Factors Regulating Arteriogenesis

Wolfgang Schaper, Dimitri Scholz

Abstract—Growth of collateral vessels is potentially able to preserve structure and a variable degree of function in subtended tissues in the presence of arterial occlusions. The process of transformation of a small arteriole into much larger conductance artery is called arteriogenesis. Small arterioles that interconnect side branches proximal from the arterial occlusion with distal ones experience increased fluid shear stress because of the increased blood flow velocity attributable to the pressure gradient along the bridging collaterals. This activates the endothelium and leads to monocyte adhesion and infiltration with the subsequent production of growth factors and proteases. Preexistent arterioles are essential. Their presence is genetically determined. Arteriogenesis is not organ- or species-specific; coronary or peripheral collateral vessels develop following the same design principles in mice, rats, rabbits, or dogs. In contrast to angiogenesis, arteriogenesis is not dependent on the presence of hypoxia/ischemia. (Arterioscler Thromb Vasc Biol. 2003;23:1143-1151.)

Key Words: factors ■ arteriogenesis

Collateral artery growth is the most important tissue-saving, organ-saving, and often life-saving adaptive process after arterial occlusion in virtually all vascular provinces of the body. Because its molecular interactions are different from those of angiogenesis, with which it has been confused for some time, a “petit committe” consisting of Werner Risau, Ramon Munoz-Chapuli, and Wolfgang Schaper coined the term arteriogenesis in 1996, and more than 60 publications and reviews have appeared since then. Arteriogenesis is the process whereby a preexisting arteriole from the resistance vessel class matures into an artery of the conductance vessel class, in analogy to angiogenesis, where a sprouting capillary originates from a preexisting capillary (Table).

Substrates
A question often discussed is whether arterial collaterals had formed de novo or whether a preexisting arteriolar network is needed that expands.1–5 In the embryo, the primary capillary network differentiates into arterioles and arteries by recruiting smooth muscle cells (SMCs). This is believed by some authors to be recapitulated in the adult; angiogenesis occurs first, ie, the sprouting of capillaries from preexisting capillaries under the influence of hypoxia/ischemia and related transcription factors (hypoxia inducible factor [HIF]), which is followed by the recruitment of SMCs from the adjacent tissue or along the vascular tree from upstream.6–9 Although this may occur in some selected instances, it is not the dominant form in collateral vessel growth in the heart or brain or the vascular periphery. First of all, the locales of arteriogenesis and angiogenesis are far apart (Figure 1). A drastic example is the occlusion of the femoral artery, where collateral vessels develop in the upper leg between proximal and distal side branches, relatively close to the site of occlusion. In contrast, ischemia and angiogenesis occur in the lower leg.
and in the foot, at a long distance from the arterial growth.\textsuperscript{10–12} In these vascular provinces, a network of preexisting arterioles exists that is arranged arcade-like for the optimal distribution of blood flow.\textsuperscript{5} Long muscles in the murine upper leg exhibit a dual arterial blood supply; different side branches enter the muscle from both ends (Figure 2) and taper down, and the arterioles from both side branches meet and connect in the middle of the muscle.\textsuperscript{5} If the arterial segment between the side branches serving the muscle is occluded, an uninterrupted (and even accelerated) blood flow is maintained, and only the direction of flow reverses in the receiving segment. The connecting segment enlarges by growth. This is the simplest model of arteriogenesis. With larger animals, similar processes reign, but the complexity increases.

Not all organs exhibit interconnecting arteriolar networks. In these cases, the differentiation of the primary capillary plexus was complete; all arteries are anatomical end arteries, and connections exist only on the capillary level. The heart of pigs, rats, and mice are cases in point. The hearts of guinea pigs, dogs, cats, and humans are endowed with interconnecting arteriolar networks, albeit at different degrees.\textsuperscript{13} The arteriogenic adaptation to coronary occlusion is directly dependent on the existence and density of the arteriolar network; no infarction develops after acute coronary occlusion in the guinea pig, but maximal infarctions develop in the pig and rat heart. Slowly progredient stenoses leading to complete occlusions of 2 of the 3 epicardial coronary arteries are tolerated in the canine heart without infarction, but a much longer period of progressive occlusion of only the small left circumflex artery is needed in the pig heart. Pig coronary collaterals that develop after amaroid constriction of the left circumflex look like giant capillaries and are almost devoid of SMCs. Because their original substrate before occlusion was capillary connections, the intravascular pressure is much lower (typical for the microcirculation) than in the arterial collaterals of the canine heart under similar conditions.\textsuperscript{14} These findings strongly suggest that vascular smooth muscle, if not primarily present, cannot be recruited. This means that de novo arteriogenesis from capillaries is extremely unlikely or infrequent.

Contrary to conventional paradigms,\textsuperscript{15–18} angiogenesis cannot replace a conducting artery. Much too many capillaries would be needed to generate the same lumen area of the artery, and the tissue volume created by millions of capillary wall tissue would in essence replace the organ to be furnished with blood. A hemangioma would be the result that actually occurs with overexpression of vascular endothelial growth factor (VEGF).\textsuperscript{19,20}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Differences Between Angiogenesis and Arteriogenesis} & \textbf{Angiogenesis} & \textbf{Arteriogenesis} \\
\hline
\textbf{Definition} & Formation of new capillaries by sprouting & Growth of collateral arteries \\
\textbf{Source} & Pre-existing capillaries & Pre-existing arterioles \\
\textbf{Location} & Lower leg & Upper leg \\
\textbf{Oxygen status} & Hypoxia & Normoxia \\
\textbf{Trigger} & Ischemia & Shear stress \\
\textbf{Cellular mechanism} & Inflammation because of ischemic focal tissue damage & Inflammation because of increased shear stress \\
\textbf{Increases blood flow maximally} & 1.5- to 1.7-fold & 10- to 20-fold \\
\textbf{Compensation for an occluded artery} & Unable & Able \\
\hline
\end{tabular}
\caption{Collateral growth occurs in preexistent arterioles. Laser Doppler image of collateral blood flow in an anesthetized mouse with exposed upper thigh skeletal muscles with chronic occlusion (7 days) of the left femoral artery. Shown are 2 preexistent arterioles that exhibit a faintly visible flow signal but a very strong signal on the occluded side, ie, the effect of growth after 7 days of occlusion. 1 indicates aorta; 2, A. iliaca; 3, A. femoralis; 4, A. pudenda externa; 5, A. profunda femoris; 6, A. tibialis posterior; and 7, A. saphena.}
\end{table}
Molding Forces

The arterial system exhibits a high degree of plasticity in relation to changes in the molding forces. These are the circumferential wall stress (CWS)\(^2\) and fluid shear stress (FSS).\(^{21}\) CWS is directly proportional to intravascular pressure and inversely proportional to wall thickness, and it is borne by the smooth muscle of the media that varies in size according to pressure. FSS with a range of 20 to 30 dynes/cm\(^2\) is a weak force compared with circumferential wall stress, which is 10\(^6\) times higher. FSS is proportional to the blood flow velocity and inversely related to the cube of the radius.\(^{21}\)

It is sensed by the endothelium, which, in response, changes the expression of growth factors, secretes NO,\(^{22,23}\) prostacyclin, and probably other transmitters, and leads, with prolonged exposure, to positive arterial remodeling. However, even small increases in the radius of collateral arteries lead to a precipitous fall of the FSS because of the cubic relationship, and the FSS-related growth ends prematurely.

The concept of FSS as a molding force was advocated 100 years ago by Thoma,\(^24\) who described a constant relationship between the velocity of blood flow and the diameter of the artery in the developing chick embryo. Murray\(^25\) proposed that the vascular system is optimally configured to minimize the amount of mechanical and metabolic work to provide adequate blood flow, and he predicted that FSS is constant throughout the vasculature and that blood flow through each vessel is proportional to that vessel’s diameter cube. In the context of our studies, Murray’s hypothesis would indeed fit, because the collateral growth stops when FSS has normalized again. Unfortunately, this occurs prematurely. However, Murray’s hypothesis cannot explain the second phase of arteriogenesis, ie, the growth of smooth muscle, which is governed by the circumferential wall stress.

Not all authors agree that FSS is the sole or even an important molding force within an arteriolar network. The group of Spaan and colleagues\(^{26,27}\) modeled flow through an electrical network with a constant pressure source with the aim to keep FSS within narrow physiological limits. The network did not remain stable because of its preference for few large connections at the expense of all others that regressed. This, the authors concluded, is not compatible with anatomical and physiological observations that show stable networks. However, in our experiments, the mathematical network behaves exactly like the collateral network, which favors large collateral vessels and induces regression of most others, a process called pruning,\(^{28–30}\) from which we conclude that FSS is indeed an important physical morphogen in arteriogenesis. Regression of a multitude of small collaterals in favor of the persistence of a few larger ones makes furthermore eminent sense in view of Poiseuille’s law that predicts substantial energy losses when the compound vascular cross-sectional area is divided among many vessels. Regression occurs by intimal proliferation.

However, both CWS and FSS play an important role in arteriogenesis. Structural dilation of transforming arterioles not only increase CWS but also, because these former microvessels have now joined, the class of macro (conductance) vessels with their higher intravascular pressure. Because normalization of CWS is reached (by increasing wall thickness) at pressure levels significantly lower than mean aortic pressure, CWS-related growth stops also before reaching optimal values. Price et al\(^31\) also advocate a concerted action of these two forces, and their findings in growing arcade networks are consistent with the hypothesis that the maintenance of mean circumferential wall stress and the pressure shear stress relationship are operative design principles for collateral arteriole development during physiological growth.

Modulation of Fluid Shear Stress

Experimental testing of the FSS hypothesis in vivo is difficult. We anastomosed the distal stump of the occluded artery side-to-side to the accompanying vein, creating an AV shunt. Because the entire shunt flow plus part of the nutritive flow to the lower leg was forced first to pass through collateral vessels, these were under very high FSS. Under these conditions, collateral flow increased 4-fold compared with the contralateral side, which was only occluded but not shunted. We found that the high FSS increased number and size of collateral vessels, and it also restarted vascular growth when it had already completed its natural course.\(^32\) Under these conditions, the maximal collateral conductance was 80% of the maximal conductance of the arterial bed before occlusion, ie, an almost complete functional restitution was reached in contrast to the 33% that is obtained spontaneously. Therapeutic interventions with growth factors are not nearly as effective as the shunting procedure.

Molecular Mechanisms of Shear Stress

Chronically increased shear stress activates endothelium in a morphologically visible way. It loses volume control and swells,
because chloride channels change their open probability.\textsuperscript{33} Inhibitors of the chloride channel also inhibit arteriogenesis.\textsuperscript{34} The location and nature of the mechanotransducer of shear stress are controversially discussed,\textsuperscript{35} and protein kinases and stretch sensitive K\textsuperscript{+} channels were studied.\textsuperscript{86} We found that high shear stress in vitro causes a transient phosphorylation of focal adhesions,\textsuperscript{11} which could also act as mechanoreceptors. By whatever way the mechanical force is transmitted from the deformed cell (membrane) to its nucleus, it will activate transcription factors, like egr-1 (upregulated during the early phases of arteriogenesis), that switch on gene expression, notably of chemokines like MCP-1 but also adhesion molecules like intracellular adhesion molecule-1 (ICAM-1), that are necessary for the docking of monocytes. Other transcription factors that are so far not structurally identified may bind to the GAGACC motif present in the promoter region of several growth factors initiating their expression.\textsuperscript{37} Shear stress is also known to release NO, but it is not known whether chronically increased shear stress will lead to chronically increased amounts of released NO. A lasting step increase in shear stress leads only to a transient increase of egr-1,\textsuperscript{38} and this may also happen with the NO response. The increased permeability of immature collaterals may have been caused by NO. Shear stress is a locally restricted force acting only at the endothelium and is not, unlike tangential or circumferential wall stress, mechanically transmitted into the medial layers, because the internal elastic lamina is a barrier, and direct connections between endothelial and SMCs are few. An endothelial-secreted chemical is the most likely transmitter of information to the smooth muscle layers. Platelet-derived growth factor is produced by activated endothelium and is a potent SMC mitogen. However, expression studies on the RNA level have, in our hands, not shown any changes related to the early stages of collateral growth. Immunofluorescence studies have shown the presence of PDGF antigen in neointima formation in canine collaterals,\textsuperscript{19} which supports findings by the Geary rabbit model (unpublished data, 2003).

Role of the Monocyte

The endothelial lining of growing canine coronary collaterals is studded with monocytes that had attached, during phase 1 of arteriogenesis, to the now much rougher surface of the swollen endothelial cells that, activated by shear stress, upregulate the monocyte chemoattractant MCP-1 and adhesion molecules\textsuperscript{11} to which the Mac-1 receptor of monocytes binds. Infusion of soluble ICAM-1 binds to circulating monocytes and prevents their adhesion to transforming arteries. The same results can be obtained with intravenous infusion of anti-ICAM-1 antibodies that also prevent monocyte attachment. Targeted disruption of the MCP-1 receptor (CCR-2) in mice prevents almost all collateral growth after femoral artery occlusion,\textsuperscript{43} but infusion of MCP-1 into the proximal stump of the occluded femoral artery led to increased monocyte influx and elicited a strong arteriogenic effect.\textsuperscript{44} We also discovered that the weak arteriogenic effects of chronically infused VEGF A is caused by the monocyte attractant effect of VEGF that binds to the VEGF receptor 1, which is exclusively present on monocytes.\textsuperscript{45} A similar effect was discovered with placenta growth factor (PIGF). The arteriogenesis-inhibiting effect of targeted disruption of PIGF in mice\textsuperscript{46} could be lifted by bone marrow transplantation, ie, an effect of monocytes.\textsuperscript{46,47} Because infusion of VEGF-E, which binds exclusively to VEGFR-2, did not influence arteriogenesis, we concluded that the effects of VEGF-A on arteriogenesis are caused by monocyte activation.\textsuperscript{48} Intravenous infusion of liposome-packaged phosphonates (alendronate) destroyed all monocytes/macrophages for a period of \(\sim 1\) week. During this time, VEGF and PIGF infusions remained completely inactive, showing again the importance of monocytes in arteriogenesis.\textsuperscript{48}

Role of Mast Cells

Mast cells aggregate in the adventitia of growing collaterals for a surprisingly long time (up to 6 weeks). They are known to secrete bFGF and VEGF\textsuperscript{50} and a host of vasoactive autacoids but also platelet activating factor and a variety of other cytokines. They have been implicated in angiogenesis, but our studies are the first to show their presence in an
arteriogenic scenario. Mast cells belong to the basophile lineage, and future studies will be directed to test their role.

**Role of Adult Stem Cells**

Adult bone marrow–derived stem cells or circulating endothelial precursor cells are much debated in angiogenesis research. The necessity of recruiting circulating cells for stable incorporation into growing collateral arteries seems remote, because local in situ proliferation occurs at a rapid rate and seems self-sufficient. The pertinent problem is that of cell-specific markers. Intimal cells, typical for the later stages of arteriogenesis and believed to have migrated from the media, exhibit greatly reduced α-smooth muscle actin and could, theoretically, have entered the subintimal space from the lumen. We tested the hypothesis by transplanting bone marrow from GFP mice into lethally irradiated hosts and followed the repopulation of the marrow with green cells. Thereafter, the femoral artery was ligated and the presence of green cells, stably incorporated into growing collateral arteries, was investigated. Although >80% of all peripheral blood white cells were green, no marrow-derived green cells could be detected in collateral arteries. This supported our view that proliferation of vascular cells in growing collateral arteries is a local and self-sufficient process that is not dependent on circulating precursors or other stem cells.

**Inflammation as a Paradigm of Arteriogenesis**

The midzone of growing collaterals is surrounded by a perivascular inflammation consisting mainly of monocytes and T-lymphocytes. These have migrated partially from the arterial lumen but also from neighboring parallel venules under the influence of cytokines. The media of these vessels is thus surrounded from both sides by inflammatory cells that secrete growth factors and proteases that are necessary to remodel the old media. Inhibition of the matrix-metalloproteinases retards arteriogenesis. T-lymphocytes are also necessary to destroy cells in the close vicinity of the collateral artery to create the space to be occupied by the greatly expanding vessel. Space-gaining erosion of bone tissue by intercostal collaterals in aortic isthmus stenosis is another well-known drastic example of the aggressiveness of arteriogenesis. The prominent intima into which phenotypically altered SMCs migrate suggests that the new artery is also remodelled from the inside out.

**Chemical Transmitters, Growth Factors, Cytokines: NO, an Enigmatic Transmitter?**

One theory of arteriogenesis predicts that the effect of FSS is transmitted from the endothelium to the underlying layer of smooth muscle. Among the known transmitters released from stressed endothelium, NO is one of the best known but also one of the most controversial. In fact, it was reported that L-NAME, an inhibitor of NO synthase, inhibits arteriogenesis more than angiogenesis. This is difficult to understand, because NO is antiproliferative in SMCs. Chronic treatment with L-NAME of femoral occluded mice retarded the recovery of blood flow, but flow was restored within 2 days after interrupting treatment, showing that the retardation was only the effect of vasoconstriction and not a delay in growth (unpublished data, 2003). NO may have a role in permeabilizing the vascular wall during the initial stages of arteriogenesis. The role of NO in angiogenesis is also controversially discussed, because it was reported that the mitogenic effect of VEGF is inhibited by L-NAME and that mice with targeted disruption of the endothelial NO synthase gene are unresponsive to exogenous VEGF.

**The Role of Growth Factors**

**Fibroblast Growth Factor**

The great life- and tissue-sparing potency of the collateral circulation was almost always taken for granted, but a pharmacological or biological approach to stimulate their development was not available until the late 1980s, when the first growth factor peptides became known. After isolation, purification, cloning, and sequencing of FGF in 1986 by Abrahams et al., we detected, isolated, and amino acid–sequenced the factor in pig heart and found subsequently that heart is the richest source of the FGF-1 mRNA. However, we could not show that FGF expression changes when a large coronary artery was slowly occluded and when a collateral circulation developed. This led us to postulate that the availability of the ligand is not important for the onset of vascular growth but rather the availability of the receptor is important. We showed that in femoral occluded rabbits, the FGF ligands 1 and 2 did not change their level of transcripts, but the FGF receptor 1 was upregulated at a critical stage of arteriogenesis, albeit only during a brief window of time. The reverse experiment whereby we infused microsphere-bound FGF-1 into the normal coronary system of pigs showed a similar result: little mitogenic effect in normal tissue where the FGF receptor is not expressed but a strong effect when a focal ischemia leads to local inflammation and receptor upregulation. The lack of a phenotype after targeted disruption of the FGF-1 and -2 ligands remains a challenge. However, the FGF family consists now of 21 members, and it seems feasible that others than FGF-1 or-2 have substituted, which remains an as-yet-unproven hypothesis. Phosphorylation of the FGF receptor 1 in double FGF-1 and -2 knockout mice would be a strong argument in favor of the presence of substituting homologs.

Newer studies with transgenic overexpression of FGF-1 in the heart and with FGF-2 in skeletal muscle have shown that FGF-1 leads to an increase in the arteriolar density and branching, whereas FGF-2 overexpression in skeletal muscle leads to increased capillary density. Because the FGF-2 transgene was expressed under the control of the phophoglyceratekinase promoter, we assumed that ischemia would increase the expression of this glycolytic enzyme and with it FGF-2. This was indeed the case. Furthermore, FGF itself increased phosphorylatekinase so that a positive feedback loop existed. Transgenic FGF-2 mice were more resistant toward ischemia. A regimen of treadmill running in these transgenic animals showed total recovery of blood flow soon after femoral artery occlusion.

**Vascular Endothelial Growth Factor**

We assumed at first that in contrast to the unchanged expression of FGF, the expression of VEGF in ischemic...
tissues is associated with arteriogenesis. Apart from the fact that collateral vessels do not develop in hypoxic tissue, VEGF A or B expression was not changed in the collateral artery tissue itself nor in the surrounding skeletal muscle.64 However, when large doses of VEGF were infused over 7 days into the proximal stump of the occluded femoral artery, a significant increase in collateral blood flow was observed, which amounted to \( \approx 60\% \) of the MCP-1 effect. As said above, this effect is most probably attributable to the monocyte-attracting effect of VEGF A.48 Infusion of soluble VEGF receptors into mice with chronic femoral artery occlusion did not inhibit arteriogenesis (Helisch, unpublished data, 2002), arguing against a role of this growth factor in arteriogenesis. Many animal studies testing the angiogenic effects of VEGF have used surrogate end points like indirect and poorly reproducible cuff blood pressure at calf level after femoral artery excision in the rabbit.65 Sometimes investigators did not realize that peripheral pressures fall when collateral flow increases, as with reactive hyperemia, which occurs after repeated inflation of the cuff. Low peripheral pressures were erroneously interpreted as representing low flows. Angiographic evidence for vascular growth is equally unreliable, because angiograms are difficult to translate into blood flow. The typically observed pruning, ie, the reduction in the number of collaterals as a function of time after arterial occlusion in favor of fewer but larger ones, would result in a decrease of the angiographic score although blood flow is increased, as with reactive hyperemia, which occurs after repeated inflation of the cuff. Low peripheral pressures were erroneously interpreted as representing low flows. Angiographic evidence for vascular growth is equally unreliable, because angiograms are difficult to translate into blood flow. The typically observed pruning, ie, the reduction in the number of collaterals as a function of time after arterial occlusion in favor of fewer but larger ones, would result in a decrease of the angiographic score although blood flow is higher. In vivo angiograms depend critically on timing and whether or not subtraction techniques were used in both control and treated populations. True end points for experimental therapeutic studies are flows and pressures in the collateral-dependent vascular bed under maximal vasodilatation and, if technically feasible, under a range of perfusion pressures.66 These criteria were almost always missing in VEGF studies. Clinical trials with VEGF were so far positive only in unblinded case reports.15,67–69

**Colony Stimulating Factor**

Colony stimulating factor acts by releasing immature monocytes from the bone marrow and by antagonizing apoptosis of macrophages, which increases the duty cycle of monocytes/macrophages. Interestingly, it has antiatherosclerotic actions and reduces cholesterol levels in human patients.70 We could show that it amplifies the effects of MCP-1 on arteriogenesis in the rabbit hindlimb model13 and that it was able to restart arteriogenesis some time after femoral occlusion when MCP-1, given alone, is inefficient. Clinical trials in coronary patients showed it to be effective in raising the fractional collateral flow reserve and reducing anginal class and total serum cholesterol.70

**Transforming Growth Factor-β**

Transforming growth factor-β is expressed in growing collateral arteries, and several downstream targets, like the nuclear protein CARP (cardiac ankyrin protein),72 are upregulated in transforming vessels. CARP is the most prominent differentially expressed gene in our subtractive hybridization and microarray studies. Infusion of the transforming growth factor-β protein into the distal stump of the occluded femoral artery73 leads to a significant stimulation of arteriogenesis.

**Role of Ischemia/Hypoxia**

Arteriogenesis and angiogenesis differ rather fundamentally in that angiogenesis occurs in hypoxic tissue, which is usually far away from the localization of collateral vessels that bridge a major arterial occlusion, and takes place in a normoxic environment.10,12 This is still a contested issue, particularly in the heart, where the distances between ischemic tissue and places of collateral vessel growth are small and where the chance of tissue contamination in expression studies is high.74 The situation is clear in the vascular periphery, where occlusion of the femoral artery creates ischemia in the foot but collaterals develop in the thigh, a large distance indeed, defying any relation. It is true that under clinical circumstances, arteriogenesis is mostly closely associated with the occurrence of ischemia but causal relations usually do not exist. In fact, arteriogenesis may continue long after tissue ischemia has abated. Studies in genetically modified mice and in different inbred and outbred mouse strains have shown that collateral arteries whose number and diameter are genetically determined develop in a normoxic milieu.12

Hypoxia stimulates the expression of the transcription factor HIF25,76 by stopping its normoxic breakdown. HIF increases the expression of the capillary growth factor VEGF,77,78 but it is very unlikely that VEGF plays a direct role in arteriogenesis, because its expression does not change, neither in arteriolar nor in the surrounding skeletal muscle tissue after femoral (or coronary) occlusion.60 Arterial tissue, including that of growing collaterals, constantly bathed in oxygen-rich arterial blood will not become hypoxic, and venous vessels do not grow despite the extremely low oxygen tension of coronary venous blood. In hindlimb ischemia studies with exogenous growth factors or their genes, the capillary density often decreases after arterial occlusion and slowly returns to subnormal values within days and weeks.79 This can hardly be called proangiogenic.

**Remodelling**

After the acute phase of arteriogenesis that is dominated by the inflammatory events, remodelling begins (phase 2 of arteriogenesis), ie, the much slower consolidation of the arterial structure after the final diameter was almost reached. A new elastic lamina is synthesized by the SMCs, and the rebuilding of the media and the formation of an intima begins with the downregulation of the tissue inhibitor of matrix-metalloproteinases (TIMP and MMP).80,81 This is followed by an upregulation of the expression and activity of the MMPs that digest the matrix and provide the space for new tissue itself nor in the surrounding skeletal muscle.64 How-
Because the thickening of the vessel wall occurs under markedly increased tangential wall stress, the intercellular connections and the communication between cells change. This is paralleled by the strongly induced expression of connexin 37 in SMCs. Connexin 37 is an early marker of arteriogenesis. It is normally not expressed in SMCs and only weakly in endothelium. The remodelling process of large collaterals is finally characterized by the significant increase in length (tortuosity) and by the formation of a substantial intima (Figure 3). At very late stages, the intima disappears in mature collaterals, probably because the longitudinal muscle had assumed first a helical and later a circumferential orientation. In very small animals, like mice, neither intima formation nor pruning is observed, most probably because the increase in new tissue mass is so small that remodelling processes are not required. However, already in the rabbit a sizeable intima is seen in hindlimb collaterals sometime after femoral artery occlusion.

It is tempting to speculate that collateral arteries develop from the inside out using the intima as a platform; this is the incubator where the growth factors are produced, where the MMPs and other proteases are activated, and where the SMCs migrate to and then proliferate, thereby weakening the media from which they leave, producing the bulge of later tortuosity.

**Late Long-Term Growth**

Arteriogenesis consists of two phases, the early inflammation dominated phase and the later phase of slow diameter increase that is devoid of overt inflammatory signs. The second phase of slow positive remodeling has its analogy in other observations, and it is probably also influenced by chronic but smaller changes in fluid shear stress. In canine hearts with experimentally occluded coronaries where the only patent one is the left anterior descending artery, which now supplies the entire left ventricle, the increase in the tissue mass supplied by the left anterior descending artery constitutes, even under resting conditions, an extra flow load that leads to an increase in lumen area, which is clearly visible several months later.

Guided by these observations, the intima formation in mature collateral arteries may be explained differently; the rather sudden decrease of fluid shear stress after the first growth-induced gain in radius explains the observed expression of PDGF-B in the endothelium and intima, attracts and induces SMCs, and causes a thick neo-intima, as described by Geary et al in their elegant work with flow changes in implanted vascular prostheses.

**Relationships Between Arteriogenesis and Atherosclerosis**

Surprisingly, arteriogenesis and atherosclerosis share many features except one: collateral vessels increase in diameter, but atherosclerotic vessels decrease. However, they share the inflammatory component, in particular the monocyte and T-lymphocyte involvement, the upregulation of MCP-1, adhesion molecules, and matrix proteases, the change in phenotype and migration of SMCs, and the formation of an intima. In small animal models of atherosclerosis (mice), the increase in the expression of ICAM, VCAM, and MCP-1 is accepted as an index of success in proatherogenic interventions. It is exactly these molecules that become first and foremost upregulated in arteriogenesis. It is also of note that most of the angiogenic growth factors are proatherogenic and prothrombotic, and it may happen that proarteriogenic therapy may stimulate atherosclerosis and that antiatherosclerotic therapy may inhibit arteriogenesis. This strange coincidence may have something to do with the restricted repertoire of the arterial system to deal with stimuli. However, the main difference may be that in arteriogenesis, small preexistent arterioles become wider under the concerted actions of physical forces, growth factors, and matrix proteases, whereas larger arteries, consisting of numerous layers of smooth muscle and larger amounts of matrix proteins, react to endothelial activation (or dysfunction) with a locally restricted intimal growth, because the thick intima and media inhibit the progression of the growth stimuli and are a barrier to the invasion of monocytes.

**Conclusion**

In contrast to angiogenesis, which is dominated by a single growth factor (VEGF), arteriogenesis relies on a complex...
interplay of many growth factors, cytokines, different cell types, a multitude of proteolytic enzymes, and, at least during the initial stages, an environment of inflammation. Tissue ischemia/hypoxia is needed for angiogenesis but not for arteriogenesis, which is governed initially by physical forces that activate the endothelium of preexistent arterioles. After peripheral artery occlusion in rabbits and mice, arteriogenesis proceeds much faster than angiogenesis because of a structural dilatation of preexisting collateral vessels followed by mitosis of all vascular cell types, which restores resting blood flow within 3 days. Recovery of dilatory reserve (maximal flow) takes longer. The slower angiogenesis is unable to significantly restore flow even if angiogenesis reduces the minimal terminal resistance of the entire chain of resistors by 50%.

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