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Does the Role of Angiogenesis Play a Role in Atherosclerosis and Plaque Instability?

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Abstract

Atherosclerosis is an inflammatory vascular disease which begins as fatty streaks underlining the endothelium of large artery walls, usually coronary or carotid. It consists of endothelial dysfunction, the accumulation of lipid and cholesterol in the intima from a young age and the recruitment of inflammatory cells to sites of damage in the artery wall. The disease progresses through several stages, eventually resulting in the formation of a cholesterol rich plaque. The role of angiogenesis in atherosclerosis has been of growing interest over the last 20 years since experimental studies have not been able to support a causal relation. Angiogenesis involves hyperplasia of the vasa vasorum in the early stages of atherosclerosis which is thought to be angiogenesis independent, followed by intimal neovascularization in the late angiogenesis dependent stages of the disease. Angiogenesis increases the supply of oxygen and nutrients to the artery wall and supports initial plaque growth. Once the atherosclerotic plaque develops, intimal angiogenesis is thought to contribute to characteristics of an unstable plaque such as fibrous cap thinning, a cholesterol rich necrotic core, excess infiltration of inflammatory cells, plaque hemorrhage and the increased risk of plaque rupture. This account discusses the evidence behind the role of angiogenesis in atherosclerosis and plaque instability.

Keywords: Atherosclerosis; Angiogenesis; Plaque instability

Introduction

Atherosclerosis is an inflammatory vascular disease contributing to the 30% of all global deaths by cardiovascular diseases such as myocardial infarction and stroke. Atherosclerotic lesions begin as fatty streaks which underline the endothelium of artery walls, primarily in large carotid and coronary arteries. As a result of endothelial dysfunction, lipid and cholesterol continuously accumulate within the intima from a young age and recruit inflammatory cells into the lesion. Atherosclerosis gradually progresses from intimal thickening into fatty streak and foam cell formation and eventually into a cholesterol rich fibrous plaque which can narrow or block the arterial lumen, hampering the blood flow [1-3]. The formation of an unstable plaque with characteristic fibrous cap thinning, a necrotic core and the excess infiltration of inflammatory cells, increases the risk of plaque rupture. The formation of new blood vessels in atherosclerosis has been of growing interest over the last 20 years since there is a great deal of uncertainty into whether it plays a beneficial or detrimental role in this disease. Angiogenesis is thought to contribute to atherosclerosis and plaque instability; however, experimental studies have not yet determined a causal relationship. Determining its role is vital for the development of treatments for atherosclerosis and this account discusses the evidence behind the controversy.

Atherosclerosis

Diagram 1 describes the timeline of events associated with atherosclerosis over 4 decades progressing from intimal thickening to plaque instability defined by the American Heart Association (AHA).

Epidemiological studies have shown that atherosclerosis can be initiated by a number of different conditions such as hypertension, diabetes, and lifestyle factors such as lack of exercise, heavy alcohol consumption, smoking and being overweight. The most common risk factor for atherosclerosis is hypercholesterolemia. Dietary lipids are transported in the blood as chylomicrons whereas endogenous lipids are transported as Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL) and High Density Lipoproteins (HDL). High levels of blood LDLs and VLDLs are associated with the increased risk of atherosclerosis as they carry cholesterol to peripheral tissues. HDLs are responsible for removal of cholesterol from peripheral tissues and therefore low levels are observed in atherosclerosis [3]. These risk factors can be controlled, however, there are also factors which increase susceptibility to atherosclerosis such as gender, genetic susceptibility and age which cannot be controlled [2]. Although, the presence of atherosclerotic lesions deep within the intima can occur from a young age, atherosclerosis is associated with aging as symptoms of the disease do not usually manifest until later in life.

Initiation of atherosclerosis

Atherosclerosis is initiated in response to endothelial dysfunction caused by the risk factors described previously. A normal adult artery develops an accumulation of lipid particles within the intima and diffuse intimal thickening from a young age (Diagram 2) [4].

The endothelium is responsible for vasodilation, suppression of smooth muscle cell proliferation and inhibition of inflammatory responses; functions which are all affected in atherosclerosis. The theory behind initiation of atherosclerosis is based on the hypothesis that risk factors enter the intimal layer of artery wall from the lumen and causes endothelial dysfunction or injury. Another theory suggests that atherosclerosis is initiated by hypoxia in the medial and adventitial layers of the artery wall through constriction of the vasa vasorum in hypertension [2]. The vasa vasorum is a small

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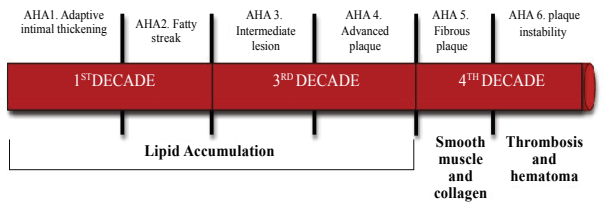

Diagram1: describes the timeline of events associated with atherosclerosis over 4 decades progressing from intimal thickening to plaque instability defined by the American Heart Association (AHA).

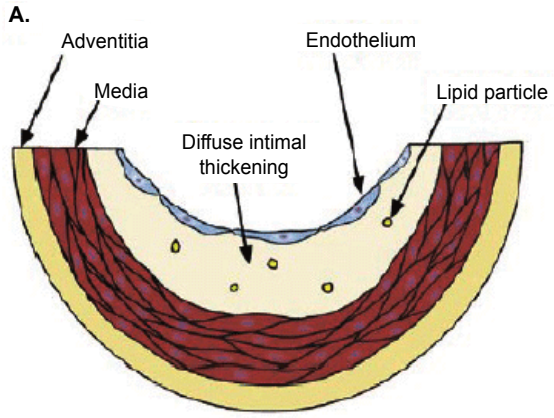

Diagram 2: Normal adult artery with diffuse intimal thickening and accumulation of lipids (Vuorio et al. [4]).

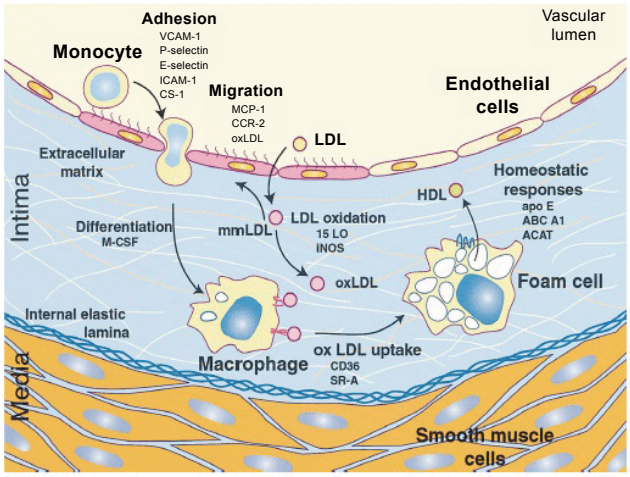

Diagram 3: Foam cell and fatty streak formation (Glass and Witztum [3]).

network of veins and arteries or simply end arteries penetrating the adventitial and outer medial layers of the vessel wall, originating from branch points at regular intervals [2,5,6]. They consist of two types of vessels; the first order vasa vasorum run longitudinally along the vascular wall and separate into second order vasa vasorum that form circumferential arches around the lumen. The diffusion distance is limited to 100μm, and so these vessels are responsible for the transport of oxygen and nutrients to the adventitial layer of vascular wall as well as the removal of waste products [6-8]. Oxygen and nutrients diffuse from the adventitia and supply the media whereas the intima receives a supply of nutrients from the lumen of the artery. Therefore, atherosclerosis occurs as a result of mild hypoxia in the vasa vasorum as well as endothelial dysfunction in the arterial lumen. The vasa vasorum plays a decisive role in atherosclerosis and its interaction with the endothelium of the host vessel results in impaired endothelial function observed in early atherosclerosis [2,9].

Foam cell and fatty streak formation

As a result of endothelial dysfunction, lipids accumulate deep within the intima of the artery wall in the first decade of atherosclerosis. This results in foam cell and fatty streak formation described in Diagram 3.

LDLs enter the intima through the endothelium and bind to the extracellular matrix. Due to the decrease in nitric oxide production or activity, LDLs are the oxidized by enzymes such as nitric oxide synthase (NOS), 15-lipoxygenase (15-LO) or by reactive oxygen species (ROS).

As a result of enzyme oxidation, 'minimal' modification (mm) LDLs and oxidized LDLs are formed. MmLDLs induce endothelial cells to express adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) which are essential for leukocyte transmigration, interactions with inflammatory cytokines as well as the formation of neovessels [1,3].

Monocytes from the blood attach to the endothelium via adhesion molecules and transmigrate to enter the sub-endothelial space. Monocytes differentiate into macrophages after migration and express scavenger receptors enabling the uptake of oxidized LDLs. The accumulation oxidized LDLs in macrophages leads to the formation of foam cells which contribute to the formation of fatty streaks (AHA 2) [1,3]. Foam cell cholesterol is esterified and stored as lipid droplets or extruded out of the cell through cholesterol transporters such as ABC-A1 and accepted by HDLs. Fatty streak have a yellow discoloration due to the accumulation of foam cells and represent the bulk of the atherosclerotic lesion, occupying up to 6 layers of the intima of the artery wall. In early atherosclerotic lesions, the distribution of fatty streaks in the vasculature is predominantly in areas where there are changes in blood flow such as bifurcations and back currents causing endothelial injury [10].

Intermediate lesion and atheroma

The third decade of atherosclerosis described in diagram 1 involves the formation of an intermediate lesion and atheroma. Diagram 4 describes the interaction between foam cells and T helper lymphocytes which cause inflammation in the vascular wall by the release of interleukins IL-4, IL-10 and IL-14. Type 2 T helper lymphocytes stimulate foam cells to secrete cytokines which leads to smooth muscle cells migration from the medial part of the vascular wall into the intima [10].

Migrated muscle cells may take up lipid droplets, proliferate and secrete fibrin, collagen and proteoglycans which make up a poorly developed extracellular matrix. The combination of these extracellular proteins and fibrinogen from the blood leads to the formation of a fibrous plaque [3,10]. Type 1 T helper lymphocytes release CD40 and interferon-γ which are both involved in inflammation within the atherosclerotic lesion [3].

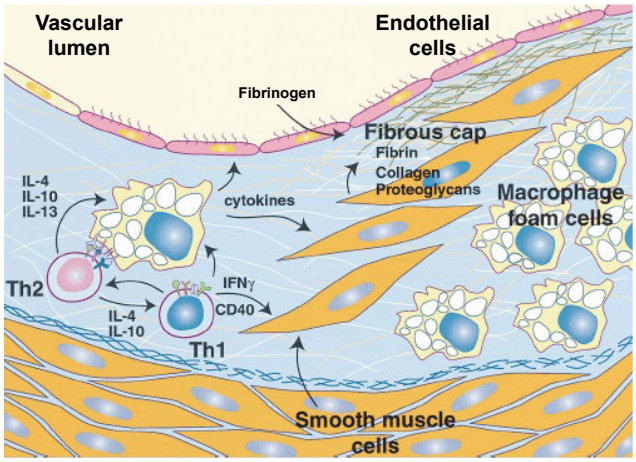

Diagram 4: Lesion progression and scavenger pathway (Glass and Witztum [3]).

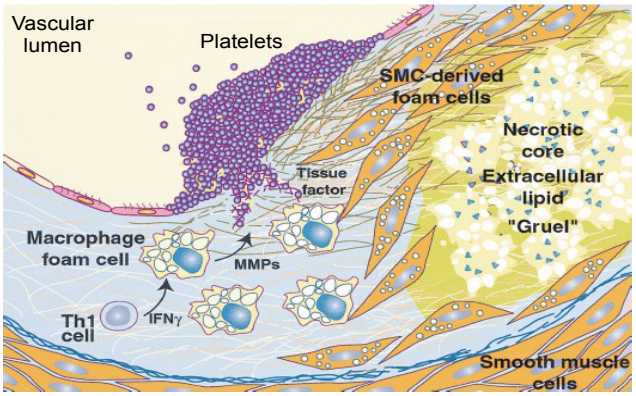

Diagram 5: Plaque instability and rupture (Glass and Witztum [3]).

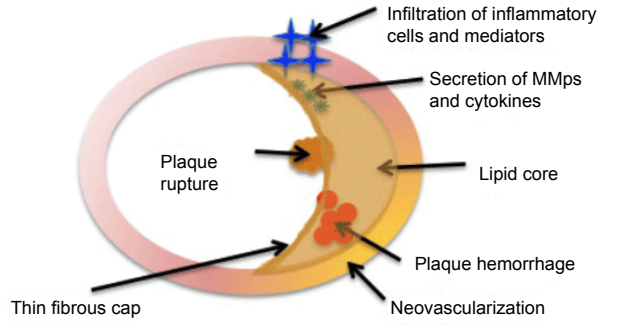

Diagram 6: Biological factors involved in plaque instability.

Fibrous plaque formation and plaque instability

The fibrous plaque consists of an accumulation of smooth muscle cells suspended in a matrix of collagen and some elastic fibers, and surrounded by a dense fibrous cap. The smooth muscle cells adopt a lacunar-shape in dense layers of collagen in the basement membrane as shown in diagram 5 [10].

As deposition of lipid and cholesterol in foam cells and the migration and proliferation of intimal smooth muscle cells continues, the size of the plaque increases and the artery lumen narrows. Hypoxia as well as oxidized LDLs within the plaque core causes necrosis of foam cells and smooth muscle cells. Necrotic cells release cholesterol, oxidized LDLs and insoluble lipids, causing their extracellular accumulation called 'gruel'. In addition to the increase in plaque size, the plaque also becomes unstable. Macrophage foam cells secrete matrix metalloproteinases degrade fibrous plaque proteins causing it to weaken and thin. There are a number of different morphological characteristics of vulnerable plaques including a thin fibrous cap, neovascularization, plaque hemorrhage, excessive inflammatory cell infiltration and plaque rupture described in diagram 6 below [3,11,12].

Angiogenesis in Atherosclerosis

Angiogenesis is the formation of new blood vessels from pre-existing vasculature which involves endothelial cell proliferation, migration, tube and lumen formation, and occasionally the recruitment of smooth muscle cells and other adventitial cells [13,14]. In atherosclerosis, an increase in pro-angiogenic factors and/or a decrease in anti-angiogenic factors stimulates angiogenesis, this is known as the angiogenic switch. In human atherosclerosis, there is an increased expression of angiostatin, an anti-angiogenic factor which causes a reduction in collateral vessel formation whereas increased expression of platelet factor 4 is associated with plaque angiogenesis [15,16].

Neovascularization is extremely rare in the absence of atherosclerosis and therefore, endothelial dysfunction is thought to occur first and angiogenesis follows [7]. In atherosclerosis, hyperplasia of the vasa vasorum in the adventitial layer of the artery wall is followed by intimal or ectopic neovascularization within the atherosclerotic plaque. A number of studies have shown that there is an increase in blood flow through arteries during atherosclerosis which cannot solely be due to the dilation of existing vessels. Therefore, newly formed blood vessels must be responsible [5,17]. Due the differences in the balance between pro- and anti-angiogenic factors in human subjects, angiogenesis is a common, but not a predictable, component of early human atherosclerosis.

Initiation of angiogenesis in atherosclerosis

Hyperplasia of the vasa vasorum and intimal neovascularization are initiated by different conditions but induced by the same pathway [18]. Vasa vasorum angiogenesis which is part of the remodeling process is induced by hypoxia in the early stages of atherosclerosis. Ectopic neovascularization on the other hand occurs in response to an increase in oxygen demand of infiltrated plaque inflammatory cells associated with the late stages of atherosclerosis [19]. Until recently, it was also thought that intimal growth greater than 500 μm reduces oxygen diffusion from the arterial lumen to the vessel wall; however, this has been shown to have little effect on hypoxia [20,21].

Oxidative stress and local hypoxia stimulate the release of hypoxia inducible factor 1α (HIF-1α) from endothelial cells [1]. In hypoxia, degradation of HIF-1α is inhibited and it enters the nucleus and induces the expression of vascular endothelial growth factor (VEGF), an important factor in the maintenance of endothelial function and integrity [1,11,22]. HIF-1α also induces transcription of other pro-angiogenic factors such as platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) [11].

The role of VEGF in atherosclerosis is primarily in the formation

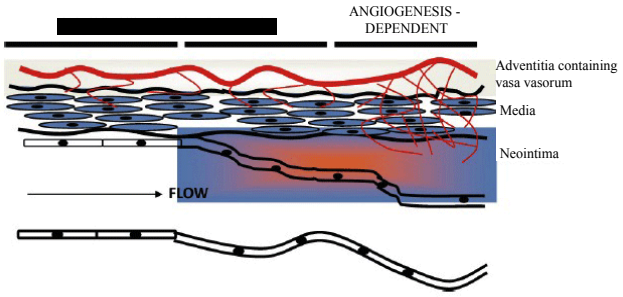

Diagram 7: Angiogenesis of vasa vasorum contributes to intimal thickening in the later stages of atherosclerosis after an angiogenesis-independent phase has occurred (Khurana et al., Mulligan-Kehoe [27,29]).

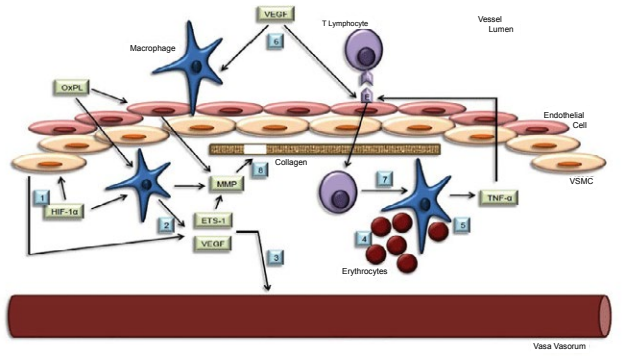

Diagram 8: Angiogenic factors involved in atherosclerosis (Armstrong et al. [45]).

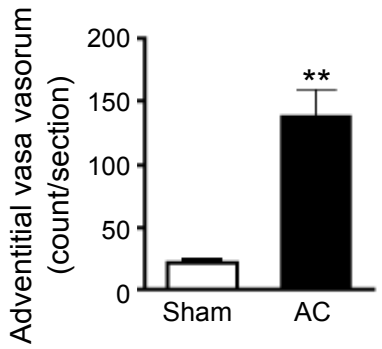

Figure 1: Adventitial vasa vasorum hyperplasia following VEGF and HIF -1α expression in constriction of the suprarenal abdominal aorta (AC) in rats (Kuwahara et al. [23]).

of new blood vessels. E26 transformation-specific sequence-1 (ETS-1) is a transcription factor that regulates VEGF-induced angiogenesis [1]. VEGF expression can also be induced in endothelial cells and macrophages by the action of oxidized phospholipids which further stimulate angiogenesis in atherosclerosis.

Vasa vasorum Angiogenesis in Atherosclerosis

As a result of hypertension, adventitial vessels are prone to collapsing during the cardiac cycle. This impairs blood flow through the vasa vasorum, leading to hypoxia or acute arterial injury which induces angiogenesis in the adventitial vasa vasorum [9,21]. In hypertensive rats, an increase in HIF-1α and VEGF expression in hypoxia is followed by hyperplasia of the vasa vasorum (Figure 1) [23].

Hypertension was induced by aortic constriction in this study which does not represent what happens in atherosclerosis. In addition to this, rats have a smaller artery diameter, a smaller thickness of the adventitial and medial layers in the artery wall as well as a smaller vasa vasorum. This questions whether these results can be used to predict the development of vasa vasorum in human atherosclerosis [14].

Angiogenesis in the adventitial vasa vasorum is associated with a decrease in mean diameter of the first order vasa vasorum and an increase in density of second order vasa vasorum. Figure 2 shows an increase in second order vasa vasorum in animals fed on a hypercholesterolemic diet for 6-12 weeks; the most common risk factor of atherosclerosis. The spatial pattern of vasa vasorum in group 1 (control) is characterized by the visible separation between first (white arrow) and second order (red arrow head) vasa vasorum. Both groups 2 and 3 on hypercholesterolemic diets show a newly formed network of vasa vasorum, predominantly second order [24]. In group 3 animals, the vessel wall area continued to increase with duration of the high-cholesterol diet, whereas the density, number and ratio of first and second order vasa vasorum were similar to that of group 2 [24].

Withdrawal of the hypercholesterolemic diet in monkeys has been shown to decrease the density of the vasa vasorum and decrease the vasa vasorum blood flow to the vascular wall [25]. This suggests that atherosclerosis is not initially dependent on hyperplasia of the vasa vasorum since it is reversible and angiogenesis may only be a pathophysiological reaction to endothelial damage. These studies were carried out on animals which have smaller vasculature than humans suggesting that the effects of hypercholesterolemia are observed faster than they would be in human atherosclerosis and animal vasculature may react differently.

Vasa vasorum angiogenesis takes place during the early stages of atherosclerosis and continues as the disease progresses. It is thought to be an adaptive response to hypoxia or endothelial damage as it increases the oxygen and nutrient supply to the arterial wall to prevent ischemia [9,19]. Although angiogenesis of the vasa vasorum has a protective role in atherosclerosis, it may also contribute to cardiovascular events. Figure 3 shows that the density of vasa vasorum is significantly higher in patients with cardiovascular events compared to patients with no complications of atherosclerosis in the early stages of the disease (AHA 0,1,2). Adventitial vasa vasorum was also higher in the advanced stages of atherosclerosis (AHA 5,6) compared to the early stages in both types of patients [21].

AHA 0,1,2 represents adaptive intimal thickening and fatty streak formation associated with the early stages of atherosclerosis and AHA 5,6 represents fibrous plaque formation and instability. An increase in the density of vasa vasorum in patients with cardiovascular events compared to those without, suggests that angiogenesis contributes to complications of atherosclerosis.

Angiogenesis of the Vasa Vasorum into the Intima

Angiogenesis of the vasa vasorum is associated with the formation of intimal neovessels. As the atherosclerotic lesion progresses, the media and sub-intimal space become sandwiched

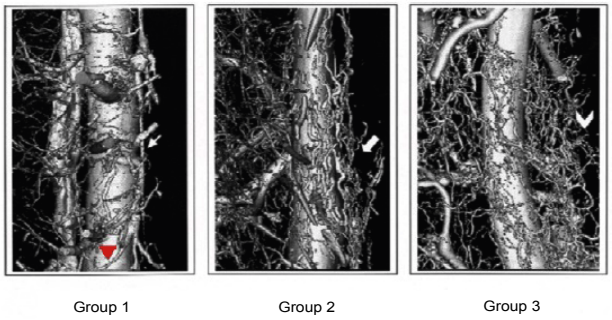

Figure 2: Left anterior descending artery from normal animals (Group 1: left panel), animals fed a hypercholesterolemic diet for 2 and 4 weeks (Group 2: middle panel) or 6 and 12 weeks (Group 3: right panel) (Herrmann et al. [24]).

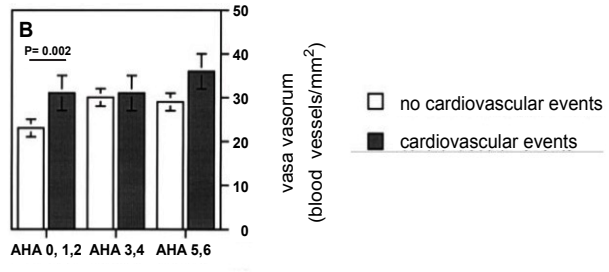

Figure 3: Adventitial vessels of vasa vasorum from patients with cardiovascular and those without at AHA0,1,2, AHA3,4, and AHA5,6 (Fleiner et al. [21]).

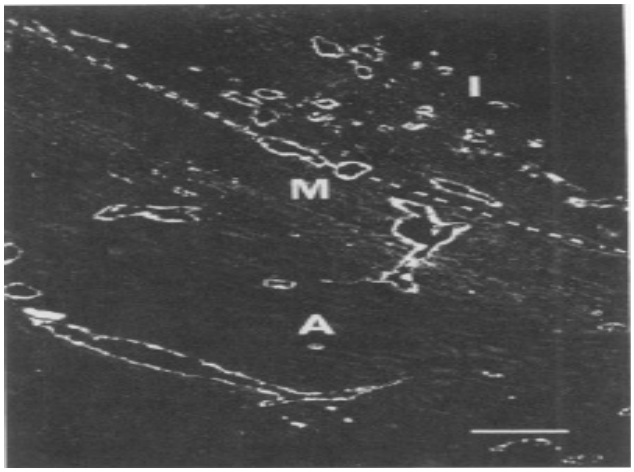

Figure 4: Microvessels extend from the coronary adventitia (A) through the media (M) and ramify within the intima (I). The dotted line represents the border between the intimal and medial layers of the coronary artery wall (Zhang et al. [30]).

between the intima and the adventitia. This causes neovessels to sprout from the adventitia through the media and grow into the intimal atherosclerotic lesion (Diagram 7) [26,27]. Angiogenesis of the 2nd order vasa vasorum through the media increases the supply of nutrients and oxygen to the atherosclerotic lesion [28,14].

Early stages of atherosclerosis are thought to be angiogenesis-independent whereas late stages of atherosclerosis is thought to be an angiogenesis-dependent [27,29]. Figure 4 shows the growth of coronary adventitial neovessels through the media and into the intima of human coronary atherosclerotic arteries [30].

This study also correlated the total number of intimal microvessels derived from the vasa vasorum with intimal thickening [30]. Although this was the first study to show a link between neovascularization and intimal thickening, they only considered intimal vessels originating from adventitia and not those originating from the arterial lumen. Approximately 96.5% of intimal neovessels come from the adventitial vasa vasorum and only 3.5% are derived from the arterial lumen [17]. The volume of vasa vasorum lumens within the intimal lesion is closely correlated with the increase in lesion volume as the disease progresses in mice (Figure 5) [31].

Angiogenesis of the vasa vasorum correlates with intimal thickening throughout atherosclerosis suggesting that it could play a pathological role in lesion progression and plaque instability [2,18,11]. This stage of atherosclerosis is said to be angiogenesis-dependent since growth of the intimal plaque depends on the supply of oxygen, nutrients, hormones and growth factors from neovessels. This study was carried out on mice and therefore questions whether this is true for human atherosclerosis.

Plaque neovessels derived from the vasa vasorum are correlated with the degree of inflammatory cell infiltration, whereas neovessels derived from the lumen are associated with intra-plaque hemorrhage [17]. Therefore, angiogenesis is correlated with characteristics of disease progression, however, the direct relationship between angiogenesis and atherosclerosis is not fully understood.

Intimal Neovascularization in Atherosclerosis

Several studies have suggested that hyperplasia of the vasa vasorum drives intimal neovascularization in atherosclerosis [32]. Other studies have shown that angiogenesis in the vasa vasorum stimulates intimal angiogenesis, but does not initiate it [33]. Intimal angiogenesis is stimulated by oxidative stress in macrophages and other inflammatory cells within the lesion.

Ectopic neovascularization is a sign of late stages of atherosclerosis (AHA 5, 6) and is thought to be dependent on angiogenesis (Figure 6). Intraplaque neovascularization does not discriminate between symptomatic and asymptomatic patients suggesting that the presence intimal neovascularization is not solely responsible for plaque instability in patients with complications of atherosclerosis [21].

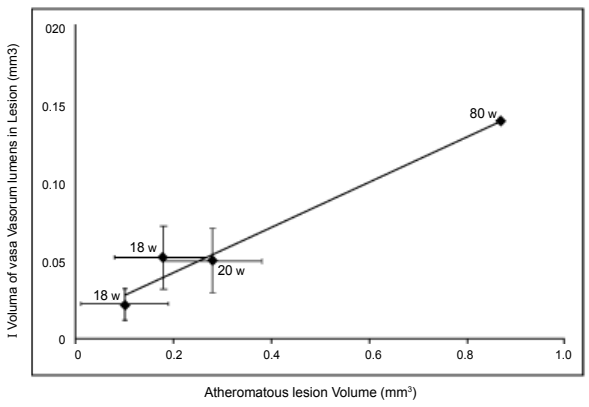

Figure 5: Correlation between lesion volume and volume of vasa vasorum in a murine atherosclerotic lesion (Langheinrich et al.[31])

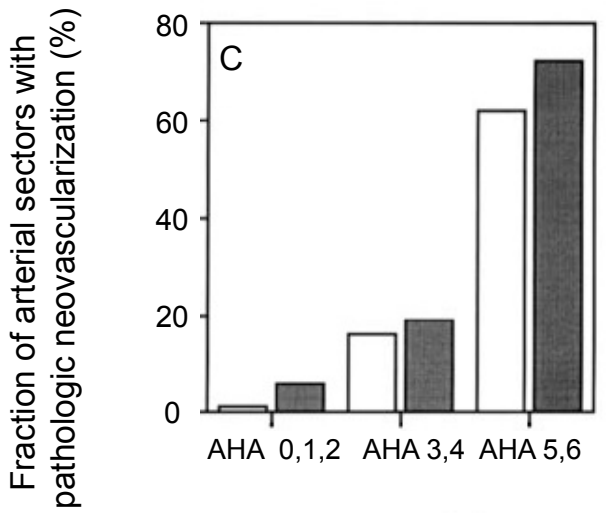

Figure 6: Intimal neovascularization is a sign of advanced lesions however, it does not discriminate between patients with cardiovascular events and those without (Fleiner et al. [21]).

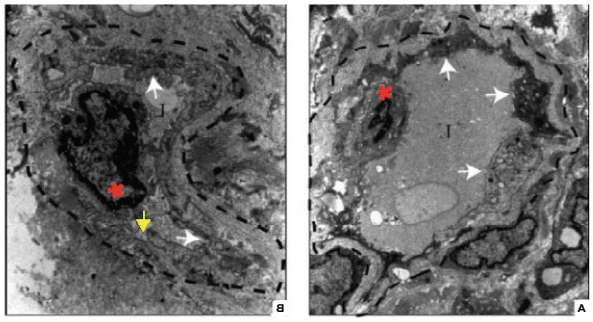

Figure 7: Ultrastructure of human coronary atherosclerotic plaque neovessels. (A) ultrastructure of adventitial vessels in the advanced coronary plaque. (B) Ultrastructure of intra-plaque microvessels (Sluimer and Daemen [20]).

There is a significantly higher density of intimal microvessels in symptomatic patients compared to non-symptomatic in AHA 5,6 stages of the disease. This suggests that angiogenesis does play a pathological role in atherosclerosis and plaque instability. Plaque angiogenesis may simply be a pathophysiological state of the arterial wall and may not be necessary for plaque instability since many murine models do not develop intra-plaque neovessels. In human plaques, angiogenesis is abundant, however, this does not suggest that angiogenesis is necessary for the development of atherosclerosis.

Angiogenic factors in atherosclerosis

Intimal neovascularization is thought to play a role in plaque stabilization by increasing the supply of oxygen, hormones and growth factors necessary for plaque growth [14,30]. Although, angiogenesis plays an initial protective role against cell damage and death by hypoxia, angiogenesis may contribute to atherosclerosis and plaque instability through the pathologies of the neovessels formed and by the delivery of factors which enhance plaque progression [34]. Diagram 8 describes how angiogenic factors may contribute to the progression of atherosclerosis and plaque instability. Steps 1-3 involve the expression of HIF-1α, VEGF, and ETS-1 in induction of angiogenesis of the vasa vasorum and within intima described earlier [1,19].

Microvascular phenotype in atherosclerosis

The ultra-structure of adventitial and intra-plaque microvessels formed during human coronary atherosclerosis have abnormal characteristics. Figure 7A shows the ultrastructure of adventitial vessels in an advanced human coronary plaque. The white arrows indicate endothelial cells displaying spikes or blebs and intracytoplasmic vacuoles. Red asterisks indicate leukocytes adhered to inner the lumen (L). Figure 7B shows the ultrastructure of intraplaque neovessels with open endothelial cell junctions, detachment of the basement membrane from endothelial cells [20].

The structures of vessels formed during atherosclerosis are thought to contribute to atherosclerosis. Endothelial cell junctions are disrupted by VEGF and caused to open. In addition to this, an increase in the ratio of angiopoietin 2: angiopoietin 1, increases permeability of neovessels in atherosclerosis [35]. Gaps in the endothelium are thought to allow extravasation of erythrocytes and increase the transmigration of leukocytes into the atherosclerotic lesion causing intra-plaque hemorrhage and inflammation.

Angiogenesis and Plaque Hemorrhage

Plaque hemorrhage is a feature of unstable plaques and it occurs more commonly in intimal neovessels originating from the arterial lumen. Although, the presence of plaque neovessels is not solely associated with symptomatic plaques, plaque hemorrhage is thought to be essential for plaque instability in atherosclerosis [36,37]. The intraplaque neovessels formed during angiogenesis are more fragile and prone to micro and gross hemorrhage than vessels which grow non-pathological conditions (diagram 8, step 4) [1]. Neovessels closer to the arterial lumen in the intima are multilobular and dilated, consisting of a single layer of endothelial cells and a lack smooth muscle cells (class II neovessels) in human (Figure 8). Normal neovessels in the media however, are smaller, more mature, and have smooth muscle cells surrounding them (class I) in human carotid atherosclerosis [37,38].

An increase in class II neovessels were observed in patients with unstable carotid atherosclerotic plaques [38]. With only a few mural cells, pericytes or smooth muscle cells, the plaque neovessels become leaky leading to the increased infiltration of inflammatory cells and increased risk of intraplaque hemorrhage [37]. It has also been reported that there is an increase in mural cells in carotid plaques from symptomatic patients compared to asymptomatic patients suggesting that fragility of microvessels within plaques may not contribute to plaque instability. In addition to this, the coronary adventitial vasa vasorum also have thin walls in normal conditions suggesting that thin-walled neovessels are unlikely to cause plaque hemorrhage [20].

The delivery of substances of the renin-angiotensin system which may cause fragile intraplaque neovessels to constrict and the increase in blood pressure could result in plaque hemorrhage. The leaky intraplaque neovessels may lead to the extravasation of red blood cells from the blood into the plaque (Figure 9) [39].

Red blood cells are rich in cholesterol and therefore, lysis adds to lipid loading of the atherosclerotic plaque [40]. Intra-plaque hemorrhage and lipid loading causes an increase in plaque volume contributing to plaque vulnerability [26]. Figure 10 shows that a high iron score is associated with a large size of necrotic core [41].

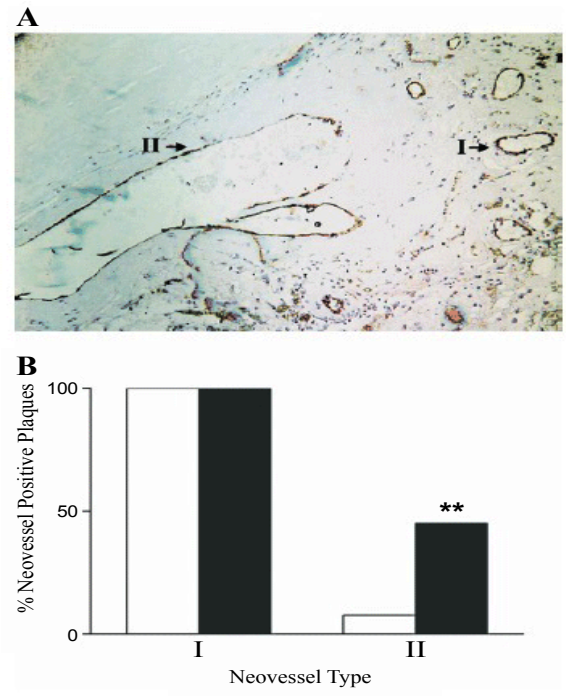

Figure 8: (A) Immature intraplaque vessels (class II) are interlobular and wider than normal vessels (class I). (B) an increase in class II vessels associated with carotid atherosclerotic plaques from symptomatic patients (black bars) compared asymptomatic (white bars) (Dunmore et al. [38]).

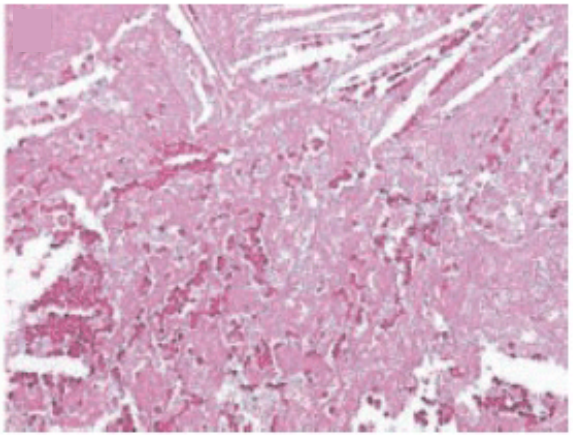

Figure 9: Human aortic plaque with intra-plaque hemorrhage. Red blood cell extravasation within lipid core (Moreno et al. [39]).

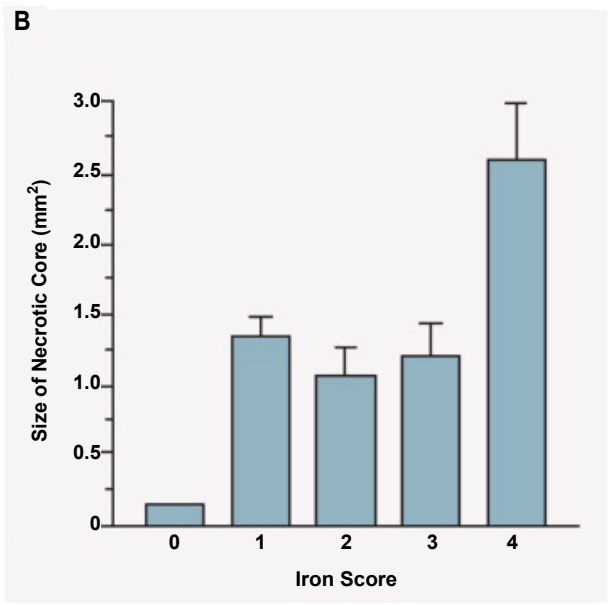

Figure 10: Iron staining within the human coronary atherosclerotic plaque:
0=No detectable staining
1=Trace staining of1 -2 macrophages
2=Mild staining of 3 -5 macrophages
3=Moderate staining of 6 -20 macrophages
4=Marked staining of more than 20 macrophages (Kolodgie et al. [14]).

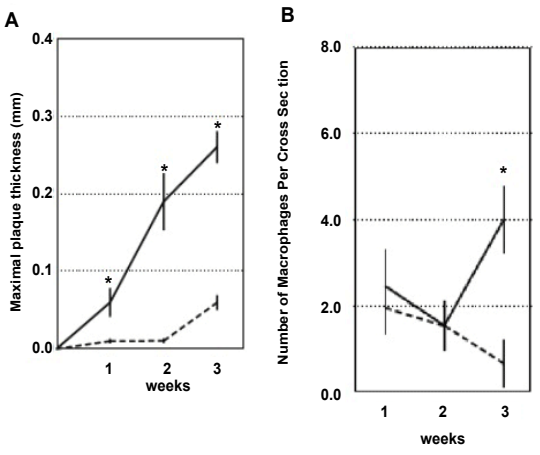

Figure 11: (A) maximal plaque thickness over 3 weeks in VEGF treated mice (solid line) and in albumin treated mice (dashed line). (B): Number of mature macrophages (Celletti et al. [43]).

The lysis of extravasated red blood cells release free haemoglobin containing iron which is involved in oxidative damage and macrophage activation [37]. This further increases the oxygen demand, enhancing the progression of atherosclerosis and stimulating further angiogenesis. Macrophages within the intima phagocytize the cell debris shed during plaque microvessel hemorrhage, including the cholesterol-rich erythrocyte membranes. Intimal macrophages develop into lipid-laden foam cells, enhancing growth of the plaque's necrotic core and size of the plaque [1]. This evidence suggests that plaque hemorrhage undoubtedly leads to an increase in plaque growth and instability, however it is not associated with an increase in the presence of plaque neovessels [11].

Studies have shown that plaque hemorrhage is not always a feature of high plaque microvessel density in human coronary atherosclerosis and therefore, vessel fragility plays an important role in triggering plaque hemorrhage rather than angiogenesis itself [5,14]. This suggests that angiogenesis does not directly cause plaque instability in atherosclerosis. It could also be argued that hemorrhage within unstable plaques would only occur if angiogenesis induced the formation of immature neovessels in the first place.

Angiogenesis and Inflammation

The first route of infiltration of inflammatory cells occurs from the endothelial surface of the arterial lumen into the intima. Angiogenesis provides a second port of entry for inflammatory cells, proliferative and chronic inflammatory factors into the intima through the disrupted endothelium of intraplaque microvessels. A characteristic of plaque instability is excess infiltration of inflammatory cells into the plaque. Inflammation is aided enhanced by angiogenic factors such as IL-8, IL-17, VEGF, TNF-α, and VCAM-1 and associated with an increase in intra-plaque neovessels [20,26],

Interleukins in atherosclerosis

Macrophages are attracted into the plaque by chemoattractants such as interleukin (IL)-8 and 17 released by inflamed tissue (Diagram 8, step 5). These interleukins contribute to angiogenesis; IL-8 stimulates endothelial cells to proliferate and IL-17 is involved in endothelial cell migration and cord formation [42]. Both of these interleukins also contribute to atherosclerosis by acting as chemoattractants and inducing the expression of adhesion molecules which increase leukocyte transmigration into the intima. IL-8 induces chemotaxis of T lymphocytes into the lesion, leading to the increase in tumor necrosis factor-α (TNF-α) expression and E-selectin. IL-17 increases the expression of VEGF, IL-8, ICAM-1 and monocyte chemoattractant protein 1 by fibroblasts as well as TNF-α and IL-1β by macrophages [42]. Excess infiltration of inflammatory cells is a feature of unstable plaques and therefore, angiogenic factors contribute to the progression of atherosclerosis.

VEGF and atherosclerosis

In addition to angiogenesis, VEGF increases vascular permeability of the vasa vasorum, artery and intraplaque neovessels as well as the infiltration of macrophages and other inflammatory cells and mediators into the intimal lesion (Diagram 8, step 6). Macrophages bind to VEGF via their cell surface VEGF receptor 1 (flt-1) [1,8]. VEGF induces inflammation within the atherosclerotic lesion by stimulating expression of E-selectin on endothelial cells causing the infiltration T lymphocytes. This infiltration of inflammatory cells is enhanced by the presence of plaque neovessels and it is associated with an increase in total plaque size. [1,43].

Figure 11 shows that treatment of VEGF for 3 weeks increases plaque size in cholesterol- fed mice deficient in both apolipoprotein E and apolipoprotein B100. VEGF treatment increased both the thoracic aorta plaque thickness via angiogenesis but also the number of macrophages within the plaque by week 3 of the cholesterol diet in mice [43].

The reliability of these results is questionable since VEGF treatments in murine models enhance plaque progression, whereas in humans, a VEGF antagonist enhances the progression of atherosclerosis and it is associated with the increased risk of thromboembolism [44]. VEGF has also been considered for treatment of cardiovascular disease as it induces the growth of collateral blood vessels although it has been found to enhance plaque progression even in human. This highlights the poor understanding of the role of angiogenesis in this disease.

The increase in vascular permeability by VEGF contributes to an increase in plaque thickness due to the infiltration of macrophages and lipids. Therefore, angiogenesis does not directly play a role in disease progression, however, factors which induce angiogenesis contribute to atherosclerosis. Although VEGF has been shown to increase inflammation in atherosclerosis which drives plaque progression and instability, it has also been shown to inhibit smooth muscle proliferation and therefore also plays a beneficial role in reducing intimal thickening in atherosclerosis [18]. Therefore, it is impossible to study the role of angiogenesis in atherosclerosis without inflammatory factors contributing to disease progression.

TNF-a and atherosclerosis

As mentioned earlier, Il-8 induces chemotaxis of T lymphocytes into the intimal lesion. T lymphocytes stimulate macrophages to release tumor necrosis factor-α (TNF-α) (Diagram 8, step 7). TNF-α synergizes with VEGF which increases the expression of E-selectin, allowing further infiltration of immune cells into the atherosclerotic plaque [1]. E-selectin adheres to inflammatory cells, enhancing transmigration into the atherosclerotic lesion [45].

TNF-α also induces the expression of VCAM-1, and ICAM-1 in smooth muscle cells and endothelial cells which are involved in angiogenesis [45]. VCAM-1 and ICAM-1 however also enhance the progression of atherosclerosis by adhering to inflammatory cells, and aiding their transmigration into the atherosclerotic plaque [45].

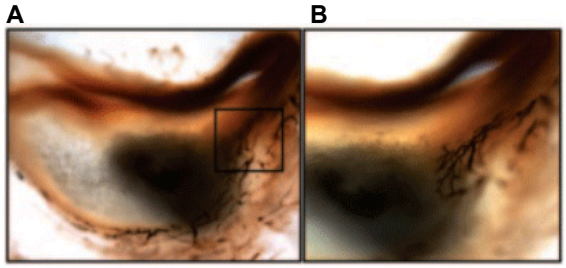

Figure 12: Intraplaque vasa vasorum entering the media into the necrotic core. (A) low power (20x) and (B) high power (40x) images of human coronary segments (Virmani et al.[40]).

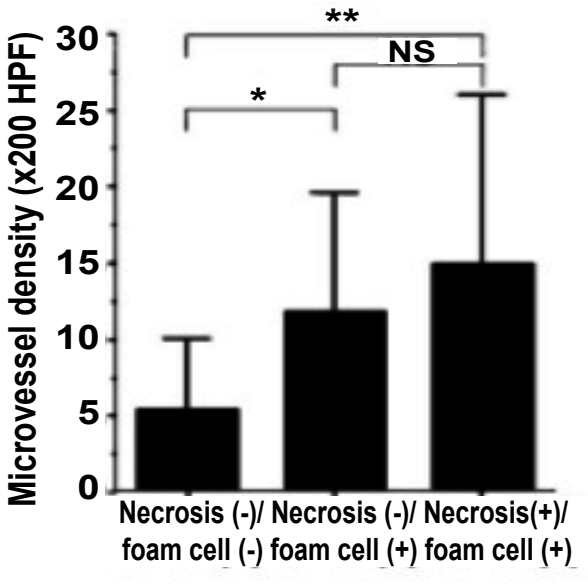

Figure 13: The correlation of microvessel density in atherosclerotic plaques with necrosis and foam cell (Hiyama et al. [48]).

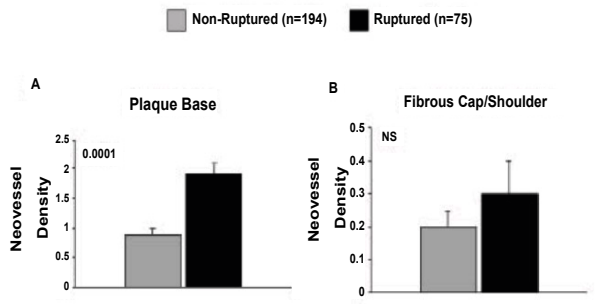

Figure 14: Neovessel density in (A) the base and (B) the fibrous cap/shoulder of human ruptured and non-ruptured plaques (Moreno et al. [39]).

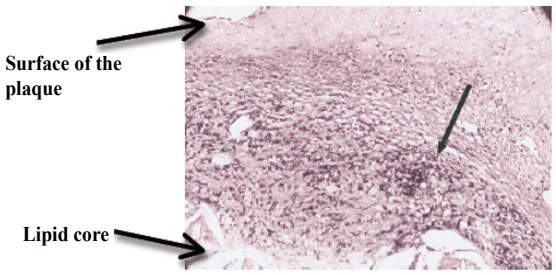

Figure 15: Intense staining of MMP-9 and MMP-9 mRNA (arrow) in the shoulder region of human carotid plaques (Loftus et al. [49]).

Although ICAM-1, VCAM-1 and E-selectin are more prevalent on endothelial cells of microvessels within the atherosclerotic plaque when compared to luminal endothelial cells, it has also been shown that they do not contribute to plaque [46,47].

Delivery of angiogenic factors into the plaque via neovessels not only stimulates angiogenesis but also induces inflammation in atherosclerosis. Therefore, angiogenesis does contribute to plaque instability through intensifying inflammation within the lesion. It has also been found that angiogenesis and inflammation in intermediate atherosclerotic lesions have a beneficial effect on arterial integrity. The role of angiogenesis in atherosclerosis is therefore unclear, however, evidence suggests that it could be initially protective, but as the plaque grows larger, it becomes a by-product of inflammation which drives plaque instability [21].

Angiogenesis and the Necrotic Core

Plaque instability is associated with an increase in size of the plaque necrotic core due to defective clearance of necrotic cells. Microvessels within the plaque have abnormal endothelial cell protrusions which can obstruct the blood flow out of the atherosclerotic lesion. This prevents clearance of necrotic cells increasing the size of the necrotic core in the plaque. Not much is known about the differentiation of microvessels of the vasa vasorum and whether lymphatic systems are responsible for the removal of necrotic cells and therefore more research is required. As the necrotic core increases in size, this induces the release of lipids from foam cells which in turn increases the infiltration of immune cells. Figure 12 shows the invasion of vasa vasorum neovessels into the necrotic core of a human coronary atherosclerotic plaque [40].

Vasa vasorum angiogenesis invades the plaque's necrotic core and acts as a delivery system into the intima, supplying LDLs and macrophages which may contribute to plaque instability [34]. It has also been shown that a high density of intra-plaque neovessels is associated with the presence of both necrotic and foam cells in unstable plaques (Figure 13) [48].

Intra-plaque angiogenesis increases the size of the necrotic core and the accumulation of foam cells in unstable plaques by the delivery of LDLs and mm-LDLs. This suggests that angiogenesis does play a role in plaque destabilization by delivery of substances which enhance plaque progression and destabilization. Fibrocalcific plaques have a very low content of lipid within the intima and the lowest density of neovascularization. This provides further evidence that intra-plaque neovessels are associated with a lipid-rich necrotic core in unstable plaques [39].

Angiogenesis and Plaque Rupture

Plaque rupture is another characteristic of plaque instability. Angiogenesis may contribute to plaque rupture through fibrous cap thinning, a decrease in tissue clearance and a decrease in wall stiffness. ETS-1 and oxidized phospholipids induce the expression of proteinases such as matrix melloproteinases (MMPs) from smooth muscle cells and endothelial cells (Diagram 8, step 8) [11] MMPs breakdown the extracellular matrix allowing neovessels to invade surrounding tissues in angiogenesis. MMPs may also contribute to plaque instability by mediating collagen degradation of the atherosclerotic fibrous cap [1].

Plaque rupture has been shown to occur at sites with a high density neovessels; the shoulder and the base regions of the atherosclerotic plaque (Figure 14) [26,39].

As a result of proteolytic enzyme activity by MMPs, plaque structures weaken contributing to plaque rupture in the shoulder and base regions [26]. The expression of MMPs in angiogenesis is induced in endothelial cells, however, intra-plaque macrophages also express and secrete MMPs. Figure 15 shows that there is increased staining of MMP-9 at the shoulder region of the atherosclerotic plaque, however, this was only found at sites associated with intense inflammation and infiltration of macrophages [49].

Therefore, MMP-9 mediated fibrous plaque thinning in the shoulder regions of carotid atherosclerotic plaques is due to infiltrated macrophages and not due to MMPs involved in angiogenesis [49]. However, it has also been found that neovessels are more abundant in sites of inflammation suggesting that they may indirectly initiate fibrous plaque thinning through the infiltration of macrophages which secrete MMPs.

Plaque rupture is also thought to be induced by a combination of high wall stresses and plaque strains. High wall stresses are caused by local increases in blood pressure, narrowing of the lumen or pulsality and plaque strains are caused by the characteristics of an unstable plaque. The effects of angiogenesis increases the wall stresses and strains within the plaque, causing the plaque to soften and increasing the risk of plaque rupture [20]. Other studies report that plaque rupture occurs at sites exposed to blood elements rather than as a result of vessel leakage [50].

Conclusion

The role of angiogenesis in atherosclerosis is both adaptive and pathological. Angiogenesis prevents ischemia in early atherosclerosis, however, the morphological characteristics of intra-plaque neovessels

and delivery of factors which enhance inflammation and plaque growth suggest angiogenesis contributes to plaque instability. The secretion of matrix melloproteinases during angiogenesis may contribute to fibrous cap thinning as well as the delivery of lipids and reduced the clearance of necrotic cells, increases the growth of the necrotic core. Angiogenesis provides a second route of entry for inflammatory cells into the intima and the ultrastructure of plaque neovessels formed, increases the risk of hemorrhage. Angiogenic factors themselves may even contribute to inflammation in atherosclerosis and this places stress and strains on the plaque, increasing the risk of rupture. However, the evidence for the above is not conclusive since angiogenesis is merely associated with these characteristics and no causal relation has been found. The scarcity of intra-plaque microvessels that develop in mice and other animal models during atherosclerosis has limited research. Data from human studies has developed our understanding of the atherosclerosis, however, these tissues are taken from autopsied specimens and prevents controlled in vivo studies. The role of angiogenesis in atherosclerosis still remains unclear and many studies suggest that angiogenesis may simply be a pathophysiological state of the artery wall or a by-product of inflammation which drives atherosclerosis and plaque instability. Future studies could look into the use drugs which block VEGFR-1 on macrophages as this would prevent pathological angiogenesis by avoiding plaque inflammation and toxicities [36].

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