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STATE-OF-THE-ART PAPER

# The Role of Monocytes in Angiogenesis and Atherosclerosis

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**New vessel formation inside the arterial wall and atherosclerotic plaques plays a critical role in pathogenesis of heart attacks and strokes. The 2 known mechanisms resulting in the formation of new vessels within the plaque are local ischemia and inflammation. Blood monocytes play an important role in both processes. First, they express receptors for vascular endothelial growth factor and some of them may serve as circulating ancestors of endothelial cells. Second, monocytes are associated with inflammation by synthesis of inflammatory molecules following their activation (e.g., after stimulation of Toll-like receptors). Neovascularization is a reparative response to ischemia, and includes 3 processes: angiogenesis, arteriogenesis, and vasculogenesis. Angiogenesis, the formation of new capillary vessels is known to occur in response to a hypoxic environment. The interaction between leukocytes and vascular wall via overexpression of various molecules facilitates the migration of inflammatory cells into the plaque microenvironment. Monocytes are intimately involved in tissue damage and repair and an imbalance of these processes may have detrimental consequences for plaque development and stability. Importantly, monocytes are comprised of distinct subsets with different cell surface markers and functional characteristics and this heterogeneity may be relevant to angiogenic processes in atherosclerosis. The aim of this review article is to present an overview of the available evidence supporting a role for monocytes in angiogenesis and atherosclerosis.** (J Am Coll Cardiol 2014;63:1–11) © 2014 by the American College of Cardiology Foundation

Atherosclerosis is the primary cause for stroke and coronary artery disease in the Western World. It is a chronic inflammatory process characterized by development of lipid rich plaques within the layers of the arterial wall (Fig. 1). Within this thickened wall is where foam cells, monocyte derived lipid laden macrophages have been recognized (1). The formation of atherosclerotic plaque is a series of events that is initiated with lipid accumulation (fatty streak) followed by monocyte infiltration and the lipid core formation. Advanced lesions can obstruct arterial lumen, but at any stage atherosclerotic plaque may be complicated by rupture causing a hypoxia/ischemia of the downstream tissues and subsequent vascular complications.

Unhealthy lifestyles, diabetes, obesity, hypertension are still common contributors to the atherogenesis and development of unfavorable events thus prompting identification of new therapeutic targets (2). This is particularly true as current treatment modalities such not all patients are suitable for adequate coronary artery bypass grafting or angioplasty. Of interest, each of the risk factors mentioned previously triggers numerous pathological pathways involving a number of molecular processes, which include lipid metabolism, coagulation, apoptosis, hypoxia, and the immune response (3).

The body's natural response to ischemia is a reparative mechanism summarized by the term *neovascularization*. Neovascularization includes 3 processes: angiogenesis, arteriogenesis, and vasculogenesis. The formation of new capillary vessels, angiogenesis, has been extensively researched and occurs in response to a hypoxic environment (4). Progression and expansion of already existing collateral smooth muscle-type vessels or arteriogenesis, is believed to be a mechanism of organ preservation in the presence of vascular occlusion. Vasculogenesis or new vessel growth derived from progenitor/stem cells has been demonstrated in both the adult and embryo (5). Understanding these processes of vessel adaptation or formation is fundamental for developing new therapeutic strategies.

Inflammation has been shown to be an essential factor accompanying both the angiogenic and atherogenic pathways (5). Monocyte-derived macrophages play a pivotal role in lipid deposition and progression of atherosclerosis, but they are also implicated in the genesis of new vessels (6). The aim of this article is to present an overview of the available evidence supporting the role of monocytes in angiogenesis.

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**Abbreviations and Acronyms**

**Ang** = angiopoietin
**$\beta_2$A** = beta adrenergic system
**bFGF** = basic fibroblast growth factor
**EPC** = endothelial progenitor cells
**HIF** = hypoxia inducible factor
**ICAM** = intracellular adhesion molecule
**IL** = interleukin
**MCP** = monocyte chemoattractant protein
**Tie** = tyrosine kinase
**TNF** = tumor necrosis factor
**VCAM** = vascular adhesion molecule
**VEGF** = vascular endothelial growth factor

## Search Strategy

We searched the following electronic databases (limiting the search from 1970 to July 2010): Pubmed, Medline, EMBASE, and Cochrane Reviews. Given the enormity of this subject area, we have focused on areas of particular relevance to angiogenesis and the role of monocytes in neovascularization. The key words used were *angiogenesis*, *neovascularization*, *vasculogenesis*, *angiopoietin*, *vascular endothelial growth factor* (VEGF), *Tie2*, *monocytes*, and *monocyte subsets*.

## Atherogenesis and Plaque Neovascularization

Atherosclerosis is characterized by monocyte adherence to endothelium cell, migration into the arterial wall, and lipid accumulation (7). The earliest detectable atherosclerotic change is pathological intimal thickening (8).

Enlargement of the plaque results in intraplaque hypoxia that triggers the inflammatory cell infiltration, thus promoting local neovascularization (9). Interestingly, although intimal thickening is believed to be an early surrogate marker for atherosclerosis, pathological neovascularization is implicated in both early and late stages of the disease (9). For example, in experimental studies on hypercholesterolemia, adventitial neovascularization in the coronary arteries has been shown to be present even before the actual plaque (protrusion into the lumen) begins to develop (9). Two instrumental factors influencing the initiation of intra-arterial neovascularization are local ischemia and either local or systemic inflammatory burden. Pathological thickening of the intima greater than 100 μl increases the distance between the lumen and the inner parts of the vascular wall, thus impairing the supply with oxygen and nutrition. As vascular disease ensures excessive vessel wall thickness, proliferation of the vasa vasorum and intimal neovascularization is observed. Indeed, the degree of adventitial neovascularization has recently been demonstrated to be associated with intima-media thickness (10).

Evidence of the role of ischemia in the initiation of angiogenesis stems from the demonstration of increased levels of hypoxia inducible factor (HIF-1), which ultimately promotes VEGF production (4,11). As a potent stimulator of angiogenesis, VEGF is consequently able to create a local pro-angiogenic environment by mobilizing endothelial progenitor cells (EPCs) (Table 1) (12). Furthermore, aggressive plaque development and accelerated neovascularization of the vascular wall have been seen following the administration of VEGF in laboratory experiments (13).

Hypoxia-independent pathways triggered by an inflammatory stimulus within the vascular wall have also been

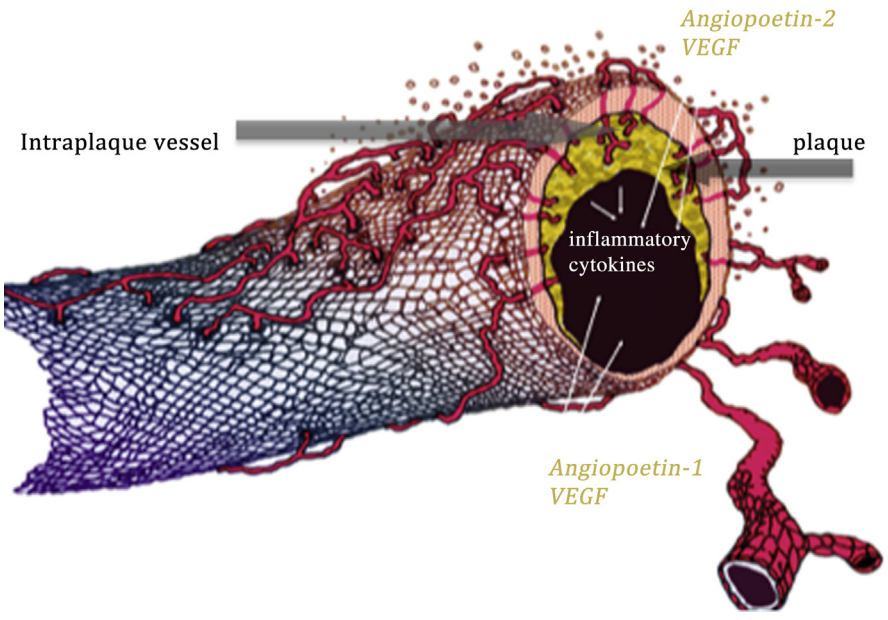


**Figure 1** Angiogenesis and Inflammation

Site of occurrence within the arterial wall/vessel. VEGF = vascular endothelial growth factor.

**Table 1 Angiogenesis Inhibitors and Stimulators**

| Stimulators | Inhibitors |
|---|---|
| VEGF | Angiostatin |
| IL-6 | TGF-β |
| TNF | Interferon |
| bFGF | IL-12 |
| PDGF | Antithrombin III |

bFGF = basic fibroblast growth factor; IL = interleukin; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

recognized as modulators of angiogenesis (14). The density of intraplaque vessels corresponds to the focal accumulation of inflammatory cells (i.e., monocytes/macrophages) forming a pathological circle: angiogenesis > mobilization of inflammatory cells > angiogenesis (15). Switching between this inflammatory/angiogenic cascade may be responsible for enhanced plaque progression related to local plaque inflammation and plaque destabilization. This hypothesis is supported by increased expression of leukocyte adhesion molecules, such as vascular adhesion molecule-1 (VCAM) and intracellular adhesion molecule-1 (ICAM), on the intimal side of vascular endothelium as opposed to the adventitial side (16). Therefore, these observations allude to the notion that the presence of these adhesion molecules on the newly formed vessels is associated with the enhanced accumulation of leukocytes (16).

While association of the development and progression of atherosclerosis with macrophages has long been recognized, the function of their blood ancestors, monocytes, was less addressed. However, a potential link between monocytes and abnormal plaque angiogenesis has a strong biological justification. Prior to discussing angiogenesis, an overall understanding of vasculogenesis (new-vessel formation) and its relationship to EPCs needs to be addressed.

## Vasculogenesis

Vascular system development in the embryo is termed *vasculogenesis*. The first endothelial and hematopoietic cells are derived from a process whereby blood islands are formed from hemangioblasts, otherwise known as mesodermal progenitors in the embryonic yolk sac. These islands of cells differentiate and proliferate from precursors of the vascular wall, angioblasts, which further give origin to endothelial cells (17). Vascular development occurs, as these endothelial cells form the first primitive tubes/vessels. Importantly, it is at this point when both VEGF and basic fibroblast growth factor (bFGF) (a critical angiogenic factor), starts to play a role in the process (18). Activation of bFGF receptors on endothelial cells by bFGF increases the endothelial cell motility, proliferation and proteinase activity (19). bFGF can also induce VEGF expression via HIF-1α activation as seen in a study by Shi et al. (20), who showed that HIF-1α induction by bFGF seemed to be an independent pathway triggering VEGF expression in breast cancer. Bovine studies have shown endothelial cell proliferation and capillary formation in the presence of bFGF and VEGF (21).

The identification of EPCs in the adult has led to efforts to understand their contribution to the adult angiogenic processes. However, it must be mentioned that there is much debate regarding methods of EPC quantification and standardizations. This is due to a significant cell overlap observed and the presence of the progenitors at different stages of maturation (22). Recently, Sozer et al. (23) have demonstrated that monocytes and EPCs share many characteristics while other less differentiated primitive cells produce endothelial cells only in vitro. Further delineation of their phenotype is required.

## Angiogenesis

The development of new vasculature, particularly the formation of new capillaries from endothelial cells that "sprout" from existing blood vessels is of importance for a number of pathological and homeostatic processes. It is also fundamental for the embryonic development. Tumor research, the important field for angiogenic studies, has not only suggested a pathological significance of the angiogenic process in cancer but also led to further efforts to investigate these processes in biological mechanisms of wound healing, ovulation, and tissue repair (24).

Naturally healthy tissue requires a supply of nutrients and oxygen. Also the restoration of tissue under ischemic conditions and tumor growth are dependent on new vessel formation for the supply of nutrients and the removal of degradation products. This understanding of an angiogenic process has led to a vast number of studies on therapeutic approaches to the management of vascular disorders. The primary pathological focus of this evidence has been on understanding the atherosclerosis-related angiogenesis in plaque formation, and the inhibition of neovascularization thereby attempting to slow the disease progression (24).

Interestingly, the main laboratory approach used for understanding of the mechanisms of atherosclerosis-related angiogenesis was based on analysis of ischemic tissues in the presence of pre-existing plaque stenosis as opposed to long-term studies on the development and progression of the disease. For example, a study by McCarthy et al. (25) suggested an association between symptomatic carotid disease (plaque) and the presence of intraplaque neovascularization. However this study recruited patients who had pre-existing carotid stenosis and did not study patients who were initially carotid plaque free and developed stenosis over a number of years (25). This lack of evidence on early changes and pathological mechanisms has driven the need for non-invasive approaches to detect neovascularization, such as carotid contrast ultrasonography.

The initiating factor of angiogenesis is a hypoxic environment, often associated with tissue inflammation (26). The entire pathway is thought to be stimulated by HIF-1α.

In the ischemic environment HIF-1α, escapes degradation due to its transcription being down-regulated and its ability to bind other factors (e.g., HIF-1β) activating target genes involved in angiogenesis (27). Interestingly a number of growth factors (e.g., platelet-derived growth factor, and bFGF) also share this regulatory hypoxic driven pathway (28). VEGF, an essential angiogenic modulator has been shown in several in vivo models to induce a strong concomitant angiogenic cascade with HIF-1 (11). VEGF expressed by macrophages and T-lymphocytes stimulates endothelial cells to produce monocyte chemoattractant protein (MCP)-1, hence attracting monocytes and enhancing cell migration by increasing the permeability of the endothelial layer (29,30). Embryologically, the absence of VEGF results in early death due to abnormal blood vessel growth, demonstrating a common link between the physiological and pathological angiogenic pathways (31).

The sprouting of new vessels from pre-existing vasculature is known as angiogenesis. Angiogenic signals from surrounding cells lead to vasodilatation and an increase in vascular permeability (27,32). Digestive enzymes such as collagenase and matrix metalloproteinases partially destroy the basement membrane (33). The plasma proteins then form a fibrin rich matrix, with a lumen forming in the proliferating capillary when the activated endothelial cells migrate towards the site (34). Ultimately, the newly formed capillaries become part of the existing circulation in a process in which shear stress is a critical factor (35).

Although inflammation plays a significant role in angiogenesis, multiple other processes are implicated in development of new vessels, including cell-to-extracellular matrix interactions, vascular wall maturation and basal lamina modifications (32,36). The behavior of endothelial cells is significantly influenced by inflammatory leukocytes able to release the number of proangiogenic factors, such as VEGF, hepatocyte growth factor, and tumor necrosis factor-(TNF)-α, and interleukin (IL)-8 (37,38).

In areas of atherogenic lesions chronic infection, cigarette smoking, free radicals, hypertension, and diabetes have all been implicated as causes in the activation of endothelial cells (35,39). The increased shear stress acting through both membrane structures and cell junction molecules, stimulate quiescent endothelial cells lining the vascular wall (40). This intracellular signaling triggers the expression of genes such as MCP-1 mRNA, involved in induction of transcription factors responsible for shear stress-mediated effects (32,34). This sequence of events in the presence of hypercholesterolemia triggers expression of adhesion molecules, particularly P-selectin, E-selectin, VCAM-1, ICAM-1, and MCP-1 release and activation of genes responsible for the expression of CCR2 (MCP-1 receptor) (32,41).

## Monocytes in Angiogenesis

Oxygen-deprived intima of the arterial wall recruits circulating monocytes via specific integrin receptors (macrophage adhesion ligand [Mac]-1), that interacts with the endothelial adhesion molecules (36,42) (Fig. 2). It has been shown that this binding predominantly occurs at the tight junctions of the endothelial cells and allows monocyte entry into the subendothelial space. VEGF, expressed by macrophages activates the production of MCP-1 by the endothelial cells and an increase in the permeability of the endothelial layer (Table 2) (35). The chronic low-grade inflammation inside the vascular wall has been shown to be associated with monocyte infiltration. The monocyte maturation to macrophages is accompanied by the production of cytokines and growth factors (36,38).

Plaque monocytes/macrophages interact with collagen and proteoglycans in the extracellular matrix by expressing proteases such as urokinase plasminogen activator (43). Urokinase plasminogen activator activates plasmin, which in turn degrades the extracellular matrix (43). The monocytes produce platelet-derived growth factor, which induces mitotic activity of the endothelial cells and vascular smooth muscle cells (44). Activated plaque monocytes/macrophages ingest the oxidized lipids and become lipid-laden "foam" cells. It is believed that "foam" cells promote vascular remodeling by stimulation of smooth muscle cell migration and a subsequent shift in endothelial function (45).

While there is a distinct relationship between monocytes and angiogenesis in the atherosclerotic lesions, controversy surrounds the origin of the native endothelial cells as well as the role of specific subtypes of monocyte populations such as CD14+/VEGFR-2+ monocytes (46). Animal studies have demonstrated that although endothelial cells play a role in the initiation of the atherosclerotic process they themselves may be bone marrow-derived as in tumor-associated blood vessels (47). Once monocytes have infiltrated the tissue layers a proportion of them will differentiate into dendritic cells triggering the activation of antigen specific T lymphocytes associated with creation of the local inflammatory environment (48).

A large proportion of circulating EPCs was found to be of monocytic origin (49). Human monocytes include a population of cells able to obtain endothelial cell phenotype in culture (50). Cultures of so-called "early" EPCs are mainly comprised of monocytes and T-cells and their formation is strictly dependent upon monocyte presence (51). Additionally, monocytes constitute the dominant population among circulating cells expressing type 2 receptor for VEGF (VEGFR2) (52). Cells bearing CD14 (a monocyte marker) are capable of improving re-endothelialization after carotid balloon injury in animals and this process depends on the levels of a major factor stimulating monocyte mobilization, MCP-1 (53). Elsheikh et al. (46) have reported that transplantation of CD14+/VEGFR2+ cells into balloon-injured femoral arteries of nude mice significantly contributed to their efficient re-endothelialization. These data support the possible involvement of monocytes in hypoxia-induced VEGF-mediated formation of vasa vasorum (Table 3).

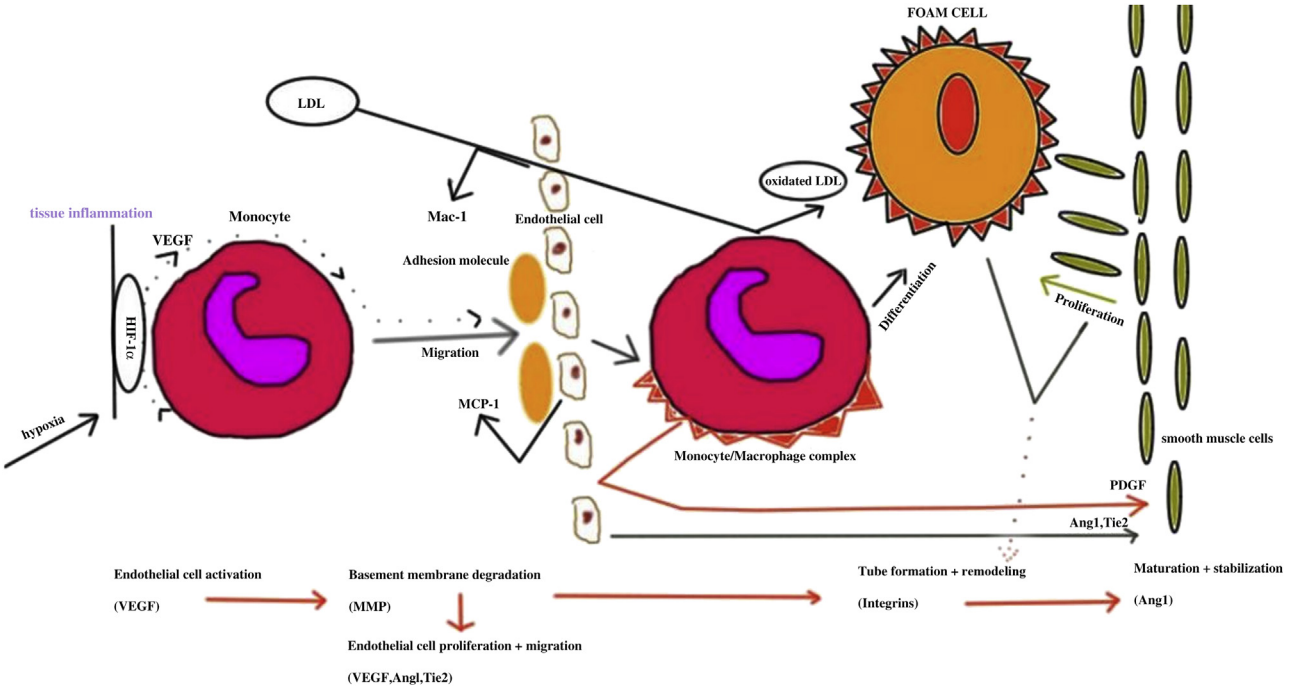


**Figure 2** **Monocytes Role in Angiogenesis**

Pathway from hypoxia to the production of the macrophage foam cell. Ang = angiopoietin; HIF = hypoxia induced factor; LDL = low-density lipoprotein; MAC = macrophage antigen; MCP = monocyte chemotactic protein; MMP = matrix metalloproteinase; PDGF = platelet-derived growth factor; Tie = tyrosine kinase; VEGF = vascular endothelial growth factor.

## Monocyte Heterogeneity

Monocyte subsets in particular are believed to play a differential role in intra-plaque angiogenesis and tissue repair (32). The subsets differ in phenotype, granulation, size, morphology, and genetic make up (54). Over the last 30 years, human monocyte subsets were distinguished based on their surface CD14/CD16 expression as "classical" CD14++CD16− cells and less frequent "nonclassical" CD14+CD16++ blood monocytes (55).

A third subset can be distinguished by surface expression of CCR2 (Table 4) (56). Interestingly, these CD14++CD16+CCR2+ monocytes phenotypically resemble the previously reported pro-angiogenic monocytes (56). For example, De Palma et al. (57) and Venneri et al. (58) demonstrated distinct pro-angiogenic properties of tyrosine kinase (Tie) 2–expressing monocytes. This conclusion lends to an earlier study by Lu et al. (59) on bone marrow derived vascular progenitors, which demonstrated blood vessel formation to be an angiogenic process (from pre-existing vessels) but also having a vasculogenic component. In other words, growth factors, cytokines, and other key proangiogenic contributions derive not only from local tissues but also from bone marrow (59).

The identification of each subset thus allows further research into their respective physiological functions. Ziegler-Heitbrock et al. (60) showed that CD14+CD16+ monocytes have some features common with mature tissue macrophages. However, animal-based studies on monocyte subsets are controversial due to substantial differences between human and murine monocyte subsets (61). Nahrendorf et al. (62) compared monocytes in a model of mouse myocardial infarction and suggested that specific signaling may depend on site and type of hypoxia/ischemia insult and time of recovery from this injury.

Although 3 monocyte subsets are now recognized, the majority of published studies only refer to 2 monocyte subpopulations (i.e., CD14+CD16- and CD14+CD16+ monocytes) without further subdivision of the CD16+ cells, and thus careful interpretation of such data is required. The CD16+ monocytes are infrequent (less than 15% in healthy humans), but their proportions are increased in patients with stenotic coronary artery disease, and myocardial infarction, being related to the up-regulation of inflammatory cytokines (63,64). These data may indicate a possible role of CD16+ monocytes for the advanced inflammation-mediated arteriogenesis/intra-plaque angiogenesis. However, the relation of monocyte populations to systemic atherosclerosis (intima-media thickness, adventitial vasa vasorum) and high-risk indices for plaque destabilization is clearly understudied. Subset

**Table 2** Examples of Animal and Human Studies Implicating Monocytes in Angiogenesis

| First Author (Ref. #) | Model | Mediating Factor | Study Design | Study Finding |
|---|---|---|---|---|
| Capoccia et al. (65) | Mouse | MCP-1 | Direct injection of bone marrow cells into blood of surgically induced hind limb ischemia. Adductor hind leg muscle was then subjected to flow cytometry, ELISA, and immunofluorescence. Bone marrow cells were also harvested and transplanted into wild-type mice. | Inflammatory subset of monocytes was selectively recruited to the site of insult in parallel with increased MCP-1 amounts. Two waves of monocyte proliferation were demonstrated in the presence of angiogenesis and inflammation. |
| Arras et al. (102) | Rabbit | TNF, bFGF | Femoral artery occlusion of rabbit hind limb for 3 and 7 days, with randomly given lipopolysaccharide. Further control animals were tested at 21 days for comparison. Carotid artery catheters were also placed for proliferation analysis. | After day 7 of induced hypoxia, maximal macrophage proliferation was present being associated with higher TNF and bFGF levels. Monocytes/macrophage activation played important role in angiogenesis and vessel growth in the presence of hypoxia. |
| Hong et al. (30) | Rat, chick | MCP-1, VEGF | Thoracic and abdominal aortas were obtained from 5-week-old rats. VEGF was analyzed by mRNA expression using PCR. MCP-1 was analyzed in vivo using chick chorioallantoic membrane. | Monocytes were implicated in angiogenesis in MCP-1–mediated manner and related to HIF and VEGFA up-regulation. |
| Cursiefen et al. (103) | Mouse | VEGF | Mouse model of suture induced inflammatory corneal neovascularization. Immunochemistry and morphometry were used to analyze angiogenesis in the cornea. | VEGF mediates the recruitment of monocytes/macrophages resulting in the initiation of neovascularization in the presence of inflammation but also amplifies the pathological process of both angiogenesis. |
| Eubank et al. (104) | Human | M-CSF, VEGF | Isolated human monocytes were stimulated with M-CSF. ELISA was used for VEGF analysis. | M-CSF enhanced production of VEGF and angiogenesis by human monocytes. |
| Venneri et al. (58) | Human | Tie2 | Healthy blood donors and surgically resected tumor tissue. Analysis performed using flow cytometry, western-blot analysis, immunohistochemistry, and migration assays. | Tie2+ monocytes were associated with angiogenesis. |

Ang-2 = angiopoietin; bFGF = basic fibroblast factor; EC = endothelial cell; ELISA = enzyme-linked immunoadsorbent assay; HIF = hypoxia inducible factor; M-CSF = macrophage colony-stimulating factor; MCP = monocyte chemotactic protein; PCR = polymerase chain reaction; Tie2 = tyrosine kinase; VEGF = vascular endothelial growth factor.

specificity may also be dependent on expression of multiple receptors and MCP-1–mediated signaling (65). Indeed, angiogenesis and monocyte subset involvement is a multiple stepwise process, which consists of 2 areas of recruitment, local and bone marrow, which are specific to the stimulating environment (65). Further studies are being performed to delineate the specific functions of each of the monocyte subsets, but their specific roles in plaque progression, stability, and rupture remains insufficiently understood at present.

## Monocytes in Atherosclerosis Progression

Uncontrolled lipid accumulation followed by rapid monocyte infiltration and phagocytosis of low-density lipoprotein-mediated by scavenger receptors subsequently results in macrophage apoptosis. This understandably increases the atherosclerotic plaque core with ensuing necrotic tissue, collagen deposition and migration of smooth muscle (32). This pattern of monocyte/macrophage deposition and removal although protective by nature is only mediated by the inflammatory reaction that it manifests itself. This unbalanced inflammation with excessive cytokine release from monocytes and enhanced monocyte expression of the Toll-like receptors promotes both angiogenesis and plaque growth and destabilization. Administration of statins to subjects with hypercholesterolemia inhibited the expression of monocyte pro-inflammatory cytokines (TNF and IL1β) and the treatment has a well documented capacity to reduce risk of unfavorable events in patients with stable coronary heart disease (66). Moreover, long-term treatment with statins can prevent progression or even lead to regression of the atheroma, although the relative magnitude of lipid-independent pleiotropic effects of the statins in the overall benefits of the drugs remain unclear.

**Table 3** Family of VEGF and Their Functions

| Type | Function |
|---|---|
| VEGFA | Angiogenesis |
| | Chemotactis |
| | Vasodilatation |
| VEGFB | Embryonic |
| VEGFC | Lymphangiogenic |
| VEGFD | Lung lymphatics |
| PIGF | Vasculogenesis |

PIGF = placental growth factor; VEGF = vascular endothelial growth factor.

**Table 4** Monocytes Subsets and Their Functions

| Monocyte subset | Expression | Primary Function |
|---|---|---|
| Mon1 (classical) | CD14++CD16–CCR2+ | Phagocytosis<br>Cytokine production |
| Mon2 (intermediate) | CD14++CD16+CCR2+ | Angiogenesis |
| Mon3 (non-classical) | CD14+CD16++CCR2– | Collagen deposition<br>Anti-inflammatory effects |

## Plaque Instability and Rupture

The volatile nature of an atheromatous plaque is responsible for approximately 60% of symptomatic carotid artery disease and about 75% of acute coronary events. Neovascularization has been implicated as a possible contributor to the process by which an asymptomatic fibroatheromatous plaque becomes a lesion vulnerable to rupture, although the precise mechanism of how this occurs remains unclear. As the plaque progresses, the adventitial vasa vasorum is the site of initiation of intraplaque vessels formation (67). Indeed, the presence of neo-vessels within the plaque has been associated to its rupture (67). In a comparison of stable plaques to those in both vulnerable and ruptured plaques, there is a 2- to 4-fold increase in the number of vasa vasorum, respectively (68). Although plaque deposits themselves may be localized and unique, the changes found in the arterial wall vascularization are known to be systemically widespread, lending to the notion of atherosclerosis being a panarterial disease (69). However the factors that trigger the change, from a nonthreatening to unstable plaque remains poorly understood.

The synthesis of pro-inflammatory molecules such as IL-6 and TNFα, mediated by stimulation of Toll-like receptors (TLR4) are upgraded by activated monocytes (51). The interaction(s) between endothelial cells and white blood cells results in an inflammatory cascade resulting from the interaction among CD14, a monocyte endotoxin receptor, acting together with a coreceptor, Toll-like receptor, leading to monocytic activation (70). This monocyte activation subsequently enhances the affinity of monocyte ligands to adhesion molecules, thus promoting monocyte–endothelium adhesion (70). This has been demonstrated by the presence of microvessels within lipid-rich plaques strongly expressing adhesion molecules (ICAM-1, VCAM-1) thereby facilitating transendothelial migration of inflammatory cells (i.e., monocytes) into the plaque microenvironment (16). This implicates the potential involvement of monocytes and their role in plaque neovascularization and plaque rupture.

## Angiopoietins

Homeostasis of the vascular system is supported by secreted glycoproteins called angiopoietins (Ang) (71). The latter function as growth factors to aid angiogenesis. However, this may not necessarily be the case for angiopoietin 1 (Ang1), which, under specific conditions, may act as an inhibitor of the angiogenic process (72).

There are 4 main ligands in the angiopoietin group (Ang1, Ang2, Ang3, Ang4) (73). Ang1 and Ang2 have been well studied and have a strong affinity to tyrosine kinase receptors (74). Both Ang1 and Ang2 can be found in high concentration in tumors, particularly angiosarcoma suggesting their role in both tumor angiogenesis and progression (75). Shim et al. (76) demonstrated differences between Ang1 and Ang2 in their response to hypoxia. Ang2 was up-regulated in the presence of ischemic tissue whereas Ang1 was mostly associated with malignancy. However, both are implicated in the angiogenic processes. The family of receptors, which primarily maintains Ang influence and ability to be expressed in endothelial cells, is a Tie2. Tie2 is involved in the stabilization of mature blood vessels, promoting the interaction between endothelial cells and supporting periendothelial cells (Fig. 3) (77).

Animal studies have shown that absence of either Ang1 or Tie2 results in incomplete vascular development and death (78). Interestingly, the interaction between Tie1 and Tie2 remains primarily unclear but it is known that none of the Ang family members directly binds Tie1, yet Tie2 inhibits Tie1-mediated regulatory control of endothelial cell function (79).

Hauer et al. (80) have demonstrated an overall reduction of experimental atheroma after Tie2 inhibition. Consequently,

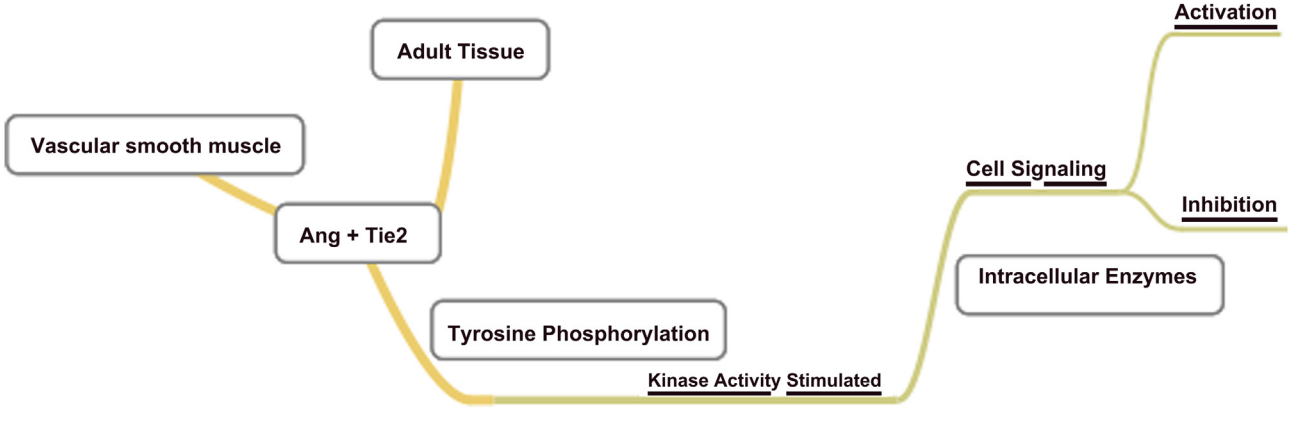


**Figure 3** Interaction of Tie2 With Vascular Smooth Muscle

Pathway from vascular smooth muscle to cell signaling and relationship to tyrosine phosphorylation. Ang = angiopoietin; Tie2 = tyrosine kinase receptor type 2.

a relationship between both Ang2 and VEGF and angiogenesis was shown in genetically modified mouse studies (81). Although the angiogenic effects were greater in the lymphatic tissue rather than in blood vessels, the studies raised interest in the development in antiangiogenic therapies (81). Recently, Saharinen et al. (82) suggested that the 2 systems (i.e., mediated by VEGF and Ang) played different roles in blood and lymphatic vessel growth.

Another study demonstrated not only a link between both VEGF and Ang2 but also a clear difference in how they regulated the angiogenic pathways (83). Indeed, Ang1 has showed an inhibitory role against the actions of Tie2 in blood vessel maturation while Ang2 expression counteracted this Ang1 effect, thus promoting vascular stabilization (84). Once again this antagonist relationship has sparked interest from both a scientific and therapeutic point of view.

## VEGF

MCP-1, although known primarily to play a role in inflammation has been shown to be a chemokine with angiogenic properties. Hong et al. (30) demonstrated that the MCP-1–mediated angiogenic cascade is maintained and modulated by VEGF (30). Further evidence to this relationship and the monocyte role in angiogenesis has been shown by in vitro treatment of human monocytes with VEGF obtained from tumor cells, resulting in both monocyte activation and migration (85).

VEGF is a pro-angiogenic growth factor primarily involved in the initiation of new capillary formation (17). VEGF is involved in embryonic angiogenesis but it is as well a potent signaling protein, which stimulates vasculogenesis and angiogenesis in the presence of injury, exercise, and formation of collaterals (86,87). There are 4 well-known VEGF derivatives plus 1 placental growth factor (Table 3, Fig. 4). Interestingly, excessive VEGF expression has been linked to the progression of malignancy, and retinal eye disease (88). VEGF main action, however, is mediated by binding of tyrosine-kinase receptors (89).

VEGFR2 is essential for endothelial cell survival (90). Absence of VEGFR2 is incompatible with development of endothelial and hematopoietic cells in animals (91). In contrast VEGFR1 is not obligatory for endothelial cell differentiation but it is required for embryonic development (92). Interestingly, a possible antagonistic relationship between VEGFR1 and VEGFR2 has highlighted the intricate relationship between promoting and maintaining vascular development in ischemia, cancer, and other pathological processes (93). Unfortunately, despite the primary role of VEGFR2 in both vasculogenesis and angiogenesis, the molecular mechanisms controlling its genetic expression are still at an early stage of recognition, representing justification for renewed focus in the critical process of protein modulation in a therapeutic respect (88). This is especially true because it has been demonstrated that tumor genesis itself involves specific angiogenic factors based on tumor type (94).

## The Adrenergic System and Angiogenesis

The adrenergic system has been shown to be implicated in regulation of expression of pro-angiogenic factors and angiogenesis (95). For instance, high norepinephrine levels are linked to increased VEGF expression (96). Although the mechanisms of norepinephrine-mediated VEGF upregulation in atherosclerosis remain unclear, a post-transcriptional mechanism has been revealed by which norepinephrine-induced HIF-1α production modulated VEGF expression in cancer cells (97).

In the absence of ischemia or even exercise, the alpha adrenergic system has been demonstrated to increase capillary blood flow via an increase in capillarity of skeletal muscles (98). The alpha adrenergic system has been shown to inhibit angiogenesis by interfering with endothelial

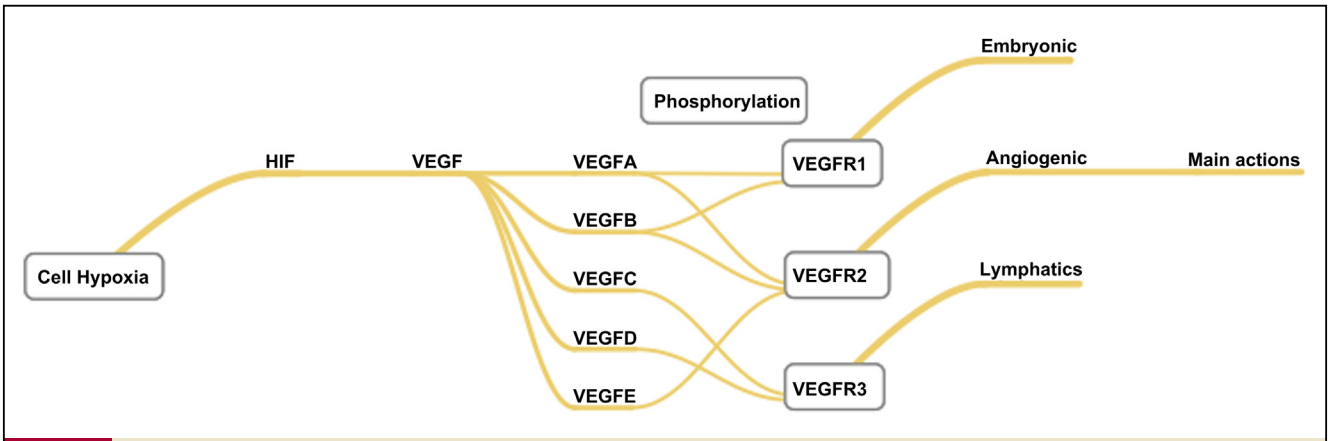


**Figure 4** VEGFs

Family of vascular endothelial growth factors (VEGFs), receptors, and their functions. HIF = hypoxia induced factor; VEGFR = vascular endothelial growth factor receptor.

cell proliferation and responsiveness to VEGF. Although expressed in many tissues, the primary role of beta adrenergic system ($\beta_2$A) receptors overall remains unclear. Leosco and colleagues (99) demonstrated the promotion of angiogenesis with exercise resulting in improved $\beta_2$A signaling. In the presence of ischemia, adrenergic down-regulation of $\beta_2$A with enhancement and preservation of capillaries along with the promotion of endothelial cell proliferation suggests a vital role in regulation of angiogenesis by the $\beta_2$A system (100). Most recently emphasis has been placed on identification of specific $\beta_2$A receptor subtypes on lipopolysaccharide laden monocytes implicated in creation of the pro-inflammatory state, mediated by cytokine modulation and cyclic adenosine monophosphate–dependent mechanisms (101).

## Conclusions

Atherosclerosis and its angiogenic component is an obvious feature of vascular disease. The regeneration of vascular beds with capillary sprouting relies on angiogenesis. Atherosclerosis has an inflammatory component, and MCP-1 plays a role in recruiting monocytes to the site of insult. Monocytes, important members of the innate immune system have been shown to play an intricate role in angiogenesis. Neovascularization is a major contributor to plaque progression. Much more research is required to establish the exact mechanisms underlying the tightly regulated angiogenic processes. One approach would be to continue to investigate the potential presence of various subtypes of monocytes and their specific receptors and roles in cardiovascular disease. This hopefully would be useful for the development of new therapeutic targets for prevention of progression of atherosclerotic disease and its complications.

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## REFERENCES

1. Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell 2001;104:503–16.
2. Cannon CP. Cardiovascular disease and modifiable cardiometabolic risk factors. Clin Cornerstone 2007;8:11–28.
3. Henry TD, Annex BH, McKendall GR, et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation 2003;107:1359–65.
4. Bjornheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall in vivo. Arterioscler Thromb Vasc Biol 1999;19:870–6.
5. Silvestre JS, Mallat Z, Tedgui A, Levy BI. Post-ischaemic neovascularization and inflammation. Cardiovasc Res 2008;78:242–9.
6. Li AC, Glass CK. The macrophage foam cell as a target for therapeutic intervention. Nat Med 2002;8:1235–42.
7. Pietsch A, Erl W, Lorenz RL. Lovastatin reduces expression of the combined adhesion and scavenger receptor CD36 in human monocytic cells. Biochem Pharmacol 1996;52:433–9.
8. de Groot E, van Leuven SI, Duivenvoorden R, et al. Measurement of carotid intima-media thickness to assess progression and regression of atherosclerosis. Nat Clin Pract Cardiovasc Med 2008;5:280–8.
9. Kwon HM, Sangiorgi G, Ritman EL, et al. Enhanced coronary vasa vasorum neovascularization in experimental hypercholesterolemia. J Clin Invest 1998;101:1551–6.
10. Magnoni M, Coli S, Marrocco-Trischitta MM, et al. Contrast-enhanced ultrasound imaging of periadventitial vasa vasorum in human carotid arteries. Eur J Echocardiogr 2009;10:260–4.
11. Kuwahara F, Kai H, Tokuda K, et al. Hypoxia-inducible factor-1alpha/vascular endothelial growth factor pathway for adventitial vasa vasorum formation in hypertensive rat aorta. Hypertension 2002;39:46–50.
12. Inoue M, Itoh H, Ueda M, et al. Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions: possible pathophysiological significance of VEGF in progression of atherosclerosis. Circulation 1998;98:2108–16.
13. Celletti FL, Hilfiker PR, Ghafouri P, Dake MD. Effect of human recombinant vascular endothelial growth factor165 on progression of atherosclerotic plaque. J Am Coll Cardiol 2001;37:2126–30.
14. Carmeliet P. Angiogenesis in health and disease. Nat Med 2003;9:653–60.
15. Moulton KS, Vakili K, Zurakowski D, et al. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc Natl Acad Sci U S A 2003;100:4736–41.
16. O'Brien KD, McDonald TO, Chait A, Allen MD, Alpers CE. Neovascular expression of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in human atherosclerosis and their relation to intimal leukocyte content. Circulation 1996;93:672–82.
17. Risau W. Mechanisms of angiogenesis. Nature 1997;386:671–4.
18. Tomanek RJ, Sandra A, Zheng W, Brock T, Bjercke RJ, Holifield JS. Vascular endothelial growth factor and basic fibroblast growth factor differentially modulate early postnatal coronary angiogenesis. Circ Res 2001;88:1135–41.
19. Klagsbrun M. Mediators of angiogenesis: the biological significance of basic fibroblast growth factor (bFGF)-heparin and heparan sulfate interactions. Semin Cancer Biol 1992;3:81–7.
20. Shi YH, Wang YX, Bingle L, et al. In vitro study of HIF-1 activation and VEGF release by bFGF in the T47D breast cancer cell line under normoxic conditions: involvement of PI-3K/Akt and MEK1/ERK pathways. J Pathol 2005;205:530–6.
21. Goto F, Goto K, Weindel K, Folkman J. Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen gels. Lab Invest 1993;69:508–17.
22. Schmidt-Lucke C, Fichtlscherer S, Aicher A, et al. Quantification of circulating endothelial progenitor cells using the modified ISHAGE protocol. PLoS One 2010;5:e13790.
23. Sozer S, Wang X, Zhang W, et al. Circulating angiogenic monocyte progenitor cells are reduced in JAK2V617F high allele burden myeloproliferative disorders. Blood Cells Mol Dis 2008;41:284–91.
24. Johnstone CC, Farley A. The physiological basics of wound healing. Nurs Stand 2005;19:59–65. quiz 66.
25. McCarthy MJ, Loftus IM, Thompson MM, et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. J Vasc Surg 1999;30:261–8.
26. Bosco MC, Puppo M, Blengio F, et al. Monocytes and dendritic cells in a hypoxic environment: Spotlights on chemotaxis and migration. Immunobiology 2008;213:733–49.
27. Kimura H, Esumi H. Reciprocal regulation between nitric oxide and vascular endothelial growth factor in angiogenesis. Acta Biochim Pol 2003;50:49–59.
28. Atkinson S, Fox SB. Vascular endothelial growth factor (VEGF)-A and platelet-derived growth factor (PDGF) play a central role in the pathogenesis of digital clubbing. J Pathol 2004;203:721–8.
29. Melter M, Reinders ME, Sho M, et al. Ligation of CD40 induces the expression of vascular endothelial growth factor by endothelial cells and monocytes and promotes angiogenesis in vivo. Blood 2000;96:3801–8.
30. Hong KH, Ryu J, Han KH. Monocyte chemoattractant protein-1-induced angiogenesis is mediated by vascular endothelial growth factor-A. Blood 2005;105:1405–7.

31. Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996;380:435–9.
32. Pamukcu B, Lip GY, Devitt A, Griffiths H, Shantsila E. The role of monocytes in atherosclerotic coronary artery disease. Ann Med 2010; 42:394–403.
33. Sengupta N, MacDonald TT. The role of matrix metalloproteinases in stromal/epithelial interactions in the gut. Physiology (Bethesda) 2007;22:401–9.
34. Ojeifo JO, Forough R, Paik S, Maciag T, Zwiebel JA. Angiogenesis-directed implantation of genetically modified endothelial cells in mice. Cancer Res 1995;55:2240–4.
35. Marumo T, Schini-Kerth VB, Busse R. Vascular endothelial growth factor activates nuclear factor-kappaB and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells. Diabetes 1999; 48:1131–7.
36. Hoefer IE, van Royen N, Rectenwald JE, et al. Arteriogenesis proceeds via ICAM-1/Mac-1- mediated mechanisms. Circ Res 2004; 94:1179–85.
37. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004;56:549–80.
38. Hoefer IE, van Royen N, Rectenwald JE, et al. Direct evidence for tumor necrosis factor-alpha signaling in arteriogenesis. Circulation 2002;105:1639–41.
39. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
40. Resnick N, Yahav H, Shay-Salit A, et al. Fluid shear stress and the vascular endothelium: for better and for worse. Prog Biophys Mol Biol 2003;81:177–99.
41. Sadhu C, Ting HJ, Lipsky B, et al. CD11c/CD18: novel ligands and a role in delayed-type hypersensitivity. J Leukoc Biol 2007;81: 1395–403.
42. Schuler P, Assefa D, Ylanne J, et al. Adhesion of monocytes to medical steel as used for vascular stents is mediated by the integrin receptor Mac-1 (CD11b/CD18; alphaM beta2) and can be inhibited by semiconductor coating. Cell Commun Adhes 2003;10:17–26.
43. Menashi S, Lu H, Soria C, Legrand Y. Endothelial cell proteases: physiological role and regulation. Baillieres Clin Haematol 1993;6: 559–76.
44. Polverini PJ, Cotran PS, Gimbrone MA Jr., Unanue ER. Activated macrophages induce vascular proliferation. Nature 1977;269:804–6.
45. Kruth HS. Macrophage foam cells and atherosclerosis. Front Biosci 2001;6:D429–55.
46. Elsheikh E, Uzunel M, He Z, Holgersson J, Nowak G, Sumitran-Holgersson S. Only a specific subset of human peripheral-blood monocytes has endothelial-like functional capacity. Blood 2005;106: 2347–55.
47. Nolan DJ, Ciarrocchi A, Mellick AS, et al. Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. Genes Dev 2007;21:1546–58.
48. Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, Muller WA. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science 1998;282:480–3.
49. Shantsila E, Watson T, Lip GY. Endothelial progenitor cells in cardiovascular disorders. J Am Coll Cardiol 2007;49:741–52.
50. Fernandez Pujol B, Lucibello FC, Gehling UM, et al. Endothelial-like cells derived from human CD14 positive monocytes. Differentiation 2000;65:287–300.
51. Shantsila E, Lip GY. Monocytes in acute coronary syndromes. Arterioscler Thromb Vasc Biol 2009;29:1433–8.
52. Romagnani P, Annunziato F, Liotta F, et al. CD14+CD34low cells with stem cell phenotypic and functional features are the major source of circulating endothelial progenitors. Circ Res 2005;97:314–22.
53. Fujiyama S, Amano K, Uehira K, et al. Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial progenitor cells. Circ Res 2003;93:980–9.
54. Yona S, Jung S. Monocytes: subsets, origins, fates and functions. Curr Opin Hematol 2010;17:53–9.
55. Grage-Griebenow E, Flad HD, Ernst M. Heterogeneity of human peripheral blood monocyte subsets. J Leukoc Biol 2001;69:11–20.
56. Shantsila E, Wrigley B, Tapp L, et al. Immunophenotypic characterization of human monocyte subsets: possible implications for cardiovascular disease pathophysiology. J Thromb Haemost 2011;9: 1056–66.
57. De Palma M, Venneri MA, Galli R, et al. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell 2005;8:211–26.
58. Venneri MA, De Palma M, Ponzoni M, et al. Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. Blood 2007;109:5276–85.
59. Lu K, Lamagna C, Bergers G. Chapter 3. Bone marrow-derived vascular progenitors and proangiogenic monocytes in tumors. Methods Enzymol 2008;445:53–82.
60. Ziegler-Heitbrock HW, Fingerle G, Strobel M, et al. The novel subset of CD14+/CD16+ blood monocytes exhibits features of tissue macrophages. Eur J Immunol 1993;23:2053–8.
61. Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu Rev Immunol 2009;27:669–92.
62. Nahrendorf M, Swirski FK, Aikawa E, et al. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med 2007;204:3037–47.
63. Schlitt A, Heine GH, Blankenberg S, et al. CD14+CD16+ monocytes in coronary artery disease and their relationship to serum TNF-alpha levels. Thromb Haemost 2004;92:419–24.
64. Tapp LD, Shantsila E, Wrigley BJ, Pamukcu B, Lip GY. The CD14++CD16+ monocyte subset and monocyte-platelet interactions in patients with ST-elevation myocardial infarction. J Thromb Haemost 2012;10:1231–41.
65. Capoccia BJ, Gregory AD, Link DC. Recruitment of the inflammatory subset of monocytes to sites of ischemia induces angiogenesis in a monocyte chemoattractant protein-1-dependent fashion. J Leukoc Biol 2008;84:760–8.
66. Ferro D, Parrotto S, Basili S, Alessandri C, Violi F. Simvastatin inhibits the monocyte expression of proinflammatory cytokines in patients with hypercholesterolemia. J Am Coll Cardiol 2000;36:427–31.
67. Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Hum Pathol 1995;26:450–6.
68. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 2005;25: 2054–61.
69. Fleiner M, Kummer M, Mirlacher M, et al. Arterial neovascularization and inflammation in vulnerable patients: early and late signs of symptomatic atherosclerosis. Circulation 2004;110:2843–50.
70. Lauener RP, Geha RS, Vercelli D. Engagement of the monocyte surface antigen CD14 induces lymphocyte function-associated antigen-1/intercellular adhesion molecule-1-dependent homotypic adhesion. J Immunol 1990;145:1390–4.
71. Barton WA, Tzvetkova D, Nikolov DB. Structure of the angiopoietin-2 receptor binding domain and identification of surfaces involved in Tie2 recognition. Structure 2005;13:825–32.
72. Metheny-Barlow LJ, Li LY. The enigmatic role of angiopoietin-1 in tumor angiogenesis. Cell Res 2003;13:309–17.
73. Thomas M, Augustin HG. The role of the angiopoietins in vascular morphogenesis. Angiogenesis 2009;12:125–37.
74. Lemieux C, Maliba R, Favier J, Theoret JF, Merhi Y, Sirois MG. Angiopoietins can directly activate endothelial cells and neutrophils to promote proinflammatory responses. Blood 2005;105:1523–30.
75. Amo Y, Masuzawa M, Hamada Y, Katsuoka K. Observations on angiopoietin 2 in patients with angiosarcoma. Br J Dermatol 2004; 150:1028–9.
76. Shim WS, Ho IA, Wong PE. Angiopoietin: a TIE(d) balance in tumor angiogenesis. Mol Cancer Res 2007;5:655–65.
77. Thurston G. Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis. Cell Tissue Res 2003;314:61–8.
78. Sato TN, Tozawa Y, Deutsch U, et al. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. Nature 1995;376:70–4.
79. Saharinen P, Kerkela K, Ekman N, et al. Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2. J Cell Biol 2005;169:239–43.
80. Hauer AD, Habets KL, van Wanrooij EJ, et al. Vaccination against TIE2 reduces atherosclerosis. Atherosclerosis 2009;204:365–71.

81. Wu X, Liu N. The role of Ang/Tie signaling in lymphangiogenesis. Lymphology 2010;43:59–72.
82. Saharinen P, Bry M, Alitalo K. How do angiopoietins Tie in with vascular endothelial growth factors? Curr Opin Hematol 2010;17:198–205.
83. Fujiyama S, Matsubara H, Nozawa Y, et al. Angiotensin AT(1) and AT(2) receptors differentially regulate angiopoietin-2 and vascular endothelial growth factor expression and angiogenesis by modulating heparin binding-epidermal growth factor (EGF)-mediated EGF receptor transactivation. Circ Res 2001;88:22–9.
84. Hawighorst T, Skobe M, Streit M, et al. Activation of the tie2 receptor by angiopoietin-1 enhances tumor vessel maturation and impairs squamous cell carcinoma growth. Am J Pathol 2002;160:1381–92.
85. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marme D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood 1996;87:3336–43.
86. Prior BM, Yang HT, Terjung RL. What makes vessels grow with exercise training? J Appl Physiol 2004;97:1119–28.
87. Miquerol L, Langille BL, Nagy A. Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression. Development 2000;127:3941–6.
88. Guo S, Colbert LS, Fuller M, Zhang Y, Gonzalez-Perez RR. Vascular endothelial growth factor receptor-2 in breast cancer. Biochim Biophys Acta 2010;1806:108–21.
89. Shibuya M, Yamaguchi S, Yamane A, et al. Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. Oncogene 1990;5:519–24.
90. Kabrun N, Buhring HJ, Choi K, Ullrich A, Risau W, Keller G. Flk-1 expression defines a population of early embryonic hematopoietic precursors. Development 1997;124:2039–48.
91. Matthews W, Jordan CT, Gavin M, Jenkins NA, Copeland NG, Lemischka IR. A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to c-kit. Proc Natl Acad Sci U S A 1991;88:9026–30.
92. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995;376:66–70.
93. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature 2000;407:242–8.
94. Hayes DF, Miller K, Sledge G. Angiogenesis as targeted breast cancer therapy. Breast 2007;16 Suppl 2:S17–9.
95. Ristori C, Filippi L, Dal Monte M, et al. Role of the adrenergic system in a mouse model of oxygen-induced retinopathy: antiangiogenic effects of beta-adrenoreceptor blockade. Invest Ophthalmol Vis Sci 2011;52:155–70.
96. Yang EV, Kim SJ, Donovan EL, et al. Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. Brain Behav Immun 2009;23:267–75.
97. Park SY, Kang JH, Jeong KJ, et al. Norepinephrine induces VEGF expression and angiogenesis by a hypoxia-inducible factor-1alpha protein-dependent mechanism. Int J Cancer 2011;128:2306–16.
98. Dawson JM, Hudlicka O. The effects of long term administration of prazosin on the microcirculation in skeletal muscles. Cardiovasc Res 1989;23:913–20.
99. Leosco D, Rengo G, Iaccarino G, et al. Exercise promotes angiogenesis and improves beta-adrenergic receptor signalling in the post-ischaemic failing rat heart. Cardiovasc Res 2008;78:385–94.
100. Iaccarino G, Ciccarelli M, Sorriento D, et al. Ischemic neoangiogenesis enhanced by beta2-adrenergic receptor overexpression: a novel role for the endothelial adrenergic system. Circ Res 2005;97:1182–9.
101. Grisanti LA, Evanson J, Marchus E, et al. Pro-inflammatory responses in human monocytes are beta1-adrenergic receptor subtype dependent. Mol Immunol 2010;47:1244–54.
102. Arras M, Ito WD, Scholz D, Winkler B, Schaper J, Schaper W. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. J Clin Invest 1998;101:40–50.
103. Cursiefen C, Chen L, Borges LP, et al. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Invest 2004;113:1040–50.
104. Eubank TD, Galloway M, Montague CM, Waldman WJ, Marsh CB. M-CSF induces vascular endothelial growth factor production and angiogenic activity from human monocytes. J Immunol 2003;171:2637–43.

**Key Words:** angiogenesis ■ angiopoietin ■ monocyte subsets ■ monocytes ■ Tie2 ■ vascular endothelial growth factor.