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REVIEW

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Vascular remodeling by intussusceptive angiogenesis

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Abstract Intussusception (growth within itself) is an alternative to the sprouting mode of angiogenesis. The protrusion of opposing microvascular walls into the capillary lumen creates a contact zone between endothelial cells. The endothelial bilayer is perforated, intercellular contacts are reorganized, and a transluminal pillar with an interstitial core is formed, which is soon invaded by myofibroblasts and pericytes leading to its rapid enlargement by the deposition of collagen fibrils. Intussusception has been implicated in three processes of vascular growth and remodeling. (1) Intussusceptive microvascular growth permits rapid expansion of the capillary plexus, furnishing a large endothelial surface for metabolic exchange. (2) Intussusceptive arborization causes changes in the size, position, and form of preferentially perfused capillary segments, creating a hierarchical tree. (3) Intussusceptive branching remodeling (IBR) leads to modification of the branching geometry of supplying vessels, optimizing pre- and postcapillary flow properties. IBR can also lead to the removal of branches by pruning in response to changes in metabolic needs. None of the three modes requires the immediate proliferation of endothelial cells but rather the rearrangement and plastic remodeling of existing ones. Intussusception appears to be triggered immediately after the formation of the primitive capillary plexus by vasculogenesis or sprouting. The advantage of this mechanism of growth over sprouting is that blood vessels are generated more rapidly in an energetically and metabolically more economic manner, as extensive cell proliferation, basement membrane degradation, and invasion of the surrounding tissue are not required; the capillaries thereby formed are less leaky. This process occurs without disrupting organ function. Improvements in our understanding of the process should enable the development of novel pro- and anti-angiogenic therapeutic treatments.

Keywords Angiogenesis · Non-sprouting angiogenesis · Intussusceptive microvascular growth · Intussusceptive arborization · Intussusceptive branching remodeling · Pruning · Sprouting

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Mechanisms of angiogenesis

Angiogenesis is the process underlying the expansion of the microvascular system during tissue growth, regeneration, and tumorigenesis (Paku and Paweletz 1991; Folkman 1995; Risau 1997; Augustin 1998, 2001; Conway et al. 2001; Jain et al. 2002; Bergers and Benjamin 2003; Carmeliet 2003). It can occur by one of two known mechanisms: sprouting or intussusception. Although both mechanisms lead to an amplification of the capillary network, they involve different cell types and are regulated by different molecules.

Sprouting angiogenesis

Sprouting angiogenesis is localized at the abluminal aspect of vessels and is characterized mainly by local vasodilation, increased vascular permeability, and cell proliferation. It is initiated by proteolytic degradation of the basement membrane, after which endothelial cells migrate into the extracellular matrix and proliferate or vice versa. The sprouts reorganize internally to form a vascular lumen and are finally connected to other

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capillary segments. Activation of sprouting is a relatively sluggish process in vivo, requiring more than 24 h, and at least 3–5 days elapse before a new capillary loop becomes perfused and is integrated into the vascular system (Ausprunk and Folkman 1977; Burger et al. 1983).

The concept of intussusception

Non-sprouting angiogenesis by intussusception was first observed in the rapidly expanding pulmonary capillary bed of neonatal rats (Caduff et al. 1986). Numerous tiny holes (1–2 μm in diameter) detected by scanning electron microscopy within vascular corrosion casts were shown to correspond to slender transcapillary (intraluminal) tissue pillars (Caduff et al. 1986; Burri and Tarek 1990). Serial sectioning of tissue followed by transmission electron microscopy revealed these pillars to arise by invagination of the capillary wall into the vessel lumen.

Four consecutive steps in pillar formation have been described: During phase I, a zone of contact is created between opposing capillary walls. Phase II is characterized by the reorganization of the inter-endothelial cell junctions and by central perforation of the bilayer. During phase III, an interstitial pillar core is formed that is invaded by pericytes and myofibroblasts that then lay down collagen fibrils. By this stage, transluminal pillars have a diameter of $\leq$2.5 μm. During the final phase (phase IV), the pillars increase in girth without undergoing any further change in their basic structure. On the basis of these morphological observations, the authors postulated that the pulmonary capillary network expanded predominantly by the insertion of transcapillary pillars, a phenomenon that had not been described hitherto; they coined the term "intussusceptive microvascular growth" (IMG) for this process, to convey the meaning that growth of the capillary network occurred "within itself" (Caduff et al. 1986; Burri and Tarek 1990). This mechanism of angiogenesis was subsequently also demonstrated in the chick chorioallantoic membrane (CAM; Patan et al. 1993, 1996) and has since been revealed to occur in many tissues and species during both normal and pathological microvascular growth. Hence, it appears to be a general phenomenon (Patan et al. 1992, 2001a; 2001b; Djonov et al. 2000a, 2000b, 2001, 2002; Burri and Djonov 2002; Kurz et al. 2003). The concept of intussusception is schematically represented in Fig. 1.

Non-sprouting angiogenesis has also been reported to occur in myocardium (van Groningen et al.1991) and skeletal muscle (Zhou et al. 1998). Although the described modes of vascular growth were referred to by different names, viz., as "longitudinal splitting" and "luminal division", respectively, they both resemble intussusception during the initial phases of conception. The transluminal pillars subsequently formed are more elongated in these muscle tissues than in lung due to the parallel arrangement of the myofibrils.

Because of the complex spatial structure of transluminal pillars, adequate visualization of the intussusceptive process eluded investigators for a considerable time. The only reliable methods of visualization are vascular corrosion casting and serial sectioning for light or

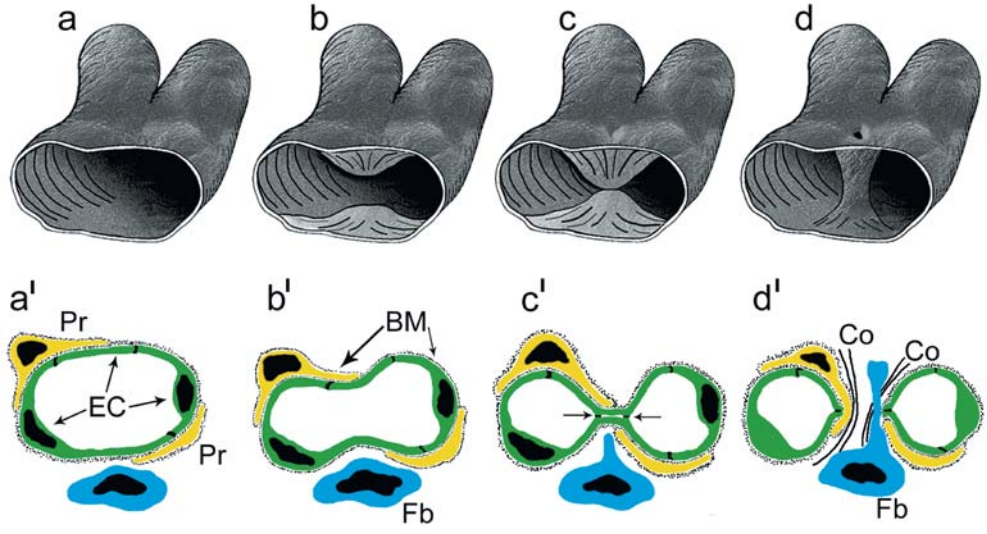

Fig. 1a–d Three-dimensional scheme depicting the generation of new vascular segments by intussusceptive growth. The process begins with the protrusion of opposing capillary walls into the vessel lumen (**a, b**). After contact has been established and "corroborated" (**c**), the endothelial bilayer becomes perforated centrally and a transluminal pillar is formed (**d**). **a'–d'** Two-dimensional representation of the events depicted in **a–d**. Endothelial cells (*EC*) situated on opposing sides of a capillary protrude into its lumen until they contact each other (**a'–c'**). Once established, this contact is "corroborated" by the formation of interendothelial junctions and then reorganized in such a manner that the endothelial bilayer is perforated centrally. The endothelial cells then retract, and the newly formed pillar increases in girth after being invaded by fibroblasts (*Fb*) and pericytes (*Pr*), which lay down collagen fibrils (*Co* in d'). For an animated version of the figure see ESM at http://dx.doi.org/10.1007/s00441-003-0784-3. **a–d** is reproduced from Kurz et al. 2003 with the authors' and publisher's permission

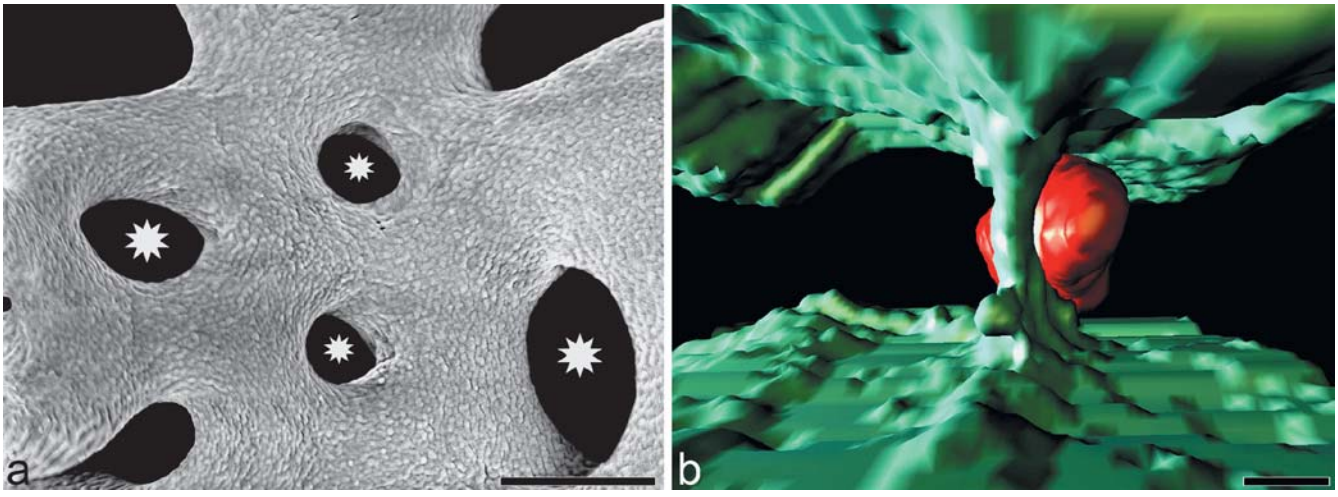

Fig. 2a, b **a** Corrosion cast of blood vessels in which newly formed transcapillary pillars appear as small holes (*stars*). **b** Three-dimensional reconstruction of a transcapillary pillar (based upon transmission electron microscopy of serial sections through a chick CAM; for methodological details, see Djonov et al. 2000a). An erythrocyte (*red*) is also represented. **b** is reproduced from Djonov et al. 2000a with the publisher's permission

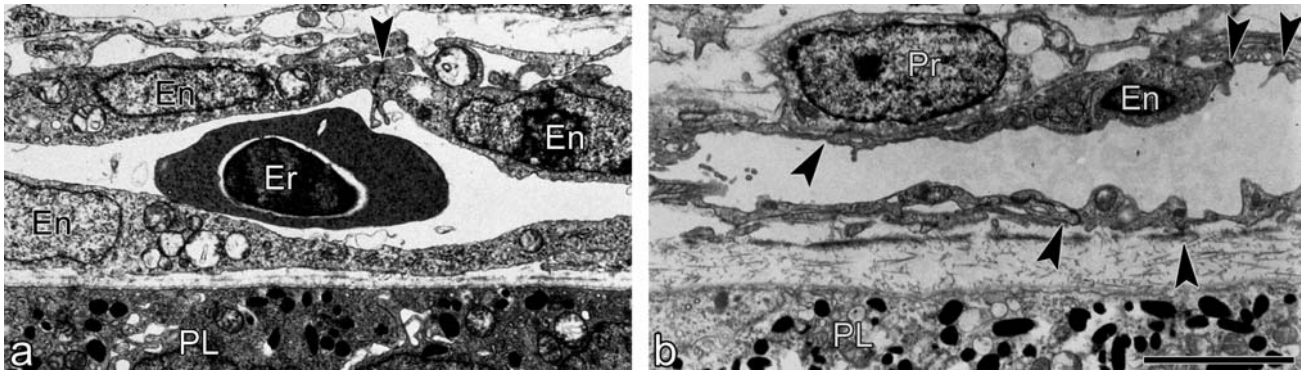

Fig. 3a, b Transmission electron micrographs (at the same magnification) of chick choroidal capillaries at days 8 (**a**) and 14 (**b**) of incubation. The thickness of the endothelium and the number of endothelial cells per unit area are dramatically reduced in **b** as a result of their attenuation and spreading during intussusceptive pillar formation (*arrowheads* positions of interendothelial cell junctions, *Er* erythrocyte, *En* endothelium, *Pr* pericyte, *PL* pigment layer). *Bar* 5 μm

transmission electron microscopy followed by three-dimensional reconstruction (Fig. 2) or confocal laser microscopy (Burri and Tarek 1990; Djonov et al. 2000a, 2000b, 2001, 2002). Other methods for three-dimensional imaging, such as nuclear magnetic resonance, micro-computer tomography, angiography, and ultrasonics do at present not have the resolution necessary (at least 1 μm) for the visualization of pillars. This circumstance may explain why intussusception was overlooked in the past.

One important characteristic of intussusceptive vascular growth is that it is achieved at an exceedingly low rate of endothelial cell proliferation. In CAMs, this rate drops dramatically between days 10 and 11, coinciding with the peak of intussusceptive pillar formation (Ausprunk et al. 1974; Kurz et al. 1995; Schlatter et al. 1997; Djonov et al. 2000a, 2000b). Similarly, in the lung vasculature, the capillary volume and surface area were earlier observed to increase 35-fold and 20-fold, respectively (Burri et al. 1974; Zeltner et al. 1987), in the absence of a change in endothelial cell number (Kauffman et al. 1974).

How can a massive expansion of the vasculature occur at such a low proliferation rate? Our comparative study of various organs before and after the onset of intussusception revealed the total endothelial cell volume to be redistributed during pillar formation by a thinning and spreading of the pre-existing cell population, as illustrated in the chick choroid (Fig. 3). The phenomenon of endothelial cell attenuation during CAM growth was first documented as an incidental finding by Ausprunk et al. in 1974. A subsequent morphometric analysis of chick CAMs revealed the thickness of endothelial cells to be reduced by more than 50% between days 10 and day 14 of incubation (Rizzo and DeFouw 1993).

Direct and definitive evidence for the existence of intussusceptive vascular growth has now been obtained (Patan et al. 1993) by using chick CAMs (Auerbach et al.

1974). This is an excellent tool for investigating normal vascular growth and remodeling processes, and for monitoring alterations induced by various pro- and anti-angiogenic factors (Ribatti et al. 2001).

The use of improved digital techniques in combination with fluorescein-isothiocyanate-dextran injection into the blood stream has enhanced the quality of earlier images. Pillar formation and remodeling have now been clearly observed, not only within capillary plexuses (Djonov et al. 2000a, 2000b), but also within small arteries and veins (Djonov et al. 2002). The combination of in vivo monitoring with histological and ultrastructural analyses of serial tissue sections has demonstrated that pillar formation requires a period of 4–5 h for completion (Djonov et al. 2002). This time can be reduced to 1 h by artificially doubling the blood flow rate (Djonov et al. 2002). Hence, in contrast to sprouting, which is characterized by extensive proliferation of endothelial cells, an increase in vascular permeability, and a duration of several days, intussusception occurs in the virtual absence of endothelial cell proliferation, is achieved at low vascular permeability levels, and requires only 4–5 h for completion. Intussusception is a widespread phenomenon that occurs in the vascular systems of all species thus far investigated.

Outcomes of intussusceptive angiogenesis

Intussusception is involved in vascular remodeling processes that have different morphological and functional outcomes. First, the sporadic occurrence of pillars within the capillary bed leads to its expansion and an increase in its complexity, namely, to intussusceptive microvascular growth (IMG). Second, pillars may arise in series and then merge to form small arteries and veins in distal parts of a vascular tree, thus leading to intussusceptive arborization (IAR). Third, pillar formation occurring within small arteries and veins can lead to remodeling via an expansion or pruning of vessel branches and an optimization of the branching geometry and of the hemodynamic conditions of the vascular tree, namly, to intussusceptive branching remodeling (IBR).

IMG: expansion of capillary plexuses

Continuous pillar formation and growth lead to a rapid expansion of the capillary plexus, thereby affording a large surface area for the exchange of oxygen, carbon dioxide, and nutrients. By these means, new segments of the capillary network arise with only little changes in the dimensions of its components (Fig. 4), viz., IMG.

IMG was first observed in the growing postnatal lung (Caduff et al. 1986; Burri and Tarek 1990) and then in the microvasculature of many other tissues and organs of several species, including the rat (Patan et al. 1992), the chick CAM (Patan et al. 1993; Djonov et al. 2000a, 2000b), retina (Djonov 2000b, 2002), and kidney (Djonov et al. 2002), in a mouse model of tissue repair (Patan et al. 2001a), in heart development (van Groningen et al. 1991), in the human endometrium (Gargett et al. 2001; Gambino et al. 2002), in cerebral vascularization after stroke (Zhang et al. 2002), and in tumor angiogenesis (Djonov et al. 2001; Patan et al. 2001a).

It is now evident that IMG represents a general and ubiquitous mechanism of capillary growth. This phenomenon explains the way in which the capillary beds of organs, which arise initially by sprouting and/or vasculogenesis, can undergo rapid expansion without any compromise in vascular physiology or function, as is reflected by the low vascular permeability conditions and

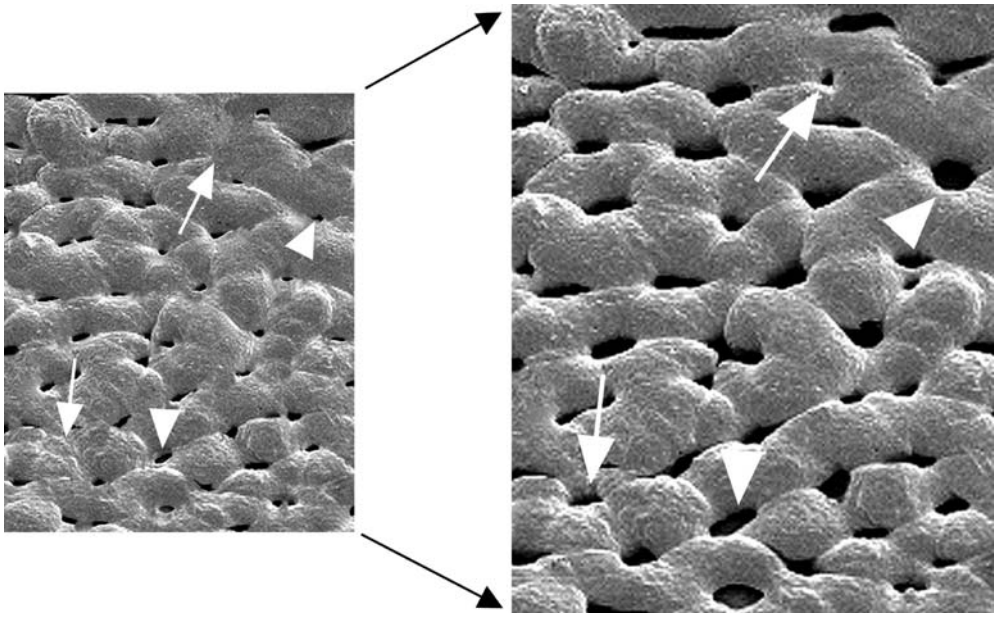

Fig. 4 Digital representation of intussusceptive microvascular growth. The capillary plexus expands by the insertion of new pillars (arrows) and by the enlargement of existing ones (arrowheads). These images are based upon observations in chick CAM corrosion casts. For an animated version of the figure see: ESM at http://dx.doi.org/10.1007/s00441-003-0784-3. These images are adapted from Djonov et al. 2002, with the publisher's permission

the low rate of endothelial cell proliferation associated with IMG.

IAR: formation of a feeding vascular tree.

As a capillary plexus grows, the perfusion distance between arteries and veins increases, which necessitates an adaptation in the system of supplying and draining vessels. Recently, intussusceptive pillar formation has been shown to be involved in the differentiation of parts of the capillary plexus into immediate pre- and postcapillary feeding vessels, viz., IAR (Djonov et al. 2000a, 2000b). IAR furnishes a mechanism whereby preferentially perfused segments of a capillary plexus can be transformed into terminal arterioles and collecting venules by changing their size and position, the number of sprays in a bunch of feeding or collecting vessels being thereby increased. IAR is initiated by the formation of serried "vertical" pillars, which demarcate future feeding vessels. These pillars undergo reshaping into narrow tissue septa that progressively fuse to form a new vascular entity. The remaining connecting bridges are "severed" by the formation of "horizontal" folds, the feeding vessels being thereby definitively separated from the capillary plexus. As a result of this process, a complex arterial and venous vascular tree arises to form a second layer (Fig. 5).

IBR: optimization of branching geometry

By means of IBR, the branching geometry of supplying vessels is adapted to optimize the pre- and postcapillary flow properties. IBR can also lead to the removal of branches (vascular pruning), thereby optimizing the efficiency of the blood supply and the hierarchy of the vascular tree.

Transluminal pillars and folds have been observed close to the bifurcation sites of arteries and veins up to 120 μm in diameter. Intravital microscopy has confirmed that these structures appear de novo, and that they are capable of rapidly changing the geometry and the hemodynamic properties at the affected branching points (Djonov et al. 2002; Kurz et al. 2003). Such pillars may have one of two fates. Those located close to a bifurcation point tend to enlarge (pillar augmentation) until their distal end contacts and merges with connective tissue in the branching angle (Fig. 6). Pillars located more than 8–10 μm away from the bifurcation point tend to elongate (pillar elongation) into a flat longitudinal fold that protrudes progressively into the lumen until this is subdivided into two distinct channels (Djonov et al. 2002). Irrespective of whether pillars undergo augmentation of elongation, these data indicate that IBR is an important morphogenic mechanism. First, it permits a narrowing of the branching angle by relocating the branching point more proximally. This may represent an important adaptive response to the continually increasing blood flow and blood pressure during embryogenesis and growth. Direct experimental evidence for this hypothesis has been furnished by Frame and Sarelius (1993) who reported the bifurcation angle of golden hamster cremaster muscle vessels to be modified in response to blood flow alterations. A 12%–14% reduction in the branching angle of retinal arteries has also been reported to occur in hypertensive human subjects (Stanton et al. 1995). Our own observations (unpublished) indicate that IBR is an important adaptive mechanism in rats suffering from

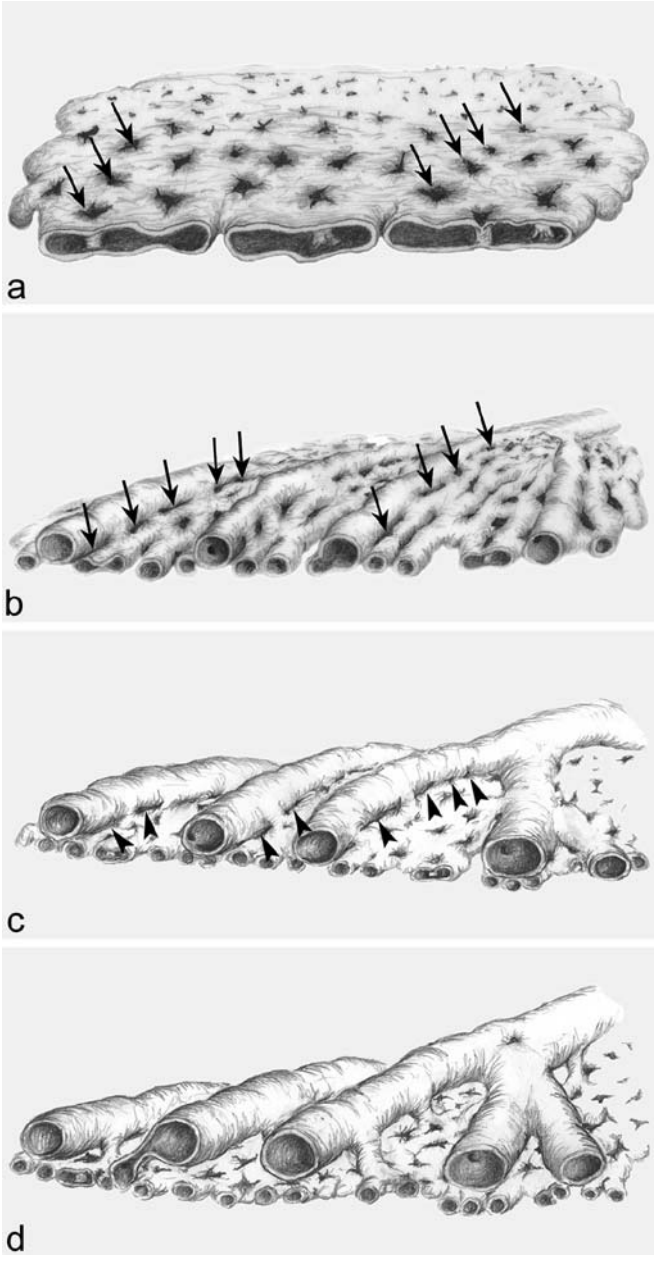

Fig. 5a–d Scheme representing intussusceptive arborization. Within a capillary plexus, a series of "vertical" pillars arises (*arrows* in **a**), which demarcates future feeding vessels. These pillars undergo reshaping and fusion to form narrow tissue septa (*arrows* in **b**). "Horizontal" pillars and folds are then formed (*arrowheads* in **c**) that separate the feeding vessels from the capillary plexus (**d**). Adapted from Djonov et al. 2000a, with the publisher's permission

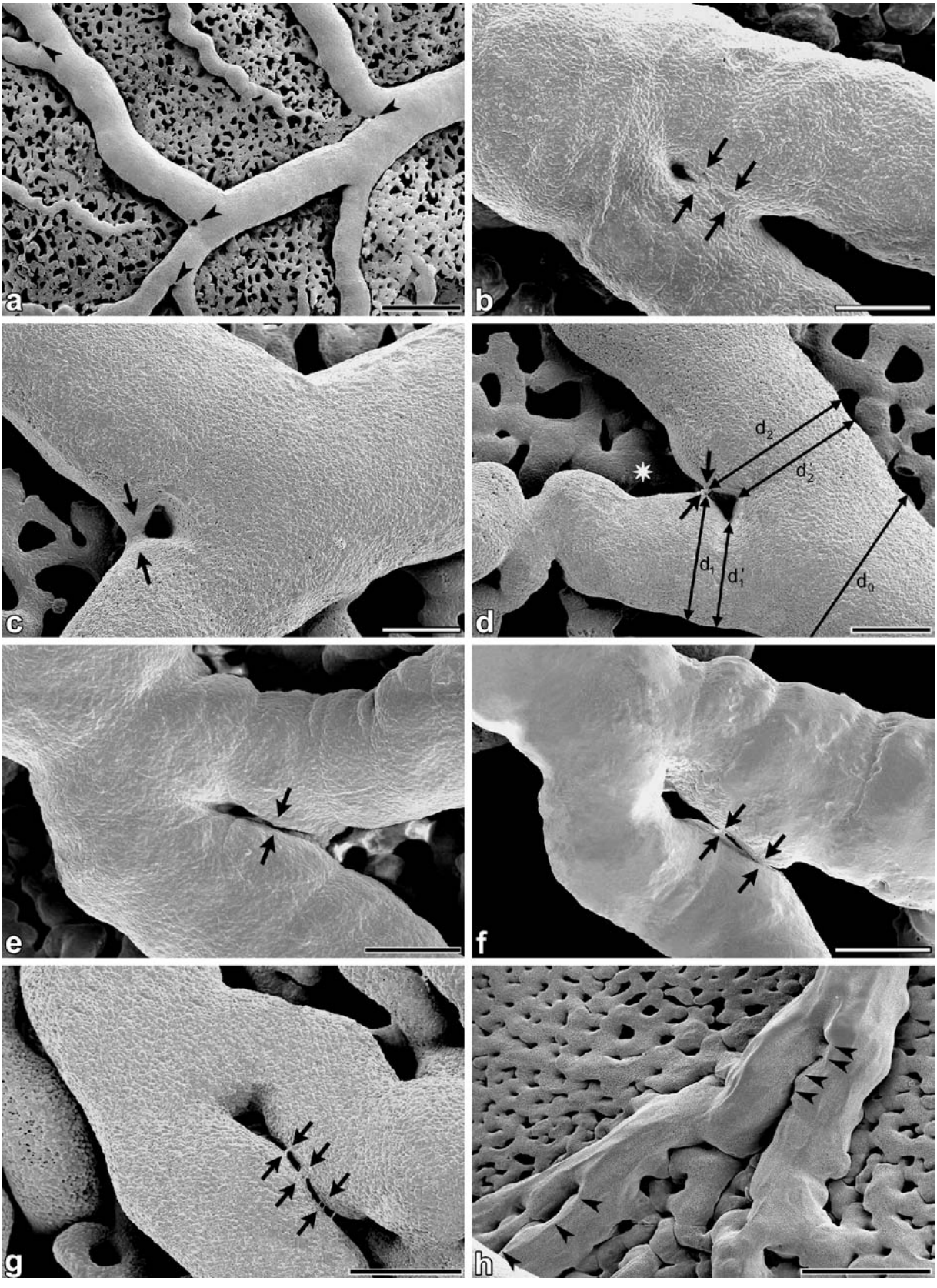

Fig. 6a–h Vascular casts of a chick CAM between days 8 and 10 of embryonic development. **a** Overview of the supplying artery and capillary plexus. Four of the five visible bifurcations exhibit a transluminal pillar (*arrowheads* holes in cast), each of which is at a different stage of pillar augmentation. **b–d** Illustration of the process of intussusceptive branching remodeling (IBR) by an increase in pillar size. During the initial stage of IBR (**b**), a pillar (hole) appears at a short distance from the bifurcation angle, from

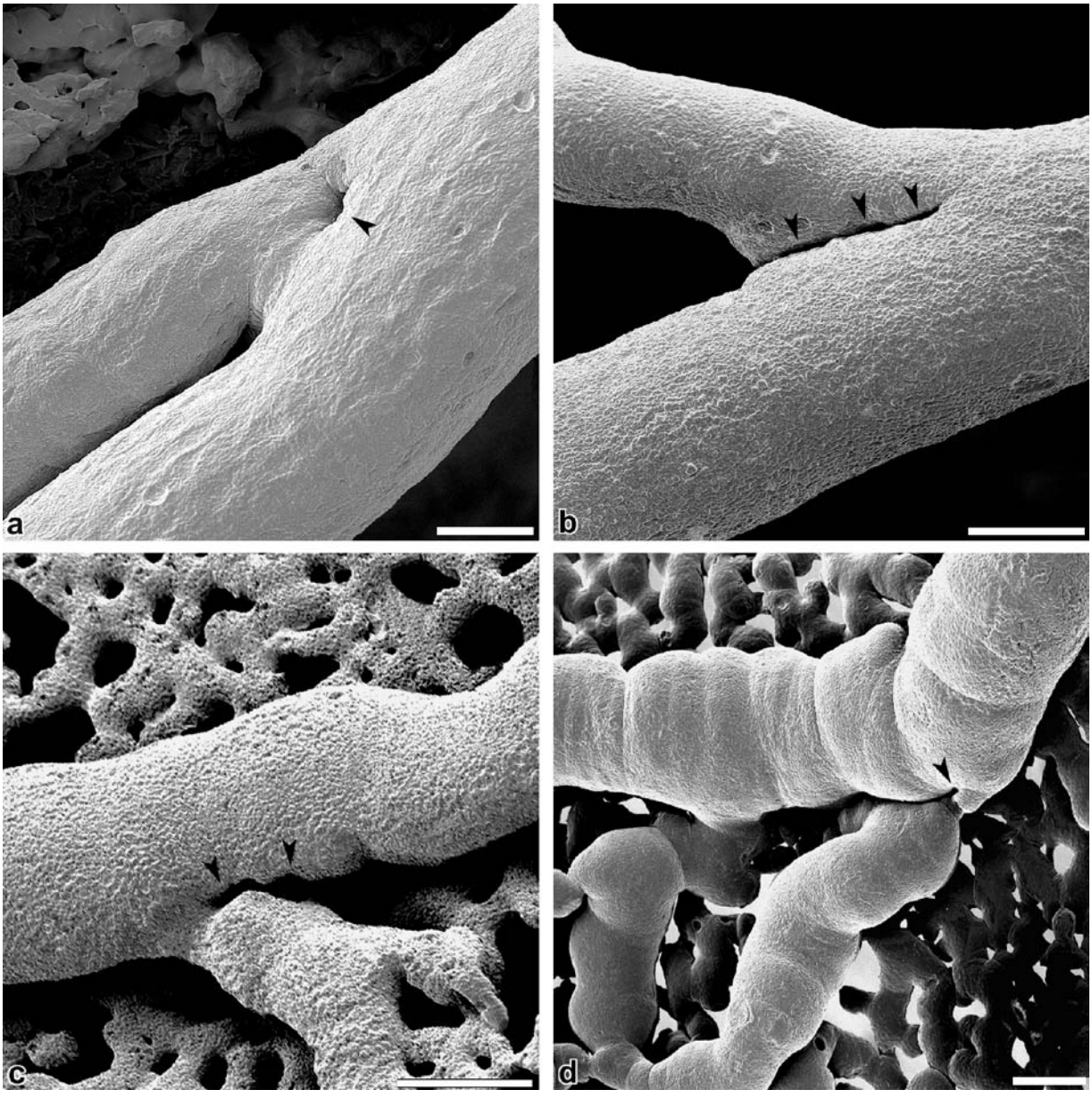

Fig. 7 Intussusceptive branching (IBR) contributes to vascular pruning. Vascular casts of feeding vessels in 12-day-old and 13-day-old CAMs illustrate the role of IBR remodeling in the pruning of unnecessary vessels. A single pillar (*arrowhead* in **a**) and then several others (*arrowheads* in **b**) arise at the bifurcation site of a feeding vessel, which then splits either partially (*arrowheads* in **c**) or completely (*arrowhead* in **d**). This event is followed by the regression of one of the daughter vessels. *Bars* 30 μm. Reprinted from Djonov et al. 2002, with the publisher's permission

which it is still separated by a patent vascular lumen (*arrows*). The pillar increases in size, which results in a narrowing (**c**) and then the complete obliteration (**d**) of the lumen. By means of this process, the pillar merges with the connective tissue (*star* in **d**) in the bifurcation angle. The *double arrows* in **d** indicate the position at which diameter measurements were made (data not shown). **e–g** Illustration of IBR by fold formation. Opposing longitudinal folds of endothelium and tissue grow into the vascular lumen in a guillotine-like (**e**) or a multifocal (*arrows* in **f, g**) manner. **h** Rows of pillars (*arrowheads*) alternating with tissue folds result in a cascade-like variant of vessel splitting. *Bars* 100 μm (**a, h**), 20 μm (**b–g**). For an animated version of IBR, see ESM at http://dx.doi.org/10.1007/s00441-003-0784-3

pulmonary hypertension. Second, IBR optimizes the hemodynamic conditions at bifurcation sites by remodeling one or both branch diameters (mainly by "pillar augmentation"). By these means, IBR yields a branching pattern that approximates to the ideal predicted by "Murray's Law" of minimal power consumption and constant shear stress (Djonov et al. 2002; Kurz et al. 2003).

During IBR, intussusceptive pillar formation does not generate new vascular segments, as it does during IMG and IAR, but adapts branching geometry to the changing hemodynamic conditions.

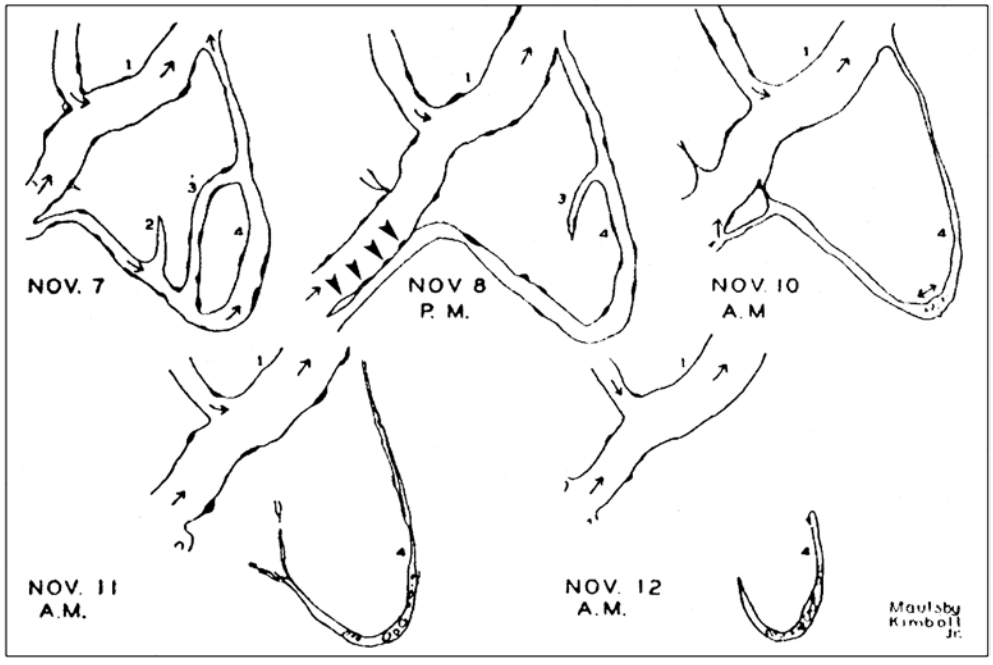

Fig. 8 By using the rabbit ear chamber as a model, Clark and Clark (1939) investigated the role of blood flow in vascular growth and remodeling. Some of their excellent drawings reveal small ellipsoidal pillars (denoted here by *arrowheads*) that split the vessel lumen in the vicinity of branching points. Fusion of these pillars leads to a separation of the lateral branch within 2 days and subsequent intussusceptive pruning and atrophy of the affected vessel. This illustration probably represents the first, albeit unknown, documentation of intussusceptive branching remodeling. Reproduced from Clark and Clark 1939 with the publisher's permission

Pruning as a result of IBR

By the successive asymmetric formation of pillars, IBR occasions the subtotal lumen obstruction of one of the daughter branches. The reduction in blood flow associated with the narrowed bore probably contributes to the regression, retraction, and ultimate atrophy of the affected branch (Fig. 7). This closing down of a vessel branch is known as vascular pruning and was first described for retinal vessels by Ashton in 1966. Pruning has recently been suggested to be controlled by specific growth factors and by oxygen tension (Risau 1997; Dor et al. 2001). We have observed signs of intussusceptive vascular pruning in most organs of the chick embryo, but it occurs in the most impressive degree in the regressing pro- and metanephrons of the kidney (data not shown). The thinning, retraction, and atrophy of vessel branches have been well described by Clark and Clark (1939). These authors demonstrated the complete separation of a side branch from the main vessel within 3 days and its disappearance by the fourth day (Fig. 8). However, they were not aware that the pruning process was initiated by the eccentric formation of pillars and folds.

Sprouting and intussusception: two complementary angiogenic mechanisms

The experimental tools commonly used in vitro and in vivo to demonstrate the process of angiogenesis, such as three-dimensional collagen gels, corneal implants, tumor implantation, wound healing, and embryonic grafting, elicit only capillary sprouting during tissue neovascularization. Indeed, intussusception would not be expected to occur in these models because of the absence of blood flow. For these reasons and perhaps also because of the visualization difficulties alluded to above, intussusception was not recognized until recently.

Structural investigations have revealed the vasculature of various organs, such as the chick CAM (Schlatter et al. 1997), the lung (Caduff et al. 1986; Burri and Tarek 1990), the heart (van Groningen et al. 1991), the endometrium (Gargett et al. 2001; Gambino et al. 2002), the eye (Djonov et al. 2000a), the kidney, and the yolk sack (data not shown), to undergo two main phases of development: The "early sprouting phase" is characterized by the appearance of multiple capillary sprouts that invade the mesenchyme and, after fusion, form the primary capillary plexus. The primitive capillary plexus can also arise directly from precursor cells by vasculogenesis, as in the area vasculosa of the chick embryo. During the "second intussusceptive phase", capillary sprouting is superseded by transcapillary pillar formation. Further vascular growth and remodeling thus occurs by intussusception. This results in a rapid expansion of the capillary network (IMG) and in vascular tree formation (IAR) and its dynamic adaptation and remodeling (IBR). The reasons underlying this switch from sprouting to intussusception are three-fold: (1) blood vessels can be formed more rapidly by intussusception than by sprouting; (2) intussusception appears to be energetically and metabolically more economical than sprouting in that it does not depend upon extensive endothelial cell proliferation, basal membrane degradation, or the invasion of surrounding tissue; (3) intussusception is characterized by "physiological" levels of transpermeability that permit vascular growth and remodeling to occur within a functionally uncompromised organ. The intussusceptive mechanisms described run

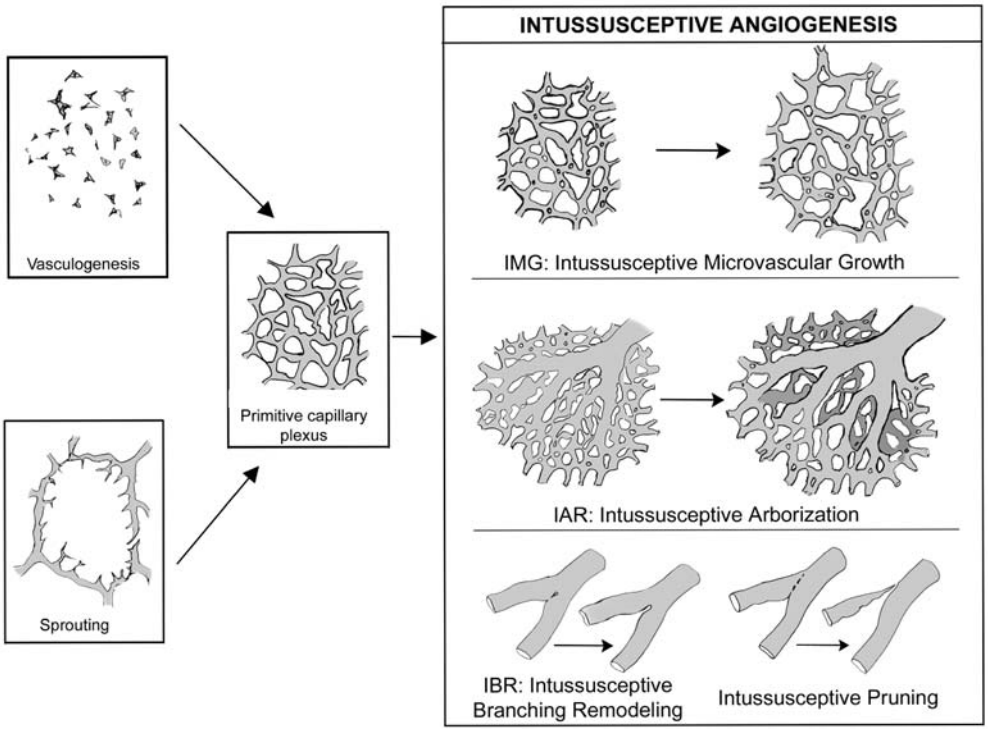

Fig. 9 Diagram summarizing intussusceptive angiogenesis (IAR). When a primitive capillary plexus is generated by vasculogenesis or sprouting, intussusception is triggered and is responsible for rapid vascular growth and remodeling. Feeding vessels are subsequently segregated from the capillary plexus by IAR. IBR optimizes branching geometry and is responsible for vascular pruning

hand in hand and represent different facets of intussusceptive angiogenesis (Fig. 9).

Regulation of intussusception

As discussed above, intussusceptive angiogenesis involves the formation of tissue pillars. By virtue of these, the vascular network expands and is remodeled to accommodate changes in functional needs. Each process is governed by a specific program that is initiated and regulated by defined molecules and cells; information concerning the identity of this is gradually emerging. Hemodynamic considerations are clearly important determinants of vascular architecture. Clamping of one of the dichotomous branches of an artery in the CAM microvasculature increases blood flow and/or pressure in its counterpart, and this has an almost immediate effect on branching morphology (Djonov et al. 2002): IBR is initiated within a few minutes, pillars are detected after 15–30 min, and the branching angles are decreased by about 20% after 40 min. Shear stress, which acts tangentially on capillary walls and is known to be modified by experimental increases in blood flow, may be responsible for these changes. Shear stress is related to the diameter of a vessel. Hence, insertion of a pillar into the blood stream near a branching point will reduce this force in post-pillar vessel segments (Djonov et al. 2002; Kurz et al. 2003). Changes in shear stress can somehow be sensed by endothelial cells and transduced by molecules such as PECAM/CD31 (Osawa et al. 2002) into their interior. This mechanotransduction system then leads to changes in the transcription rate of many proteins, such as eNOS, adhesion molecules, and angiogenic factors (Fisher et al. 2001; Zakrzewicz et al. 2002).

During arteriogenesis, physiological or pathophysiological adaptations to changes in shear stress have been reported to involve interactions between pericytes, macrophages, and endothelial cells (van Royen et al. 2001), and it is not unreasonable to assume that the former two cell types play an important role in intussusceptive growth remodeling. Indeed, morphological analyses have shown that pericytes and/or periendothelial cells are recruited during the initial and final phases of vascular pillar formation in several organs (Djonov et al 2000a, 2002). We postulate that the recruitment of pericytes contributes either to the synthesis and mechanical stabilization of the transcapillary pillar core or to the maintenance of a low vascular permeability during intussusception.

Angiopoetins and their Tie-receptors (Folkman and D'Amore 1996), PDGF-B (Hellstrom et al. 1999), and ephrins and their Eph-B receptors (Gale et al. 2001; Shin et al. 2001) are believed to be involved in the induction of sprouting angiogenesis and may also influence vascular remodeling. The same factors and receptors could likewise mediate the endothelial-to-pericyte and endothelial-to-endothelial interactions observed during intussusceptive angiogenesis, particularly with respect to angiopoietin-2 and PDGF-B, both of which are essential for pericyte recruitment in the retina (Benjamin et al. 1998), brain (Hellstrom et al. 1999), and placenta (Ohlsson et al. 1999). Notably, the injection of PDGF-B into fully developed CAMs leads to the formation of abundant large pre- and postcapillary microvessels but not to the

expansion of capillary meshes (Oh et al. 1998). Furthermore, the vasculature of knockout mice lacking angiopoietin-1 and Tie-2 remains at a primitive stage of development and fails to undergo further remodeling (Suri et al. 1996). In contrast, over-expression of angiopoietin-1 or of angiopoietin-2 in combination with VEGF is associated not only with the formation of "large" vessels, but also with the presence of abundant small holes in the capillary plexus (Thurston et al. 1999), a finding that is symptomatic of intussusception.

Vessels remain in an immature state if VEGF is maintained at a constant level (Alon et al. 1995), whereas down-regulation of this factor is associated with pruning. VEGF not only promotes the formation of new capillary segments, but also plays a crucial role in blood vessel maturation via the recruitment of pericytes and smooth muscle cells (Grosskreutz et al. 1999; Dor et al. 2003). When newly formed vessels are denuded of these cells, the vascular segments become VEGF-independent and fail to mature (Benjamin et al. 1998). VEGF is a highly potent and universal regulator of vascular responses to oxygenation levels, namely, to hypoxia, normoxia, or hyperoxia (Dor et al. 2001, 2003), and different ranges of oxygen concentration could conceivably yield "angiogenic doses," "maintenance doses", or "submaintenance dose" of VEGF with the corresponding angiogenic responses (Dor et al. 2001). Various angiogenic molecules, hemodynamic parameters, and/or oxygen tension thus appear to be responsible for initiating and coordinating intussusceptive growth. However, the precise molecular and morphological mechanisms involved remain to be elucidated.

Final remarks

Sprouting angiogenesis was first detected at the beginning of the nineteenth century. Since then, vascular research has meandered from purely descriptive observations in vivo to mechanistic hemodynamic interpretations, and from physiological explanations and structural analyses to the nearly boundless field of cell and molecular biology. By contrast, intussusceptive angiogenesis has a short history, and we are only now beginning to understand its significance. Nevertheless, our recent morphological findings are strongly suggestive of its playing an essential role in an organism's development and growth, in tissue repair and remodeling, and in tumor angiogenesis. Indeed, we have demonstrated that, after the initial phase of primitive capillary plexus formation by vasculogenesis and/or sprouting, additional vascular growth and development of complex vascular beds, including their continuous remodeling and adaptation, occur predominantly by intussusception. Bearing in mind that most of the pro-angiogenic (heart and limb ischemia) and anti-angiogenic (tumors) therapeutic approaches that are currently applied act within complex vascular beds, this finding is of great clinical relevance.

As our understanding of the regulatory mechanisms underlying intussusceptive angiogenesis improves, we should be in a better position to elaborate novel treatment strategies taking into account the dual exitence of sprouting and intussusception in vascular biology.

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