Atherosclerotic Plaque Progression and Vulnerability to Rupture

Angiogenesis as a Source of Intraplaque Hemorrhage

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Abstract—Observational studies of necrotic core progression identify intraplaque hemorrhage as a critical factor in atherosclerotic plaque growth and destabilization. The rapid accumulation of erythrocyte membranes causes an abrupt change in plaque substrate characterized by increased free cholesterol within the lipid core and excessive macrophage infiltration. Neoangiogenesis is associated closely with plaque progression, and microvascular incompetence is a likely source of intraplaque hemorrhage. Intimal neovascularization is predominantly thought to arise from the adventitia, where there are a plethora of pre-existing vasa vasorum. In lesions that have early necrotic cores, the majority of vessels invading from the adventitia occur at specific sites of medial wall disruption. A breech in the medial wall likely facilitates the rapid in-growth of microvessels from the adventitia, and exposure to an atherosclerotic environment stimulates abnormal vascular development characterized by disorganized branching and immature endothelial tubes with "leaky" imperfect linings. This network of immature blood vessels is a viable source of intraplaque hemorrhage providing erythrocyte-derived phospholipids and free cholesterol. The rapid change in plaque substrate caused by the excessive accumulation of erythrocytes may promote the transition from a stable to an unstable lesion. This review discusses the potential role of intraplaque vasa vasorum in lesion instability as it relates to plaque rupture. (***Arterioscler Thromb Vasc Biol*****. 2005;25:2054-2061.)**

Key Words: angiogenesis ■ plaque rupture ■ sudden coronary death ■ free cholesterol ■ hemorrhage

The causes of coronary lesion progression from an asymptomatic fibroatheromatous plaque to a lesion at high risk for rupture (thin cap fibroatheroma or "vulnerable plaque") are not fully understood. Recently, our laboratory showed that intraplaque hemorrhage is an important process in the progression of asymptomatic plaques into high-risk unstable lesions.[1] Red blood cell (RBC) membranes are rich in phospholipids and free cholesterol, and their accumulation within plaques plays a key role in promoting lesion instability through necrotic core expansion and inflammatory cell infiltration. The source of RBCs within coronary lesions is likely provided by inherently leaky immature blood vessels that surround and invade the plaque. Understanding the mechanisms by which plaque angiogenesis and hemorrhage occurs may ultimately help prevent the transition from a stable to an unstable lesion.

Plaque Rupture Is the Dominant Cause of Acute Coronary Thrombosis

Plaque rupture is the principal cause of luminal thrombosis in acute coronary syndromes occurring in 75% of patients dying of an acute myocardial infarction.[2] The plaques that are vulnerable to rupture are characterized by the same histopathologic signatures, except that they still have an intact fibrous cap, albeit thin.[3–5] The fibrous cap is focally interrupted in plaque ruptures, allowing circulating blood to come in direct contact with the thrombogenic contents of the lipid-rich core, leading to thrombosis and acute coronary syndromes. Ruptured plaques possess a large necrotic core with an overlying thin-ruptured fibrous cap heavily infiltrated by foamy macrophages (Table 1; also see the online supplement, available at http://atvb.ahajournals.org). In ruptured lesions, the necrotic core occupies approximately one third to one half of the total plaque area, whereas in the majority of unruptured vulnerable plaques, it occupies less than one fourth of the lesion (Table 1).[6] This observation suggests that the progressive necrotic core expansion precedes plaque rupture.

Plaque Progression as Evidenced by Morphology

Pathologic intimal thickening (PIT) constitutes the earliest atherosclerotic change and is characterized by surface smooth

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TABLE 1. Comparison of Necrotic Core Size, Number of Cholesterol Clefts, Macrophage Infiltration, Number of Vasa Vasorum, and Hemosiderin-Laden Macrophages in Culprit Plaques

Plaque Type	Necrotic Core, %	No. Cholesterol Clefts, %	Macrophage Infiltration of Fibrous Cap, %	Mean No. Vasa Vasorum	Mean No. Hemosiderin-Laden Macrophages
Rupture	34±17*	12±12*	26±20*†	44±22*†	18.9±11*†
TCFA	24±17	8±9	14±10*	26±23*	4.4±3.6*
Stable	12±25*	4±6*	3±0.7†	13±9†	5.0±9.3†
P value	0.01*	0.04*	0.005*	0.07*	0.001*
			0.0001†	0.01†	0.03†

Values represent the means±SD.
TCFA indicates thin-cap fibroatheroma (vulnerable plaque).
Modified from Virmani et al[4] and Kolodgie et al.[5]

muscle cells (SMCs) overlying relatively acellular lipid-rich pools. The pools of accumulated lipid likely occur from the loss of SMCs.[7,8] The proteoglycan matrix within the lipid pool remains intact with a paucity of free cholesterol and the beginnings of focal-speckled calcification. When macrophages infiltrate the lipid pool, the entrapment and death of these cells is thought to be responsible for the conversion of PIT into early fibroatheroma. The combination of greater macrophage infiltration and apoptotic death together with hypoxia-induced necrosis promotes development into the late fibroatheromatous lesion. As the plaque enlarges, the ensuing hypoxia or inflammatory cell infiltration is thought to promote neovascularization (Figure 1). These nascent immature blood vessels are inherently leaky and permit extravasation of erythrocytes into the plaque, further contributing to necrotic core enlargement (Figure 2).

Necrotic Core Enlargement Is Critical for Plaque Rupture

It has been shown that macrophage infiltration is the first step toward the eventual formation of an atherosclerotic plaque.[9] In vitro studies have shown that low-density lipoprotein (LDL) uptake by macrophages is facilitated by a 2-step oxidation process, beginning with mild oxidation of lipid[10,11] followed by apolipoprotein B oxidation, a modification required for scavenger receptor recognition, which is unaffected by the cholesterol content of the cell.[12] Cultured macrophages exposed to oxidized LDL are richer in free cholesterol than cholesterol esters. The threshold level of free cholesterol in macrophages is in part regulated by a re-esterification process involving acyl coenzyme A:acylcholesterol transferase, or ACAT1.[13] Manipulating the activity or expression of ACAT1 in culture[14,15] or animal models[16] favors the accumulation of free cholesterol. The formation of necrotic core is attributed mostly to the death of macrophages.[13,17] As plaques progress from fatty streaks to those with necrotic cores (gruel plaques), the free cholesterol content of the lesion increases, whereas cholesterol esters decrease.[18] The increase in free cholesterol may be closely associated with lesion instability. In a study by Felton et al of human aortic atherosclerotic plaques, the progression from nondisrupted to disrupted lesions is accompanied by increased free cholesterol, cholesterol esters, and free-to-esterified cholesterol ratio in the necrotic core; the triglyceride content is unchanged.[19] The influence of lipid composition on plaque instability in coronary sudden death is also apparent from our own studies, in which the percentage of cholesterol clefts are greater in lesions with rupture compared with eroded or stable plaques (Table 1).[4]

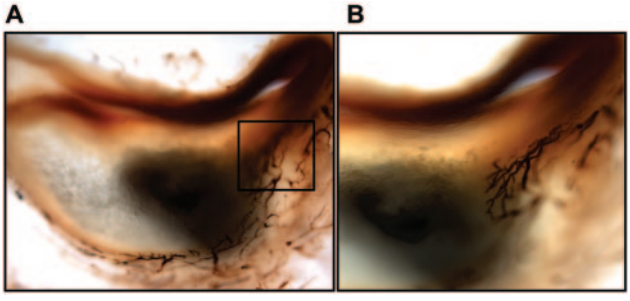

Figure 1. Demonstration of intraplaque vasa vasorum. A and B, Low- (×20) and high-power (×40) images of a coronary segment (150-μm thickness) stained with the endothelial marker *Ulex europaeus* I. Note the delineation of vasa vasorum in the adventitia extending through the medial wall into the intimal layer. B, Multiple branches of vasa vasorum are found infiltrating the border area of the necrotic core. The vasa vasorum at these sites are delicate because they are largely unsupported by matrix, which poses a high potential for leakage.

It is generally accepted that apoptotic macrophages are a likely source of free cholesterol in plaques; however, it is entirely feasible that free cholesterol within the necrotic core could be derived from other sources, including erythrocyte membranes.[13] In a recent investigation of thromboembolic pulmonary hypertension, Arbustini et al showed that necrotic cores in intimal plaques in large pulmonary arteries contain RBCs (as demonstrated by anti-glycophorin A staining) and macrophages. In addition, we[21,22] and others[20] have observed extravasated erythrocytes in disease processes outside the coronary circulation are accompanied by deposits of free cholesterol and foamy macrophages. It is well appreciated that the cholesterol content of erythrocyte membranes exceeds that of all other cells in the body, with lipid constituting 40% of the weight.[23,24] Moreover, erythrocyte membrane-derived cholesterol is elevated in patients with hypercholesterolemia and is sensitive to short-term statin therapy.[25,26]

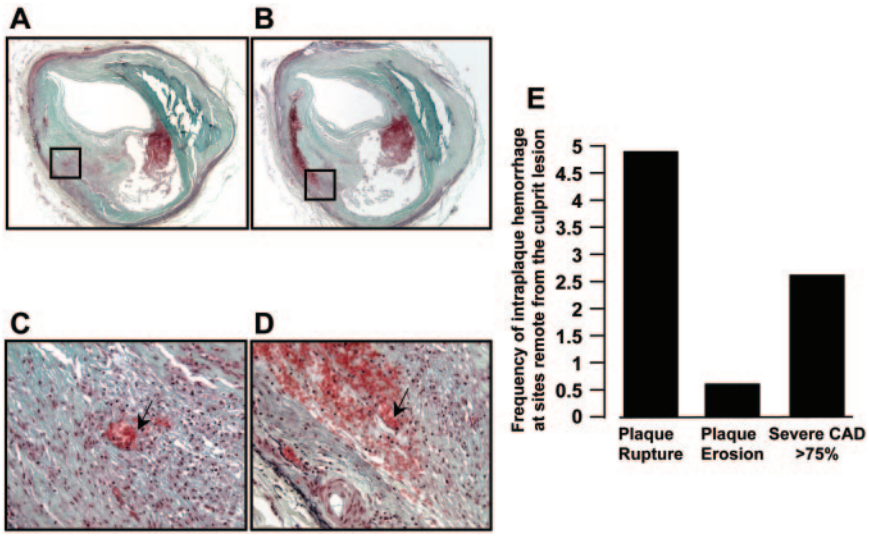

Figure 2. Recent intraplaque hemorrhage in a thin-cap fibroatheroma. Low- (A and B; ×20) and higher-power views represented by the black boxes (C and D; ×200) of a coronary artery with a thin-cap fibroatheroma ("vulnerable" plaque) and recent intraplaque hemorrhage. A and C show the spillage of erythrocytes from surrounding intraplaque vasa vasorum (arrow). B and D, Serial section of the lesion in A, showing an expanded area of hemorrhage with the higher-power image demonstrating a large pool of extravasated erythrocytes surrounding proximate microvessels (arrow). Coronary sections were stained with Movat Pentachrome. E, Bar graph representing the frequency of intraplaque hemorrhages at other coronary lesion sites in patients dying from plaque rupture, erosion, or severe coronary disease. Notably, patients dying from rupture had the highest number of remote sites with intraplaque hemorrhage. CAD indicates coronary artery disease.

Because RBCs are not capable of synthesizing lipids "de novo," most of the membrane lipid content originates from an exchange with plasma lipoproteins. The level of sphingomylenase activity in plaques should be sufficient to catalyze the release of cholesterol from RBC membranes.[27,28] In addition, excess membrane cholesterol can phase separate and form metastable membrane domains consisting of pure cholesterol arranged in a tail-to-tail orientation, creating a nidus for nucleation to crystalline cholesterol.[29]

In the early to mid-20th century, several leading pathologists forwarded the hypothesis that intraplaque hemorrhage is a major contributor to the progression of coronary atherosclerosis; however, the precise nature of this relationship was not well understood.[30–32] Recent studies from our laboratory suggest that plaque hemorrhages are more frequent in the coronary vasculature in patients dying from rupture compared with plaque erosion or stable lesions with a >75% cross-section area of luminal narrowing (Figure 2).[6] In an effort to further understand the influence of intraplaque hemorrhage on lesion progression, we examined various types of human coronary plaques for hemorrhagic events.[1]

In a relatively large series of human coronary plaques from sudden coronary death victim, there was a greater frequency of previous hemorrhages in coronary atherosclerotic lesions prone to rupture (as detected by glycophorin A) relative to lesions with early necrotic cores or plaques with PIT.[1] Importantly, the degree of reactive glycophorin A staining and the level of iron deposits in the plaque corresponded to the size of the necrotic core, and changes in these variables paralleled an increase in macrophage density, suggesting that hemorrhage itself serves as an inflammatory stimulus (Table 2).[1] By contributing to the deposition of free cholesterol, macrophage infiltration, and enlargement of the necrotic core, the accumulation of erythrocyte membranes within an atherosclerotic plaque may represent a potent atherogenic stimulus. These factors may increase the risk of plaque destabilization.

Erythrocyte Membrane-Derived Free Cholesterol and Plaque Progression

As proof of concept, we developed an animal model of simulated intraplaque hemorrhage to assess the role of erythrocytes in lesion progression.[1] The direct injection of packed erythrocytes (25 to 50 μL) into quiescent aortic atherosclerotic plaques produced excessive macrophage infiltration along with free cholesterol crystals, and iron colocalized to areas of RBCs. In contrast, control (noninjected lesions) showed the characteristics of a regressed lesion with far fewer lesional macrophages and free cholesterol. Neutral lipids identified by oil red O were also significantly greater in plaques with injected erythrocytes when compared with controls. Thus, the animal studies offer further evidence that

TABLE 2. Morphometric Analysis of Plaque and Hemorrhagic Events in Human Coronary Arteries From Sudden Death Victims

Plaque Type	GpA Score	Iron Score	Necrotic Core, mm^2	MΦ, mm^2
PIT, "no" core (n=129)	0.09±0.04	0.07±0.05	0	0.002±0.001
Fibrous cap atheroma, "early" core (n=79)	0.23±0.07	0.17±0.08	0.06±0.02	0.018±0.004
Fibrous cap atheroma, "late" core (n=105)	*0.94±0.11	*0.41±0.09	*0.84±0.08	*0.059±0.007
Thin cap atheroma (n=52)	*1.60±0.20	*1.24±0.24	*1.95±0.30	*0.142±0.016

Values are reported as the mean±SEM; *$P<0.001$ vs early core.
The No. in parentheses represents the No. of lesions examined, which totaled 365.
GpA indicates glycophorin A; MΦ, macrophages.
Reproduced with permission from Kolodgie et al.[1]

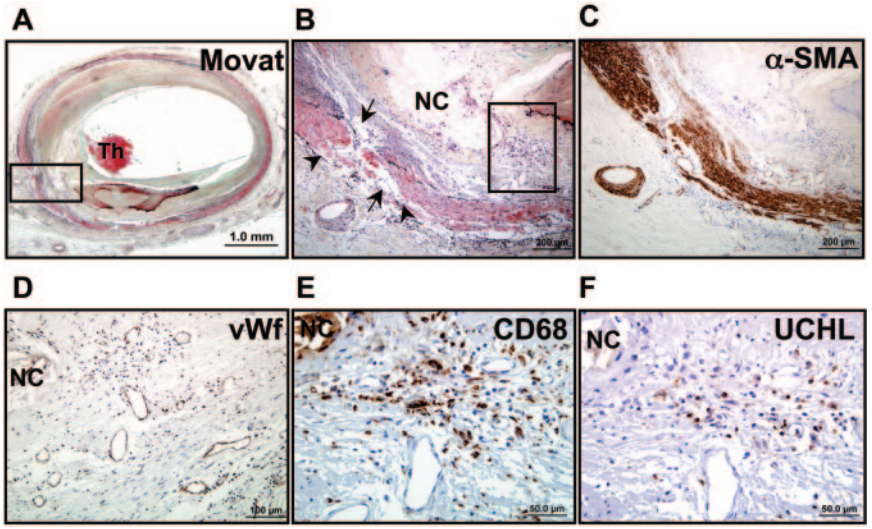

Figure 3. Coronary artery section demonstrating angiogenesis in plaque rupture. A shows a coronary artery intraluminal thrombus (Th) precipitate by plaque rupture (Movat Pentachrome; ×20). B, Higher-power magnification corresponding to the area outlined by the black box in A showing an area of medial degeneration (arrowheads) with infiltrating vasa vasorum (arrows). C, The area of medial degeneration is shown by the loss of α-actin (α-SMA)–positive SMCs. D through F, Higher-power images corresponding to the area in B stained by markers for endothelium (vWF), macrophages (CD68), and T lymphocytes (UCHL), respectively. The vasa vasorum are numerous and surrounded by perivascular -lymphocytes and macrophages. Select vessels are surrounded by α-actin–positive spindle shaped cells, in particular those located close to the media, whereas others are only positive for vWf. NC indicates necrotic core.

episodic hemorrhages in plaques contribute to accumulated free cholesterol and macrophage infiltration.

The contribution of erythrocyte membrane cholesterol to necrotic core volume is predicted to be substantial because intraplaque hemorrhage is thought to occur repeatedly over years. On the basis of a liquid volume of cholesterol in a single RBC of 0.378 μm^3 and a hematocrit of ≈50%, an accumulation of 100 μL whole blood at a 10% cholesterol exchange efficiency would add ≥0.2 mm^3 to the total necrotic core volume (T.N.T., S.P.W.). This calculation represents bleed volumes of only 0.137 μL per day whole blood (0.068 μL packed RBCs) repeated over a 2-year period. Like internal bleeds, the bulk of the erythrocyte would be degraded over days, and because membrane cholesterol fraction cannot be metabolized internally, it would be available for absorption into the necrotic core. Moreover, the uptake of erythrocyte-derived cholesterol by macrophages, in turn, would inevitably give up cholesterol to the core by apoptotic cell death. Consistent with this notion, recent MRI data of carotid plaques over an 18-month period showed evidence of intraplaque hemorrhage as contributing factor to necrotic core volume and lesion bulk.[33] Further, patients with intraplaque hemorrhage at baseline showed a far greater susceptibility to repeat plaque hemorrhages.[33] Therefore, accumulated RBC-derived cholesterol may represent a critical transition promoting the conversion of a stable plaque to an unstable phenotype.

Association of Plaque Hemorrhage and Vasa Vasorum

Pathologic examination of unstable lesions has demonstrated that intraplaque hemorrhage and plaque rupture are associated with an increased density of microvessels.[34–39] The concept of how RBCs precisely leak into the necrotic core is poorly understood. Our laboratory has described diffuse perivascular staining of von Willebrand factor (vWF) within plaque vasa vasorum and evidence of erythrocyte membranes within necrotic cores (please see online supplement).[1] This finding suggests that microvascular disruption or leakiness may promote lesion progression by providing erythrocyte-derived cholesterol. In addition to leaky vasa vasorum, plaque fissuring can also account for the accumulation of erythrocytes, which has also been described to occur in the coronary vasculature of patients dying from sudden coronary death. (please see online supplement).[40]

More than 100 cases of sudden coronary death with serial sectioning of selected plaques were examined in our laboratory to better understand the relationship of intraplaque vasa vasorum in ruptured lesions. The number of vasa vasorum was increased 2-fold in vulnerable plaques and up to 4-fold in ruptures compared with stable plaques with severe luminal narrowing (Table 1). Moreover, it was found that the invasion of intraplaque vasa vasorum from the adventitia follows a distinct pattern of arborization. The entrance into the intimal space from the adventitia occurs specifically at breakpoints in the medial layer below sites of early necrotic core formation (Figure 3). The vessels divide as they approach the core with secondary and tertiary branches surrounding its abluminal surface (Figures 1 and 3). Microvessels close to the medial wall appear to be well formed because they are typically accompanied by surrounding SMCs. This is in contrast to intimal vessels near the lumen, which appear immature. Increased numbers of T cells are commonly found at breaks in the medial wall and base of the necrotic core compared with other regions of the plaque. It is plausible that T helper cell–driven immune responses possibly through interferon-γ may inhibit SMC proliferation, contributing to medial disruption and absence of SMCs in perforating neovessels.

The Vasa Vasorum in Atherosclerosis

The intima of normal human coronary arteries lack vasa vasorum, whereas the adventitia and outer media possess a vascular network.[41] In such vessels, endothelial cell turnover is low (labeling index <0.25%).[42] Studies more than a century ago by Koester[43] originally describe increased numbers of arterial microvessels in human atheroma, which have later been confirmed by several investigators.[31,32,44] In more recent reports of human atherectomy specimens, endothelial cell proliferation is modest, although when neovascularization is observed, the proliferation indices as high as 43.5%

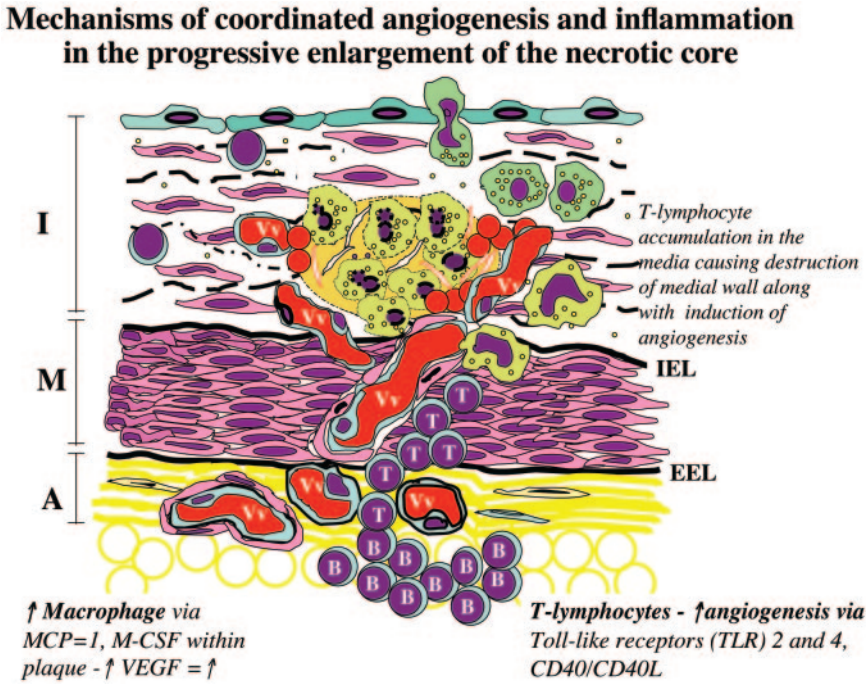

Figure 4. Mechanisms of coordinated angiogenesis and inflammation in the progressive enlargement of the necrotic core. The diagram illustrates the origin of intraplaque vasa vasorum infiltrating from adventitial vessels through a disrupted medial wall. The vasa vasorum display varying degrees of maturation with some neovessels surrounded by SMCs. The inflammatory response in the adventitia is characterized mostly by B cells; however, as the vessel traverses the media, it becomes surrounded mainly by foci of T lymphocytes and perivascular macrophages as they approach the necrotic core. Leaky or ruptured vasa vasorum result in intraplaque hemorrhages and an accumulation of free cholesterol within the necrotic core derived from erythrocyte membranes (depicted as cholesterol clefts outlined in red). It is conceivable that T lymphocytes via CV40/CD40L or TLRs induce angiogenesis by the release of angiogenic growth factors from macrophages. I indicates intima; M, media; A, adventitia; EEL, external elastic lamina; IEL, internal elastic lamina; B, B lymphocytes; T, T lymphocytes; Vv, vasa vasorum.

may be reached.[45] Barger et al demonstrated a rich network of vasa vasorum within human atherosclerotic coronary vessels.[46] In an animal study, Heistead and Armstrong reported a 5-fold increase in intima/media blood flow from proliferating microvessels in monkeys fed high cholesterol for 17 months.[47] Casting studies and confocal microscopy demonstrate that intimal microvessels arise more frequently from the dense network of vessels in the adventitia adjacent to a plaque rather from the arterial lumen.[48] Ongoing studies in our laboratory show a correlation of angiogenesis with the extent of chronic inflammation, granulation tissue, and atherosclerotic changes (please see online supplement).

Most of the intraplaque vasa vasorum are endothelialized, but only a few have mural pericytes and SMCs. Lack of mural cells and poorly formed endothelial cell junctions probably contribute to the leakiness of the intraplaque vasa vasorum.[1,36,38,49] Porous microvessels may result from release of angiogenic factors from the closely associated macrophages. It has recently been shown that a platelet-derived growth factor-β (PDGF) gradient is involved in the recruitment of mural cells; a deficiency of PDGF receptors may impair close contact with endothelial cells forming a network of immature blood vessels.

Vasa Vasorum Heterogeneity Among Different Vascular Beds

In human atherosclerosis, arterial neovascularization and inflammation were reported recently to be significantly greater in patients with signs of symptomatic than asymptomatic atherosclerotic disease in iliac, carotid, and renal arteries. Vasa vasorum correlated with intimal macrophage content; moreover they were 2- to 4-fold higher in individuals with previous cardiovascular events than those with a negative clinical history.[39] Notably, this relationship was preserved in diabetic patients as well. This quantitative analysis of angiogenic and inflammatory events in the arterial vasculature provides important evidence of a more widespread involvement of neovascularization and inflammation, which likely also involves the coronary bed.

An increased heterogeneity of vasa vasorum among different vascular beds may explain the propensity for the differential expression of atherosclerotic disease at varied anatomic locations. In normal swine coronary arteries, the density of vasa vasorum measured by micro-CT was highest in coronary arteries (2.91 ± 0.26 vessels/mm^2) versus renal (1.45 ± 0.22 vessels/mm^2), carotid (0.64 ± 0.08 vessels/mm^2), and lowest in femoral arteries (0.23 ± 0.05 vessels/mm^2).[54] The relationship between the adventitial vasa vasorum and the development of atherosclerosis was further supported by the observation that the internal mammary artery, a vessel with low incidence of atherosclerosis, shows significantly lower vasa vasorum density. Furthermore, the contribution of vasa vasorum to vascular disease may also be mediated by their physical fragility. Interestingly, the variability in vasa vasorum density among vascular beds was primarily accounted for by the second order vasa vasorum. These microvessels are smaller in diameter than the first-order vasa vasorum, less mature, and therefore might be more susceptible to hemorrhage.

Emerging Mechanisms of Plaque Angiogenesis

Angiogenesis depends on the combined action of various cytokines and growth factors secreted by infiltrating inflammatory cells. Neovascularization commonly accompanies chronic immune and inflammatory responses characterized by prominent T-cell and macrophage infiltration (Figure 4).

T-Cell–Mediated Signaling

Varying degrees of T lymphocytes are consistently present in areas of neoangiogenesis specifically within the deep intima

and below the necrotic core and shoulder regions. These morphological observations strongly suggest that T lymphocytes likely play an important role in the development and maturation of intraplaque vasa vasorum. Activated T cells are a known source of angiogenic factors, including vascular endothelial growth factor (VEGF), and can stimulate angiogenesis in association with early lymphocyte recruitment.[55] The dependency of T cells in mediating angiogenic responses was demonstrated recently in CD4 knockout mice exposed to acute hindlimb ischemia.[56] Inflammation and collateral development in response to ischemia were significantly impaired in CD4−/− versus C57BL6 wild-type mice. Moreover, rescue experiments involving the infusion of spleen-derived purified CD4-positive T cells in CD4 null mice increased macrophage recruitment, resulting in blood flow recovery, limb salvage, and reduced muscle atrophy. These data highlight the importance to CD4+ cells as initiators of angiogenic responses in addition to their importance in the accumulation of macrophages, which then secrete a broad array of cytokines and growth factors, including VEGFs, which facilitate angiogenic growth.

Role of CD40/CD40 Ligand

Several reports have established interactions between CD40 ligand (CD40L) and CD40 involving pluripotent functions on inflammation, including the production of cytokines and chemokines, as well as the angiogenesis factor VEGF, by endothelial cells. Activated human T cells are reported to mediate contact-dependent expression of matrix metalloproteinases (MMPs) in endothelial cells through CD40L/CD40 signaling.[57] These interactions through CD40/CD40L were able to induce an angiogenic response in endothelial cells cultured in 3D fibrin matrix gels, which were sensitive to MMP inhibition. More recently, ligation of CD40 resulted in the expression of several angiogenic factors, including VEGF[58] and fibroblast growth factor-2 and the receptors Flt-1 and Flt-4.[59] These studies and others[60–63] provide support for a proangiogenic function of CD40L–CD40 interactions.

Toll-Like Receptors

The role of cytokine-driven inflammation and tissue destruction is becoming recognized as a major determinant of lesion instability.[64] Production of these cytokines is initiated by signaling through Toll-like receptors (TLRs) that recognize host-derived molecules released from injured tissues and cells. TLRs activate the proinflammatory transcription factor nuclear factor κB (NF-κB) and the mitogen-activated protein kinase pathway, resulting in the production of cytokines that augment local inflammation. Signaling through TLRs is facilitated by the adapter molecule protein myeloid differentiation factor 88 (MyD88) and other homologous MyD88 adapter–like proteins expressing shared homology with the intracellular signaling domains of the interleukin-1 (IL-1) receptor that are selectively involved in the various phases of NF-κB activation.[65] In human atherosclerotic plaques, TLR1, TLR2, and TLR4 are shown to be upregulated in the endothelium and in areas infiltrated with inflammatory cells at the mRNA and protein levels. These receptors primarily colocalized with cells expressing macrophage and endothelial cell markers, although some T cells expressed TLR2 and TLR4.[66] In addition, adventitial fibroblasts and dendritic cells express functional TLR4 receptors and are able to produce a variety of cytokines after TLR4 activation.[67]

It has been suggested that TLRs or adapter molecules such as MyD88 highly influence atherosclerotic lesion bulk and progression. In a recent study by Michelsen et al, genetic deficiency of TLR4 or MyD88 in apolipoprotein E null mice resulted in a significant reduction of aortic atherosclerosis despite continued hypercholesterolemia.[68] The reduction in atherosclerosis was associated with lower levels of circulating proinflammatory cytokines IL-12 or monocyte chemoattractant protein 1 accompanied by reduced numbers of plaque macrophages and expression of endothelial leukocyte adhesion molecules. Importantly, human studies suggest TLR4 expression is upregulated in lipid-rich human plaques when compared with fibrous plaques.[69] Moreover, the capacity of innate immune system to elicit inflammation reactions in response to endotoxins is impaired in patients with TLR4 polymorphisms. These studies and others suggest that TLRs may be essential for promoting the inflammatory component of atherosclerotic disease.

Despite the effects of inflammatory cell activation on angiogenic responses, activation of TLRs may directly affect VEGF production. Activation of the cell surface G-protein–coupled adenosine receptors in murine macrophages produces, in addition to anti-inflammatory activity, an upregulation of VEGF.[70,71] Although treatment of macrophages with adenosine agonists produces only a modest increase in VEGF, stimulation of the adenosine (A_{2A}AR) receptor in the presence of TLRs results in increased VEGF secretion to a level similar to that produced under hypoxia and perhaps is the most potent inducer of VEGF expression.

The role of CD40/CD40L and TLR in the promotion of plaque angiogenesis is, at this moment, highly speculative. Experimental studies in animal models of atherosclerosis (in mice) have clearly shown a role for CD40/CD40L or TLR pathway in the development or progression of atherosclerosis; as yet, no study has shown an alteration of plaque or adventitial angiogenesis after inhibition of CD40/CD40L or inhibition of TLR signaling. Further, the presence of intraplaque vasa vasorum in mice is controversial.[72,73]

Conclusions

Observational studies of necrotic core progression identify intraplaque hemorrhage as a critical factor in atherosclerotic plaque growth and destabilization. The rapid accumulation of erythrocyte membranes causes an acute change in plaque substrate characterized by increased free cholesterol within the core and excessive macrophage infiltration. Neoangiogenesis is closely associated with plaque progression and is likely the primary source of intraplaque hemorrhage at sites of microvessel incompetence. Focal collections of T-cell– and macrophage-derived angiogenic factors contribute to: (1) the arborization of vasa vasorum around the necrotic core; (2) the formation of immature vessels; and (3) loss of basement membrane around functional capillaries. This process initiates leakage of RBCs into the plaque and induces a cycle of inflammation and neovascularization. Understanding the

mechanisms of angiogenic growth within the neointima of atherosclerotic lesions may lead to the development of new therapies designed to stabilize plaques.

Acknowledgments

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