Influence of Mechanical, Cellular, and Molecular Factors on Collateral Artery Growth (Arteriogenesis)

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Abstract—Growth of collateral blood vessels (arteriogenesis) is potentially able to preserve structure and function of limbs and organs after occlusion of a major artery. The success of the remodeling process depends on the following conditions: (1) existence of an arteriolar network that connects the preocclusive with the postocclusive microcirculation; (2) activation of the arteriolar endothelium by elevated fluid shear stress; (3) invasion (but not incorporation) of bone marrow–derived cells; and (4) proliferation of endothelial and smooth muscle cells. Most organs of most mammals including man can rely on the existence of interconnecting arterioles in most organs and tissues with heart being the exception in rodents and pigs. Arterial occlusion lowers the pressure in the distal vasculature thereby creating a pressure gradient favoring increased flow through preexisting collaterals. This increases fluid shear stress leading to endothelial activation with cellular edema, upregulation of adhesion molecules, mitogenic-, thrombogenic-, and fibrinolytic factors, leading to monocyte invasion with matrix digestion. Smooth muscle cells migrate and proliferate and the vessel enlarges under the influence of increasing circumferential wall stress. Growth factors involved belong to the FGF family and signaling proceeds via the Ras/Raf- and the Rho cascades. Increases in vascular radius and wall thickness restore fluid shear stress and circumferential wall stress to normal levels and growth stops. Although increases in collateral vessel size are very substantial their maximal conductance amounts to only 40% of normal. Forced increases in FSS can reach almost 100%. (Circ Res. 2004;95:449-458.)

Key Words: arteriogenesis ■ shear stress ■ monocytes ■ vascular remodeling ■ leukocytes

After birth, blood vessel growth proceeds mainly by two different processes. Angiogenesis describes the growth of new capillaries by sprouting or intussusception and will be reviewed elsewhere in this issue. The driving force for angiogenesis is ischemia. In contrast, arteriogenesis is based on growth and remodeling of preexisting collateral anastomoses. These arterioarteriolar connections belonging to the arcade-like microvascular blood flow distribution system are recruited to function as collateral vessels after the occlusion of a major artery. The initial triggers of arteriogenesis are physical forces like fluid shear stress. Collateral vessel growth includes attraction and invasion of circulating blood cells, proliferation of vascular wall cells, and remodeling processes with digestion and rearrangement of the extracellular matrix and elastic lamina. This review will focus on the mechanisms involved in arteriogenesis.

It has become common knowledge for many years that blood vessels regress when not constantly perfused, that they...
enlarge when chronically exposed to high flows, and that their walls become thicker with high pressures. This gives a hint that different physical forces lead to a different outcome. However, the exact cellular and molecular mechanisms responsible for the remodeling of the vascular system are still not completely unraveled. Mature collateral vessels differ only in minor histological aspects from normal arteries of the conductance type: they are muscular and contain more collagen and exhibited transiently during the growth process a significant intima consisting of smooth muscle cells in the synthetic and proliferative phenotype. However, they differ markedly in their anatomical appearance: they are sometimes excessively tortuous. In the reentry region, they join up with the distal part of the occluded artery at nonphysiological angles, which adds to the resistance toward flow. Collateral arteries can develop relatively quickly provided a preexistent network of arterioles had existed before occlusion of the artery but they can also quickly regress when the occluded artery is opened up again. This may also be the case when the subtended tissue had atrophied or is not used to full potential like in the peripheral circulation supplying the muscles of the leg. Most often, an occluded artery is not replaced by one single large collateral vessel but rather by several smaller ones. But this arrangement is inefficient because according to the Poiseuille’s Law the energy losses created by the resistance of the contributing vessels are additive. During the course of collateral artery development many of the smaller contributing vessels regress, whereas the larger ones increase in diameter and make the system more efficient. However, no ideal adaptation is reached. At optimal conditions (no tissue loss after arterial occlusion), collateral vessels recover only approximately 40% of the maximal conductance (flow at a given blood pressure at maximal vasodilatation). This was shown for the canine heart and for the peripheral circulation in pigs, rabbits, and mice. Many efforts were made to improve this rather limited recovery by the application of growth factors or their genes. However, improvement by only a few percentage points and only during a narrow window of time after arterial occlusion was achieved. As early as one week after occlusion the system becomes unresponsive to exogenous growth factors because the growth factor receptors had been downregulated. Normal arteries are, of course, totally immune against exogenous growth factors. With these restrictions and imperfections of the collateral circulation in mind one has to ask the question whether interventions to stimulate arteriogenesis have a chance of success. If the degree of adaptation by collateral vessels was already imperfect in young animals, what would the chance of success be in elderly human patients experiencing atherosclerosis and/or diabetes mellitus?

One of the most impressive observations of growing collateral arteries is their typical corkscrew-like pattern, which is a consequence of growth in length between two fixed points. Assuming that a combination of growth factors and genetically altered stem cells would indeed restart the growth cycle, would that further increase collateral tortuosity and length? If so, the system may be self-inhibiting because both would increase resistance, thus negatively influencing blood flow conductance. The effect of tortuosity on resistance can be expressed by the Dean number (De), which was developed from the Reynolds number (the Reynolds number describes at which point laminar flow turns into turbulent flow) as a correction for curvature flows:

\[ De = \frac{D V p}{\mu} \left( \frac{L}{2R} \right)^{1/2} \]

where \( D \) indicates diameter; \( V \), velocity; \( p \), density; \( L \), length; \( \mu \), viscosity; and \( R \), radius of curvature. turbulent flow is not present in small animals because blood flow velocities are too low and the Reynolds number does not achieve a critical value. However, the Dean number nevertheless can be used to estimate the influence of length \( L \) and tortuosity \( \tau \) on collateral blood flow.

**Physical Forces**

On occlusion of an artery, the pressure in its distal stump falls to very low levels. When a vascular connection like a preexistent collateral anastomosis exists between the high-pressure proximal to the occlusion with the low-pressure region distal from the occlusion, a steep pressure gradient develops which increases blood flow through these connections (Figure 1). Hence, the collateral vessel wall is now exposed to various pronounced mechanical forces: increased blood flow directly augments fluid shear stress (FSS), i.e., the viscous drag that flowing blood exerts on the endothelial lining. Assuming Newtonian fluid dynamics, FSS can be estimated using the following equation:

\[ \tau = \frac{4 \eta Q}{\pi R^3} \]

The equation that already includes blood viscosity (\( \eta \)) and the internal radius of a vessel (\( R \)), demonstrates that increased blood flow (\( Q \)) will directly result in increased FSS (\( \tau \)).

Furthermore, the wall of the collateral arteriole is influenced by pressure-related forces like longitudinal-, circumferential-, and radial wall stresses. The distention of the vessel wall, structurally weakened by matrix digestion, by the intravascular pressure, increases the circumferential wall stress, a known activator of smooth muscle cells (SMCs) proliferation.

In contrast, FSS is a relatively weak force, more than two orders of magnitude lower than the pressure-derived forces.
acting on the arterial wall. The difference is so impressive that other authors questioned the morphogenic force of FSS or posed the hypothesis that FSS can act only in concert with the pressure-dependent forces.9

So far the influence of FSS on arteriogenesis, although highly suggestive, had remained conjectural and the evidence correlative, especially because FSS is almost impossible to measure in small collaterals. Our group recently proposed a decisive experiment where FSS was suddenly increased some time after femoral artery occlusion without changes in the other forces. The experiment consisted of the drainage of most of the collateral blood flow into the venous system by creating an arteriovenous anastomosis between the distal stump of the occluded femoral artery and the accompanying vein.10 Thereby, the reentrant pressure was “clamped” near venous levels and early pressure rises were prevented during the course of collateral artery enlargement. The chronically increased FSS in this experimental setting led to a strongly amplified recapitulation of the endothelial activation as the primary physiological response to FSS and subsequently to monocyte invasion. Finally, collateral artery growth was markedly improved. Normal maximal conductance was indeed reached, thereby answering one of the above hypotheses in the affirmative: yes, it is possible to functionally replace an occluded artery completely by collaterals.

The question how the endothelial cell senses FSS and transforms the signal into a change in gene expression is also not yet completely decided. Ingber’s “tensility” model suggests an elegant explanation.11–13 The tight coupling of the cell membrane with the cytoskeleton forms a tensility architecture so that the entire cell, when deformed by FSS, acts as a sensor. In addition, the model includes a hypothesis that the cytoskeleton orients much of the cell’s metabolic and signal transduction machinery like induction of gene expression and cell differentiation.

However, before the tensility structure is deformed, the FFS has to be detected by sensitive structures like integrins, tyrosine kinase receptors, caveolae, and several ion channels within the endothelial cell membrane.14–16 An intermediary function is suggested for the actin filaments of the cytoskeleton. It was shown that the endothelial cytoskeleton is at least indirectly (eg, via a signaling cascade including G-proteins and MAPKinas) connected to all shear stress receptors,17,18 which were shown to signal to several endothelial compartments including the nucleus.19,20 More than 40 genes have been reported to contain shear stress responsive elements (SSRE) within their promoter. The biomechanical stimulus finally leads to marked alterations in the expression of numerous genes.21–24 Reproducing these in vitro finding in suitable animal models is lagging behind.

Several questions need to be addressed at this point: the cytoskeletal and contractile apparatus of both endothelial and SMCs are rapidly downregulated (resulting in a complete phenotype change) and would not be able to sense FSS after a few days. Another problem is that the collateral endothelium is deformed by FSS, but because of the weakness of this force, it cannot mechanically transmit the disturbance to the underlying layer of smooth muscle from which it is shielded by the extracellular matrix and the internal elastic lamina. A transmission of paracrine signals via junctions between the endothelium and smooth muscle can also be excluded because of the lack of such structures. Of course, diffusion signal transmission is possible and well known for vasoactive agents like sympathomimetics or vasodilators. But growth factors, cytokines, and proteases, necessary for collateral growth, have initially only limited access to SMCs, which is the cell population that expands most in arteriogenesis. Therefore, the development of collateral arteries requires the tear-down of the barriers to signal transmission.

FSS Activates Endothelium: Prerequisite for Cell-to-Cell Interactions at the Collateral Vessel Wall

As mentioned earlier, the onset of FSS induces multiple responses at the collateral endothelium. One of the early signs of endothelial activation is cell swelling. To antagonize this, endothelial cells open ion channels, allowing the efflux of osmolytes and returning cell volume to its normal value. Activation of volume regulated endothelial chloride channels (VRACs) seems to play a key role in this process.25 When VRACs were blocked using mibefradil, originally developed as a selective T-type calcium channel blocker, but also having inhibitory activity on VRACs, collateral artery growth was decreased. Furthermore, as a result of VRAC activation, the driving force for Ca2+ entry into the cell increases, representing a possible mechanism for the antiproliferative effect of chloride channel blockers like mibefradil.26 Moreover, the blockade of VRACs may induce an increase in the intracellular pH. This alkalization in turn may inhibit the cell cycle–dependent kinases and trap the proliferation cells in the G0/G1 stage.27 However, because no inhibitors completely selective for VRACs are available, mechanistic explanations remain, at least partially, on a hypothetical stage.

Although the physiological meaning of the endothelial swelling for the arteriogenic process is not completely elucidated, it has become evident that the induction of expression of multiple genes mainly initiates a machinery to trigger attraction and adhesion of circulating blood cells. Mostly genes are upregulated that code for chemoattractant or activating cytokines including growth factors or for adhesion molecules.28–35 Thus, the collateral endothelium converts from a quiescent vessel layer with very low adhesion tendency into a highly activated one, which is now supporting attraction, activation, and adhesion of leukocytes (of which monocytes and lymphocytes are examined best). Furthermore, surface expression of adhesion molecules like selectins, intercellular adhesion molecules (ICAM-1 and ICAM-2), and vascular cell adhesion molecules (VCAM-1) on endothelial cells is not only increased but they are also clustered in focal adhesion complexes. In previous studies, it was demonstrated that factors like VEGF and MCP-1, released by endothelial cells, have the ability to stimulate integrin expression on monocytes and increase cell adhesion.36–38 In addition, a rapid conformation change converts the integrins into an active state.

Mainly two integrins are responsible for the interaction of monocytes with endothelial cells: the heterodimeric proteins Mac-1 and LFA-1, which belong to the group of β2-integrins.
Both molecules have the β₂-subunit in common. The second subunit of Mac-1 is the α₅β₂-integrin and of LFA-1 is the α₅β₂-integrin. The monocyte interaction with the collateral endothelium is a complex multistep process (Figure 2). After the initial monocyte interaction with the vascular endothelium, which is called “rolling” and is mediated by several selectins, the tight monocyte adhesion to the collateral endothelium is triggered by Mac-1 and LFA-1. These integrins interact with their corresponding adhesion molecules on the endothelial cell surface, preferably ICAM-1, ICAM-2, and VCAM-1, now preferably clustered in focal adhesion complexes. In immunohistological studies, we could show that expression of ICAM-1 was markedly increased on endothelial cells of activated collateral arterioles. Monocytes Invade the Collateral Vessel Wall

The adhesion of blood monocytes to the activated collateral endothelium is only the first step in the invasion of the collateral vessel wall. This is followed by the invasion of monocytes that provide the logistics for the transformation of a 40-μm arteriole with only 1 to 2 layers of smooth muscle into an artery of up to 20-fold larger in diameter and up to 50-fold larger in tissue mass. Monocyte transmigration through the endothelial lining is again mediated by the same molecules: interaction between integrins on monocytes and ICAM-1, ICAM-2, and most likely VCAM-1 (see later) on endothelial cells is used to guide the monocytes through the vascular endothelium into deeper regions of the vessel wall, mostly adventitia, and the perivascular space. Using monoclonal antibodies against either ICAM-1 or β₂-integrins, monocyte adhesion and transmigration were blocked in vitro and collateral artery growth in vivo. The importance of VCAM-1 in this process is currently not completely known. Like for ICAM-1, an increased expression of VCAM-1 was detected in the rabbit hind limb model on the endothelium of collateral arterioles three days after femoral artery occlusion. Upregulation of VCAM-1 was also found in vitro on cytokine-treated endothelial cells. Indeed, VCAM-1 may participate in leukocyte-endothelium interaction by binding to its counterreceptor VLA-4 (α₄β₁-integrin). However, in our in vitro experiments, both interaction steps were completely inhibited by blocking the β₂-ICAM-1 pathway. Thus, the role of VLA-4 and VCAM-1 for monocyte adhesion to the collateral endothelium and their subsequent transmigration remains open and needs to be further investigated. On the other hand, VCAM-1 was found to be overexpressed in all layers of the collateral vessel wall under conditions of markedly elevated and chronically acting FSS (Figure 3).

Monocytes/macrophages adhere first to the endothelium but are later recruited from venules and accumulate in the perivascular space of growing collateral vessels. This starts at ~12 hours after occlusion and peaks between one and three days. On their migration from the intraluminal side of the collateral arteriole toward deeper vessel wall regions, monocytes have to overcome barriers, ie, the internal elastic lamina and the extracellular matrix. Monocytes are potent producers of proteases like matrix-metalloproteinases and u-PA. Their proteolytic activity could open these barriers and create the gaps by which monocytes could invade the vascular wall. This results in the required tear-down of the barriers to signal transmission and may establish the possibility for paracrine signaling between the endothelium and the smooth muscle cells. In parallel with that, SMCs develop an intercellular

![Figure 2](image1.png) **Figure 2.** Monocytes in collateral artery growth. This series of electron microscopic figures shows subsequent steps of the collateral vessel wall invasion by blood monocytes (by Jutta Schaper)

![Figure 3](image2.png) **Figure 3.** Upregulation of VCAM-1 expression in the collateral vessel wall in the rabbit shunt model. In addition to VCAM-1 expression (green) by the endothelium, chronically increased fluid shear stress also induces VCAM-1 expression in the adventitia. Nuclei are stained red; yellow color represents an overlay between red and green signals. Adapted from Pipp et al, with permission.
signaling system de novo: connexin37, which is a highly specific marker for developing collateral vessels.

**Circulating Blood Monocytes Are Critical Mediators of Arteriogenesis**

Although the presence of adherent monocytes on activated collateral endothelium was well described,47 the question remained whether the accumulated macrophages originated from the blood or from tissue-resident macrophages as suggested by one group.48 One of the methods to solve the problem was to manipulate monocyte blood counts.43 Four days after bolus injection of the cytoplastic agent 5-fluorouracil (5-FU) monocytes were almost depleted from the blood of treated mice. When the femoral artery in those mice was ligated during the monocyte depletion, blood flow recovery to the foot (an attempt to quantify arteriogenesis) was markedly reduced. Two weeks after 5-FU treatment, a rebound reaction was observed leading to a several-fold increase of blood monocyte numbers in treated rabbits and mice. When the femoral artery was ligated during the monocyte rebound phase, arteriogenesis was enhanced, demonstrating the important role of blood monocyte concentration for the progress of arteriogenesis. Similar results were obtained when monocyte depletion was rescued by the injection of isolated blood monocytes from donor mice. A similar and even more definite observation was made when rabbits were injected with liposomes containing cytotoxic bisphosphonates.49 Monocyte depletion by this treatment was more complete than after 5-FU injection, and hence, arteriogenesis was completely abolished. Postmortem angiograms taken seven days after femoral artery ligation revealed that the collateral vessel network in treated rabbits was similar to acutely ligated animals. However, when the femoral artery was ligated 3 days after injection of the liposomes (after monocytes blood concentrations returned to almost normal levels), no inhibition of collateral artery growth was visible (unpublished data). This shows that at least in the early stages of arteriogenesis monocytes are essential.

We were also interested in the pathway involved in monocyte attraction. What do mediators signal to the monocytes to travel to the collateral vessel wall and to where do they have to migrate? An approach to this question was provided by observations obtained in the rabbit hind limb model by using osmotic minipumps to locally deliver test substances into the collateral system. In these experiments, the most distinct enhancement of arteriogenesis was achieved with pumps either containing a chemotactic agent for monocytes or increasing their activation. The most potent substance that we found to stimulate collateral vessel growth was the monocyte chemotactant protein-1 (MCP-1). When MCP-1 was locally infused into the collateral network after occlusion of the femoral artery in the rabbit hind limb, a dramatic improvement of the growth process was detected seven days after occlusion,50 which was confirmed later.6 In contrast, in MCP-1 gene–deficient mice, recovery of blood flow in the hind limb was reduced, which could be rescued by local delivery of MCP-1.52 Furthermore, the inhibition of arteriogenesis in these mice correlated with reduced monocytes accumulation around the growing collateral vessels, although still a significant number of macrophages were present. To achieve further insights into the pathway of monocyte attraction and invasion, we investigated collateral artery development in mice that were gene-deficient for the chemokine receptor-2 (CCR-2).53 This receptor is known as the major functional receptor for MCP-1, although most likely not the only one of biological relevance.54,55 Using these mice on both the BALB/c and the C57BL/6 background, the importance of the MCP-1–CCR2 pathway in arteriogenesis was impressively demonstrated, and it was shown that this pathway is responsible for the recruitment of monocytes during early phases of arteriogenesis56: the data displayed a dramatically reduced recovery of pedal blood flow after femoral artery ligation. This not only correlated with other physiological parameters like the reduction in hemoglobin oxygen saturation in the foot but was also reflected by functional parameters: Because of the reduced blood supply to the distal regions of the leg, the active movement of the limb, assessed in a score, was significantly impaired. Furthermore, histological morphometry of the collateral vessel size confirmed the physiological data by showing decreased collateral vessel diameters in the CCR2 gene–deficient mice compared with controls. Finally, using well-established monoclonal antibodies against monocytes/macrophages, it was shown that accumulation of those cells in the perivascular space around collateral arteries was lacking in the CCR2 knockout group.

**Cell Proliferation and Remodeling Are Major Parts of Arteriogenesis**

Growth processes of differentiating collateral arteries are dominated by the proliferation of smooth muscle cells, adventitial fibroblasts but also of endothelial cells. This is accompanied by extensive tissue remodeling (Figure 4).

Proliferation of vascular cells is initiated as early as 24 hours after experimental occlusion of the femoral artery in the rabbit model and peaks at days 3 to 7. Weak but markedly above normal mitotic activity is still observed at 3 weeks.40 The signaling cascade uses the mitogen activated protein kinases with activation of the RAS-ERK- and the Rho pathways. Studies with rabbit SMCs in culture make the involvement of the FGF family of growth factors highly likely. No evidence was found so far for the involvement of the PDGF family of growth factors.

Proliferation activity follows the invasion of monocytes with a lag time of about one day. EC mitosis precedes that of SMCs by a few hours and growth factors are released from the matrix and from monocytes during that time.57 In addition to their direct mitogenic activity, these growth factors also influence the transcription of secondary growth factors like FGF-7, inactivation of the MMP inhibitor TIMP, and down-regulation of the extracellular matrix component elastin. The increase in cell mitosis also coincides with a morphological change in smooth muscle cells: the appearance of a prominent rough endoplasmic reticulum and many free ribosomes indicates that smooth muscle cells are transformed from the contractile into the proliferative/synthetic phenotype.40 During this phase, a neointima forms composed of smooth muscle cells in which, like in the earlier degradation of the
internal elastic lamina, matrix-metalloproteinases (MMPs) are involved. Several MMPs are known to be expressed by monocytes/macrophages, and we could demonstrate that stimulation of arteriogenesis with MCP-1 locally augments MMP-1, MMP-2, MMP-3, and MMP-9 expression and activity. Furthermore, increases in the expression of MMPs, probably produced by macrophages, were also observed in the perivascular space of growing collaterals. This indicates that MMPs participate in the digestion of the extracellular matrix and even of skeletal muscle cells (by T-lymphocytes) to create additional space for the growing collateral vessel.

The increase of collateral vessel diameter reduces FSS, which is inversely related to the cube of the vascular radius. The normalization of FSS is the signal for maturation. The initial thinning of the tension-bearing vessel wall had increased the circumferential wall stress for the smooth muscle cells, a proliferative stimulus. This leads to increased wall thickness by SMC proliferation, which acts via negative feedback to normalization of circumferential wall stress and terminates the remodeling phase. The transformation of a small microvascular resistance vessel into a large conductance artery is now completed.

Role of Other Nonvascular Cells in Arteriogenesis

Although multiple studies have shown that monocytes occupy a key function in arteriogenesis, several reports indicate that additional proinflammatory cells participate in the complex orchestration of the process. Mast cells were found in the wall of growing collateral vessels. Similar to monocytes, these cells or their precursors derive from the blood stream and invade the vascular wall. Mast cell precursors produce MMP9, which may be essential for mast cell migration into the vessel wall. They may directly stimulate proliferation of smooth muscle cells by the release of growth factors (eg, FGF-2 and TGF-β). They may also support arteriogenesis indirectly by inducing monocyte chemotaxis and their differentiation into macrophages via the release of inflammatory cytokines like MCP-1 and TNF-α. By the release of GM-CSF, they may in addition prolong monocyte survival of macrophages. Mast cells can stimulate monocytes/macrophages to release interleukins, particularly IL-1, which can stimulate production of MMPs in a variety of cells, or they may directly release MMPs or serine protease capable of degrading ECM-components. However, to what extent mast cells support arteriogenesis is unknown.

Lymphocytes were frequently observed in the wall of growing collateral vessels. So far, only one report by the Epstein group had focused on their possible role for arteriogenesis. Using mice with targeted disruption of the gene for the T-cell antigen CD4, they showed a reduction of blood flow recovery in the mouse hind limb after femoral occlusion. When the deficiency of CD4-positive cells was rescued by the injection of isolated T-cells, blood flow recovery was increased, which coincided with an increased macrophage accumulation. Hence, the authors suggest that lymphocytes may support arteriogenesis by participating in monocyte recruitment to the collateral vessel wall.

Bone Marrow–Derived Cells

Recently, the concept that vasculogenesis (growth of new blood vessels originating from stem cell–responsive angioblasts) is restricted to embryonic life has been questioned. It has been proposed that circulating bone marrow–derived stem or endothelial progenitor cells are incorporated into sites of pathophysiological revascularization, mostly into capillaries and transplantation of those cells augmented perfusion recovery and organ functions. In these reports, the contribution of the transplanted cells to the endothelium of growing vessels varied between almost no incorporation and values of above 50%. However, several reports were published questioning the transdifferentiation of bone marrow–derived cells in adult organisms. In a mouse hind limb ischemia model with reconstituted GFP-positive bone marrow, we failed to colocalize GFP signals with endothelial or smooth muscle cell markers but observed strong accumu-
lations of GFP-positive cells around growing collateral arteries (Figure 5). These growth factors and chemokines expressing cells were mainly identified as leukocytes, which is in line with a previous study, suggesting that endothelial progenitor cells originate from monocyte/macrophage lineage and secrete angiogenic growth factors. Further support was published by Kinnaird and coworkers, showing that mesenchymal stem cells may enhance collateral artery growth through paracrine mechanisms. Hence, bone marrow-derived cells may support collateral artery growth rather by releasing arteriogenic substances than by incorporating into growing collateral arteries.

**Will There Be a Cure?**

The therapeutic augmentation of blood vessel growth in patients experiencing ischemic diseases has become a challenging goal for clinical research. In contrast to promising results from animal studies and from a number of clinical phase I trials (see summaries), two larger controlled studies that tested VEGF-A and FGF-2 failed to show significant improvements. Two major reasons may apply: whereas in animal models time points of treatments can be optimized, this is impossible in humans where the moment of occlusion is unknown or had passed or, as in acute MI, is no longer suitable for arteriogenic treatment. One has to keep in mind that growth factor receptors exhibit only a brief window of activity and that cells in G0 need 24 hours after onset of stimulation to proceed with the cell cycle. If treatment starts later than a week after occlusion in the rabbit, no additional effect over spontaneous growth is achieved. Furthermore, the right form of application has yet to be found. A one-shot approach (ie, VEGF protein injected into the coronary artery) may not provide the necessary contact time and nonimmunogenic, safe, and efficient gene transfer methods have not yet been developed.

Second, the majority of experiments have been performed with young healthy animals but target groups for arteriogenic therapy are elderly patients experiencing cardiovascular diseases, ie, atherosclerosis. On the other hand, even young animals replace only 40% of their maximal conductance by...
collateral vessels. Any treatment in animals by drugs, growth factors, combination of factors, or other intervention to increase conductance to 100% in animals would constitute a reasonable basis for therapeutic trials in patients.

The effects of age on arteriogenesis are not conclusively known. Our own earlier studies with old male beagle dogs showed no difference in the ability to develop a coronary collateral circulation after progressive coronary artery occlusion.

One promising therapeutical approach seems to emerge from a recently published clinical study where improvement of coronary collateral flow was observed in patients scheduled for angioplasty but instead received GM-CSF treatment. GM-CSF is known to decrease macrophage apoptosis, thus prolonging their lifespan. Furthermore, it supports mobilization of mononuclear cells from the bone marrow. Hence, the observed effects of GM-CSF may be secondary to an increased monocyte recruitment and macrophage presence in the growing collateral vasculature.

Conclusions

Knowledge about arteriogenesis has steadily progressed during recent years and the interplay of physical forces with cellular and molecular mechanisms is now better known. However, a molecular concept for the treatment of patients that is superior to surgery and angioplasty is still missing. A milestone to be reached in the future is the development of an intervention that reactivates growth factor receptors in collateral vessels so that mixtures of growth factor peptides or genes can become active again. Bone marrow-derived cells, like monocytes, may have the potential to produce the right mixture of factors at the right time and at the right place. However, the application of fragile cells into unyielding tissue poses problems.

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