In mature human plaques these mechanisms have not been investigated thus far, but a recent study in our laboratory revealed a relationship between the direction of arterial flow and local differences in macrophage and smooth muscle cells densities in entire carotid artery plaques which were taken at autopsy. Macrophage contents, visualized by immunostaining with anti-CD68, were found to be significantly higher in the upstream parts of plaques than in their downstream parts. In contrast, in downstream (distal shoulders) the smooth muscle cells densities were larger than upstream [85]. These findings strongly suggest that flow dynamics may influence plaque stability not only in a purely mechanical way (as a rupture trigger) but also through influencing the cellular composition of plaques. In carotid plaques, rupture sites are also associated with plaque inflammation [31] and indeed, in 70% of the ruptured plaques that were encountered in our series, the rupture site was found in the upstream shoulder.

## 6. Conclusions

The onset of plaque rupture is a complex process. Lipid related inflammation in the plaque leads to degradation and weakening of the plaque tissue. On the other hand, smooth muscle cell proliferations and collagen synthesis exert reparative and stabilizing effects. These biologic features of the plaque determine to a large extent whether or not a plaque will be vulnerable, and set the stage for rupture triggers to induce a rupture event. New insights have emerged on a possible role for inflammation and repair also in local arterial wall remodeling in terms of dilation and shrinkage, with consequences for the geometry of the entire vessel [75].

The inflammatory process in plaques most likely represents a protective phenomenon which serves to eliminate accumulations of noxious agents from the arterial wall. For example, phagocytosis of lipids is basically a protective

mechanism, but unlimited uptake and foam cell death may lead to expansion of the soft atheroma. Another example is provided by the T-cell cytokine IFN-gamma. It may inhibit growth of native plaques and restenosis lesions by inhibiting smooth muscle cells proliferation and collagen synthesis [49]. However, in the advanced lipid plaque which needs the support of an intact fibrous cap the same effect appears to be dangerous. Therefore, although basically protective, in these advanced plaques the inflammatory process has a worse side-effect: destabilization and plaque rupture.

Molecular and cellular mechanisms that underlie risk factor dependent differences in plaque composition, and related types of plaque disruption are not sufficiently understood. Nevertheless, it appears that the various risk factors may influence the balance between stabilizing and destabilizing mechanisms in the plaque each in their own way. This notion may illustrate the value of a proper understanding of atherosclerotic plaque pathology for patients with acute ischemic syndromes.

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