2. ATHEROTHROMBOSIS: PATHOGENESIS OF CARDIOVASCULAR DISEASE

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2.1 Introduction

In humans atherothrombosis – atherosclerosis superimposed by thrombosis - usually develops over many years, even decades. Early lesion formation may even occur in adolescence. Lesion progression depends on genetic make-up, gender and certain well-recognised risk factors such as smoking, hypertension, hyperlipidemia and diabetes, as well as a number of non-classical risk factors that are currently the subject of intense investigation. Clinical manifestations of atherothrombosis are very different, on various locations of the arterial bed and often precipitate suddenly, sometimes with no prior warning. On the other hand, some individuals with the disease may never experience symptoms, and some may endure chronic stable manifestations without acute complications.

Atherothrombosis is a progressive disease characterised by the accumulation of lipids, fibrous material, and minerals in the arterial wall leading to narrowing of the arterial lumen. Arterial stenosis by itself may remain silent for decades and seldom cause acute vascular events. Usually because of a physical disruption, thrombus forms at the site of atherosclerotic lesion. This thrombotic complication of atherosclerotic lesion – atherothrombosis, causes most morbidity and mortality in the developed countries and will soon become a leading cause of loss of productive years world wide. Atherothrombosis can cause acute heart attack, a leading diagnosis in hospitalised adults in the developed world, stroke, the disease which devastates quality of life and leads to loss of independence or critical limb ischemia, which limits the mobility and places limbs in jeopardy due to gangrene.

In the genesis of atherothrombosis three stages can be distinguished:

(i) initiation of the atherosclerotic lesion, which is characterised by adhesion and invasion of mononuclear leukocytes to the arterial intima, their accumulation of lipids and transformation into foam cells forming a fatty streak;

(ii) progression of the atherosclerotic lesion into a fibrous plaque involving accumulation of smooth-muscle cells which elaborate extracellular matrix macromolecules;

(iii) thrombotic complications of the lesion, with thrombus formation because of a physical disruption of plaque's protective fibrous cap; this permits contact between blood and the highly thrombogenic material located in the lesion's lipid core. The following paragraphs discus the mechanisms involved in these three stages of atherothrombosis in more detail.

2.2 Initiation of the atherosclerotic lesion

Previously considered as a bland accumulation of lipids, connective tissue, and calcium, current evidence supports a central role for inflammatory processes in the pathogenesis of atherothrombosis. The inflammatory response involves not only the cells of the arterial wall: endothelial and smooth muscle cells, but also cells derived from blood - mononuclear leukocytes: monocytes and lymphocytes.

Under macroscopic examination, the earliest recognisable atherosclerotic lesion is denoted as a fatty streak. The fatty streak is slightly yellow and demonstrates longitudinal orientation at the branch points of arteries. It is essentially an aggregation of lipid-laden macrophages, derived from monocytes and known as foam cells, and T-lymphocytes. Fatty streaks contain free and esterified cholesterol mostly derived from plasma lipoproteins.

One of the earliest events in the formation of an atherosclerotic lesion is recruitment of mononuclear leukocytes to the arterial intima, mediated by specific leukocyte adhesion molecules expressed on the surface of vascular endothelial cells. Adhesion molecules comprise two families: a family of selectins and a family that shares structural similarity with immunoglobulins. Selectins mediate rolling or transitory contact of leukocytes with the endothelium. Endothelial cells overlying human atherosclerotic lesion express one member of the selectin family, P-selectin, in contrast to those in normal vessels. The other major group of endothelial leukocyte adhesion molecules, the immunoglobulin superfamily, mediates more sustained sticking of leukocytes to the endothelium than do the selectins. One member of the immunoglobulin superfamily - vascular cell adhesion molecule-1 (VCAM-1) is of special interest with regard to early atherosclerosis. It binds to a ligand which is expressed by monocytes and lymphocytes, recruited to the intima during early atherogenesis.

Once adherent, the leukocytes enter the artery wall. Current evidence suggests that certain chemo-attractant chemokines, such as macrophage chemo-attractant protein-1 (MCP-1), direct the migration of leukocytes into

the intima. Vascular cells produce chemokines when exposed to the inflammatory mediator interferon-?, a molecule elaborated by activated T-lymphocytes, and perhaps macrophages as well.

Factors, which signal the focal increase in adhesion molecules and cytokine expression at sites of the athero-sclerotic lesion predilection, are modified lipoproteins containing various oxidised phospholipids. Regulation of the expression of adhesion molecules occurs by negative control as well as at the level of gene transcription. For example, the well known endogenous mediator nitric oxide (NO), usually thought of as a vasodilator, can reduce leukocyte adhesion to arteries. Additionally, NO can counteract the induction of VCAM-1 expression by endothelial cells stimulated by such inflammatory cytokines as interleukin-1 (IL-1) or tumour necrosis factor-? (TNF-?). Thus NO acts as an anti-inflammatory mediator as well as a vasodilator.

Local shear stress alterations may also influence adhesion molecules either directly or indirectly. In areas of normal arterial blood flow, laminar shear stress augments the activity of endothelial NO synthase, the enzyme that produces endogenous •NO. Thus, the endogenous anti-inflammatory action of NO should operate at sites of undisturbed arterial flow. Local formation of NO should limit the ability of atherogenic stimuli. Disturbed flow at sites prone to early lesion formation, such as branches and bifurcations, probably attenuate this endogenous anti-inflammatory pathway. This explains why lesions tend to form in regions of disturbed blood flow such as branch points or near flow dividers in arteries.

Once mononuclear leukocytes collect in the intima, they typically accumulate lipid and become macrophage foam cells, the hallmark of the early atheromatous precursor, the fatty streak. These early lesions, although present in half of the autopsy speciment from children and adolescents do not typically cause thrombotic complications, but in many cases progress to form intermediate and advanced lesions.

2.3 Progression of the atherosclerotic lesion and formation of fibrous plaque

Accumulation of macrophage foam cells may be reversible and does not by itself cause clinical consequences. However, macrophage accumulation within the arterial intima sets the stage for progression of the lesion and its evolution into a more fibrous and eventually more complicated plaque that can indeed cause clinical disease. Accumulation of smooth muscle cells, and their elaboration of extracellular matrix macromolecules, may contribute importantly to formation of the fibrous plaque during further lesion progression. These advanced lesions have usually a fibrous cap made up of smooth muscle cells, collagen fibriles and proteogycans. The cap is surrounded by a cellular layer composed of smooth muscle cells, macrophages and T-lymphocytes. Beneath the fibrous cap lies a core that contains intact foam cells, cellular debris, extracellular lipids, cholesterol and cholesteryl esters, calcium deposits and components of blood.

Endothelial cell injury causing adherence, degranulation of platelets and release of platelet-derived growth factor (PDGF) is considered responsible for smooth muscle cell proliferation and extracellular matrix accumulation. Repeated endothelial cell injury followed by platelet adherence to the endothelium and macrophage migration into the subendothelial space supports the prominent role of thrombosis in the progression and complication of plaques. However, there is current evidence that plaques can form also in the absence of actual injury of endothelial cells. Mononuclear phagocytes, precursors of the plaque's characteristic foam cells, can insinuate themselves between intact endothelial cells and enter the intima by diapedesis. Endothelial calls or infiltrating leukocytes may themselves produce mediators such as PDGF and other growth factors such as heparin-binding epidermal growth factor, forms of fibroblast growth factor, and insulin-like growth factors. During later phases of lesion formation, platelets can indeed release fibrogenic mediators at sites of desquamation of endothelium causing mural microthrombi.

Inflammatory cytokines may possibly also be involved in growth factor expression by endothelial cells and leukocytes. For example, IL-1, a prototypic cytokine, increases production of PDGF A-chain by human vascular smooth muscle cells. IL-1 can also augment basic fibroblast growth factor expression by human smooth muscle cells. These examples illustrate how cytokines can elicit secondary expression of a variety of growth-promoting genes by vascular cells and leukocytes. Smooth muscle cell accumulation depends on the equilibrium between growth-stimulatory and growth-inhibitory stimuli, both limbs of control that are tightly regulated themselves. Smooth muscle cells receive growth stimulatory signals as well as those that promote their proliferation. Transforming growth factor–b (TGF-b) can inhibit smooth muscle cell proliferation whilst at the same time stimulating their production of extracellular matrix. Interferon-g, a cytokine derived from activated T lymphocytes, can inhibit smooth muscle cell proliferation and matrix synthesis. Endogenous heparin sulphate glycosaminoglycans can also limit smooth muscle cell division.

Progressing lesions often accumulate calcium. Far from being a passive or inevitable degenerative process, lesion mineralization also appears to depend upon closely controlled or positive and negative loops. Recent work has characterised the expression by vascular smooth muscle cells of proteins involved in bone formation and mineralization. For example, smooth muscle cells can express osteopontin.

In contrast to the early atherosclerotic lesion, that does not change the calibre of the arterial lumen, fibrous plaques protrude into the lumen leading to arterial stenosis, that can eventually limit blood flow and cause ischemia.

2.4 Plaque disruption and thrombotic complications of atherosclerotic lesion

Arterial stenosis by itself seldom causes acute vascular event. Indeed, sizeable plaques may remain silent for decades or produce only stable symptoms such as angina pectoris precipitated by increased demand. However, seemingly without warning, such stable lesions may cause the dreaded acute manifestations of atherothrombosis, such as acute myocardial infarction or stroke. Thrombosis actually causes most of the acute manifestations of atherosclerosis. Formerly, it was presumed that arteries with critical stenosis tend to thrombose and precipitate acute manifestations of atherothrombosis. We have now learned that the degree of luminal obstruction by a plaque has little relation to its likelihood of causing thrombosis. The majority of acute myocardial infarctions result from plaques that cause less than a 50% stenosis of the artery, as assessed by arteriography.

2.4.1 The mechanism of thrombosis

Thrombus formation usually occurs because a physical disruption of the atherosclerotic plaque. Plaque disruption takes two major forms:

(i) a superficial erosion of the intimal surface and

(ii) a rupture of the plaque's fibrous cap.

In the case of superficial erosion, platelets can contact subendothelial basement membrane and collagen within the plaque, which may trigger platelet aggregation. In the case of the plaque rapture, blood coagulation factors come into contact with the plaque's lipid core, which is rich in tissue factor, considered the major procoagulant in this situation. In both scenarios, a mixture of systemic "fluid phase" blood constituents such as fibrinogen and components of fibrinolysis (tissue-type plasminogen activator: t-PA and its inhibitor: PAI), and "solid state" factors including tissue factor, cell surface urokinase plasminogen activator (u-PA) and vitronectin-bound PAI come into play.

Thrombus formation within the arteries depends on the local balance between procoagulant and fibrinolytic factors. In normal haemostasis, fibrin formation (coagulation) and dissolution (fibrinolysis) require the sequential activation of zymogens, thus producing the active serine proteinases, thrombin and plasmin, respectively. Fibrinolytic activity is generated on a surface, where fibrin and cell surface receptors serve to bind plasminogen and t-PA and so localise proteolytic activity. Fibrin binds both plasminogen and t-PA, promoting their interaction and plasmin generation on its surface, where it is protected from antiplasmin. t-PA also has a surface receptor on human endothelial cells and vascular smooth muscle cells. The other plasminogen activator, u-PA does not bind to fibrin, but it does have a well-characterised cell surface receptor, urokinase plasminogen activator receptor (u-PAR). Thus, receptor bound u-PA and plasminogen can organise themselves on the cell surface in a configuration that promotes interaction and can locally generate enhanced proteolytic activity.

In superficial plaque erosion, exposure of subendotehlial collagen can promote platelet aggregation. The fluid-phase balance between fibrinogen, inhibitors of fibrinolysis and platelet agreggability clearly determine the consequences of intimal erosion. An unfavourable balance will promote occlusive thrombus accumulation. A favourable balance will limit the clot to a non-occlusive mural thrombus or a transient one due to robust fibrinolysis. Fibrinolysis, of course, also depends on both fluid phase plasminogen and solid state t-PA and u-PA localized on the surface of endothelial and other atheroma-associated cell types.

The fluid phase determinants probably apply equally to erosion and rapture. However, the "solid state" determinants play a particularly important role in the mechanism of thrombosis following plaque rapture. Plaque rapture through the fibrous cap exposes highly thrombogenic material including tissue factor, collagen filaments, and crystalline surfaces, all of which promote coagulation. Tissue factor, a transmembrane protein, binds factor VIIa and factor X and accelerates their enzymatic activity by several orders of magnitude. Strong evidence supports the view that tissue factor, particularly that expressed on macrophages, is the principal thrombogenic factor in the plague's lipid-rich core. Additionally, smooth muscle cells underlying the endothelium can also express tissue factor, further contributing to thrombin formation. Tissue factor actions lead to generation of factor Xa and prothrombin conversion to thrombin. The serine proteinase thrombin, in turn, converts fibrinogen to fibrin and stimulates platelet aggregation.

2.4.2 Determinants of plaque stability

Because of the critical role of plaque rupture in acute thrombosis, the biomechanical strength of the plaques fibrous cap is considered an important determinant of the stability of particular lesions. Since collagen accounts for most of the tensile strength of the plaque's fibrous cap, the metabolism of the macromolecules of the extracellular matrix delineates the mechanism of rupture of the atherosclerotic plaque. The amount of collagen in the lesion's fibrous cap depends upon its rate of biosynthesis by the arterial smooth muscle cell. Certain factors released from degranulating platelets, including TGF-b or PDGF, stimulate collagen synthesis by vascular smooth muscle cells. In contrast, interferon-g, which is produced by activated T lymphocytes, markedly inhibits interstitial gene expression and protein synthesis in these cells. This latter finding has particular bearing on the pathophysiology of plaque rupture because T lymphocytes accumulate at sites where plaques rupture and cause fatal thrombosis.

In addition to synthesis, degradative processes can influence the level of collagen in the plaque's fibrous cap and thereby affect its tensile strength. Several specialised enzymes can degrade collagen, elastin and other structurally key components of the extracellular matrix. Enzymes of the matrix metallo-proteinase (MMP) family can attack interstitial collagen fibrils, molecules ordinarily exceedingly resistant to proteolytic degradation. Activated macrophages within plaque can elaborate a number of these matrix-degrading enzymes: MMPs, elastases, and cathepsins S and K. Experiments on cultured mononuclear

phagocytes and resident cells of the artery wall have shown that inflammatory mediators such as cytokines augment the expression of MMP genes. Thus, members of several proteinase families may participate in degradation of structurally important constituents of the arterial extracellular matrix. As in the case of many protease cascades in biological control, these protease families have endogenous inhibitors. Tissue inhibitors of MMP (TIMP) have been localized in human plaques.

Besides a thin and collagen-poor fibrous cap of atherosclerotic lesion other features are characteristic of so-called vulnerable plaques. For example, plaques that have actually ruptured and cause thrombosis usually also have large numbers of macrophages and T-lymphocytes along with a few smooth muscle cells. Possibly smooth muscle cell death, perhaps by apoptosis or programmed cell death, may contribute to reduced smooth muscle cell number in vulnerable plaques. Indeed, some smooth muscle cells in plaques have fragmented DNA and other features characteristic of programmed cell death. In vitro studies have shown that inflammatory cytokines found in plaques can trigger the apoptotic programme in human vascular smooth muscle cells

2.5 Conclusion

This paper gives some examples of how recent progress in the cellular and molecular mechanisms of atherothrombosis has increased understanding of this disease at several levels. We have learned how the balance between positive and negative regulation factors can critically influence all stages of atherothrombosis. Induction of leukocyte adhesion molecules by cytokines and inhibition by NO exemplify this balance in processes pivotal to lesion initiation. Progression of lesions from fatty streaks to fibrous plaques depends upon a balance between smooth muscle growth and death; each of these processes is in turn dependent upon a balance between positive and negative stimuli. An altered balance between extracellular matrix synthesis and degradation, or matrix-degrading proteinases and their inhibitors can weaken the plaques fibrous cap or favour endothelial detachment that predisposes to the acute thrombotic complications of atherosclerosis. Interactions of systemic and local haemostatic components promoting thrombus formation are described.

Literature

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