REVIEW

OPEN

Plaque angiogenesis and its relation to inflammation and atherosclerotic plaque destabilization

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Purpose of review
The review discusses the recent literature on plaque angiogenesis and its relation to inflammation and plaque destabilization. Furthermore, it discusses how plaque angiogenesis can be used to monitor atherosclerosis and serve as a therapeutic target.

Recent findings
Histopathologic studies have shown a clear relationship between plaque angiogenesis, intraplaque hemorrhage (IPH), plaque vulnerability, and cardiovascular events. Hypoxia is a main driver of plaque angiogenesis and the mechanism behind angiogenesis is only partly known. IPH, as the result of immature neovessels, is associated with increased influx of inflammatory cells in the plaques. Experimental models displaying certain features of human atherosclerosis such as plaque angiogenesis or IPH are developed and can contribute to unraveling the mechanism behind plaque vulnerability. New imaging techniques are established, with which plaque angiogenesis and vulnerability can be detected. Furthermore, antiangiogenic therapies in atherosclerosis gain much attention.

Summary
Plaque angiogenesis, IPH, and inflammation contribute to plaque vulnerability. Histopathologic and imaging studies together with specific experimental studies have provided insights in plaque angiogenesis and plaque vulnerability. However, more extensive knowledge on the underlying mechanism is required for establishing new therapies for patients at risk.

Keywords
angiogenesis, atherosclerosis, inflammation, intraplaque hemorrhage, neovessel maturation

INTRODUCTION

The majority of acute cardiovascular events in patients is caused by occlusive thrombosis because of rupture or erosion of an atherosclerotic plaque [1]. Plaques that are most at risk are characterized by large necrotic cores with a thin fibrous cap [1,2▪▪]. A high-inflammatory content with increased protease activity critically determines the risk of rupture [3]. Hypoxia in atherosclerotic plaques is now widely recognized, because of the use of specific probes in imaging studies [4]. Plaque angiogenesis is a physiological response to facilitate the increased oxygen demand in the plaque but can have adverse effects by facilitating intraplaque hemorrhage (IPH) and influx of inflammatory mediators (Fig. 1a). IPH as a result of immature plaque neovessels is clearly associated with subsequent ischemic events [5–8]. Inflammatory cell, endothelial cell, and pericyte interactions can provide insight in the biological mechanisms of plaque angiogenesis. In this review, we focus on plaque angiogenesis, the relation with inflammatory mediators, and the subsequent effects of IPH on plaque instability, both in experimental models and in humans. We will further discuss options to target plaque angiogenesis, for imaging and therapeutic purposes.

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KEY POINTS

- Plaque angiogenesis and IPH are associated with cardiovascular events.
- IPH is the result of immature neovessels and contributes to plaque destabilization.
- Inflammatory mediators of plaque progression and vulnerability are found in the close proximity of plaque neovessels.
- Plaque angiogenesis can be used for imaging of plaque vulnerability and may serve as a therapeutic target.

HYPOXIA OF THE ATHEROSCLEROTIC PLAQUE

Nourishment and oxygenation of arteries is to a certain extent possible by diffusion from the luminal blood. Larger arteries (vessel size >0.5 mm) need vasa vasorum, a specialized microvascular network, to meet these demands [9]. Atherosclerotic lesion formation increases the vessel wall thickness resulting in regional limited oxygen exchange. Vascular cells respond to hypoxic conditions with changes in cell metabolism, angiogenesis, apoptosis, and inflammatory responses comparable to cells in tumors [10▪]. Local hypoxic regions and hypoxic cells in atherosclerotic lesions are demonstrated in humans and experimental models [11–14]. Increased oxygen consumption by high metabolic active cells such as macrophages further depletes the oxygen availability, creating a hypoxic environment in the atherosclerotic lesion [13]. In macrophages, hypoxia not only affects the metabolism [15,16▪] and lipid uptake [17], but also results in an increased inflammatory response characterized by increased IL-1β and caspase-1 activation [18]. Hypoxia also augments the thrombogenic potential of atherosclerotic plaques via upregulation of tissue factor [19▪]. The importance of hypoxia in enhancing atherosclerosis and plaque destabilization is excellently reviewed in this journal by Marsch *et al.* [4]. These authors also observed that hypoxia replacement therapy in mice affected efferocytosis resulting in reduced atherosclerosis [20].

Adaptation to the hypoxic environment is directed by the oxygen-sensitive transcription factor hypoxia-inducible factor (HIF)-1. HIF-1 consists of a heterodimer of the rapidly modified and degraded HIF1-α and the constant available HIF-1β. Prolyl hydroxylase domain-containing protein 2 is essential for degrading HIF-1-α and blockade of prolyl hydroxylase domain-containing protein 2 improves angiogenic responses [21]. HIF-1 induces transcription of hypoxia responsive genes such as fibroblast growth factor, cytokines, angiopoietins (ANG), and in particular vascular endothelial growth factor (VEGF) [22]. The role of HIF-1 in atherosclerosis is not univocal. Silencing of HIF-1-α in macrophages reduces proinflammatory factors and increases macrophage apoptosis [16▪]. Hyperlipidemia impairs angiogenesis in a HIF-1β and NF-κB-dependent manner [23,24]. HIF-1α inhibition abrogates the macrophage-dependent proangiogenic effect of oxidized LDL in an in-vivo angiogenesis assay [25]. Specific knockdown of HIF1-α in endothelial cells reduces atherosclerosis via reduced monocyte recruitment [26], whereas knockdown in antigen presenting cells results in aggravation of atherosclerosis via T-cell polarization [27].

PLAQUE ANGIOGENESIS

As early as 1936, small blood vessels entering an atherosclerotic plaque were described by the pathologist Paterson [28]. The pathological effect of plaque angiogenesis by facilitating inflammatory influx and IPH is well established [29–31]. A direct relationship of plaque angiogenesis and hypoxia was shown by Sluimer *et al.* [12]. Plaque angiogenesis is frequently associated with shoulder regions and gradually increases with lesion progression [31]. The majority of the neovessels in the plaque originate from adventitial vasa vasorum [29,32]. Pathophysiological studies have reported increased vasa vasorum densities in vulnerable lesions [9,32] as well as in aortic aneurysms [33]. There is experimental evidence that sprouting of vasa vasorum precedes lesion formation [34] and is more associated with adventitial inflammation and perivascular fat [30,35] and less with hypoxia. It is therefore proposed that vasa vasorum hyperplasia and plaque angiogenesis are based on two different mechanisms [9,36].

To initiate vessel sprouting, endothelial cells switch from a quiescent state to a highly migratory and proliferative state (Fig. 1b). This switch is codetermined by the metabolic status of the endothelial cells [37]. VEGF-A, the most prominent VEGF, is highly upregulated in various cell types in atherosclerotic plaques and enhances atherosclerosis [38]. VEGFR2 is the main receptor for VEGF-A, because of its strong tyrosine kinase activity. VEGF-bound VEGFR2 becomes internalized, under the influence of the urokinase plasminogen activator receptor, and continues to signal from the endosomal compartments in endothelial cells [39]. Vessel wall enveloping pericytes detach in response to ANG-2 and liberate themselves from the basement membrane by proteolytic degradation. Plasma

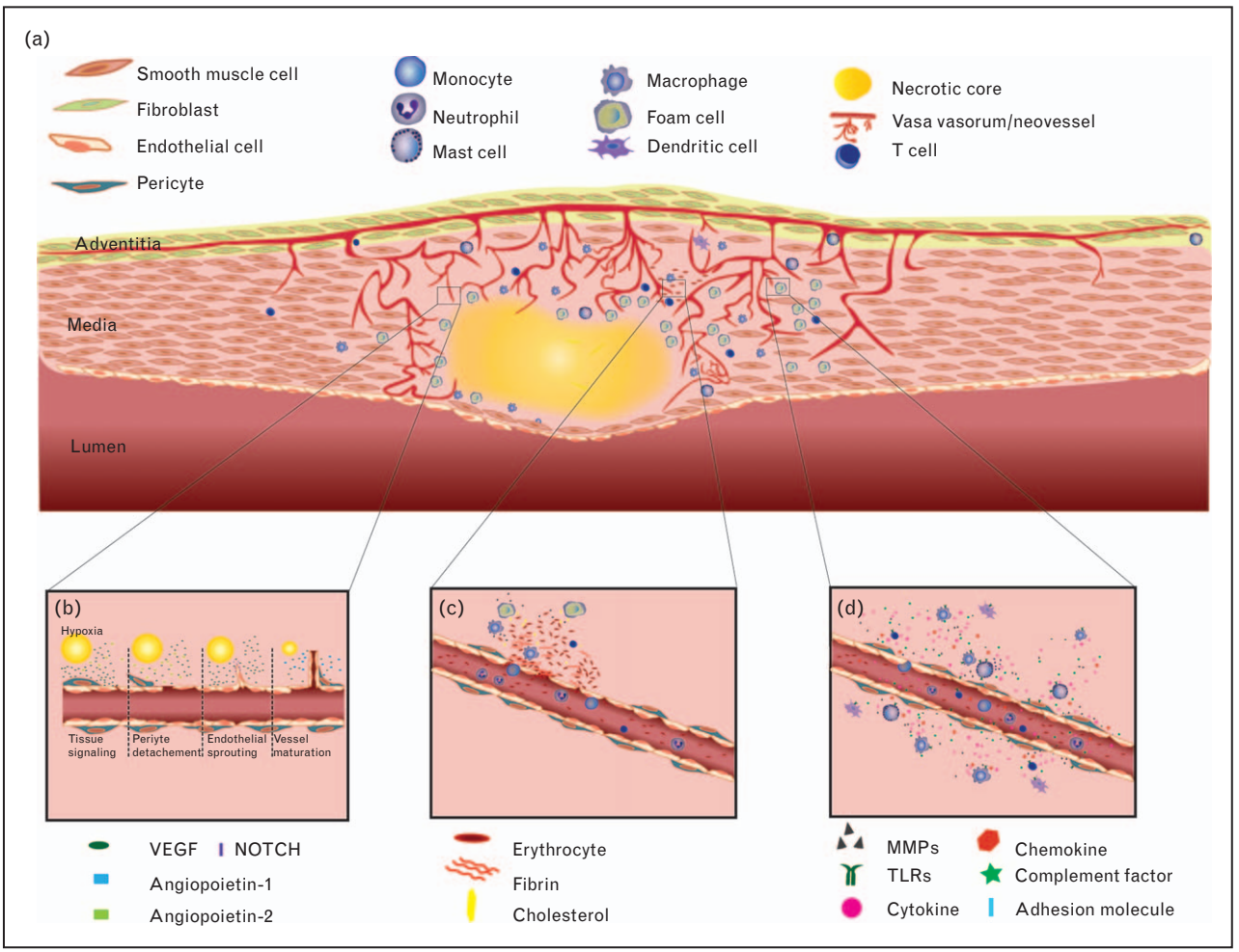

FIGURE 1. Plaque angiogenesis. (a) Plaque neovessels grow predominantly from vasa vasora in the adventitia. (b) Upon hypoxia and under influence of a gradient of VEGF, endothelial cells form a tube-like structure. To become a functional vessel, the vessel has to be stabilized by pericytes. (c) Inflammatory cells interact with endothelial cells and pericytes via inflammatory factors (d) Intraplaque hemorrhage, the extravasation of erythrocytes and inflammatory cells occurs due to immature leaky neovessels. MMPs, matrix metalloproteases; TLRs, toll like receptors; VEGF, vascular endothelial growth factor.

proteins leak into the extracellular space and create an environment into which endothelial cells can migrate in response to integrin signaling. Leading tip cells and the following stalk cells form a tube-like structure under the control of VEGF receptors, neutroplins, and NOTCH ligands [40].

For a vessel to become functional it has to become mature and perfused, otherwise it will regress. Fusion of sprouting neovessels, which is necessary to form a vascular network, is controlled by macrophages [41,42]. Basement membranes and pericytes stabilize the endothelial tubes and help in regulating the capillary diameter and vessel permeability [43▪]. ANG-1 and NOTCH stimulate basement membrane deposition [44,45]. Together with platelet-derived growth factor-B and transforming growth factor-β these factors attract pericytes and stimulate them to envelope the nascent blood vessels [44,46]. Akt signaling in endothelial cells is crucial for the interaction with pericytes [47]. Defective pericyte coverage or pericyte loss leads to abnormal capillary function [48] and consequently to leaky blood vessels. ANG and their receptor Tie-2 are essential for the maintenance of pericyte and endothelial cell integrity with a stabilizing role for ANG-1 and a destabilizing role for ANG-2. In atherosclerotic lesions with a high neovessel density, the local balance between ANG-1 and ANG-2 is in favor of ANG-2 [49]. Furthermore, inhibition of ANG-2 in mice reduces early atherosclerosis plaque development [27].

INTRAPLAQUE HEMORRHAGE

Neovessels in vulnerable plaques appear abnormal with an excess in disorganized vessels that are

enlarged and facilitate extravasation of erythrocytes [50,51]. A profound relationship between IPH and cardiovascular outcome is well established [7,52]. Plaque angiogenesis and IPH are also associated with an increased risk for secondary events [52]. Histopathologic analysis of IPH is based on extravasated erythrocytes and intramural fibrin around immature neovessels (Fig. 1c). Older hemorrhages can be identified by residual iron or glycophorin [29]. Neovessel leakages enrich plaques with blood-borne components such as erythrocytes, inflammatory cells, lipoproteins, and plasma constituents affecting pathological processes such as cholesterol retention, oxidative and proteolytic activities. Membranes of circulating cells and especially erythrocytes contain significant amounts of free cholesterol, which contribute to lipid accumulation in the plaque and can trigger inflammatory responses [29]. Hemoglobin and iron released from erythrocytes are pro-oxidant and capable of causing lipid oxidation and tissue damage [53]. Hemoglobin can trigger an atheroprotective macrophage subset, which has antioxidant and foam-cell protective effects [54–56]. IPH is, however, considered proatherosclerotic, as most macrophages associated with IPH have significant protease activity especially from excreted matrix metalloproteases (MMPs) [57]. Furthermore, intramural fibrin deposits are cleared by fibrinolytic factors, such as tPA, uPA, and plasmin, triggering a proinflammatory, proatherosclerotic response [58].

INFLAMMATORY CELL INFLUX

Inflammatory cells such as macrophages and mast cells are found in the local presence of plaque neovessels [41,58]. These cells are rich sources of cytokines, growth factors, and MMPs, and can influence both plaque stability and plaque angiogenesis [41,59▪]. Inflammatory cells can leak from immature neovessels, but active attraction of these cells via release of specific mediators such as complement factors and TLR ligands also occurs [60▪,61,62]. Furthermore, immature neovessels are known for their active extravasation of leukocytes [62]. The mechanisms involved in trafficking of leukocytes across the endothelium are well studied and involve integrin and adhesion molecule interactions [63,64]. Inflammatory mediators are released by inflammatory cells, neovessel associated endothelial cells, and pericytes. These cell types also respond to the same inflammatory mediators resulting in crosstalk and intertwined responses enhancing their (adverse) effects (Fig. 1d).

Various subsets of macrophages have different roles in atherosclerosis [56,65]. M1 macrophages display a proinflammatory role, including proinflammatory cytokine secretion and phagocytosis, enhancing plaque vulnerability. Whereas, repair-associated M2-like macrophages are linked to neovessels and promote angiogenesis, in part by elevated VEGF secretion [66,67▪]. Endothelial progenitor cells of monocytic origin are mobilized by VEGF and contribute to plaque angiogenesis [41].

A clear relationship between cardiovascular events and plaque neovessel-associated mast cells is established [58]. Mast cells contain and excrete numerous factors, such as tryptase, chymase complement factors, and TLRs, that can influence angiogenesis and plaque vulnerability [59▪,68]. In a murine vein graft model in which plaque angiogenesis prominently occurs [69], complement factor 5a [70] and TLR4 analogue radioprotective 105 [71] influence plaque stability, including IPH, in a mast cell-dependent way.

ANIMAL MODELS FOR RUPTURE, INTRAPLAQUE HEMORRHAGE AND PLAQUE ANGIOGENESIS

Complex atherosclerotic lesions with plaque rupture and subsequent thrombosis are rare in experimental animals [72,73]. Especially, plaque neovessels are seldom observed. However, various mouse models of plaque rupture and especially IPH have been described [74,75▪]. Moulton *et al.* [76] were the first to show a reduction of atherosclerotic lesion formation in apolipoprotein E deficient ($ApoE^{-/-}$) mice after treatment with the angiogenesis inhibitors endostatin, TNP-470, and angiostatin [77]. Hartwig *et al.* [75▪] performed a comparative study on models for vulnerable plaques and found that hypercholesterolemic mice with a shear stress modifier device best replicated human-like lesions with IPH. In $ApoE^{-/-}$ mice with a mutation in the fibrillin-1 gene, highly unstable plaques with plaque angiogenesis and plaque rupture were observed. This model was further associated with myocardial infarction, stroke, and sudden death [78▪▪]. Stress enhanced the phenotype, whereas treatment with atorvastatin prevented mortality and morbidity [79,80]. Human atherosclerotic lesions in saphenous vein bypass grafts are vulnerable and have a higher risk to disrupt than native atherosclerotic lesions [2▪▪,81,82]. Atherosclerotic lesions in vein grafts in hypercholesterolemic mice demonstrate plaque angiogenesis and IPH (Fig. 2) [69]. Moreover, dissections and erosion are frequently observed. Disruption of these lesions could be prevented by inhibiting protease activity by overexpressing tissue inhibitor of MMP-1 [69]. Experimental models that completely resemble human lesions will likely never occur, especially taken into account that human

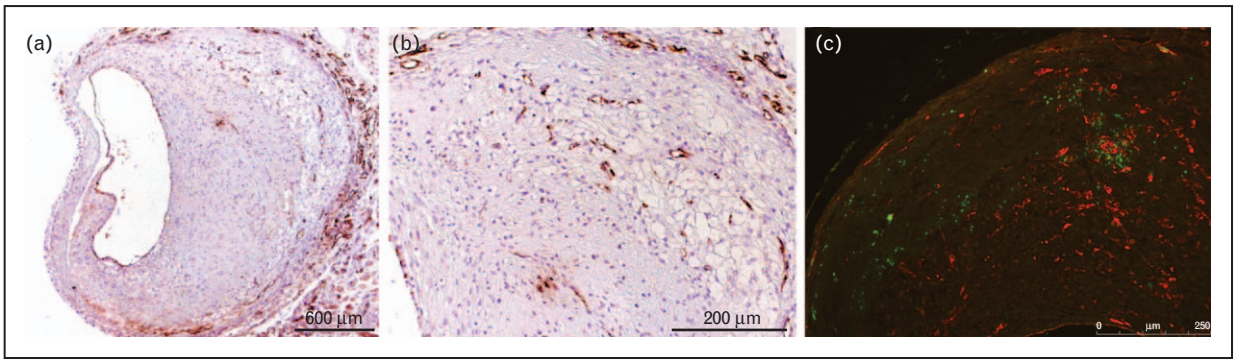

FIGURE 2. Vein graft lesions in hypercholesterolemic mice. (a) Atherosclerosis in a vein graft lesion is accompanied by CD31 positive neovessels (b) Magnification of a part of (a); CD31 positive neovessels in a region of foam cells. (c) Intraplaque hemorrhage in a vein graft lesion; neovessels (red) and erythrocytes (green).

lesions develop over decades and animal atherosclerotic plaques in months. However, specific features of vulnerable lesions can be studied in animal models. Especially, mice carrying the fibrilin-1 mutation and the vein graft model are very suitable to study plaque rupture and plaque angiogenesis.

LYMPH ANGIOGENESIS IN ATHEROSCLEROSIS

The lymphatic system forms a blind-ended network from the extracellular space to the blood circulation and consists of lymphatic vessels, lymph nodes, and associated lymph organs [83,84]. The lymphatic system actively regulates tissue fluid homeostasis, absorption of gastrointestional lipids [85], and trafficking of cells and antigens to lymph nodes [86]. VEGF-C, VEGFR-3, and lymphatic vessel endothelial hyaluron receptor 1 (LYVE1) are involved in lymph angiogenesis and maturation of the lymphatic system [84]. Lymphoid-homing chemokines CCL21, CCL19, and plasmalemma vesicle-associated protein expressed by lymphatic endothelial cells actively guide CCR7 expressing leukocytes or antigen-presenting cells to the lymph nodes [87]. CCR7 deficient mice on an atherosclerotic background showed retention of lymphocytes and exacerbated atherosclerosis [88]. Interestingly, in aged $APOE^{-/-}$ mice adventitial lymphatic lymphatic vessel endothelial hyaluron receptor 1 + vessels seem to regress, which coincides with increased levels of VEGFR-2, which could trap VEGF-C [89]. Upon chronic inflammation, tertiary lymphoid organs (TLOs) can emerge on the adventitial side of plaques [90]. These TLOs contain, in contrast to secondary lymph nodes, high densities of regulatory T cells [90] and B cells [91], and seem to be associated with atherosclerosis protection [92▪]. These intriguing findings bring new insights in the dynamic role of the adventitial lymphatic system in atherosclerosis.

IMAGING OF VULNERABLE PLAQUES

In-vivo imaging technology now offers opportunities to identify vulnerable atherosclerotic lesions in patients by analysis of major plaque components, aiming at prevention of acute events. Computerized tomographic angiography, MRI, (intravascular) ultrasound imaging, and optical coherence tomography can identify thin-cap plaques, calcification, and outward remodeling. Various established techniques and new approaches are extensively reviewed [93,94,95▪▪,96]. Contrast agents, tracers, and targeted imaging probes can enhance the resolution and/or specificity of the imaging strategy [97–100]. Especially, (18)[F]-fluorodeoxyglucose PET imaging has emerged as a promising imaging strategy, because of recognition of plaque glycolysis, plaque inflammation, and hypoxia, all major stimuli for plaque angiogenesis and vulnerability [16▪,101]. A more specific approach to image plaque angiogenesis is the use of endothelial-specific targeting. Several specific probes have been shown to be successful in experimental models [102–104]. These novel imaging techniques can be used to perform functional imaging and in-vivo microscopy of plaque components in greater detail than ever before, allowing longitudinal analyses and investigation of the efficacy of antiatherosclerotic strategies.

TARGETING OF PLAQUE ANGIOGENESIS

Plaque angiogenesis is a physiological process in which the associated proinflammatory and proatherosclerotic effects are detrimental for plaque vulnerability. Plaque angiogenesis is therefore considered a potential therapeutic target comparable to tumor angiogenesis [105]. Antiangiogenic therapies are effective in experimental atherosclerosis [76,77], whereas inhibition of VEGF increased atherosclerosis [106]. Unfortunately, antiangiogenesis strategies

showed minor survival benefits in cancer patients, even increased hypoxia and tumor progression was observed [10▪]. Antiangiogenic strategies to improve vessel maturation are a potential alternative [10▪,107,108▪▪], including targeting pericytes [43▪,109]. This seems an elegant strategy, but there are still many unresolved issues that need to be clarified.

CONCLUSION

It is well established that intraplaque angiogenesis and IPH are crucial processes for plaque instability, however, the pathophysiology of these processes are not yet resolved. The increased knowledge of plaque angiogenesis as well as the improved animal models in which plaque angiogenesis can be studied, and the potential of antiangiogenic approaches in the tumor field, suggest that antiangiogenic treatments in atherosclerosis will be defined in the near future.

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Conflicts of interest

There are no conflicts of interest.

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- ▪▪ of outstanding interest

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