

Arteriogenesis versus angiogenesis: similarities and differences

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Abstract

Cardiovascular diseases account for more than half of total mortality before the age of 75 in industrialized countries. To develop therapies promoting the compensatory growth of blood vessels could be superior to palliative surgical interventions. Therefore, much effort has been put into investigating underlying mechanisms. Depending on the initial trigger, growth of blood vessels in adult organisms proceeds *via* two major processes, angiogenesis and arteriogenesis. While angiogenesis is induced by hypoxia and results in new capillaries, arteriogenesis is induced by physical forces, most importantly fluid shear stress. Consequently, chronically elevated fluid shear stress was found to be the strongest trigger under experimental conditions. Arteriogenesis describes the remodelling of pre-existing arterio-arteriolar anastomoses to completely developed and functional arteries. In both growth processes, enlargement of vascular wall structures was proposed to be covered by proliferation of existing wall cells. Recently, increasing evidence emerges, implicating a pivotal role for circulating cells, above all blood monocytes, in vascular growth processes. Since it has been shown that monocytes/macrophage release a cocktail of chemokines, growth factors and proteases involved in vascular growth, their contribution seems to be of a paracrine fashion. A similar role is currently discussed for various populations of bone-marrow derived stem cells and endothelial progenitors. In contrast, the initial hypothesis that these cells -after undergoing a (trans-)differentiation- contribute by a structural integration into the growing vessel wall, is increasingly challenged.

Keywords: arteriogenesis • angiogenesis • monocytes • bone-marrow • fluid shear stress

Introduction

Occlusive vascular diseases remain the most important causes for death and morbidity in industrialized societies. Treatment of end-stages such as myocar-

dial infarction, peripheral artery disease, stroke and others is usually limited to angioplasty, surgery, *e.g.* revascularization and in some cases limb amputation and bypasses, and other palliative interven-

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tions. For a long time, it has been known that patients with ischemic vascular diseases spontaneously develop collateral vessels bypassing the occlusion side [1–3]. Furthermore, in the last years, great efforts have been made on developing therapeutic approaches to stimulate self-curing mechanisms within the body. In particular, investigators focussed on discovering ways to activate the growth of compensatory blood vessels which may help to cure flow deficits and, hence, preserving tissue survival and organ function. Research has been concentrated on two distinct types of vessel growth to reduce ischemia, angiogenesis and arteriogenesis. However, due to physical laws of fluid mechanics only the latter provides an equivalent response to blood flow deficits caused by arterial occlusions. This article reviews differences between both angiogenesis and arteriogenesis but also indicates common mechanisms. Furthermore, it addresses the question whether there might be a *de novo* artery formation as response to ischemia. Finally, the importance of circulating blood cells such as monocytes and the potential role of bone marrow derived cells are discussed.

Angiogenesis (Fig. 1) is defined as sprouting of new capillaries (intussusception is described as an alternative mechanism [4]) from pre-existing vessels resulting in new capillary networks [5]. These capillary networks consist of endothelial cell tubes lacking additional wall structures including smooth muscle cells or adventitial stabilizing structures and cells. The driving force for angiogenesis is hypoxia in the surrounding tissue. Sprouting of capillaries leads to an increase of their density which is equivalent to a decrease of interspaces between neighbouring vessels. Since effective diffusion is limited to short distances, angiogenic growth increases blood perfusion of hypoxic tissue and is necessary to maintain or restore local oxygen and nutrition supply, provided the feeding arteries are not stenosed.

In contrast, arteriogenesis (Fig. 1) describes the growth of functional collateral arteries from pre-existing arterio-arteriolar anastomoses [6]. Growth of these “natural bypasses” is controlled by a complex cascade of subsequent mechanisms. Initial triggers are physical forces such as altered shear forces which appear within the collateral arteriole after a blood flow increase. The latter is caused by the large pressure difference in the pre-existing arterioles connecting upstream with downstream

branches (relative to the point of occlusion) as the result of an arterial occlusion. It comprises the induction of vascular wall cell proliferation and migration and includes wall remodelling processes. In consequence, the structural enlargement of collateral arterioles to arteries proceeds as an active growth rather than by a passive dilatation caused by the altered blood pressure [7–9]

The initial trigger defines the mechanism

Angiogenesis and arteriogenesis are initiated by distinct initial triggers. Especially during tissue expansion such as in growing tumors but also in ischemic tissues, adaptive growth of capillaries is initiated as a reaction to hypoxia [10–12]. Oxygen tension plays a key role in the expression of a number of genes, including the vascular endothelial growth factor (VEGF) family. As a mediator of reactions to hypoxia, a transcription factor named hypoxia-inducible factor-1 (HIF-1) was described [13]. HIF-1 is a heterodimeric protein containing two subunits, HIF-1 α and HIF-1 β [14]. In response to hypoxia, HIF-1 binds to specific enhancer elements in the promoter region of target genes. Consequently, transcription of the potent angiogenic factor VEGF-A is markedly increased under hypoxic conditions. As a strong mitogen, VEGF-A subsequently induces proliferation of endothelial cells but also endothelial permeabilization. Since several other growth factors including TGF- β , FGF and PDGF were shown to up-regulate VEGF expression, [15] a co-operative regulation between hypoxia and locally secreted factors (which in part are also under hypoxic regulation) may occur to trigger capillary tube formation.

In contrast, arteriogenesis is induced independently of the presence of hypoxia. In fact, the occlusion of an artery, and therefore the region in which bypassing collaterals grow, is much more proximal to the hypoxic zone. In this context, Deindl *et al.* could illuminate this topic by investigating mRNA expression in tissue derived from the rabbit ischemic hind limb model [16]. Analysing several time points after occlusion of the femoral artery they neither could detect an increased expression level of HIF-1 α -mRNA nor an up-regulation of the HIF-1-controlled VEGF-gene expression. In a

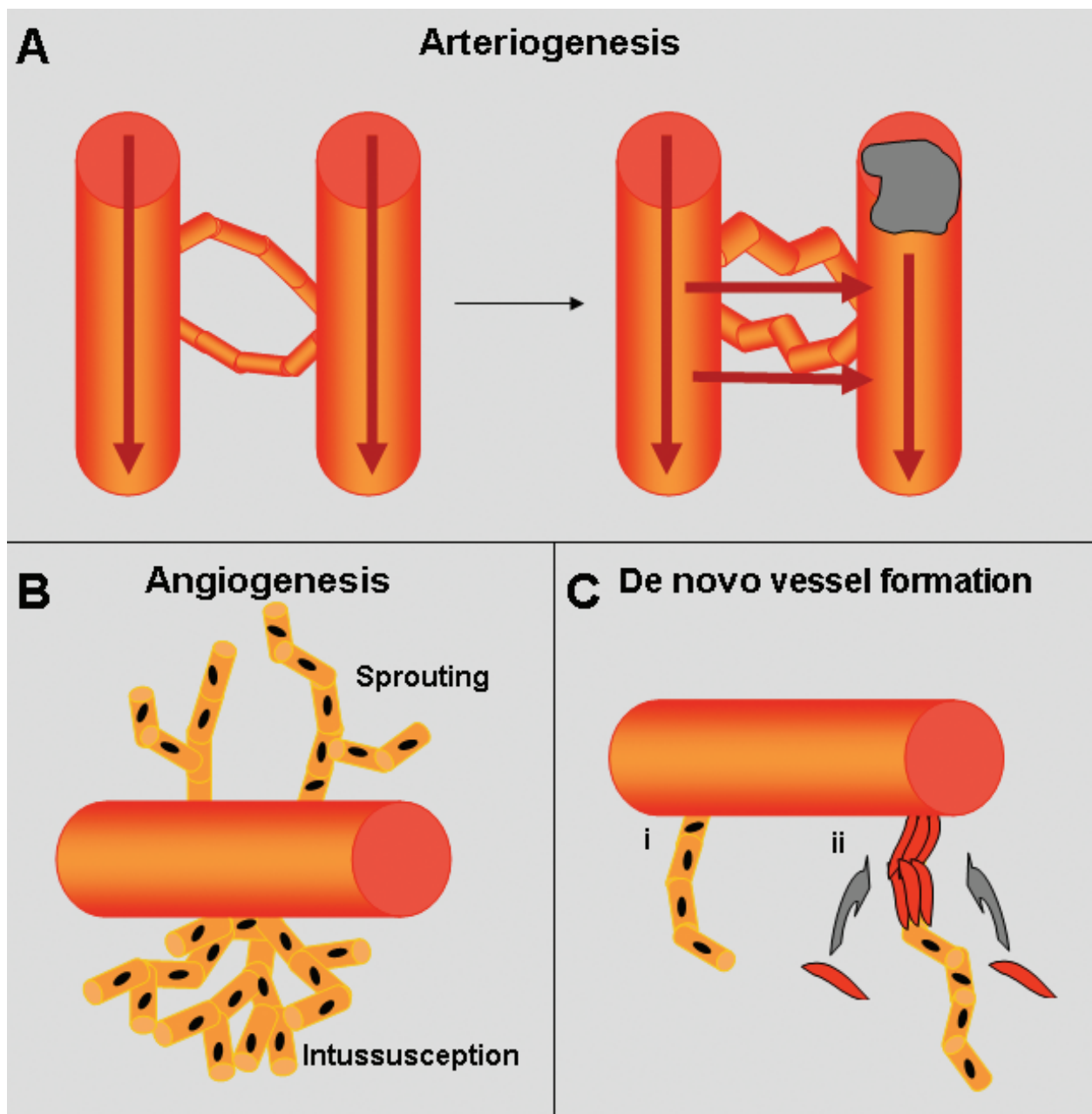


Fig. 1 Scheme showing different vascular repair mechanisms. **A:** Arteriogenesis is induced after stenosis or occlusion of a major artery (indicated by the grey spot). Fluid shear stress, caused by altered blood flow through pre-existing collateral anastomoses (slim arrows) serves as initial trigger. Growth of collaterals proceeds by remodelling of pre-existent arterioles. **B:** Angiogenesis describes the growth of capillaries from pre-existing vessels. It proceeds either by sprouting of endothelial cell or by intussusception. The initial trigger is hypoxia. **C:** Hypothetical mechanism for a de novo blood vessel formation. According to this idea, endothelial tubes are produced as a first step (i). Maturation to arterioles proceeds by the recruitment of smooth muscle cells or progenitors (ii).

recent study, it was shown by nuclear magnetic resonance imaging that especially in C57BL/6 mice collateralization occurs in well oxygenized tissue [17]. However, since both studies were performed

in the hind limb model, one cannot exclude that in other organs respectively tissues including the myocardium, collateral growth may take place under hypoxic conditions. To which extent this

potential presence of hypoxia would contribute to collateral growth remains enigmatic but one could assume a simultaneous growth of capillaries and collaterals under the mentioned circumstances.

Arteriogenesis is triggered by physical forces

It has been known for many years that blood vessels can adapt to altered flow situations. For instance, arteries can regress when not constantly perfused, enlarge on chronically increased flows or increase the wall thickness under high blood pressures. Not surprisingly, physical forces previously also have been identified as pivotal triggers for arteriogenesis. One could assume that the predominant forces are pressure-related forces such as longitudinal, circumferential and radial wall stresses caused by increased blood pressure. Circumferential wall stress which triggers proliferation of vascular smooth muscle cells (SMC), [18] likely is increased by elevated blood pressure within the collateral network and may contribute to the remodelling process. The fact that a recognizable proliferation of SMC within collateral arterioles is not detectable before three days after femoral artery occlusion, suggests that a different physical force might be the major trigger for the initiation of arteriogenesis. Although a weaker force than pressure-related ones, our group favoured fluid shear stress (FSS) as a major candidate. Unfortunately, a direct proof was missing (FSS cannot be quantified in small pre-existing collaterals due to technical limitations) and available models therefore allowed only correlative evidence. An adaptation of the ischemic hind limb model -first established in pigs, but now also practised in rabbits- provided a great progress in this question (Fig. 2) [19]. The clue was to redirect the blood flow after leaving the collateral network directly to the venous system by creating an arteriovenous anastomosis between the distal stump of the occluded femoral artery and the accompanying vein (Fig. 1). The creating of this anastomosis in addition to the femoral artery ligation provided two major beneficial consequences. First, the collateral system was connected to the low venous pressure system, preventing early blood pressure rises. Second, the shunt minimized systemic flow resis-

tances, normally caused by the distal arteriolar and capillary systems, thereby chronically increasing collateral blood flow and consequently FSS at the endothelium. Moreover, the shunt prevented a drop of FSS during later phases of arteriogenesis. This reduction of FSS normally is caused by increasing diameters of the growing collaterals and probably (as a self-inhibiting element) is responsible for an untimely termination of collateral growth. Using this model of chronically increased FSS, the extent of blood flow which was present within the collateral system one week after femoral artery occlusion was close to physiological limb blood flows. Furthermore, we noticed a dramatically increased collateral growth enhancement in the shunt model compared to experiments in which infusions of various substances were investigated. Taken together, the experiments impressively demonstrated that - despite being the weaker physical force-, FSS is the pivotal early trigger of arteriogenesis.

Activation of collateral endothelium as basis for cell recruitment

An important question that has been addressed after achieving these insights was: How is FSS as initial arteriogenic trigger transmitted onto the molecular and cellular level? Molecular mechanisms including gene expression profiling patterns are currently intensively investigated. The primary physiological response to FSS is an activation of endothelial cells. Sensors for FSS may be integrins on the cell surface by which the endothelium is anchored to the extracellular compartment of the vascular wall, and in addition tyrosine kinase receptors and ion channels [20–22]. The cytoskeleton may have an intermediate function by transmitting the signal into the nucleus [23]. A large number of genes have been reported to be controlled by shear stress responsive elements (SSRE) in their promoter, and a marked influence of FSS on expression of these genes has been shown [24–26] In this context, the most exciting current topic in our model is to unravel potential mechanistic differences between a “normal” collateral growth (after an arterial occlusion) such as in the ischemic hind limb model (in which FSS already is the predominant trigger) and the markedly enhanced collateral growth under chronically

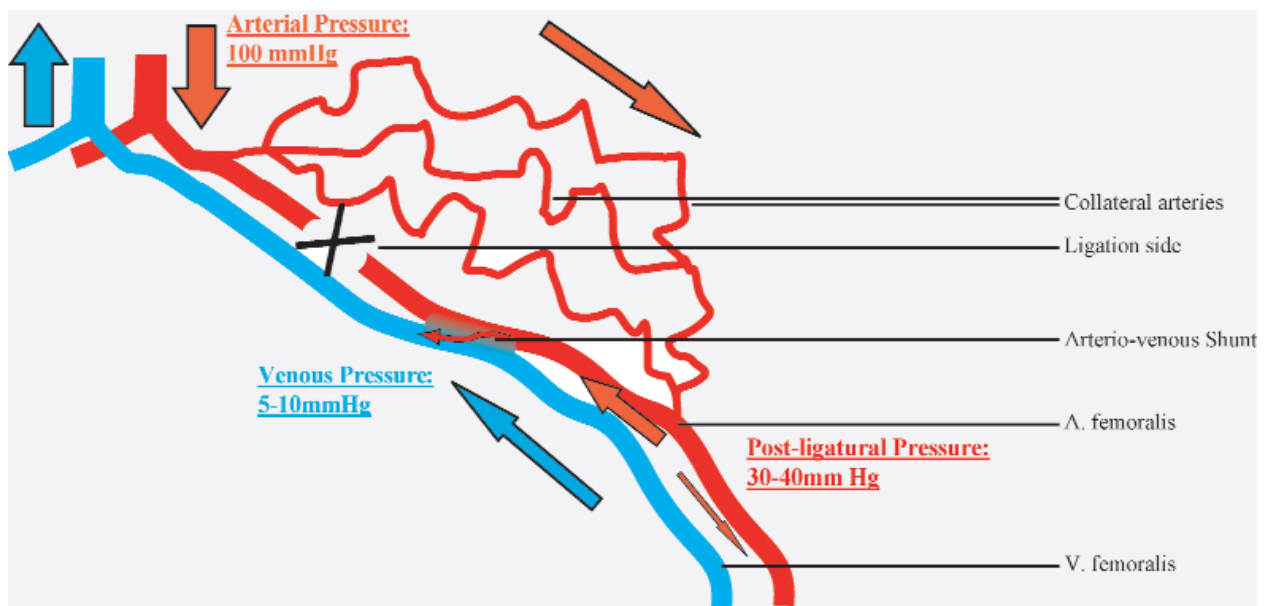


Fig. 2 Modified ischemic hindlimb model for chronically enhanced fluid shear stress. In addition to the femoral artery ligation (X) an arterio-venous shunt is created by a side-to-side anastomosis providing an arterial connection to the venous system. As a consequence, collateral blood pressure is reduced due to the venous connection. Additionally, the bypass of the distal arterial system (with high resistance) enables enhanced collateral blood flow, thereby increasing fluid shear stress along the collateral endothelium.

increased FSS as existing in the shunt model. Currently, gene profiling studies to investigate this question are ongoing.

From previous investigations it is known that endothelial cells in the collateral wall are activated in response to FSS. Besides an initial cell swelling, [27, 28] this activation is indicated by a number of processes conditioning for attraction of circulating cells. Upregulated genes encode for chemoattractant or activating cytokines or for adhesion molecules [29]. The monocyte chemoattractant protein-1 (MCP-1) is of major importance for monocyte recruitment. By transferring MCP-1 to the cell surface where it is immobilized by proteoglycans, activated endothelial cells build up a chemotactic gradient. When this chemotactic gradient was increased by chronic local infusion of MCP-1, arteriogenesis was markedly enhanced to magnitudes which were above all tested growth factor treatments [30, 31]. In addition, changes in the expression and conformation of adhesion molecules transfer the collateral endothelium from a quiescent vessel layer into a “sticky” surface, now supporting attraction, adhesion and invasion of leukocytes. Expression of selectins, intercellular adhesion molecules (ICAM-1

and -2) and vascular cell adhesion molecules (VCAM-1) not only is increased [7] but they are also clustered in focal adhesion complexes.

We and others have intensively investigated the role of circulating blood monocytes and arteriogenesis. Beginning in the mid-seventies, where electron microscopic images of heart collaterals in dogs showed massive adhesion of monocytes at the activated endothelium [32] important parts of the mechanism have been unravelled. Being attracted by MCP-1 and probably other chemoattractants, their binding to the collateral surface is mediated by integrin receptors such as Mac-1 and LFA-1. These heterodimeric molecules are counterparts of ICAM-1, -2 and VCAM-1 and their expression on monocytes can be upregulated by growth factors (*e.g.* VEGF, PIGF, TGF- β) and chemokines such as MCP-1 [31, 33, 34]. After adhesion, a (trans-) migration into deeper parts of the collateral wall and surrounding areas can be observed. Interestingly, the monocyte accumulation is not characterized by a uniformly distribution but rather by an appearance of monocyte clusters which may be one explanation for the typical corkscrew pattern of mature collaterals. Monocytes or -after their mat-

uration- macrophages seem to play a central role in the induction of proliferation of vascular wall cells as well as in vascular wall remodelling. For example, monocytes potently express proteases such as matrix-metalloproteinases and uPA [35, 36]. The proteolytic degradation extracellular structures during their migration through the collateral wall may generate the proliferation signal for SMC; proliferation and migration of SMC is initiated by elastin-derived fragments which appear during proteolytic cleavage of the elastic lamina. Furthermore, it has been shown that the release of growth factors such as FGF-2 by macrophages, directly enhances proliferation within the collateral wall [37]. Taken together, these examples may not completely elucidate all monocyte activities in the growing collateral but may indicate their pivotal contribution. Indeed, in two studies where blood monocyte concentrations were pharmacologically manipulated we could demonstrate that even the monocyte concentration in the blood is important for the dynamics of arteriogenesis [31, 38].

Alternative sources for monocytes/macrophages have been suggested such as proliferation of tissue-resident monocyte progenitors [39]. However, considering the fast recovery of blood flow deficits -for instance, in the mouse model as early as three days after occlusion a marked recovery of blood flow can be detected- one can conclude that recruitment of tissue resident cells (and their subsequent proliferation to achieve required cell numbers) may not represent a substantial pathway for monocyte recruitment. This is also supported by the fact that in contrast to blood and subsequently to bone marrow which together act as almost unlimited sources for monocytes, equivalent skeletal-muscle-resident cells are only a rare population.

As mentioned before, the pathway by which monocytes are recruited to collateral arterioles includes MCP-1. Not surprisingly, the major MCP-1-receptor, CC-chemokine receptor-2 (CCR2) is involved as well [40]. The fact, that the CCR-2 is not only expressed on monocytes but also on other cells such as activated T-cells, suggests that such cells are recruited by the same pathway. An previous observation that lymphocytes as a second blood-derived cell population appear in proximity to growing collaterals, was investigated in more details by Stephen Epstein's group [41]. They found that in mice with a genetic deficiency of the

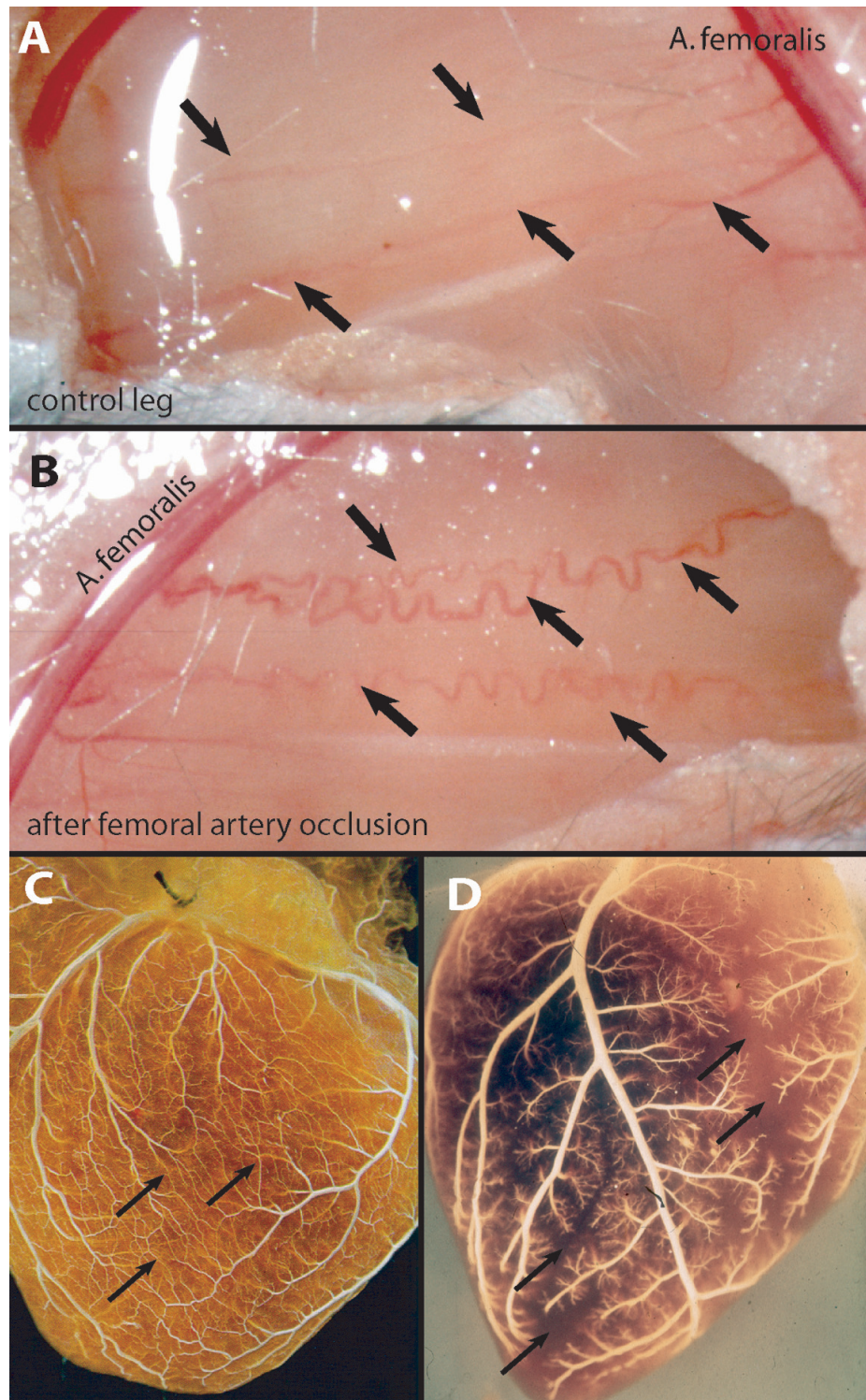
T-cell marker CD4 (CD4^{-/-}), arteriogenesis was markedly inhibited in the hind limb ischemia model which could be rescued by an injection of purified CD4-positive cells. Furthermore, the lack of CD4-positive T-cells led to a reduced inflammatory response in this model including a consistent reduction in the number of monocytes/macrophages which were detected in growing collaterals of CD4^{-/-} mice. Epstein's group concluded that T-cells contribute to arteriogenesis by releasing chemoattractive cytokines, hence supporting monocyte recruitment and supporting their paracrine activity within the growing collateral.

Contribution of stem and progenitor cells to compensatory vascular growth

A number of studies by Asahara and colleagues which first suggested the existence of circulating endothelial progenitor cells within the blood and later their contribution to compensatory vessel growth [42, 43] were later on followed by hundreds of studies, demonstrating the contribution of all kinds of adult stem or progenitor cells to different kinds of vessel growth (reviewed in [44]). Experiments were performed in different species such as mice, rats and rabbits. Furthermore, different cell populations (from mononuclear fraction of bone marrow cells and defined stem cell populations to progenitors isolated from blood) were tested in pre-clinical studies. More complicating, some studies focussed on discovering "physiologic" mechanisms while others were designed to evaluate therapeutic potencies of these cells.

Initially, data prevailed which suggested that bone marrow derived stem cells or progenitors are incorporated into the wall of growing blood vessels, predominately as components of the endothelium or vascular smooth muscle layers. Using laser scanning confocal microscopy to investigate tissue sections derived from different re-vascularisation models, our group was not able to confirm such observations [45]. By reconstituting bone marrow of lethally irradiated mice with bone marrow from GFP-transgenic donors (it is important to mention that mice with bone marrow transplants did not show any functional defects with respect to the applied models) we could indeed witness the

Fig. 3 Different examples for collateral arteries. **A.** Mouse hindlimb, left leg with acute femoral artery occlusion, only. Some pre-existing superficial collateral arterioles can be observed. **B.** Corresponding collaterals in the right hindlimb after femoral artery occlusion (one week). Increase in calibre and typical corkscrew pattern can be observed. **C.** Pre-existing collateral anastomoses (indicated by arrows) in the dog heart. **D.** In contrast to the dog, the pig heart does not show pre-existing collateral arteriolar anastomoses (arrows indicate area where collateral would be expected).



recruitment of bone marrow derived cells to growing vessels. However, no co-localization of GFP - demonstrating that these cells originated from bone marrow - with any vascular marker was found. Instead, these cells expressed markers which are typical for leukocytes or fibroblasts. Currently,

increasing numbers of data suggest a paracrine role of adult stem cells for vessel growth processes delivering growth factors and chemokines or acting as monocyte progenitors [46–48]. However, since mechanisms are still not clear, insufficient study designs may have been an important reason for the

fact that most clinical studies could not proof therapeutic use of such a treatment.

Just remodelling or *de novo* formation of arteries?

The remodelling from a small pre-existing arteriole to a large collateral artery with the ability to compensate for flow deficits is facilitated by a cascade of complex processes [49]. Briefly, growth factors released from macrophages induce proliferation of endothelial and smooth muscle cells. Degradation of extracellular structures leads to the release of additional matrix-bound growth factors. While elastin as present in the elastic lamina prevents SMC from proliferation, its degradation product, elastin-fragments stimulate their proliferation [50]. SMC migrate and rearrange according to the increasing vessel lumen and wall thickness. The remodelling finally enters a maturation state when both elastic lamina and extracellular matrix components are rearranged. It is important to note that the collateral growth process follows a fast kinetics. Data from the mouse model demonstrate that already three days after femoral artery occlusion a partial blood flow recovery is detectable highlighting the ability of the system for an immediate response to the stimulus. Moreover, it shows that the degradation of extracellular structures during early phases of the remodelling process does not lead to a reversible functional defect of the growing collateral.

The question whether there is an -physiologically relevant- *de novo* formation of collaterals has to be enlightened with respect to the rapid conversion of small pre-existing arteriole to large arteries (see Fig. 3). Interestingly, the topic has been discussed for decades. William F.M. Fulton, one of the most important contributors to the field, previously reviewed this discussion and concluded that the question was markedly influenced by the applied imaging techniques [51]. Accordingly, the kind of contrast agent and its application as well as the imaging method (radiography) determined whether anastomoses with diameters of less than 20 μm were detected. Fulton concluded that the success of describing these anastomoses was markedly based on the investigator's study design. More information is provided from the pig heart

in which arteries are sought to be "end arteries" without pre-existing arteriolar anastomoses (also in rat and rabbit heart, whereas the dog heart shows pre-existing collateral anastomoses) [52]. Attempts to induce collateral artery growth by gradually increasing occlusion of a coronary artery in the pig heart failed whereas it was successfully demonstrated in the dog heart. It can be concluded that the failure to induce true arteriogenesis in pigs was due to the lack of pre-existing arterio-arterial connections. In contrast, in several species including mice and rabbits pre-existing collateral anastomoses can be found in the periphery and, not surprisingly, arteriogenesis is present in various experimental settings in these animals. Moreover, the conversion to functional arteries, often with a typical corkscrew pattern, can be impressively observed in these models. However, it was reported that in the pig model some so-called "collaterals" with much smaller calibres compared to the dog heart become detectable, interestingly without the presence of proliferation SMC [53] and angiogenesis was induced, most likely in response to ischemia.

Another interesting mechanism was proposed as an alternative pathway by which a *de novo* vessel formation could be at least experimentally initiated (Fig. 1c) [54]. According to this approach, the monocytes/macrophages use their proteolytic activity provided by matrix-metalloproteinases and other proteases to drill tunnel-like structures into the extracellular compounds which then might be colonized by circulating endothelial progenitors or endothelial cells derived from transdifferentiation of monocytes [55, 56]. Although in principal being in line with monocyte biology and perhaps emerging as a new mechanism, it is not clear if such vessels become and remain capillaries or if they undergo an arterialisation such as previously proposed by Carmeliet [57]. He proposed that pericytes which might best defined as smooth muscle cell progenitors are attracted to such endothelial tubes and form the nucleus for a smooth muscle layer. The fact that a CD34⁺ cell line derived from cord blood showed myogenic plasticity, could indicate a hint towards a mechanism [58]. However, if such a process analogously to early artery formation during embryo development also proceeds in the adult organisms, is still not clear.

Summary

In summary, the remodelling of pre-existing collateral anastomoses to functional arteries termed arteriogenesis is the only physiologically relevant way of blood vessel growth which has the ability to compensate for an occlusion or stenosis of a major artery. The driving force for arteriogenesis is altered fluid shear stress which initiates a complex cascade of molecular and cellular events leading to increased vessel lumen and wall thickness. Monocytes play a pivotal role in arteriogenesis by releasing growth factors, proteases and chemokines, hence mediating cell proliferation and migration as well as structural remodelling of the extracellular compartment. In addition, other circulating cells such as T-cells and bone marrow-derived cells contribute to collateral growth, most likely in a paracrine fashion. Direct signal transmission between shear-activated endothelium and smooth muscle cells may also exist next to the monocyte-based mechanisms and are presently under investigation in our group. They may be easier to use for treatment purposes than monocytes that play also a role in the genesis of atherosclerosis.

Furthermore, depending on the pathology of the impaired blood supply we most likely may need the therapeutic restoration either as a macrovascular repair (arteriogenesis; in case of major arterial stenosis or occlusion) or - in the case of ischemic tissue - as a microvascular repair *via* angiogenesis.

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