

Role of pericytes in vascular immunosurveillance

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1. ABSTRACT

Pericytes build together with endothelial cells the microvascular vessel wall. They have been mainly implicated in angiogenesis and maintenance of the blood brain barrier, but there is accumulating evidence for an immunological function of pericytes. Occupying a strategic position between the blood stream and the interstitial space, pericytes are able to sense environmental cues from both sides in an organ-specific manner and serve as gatekeepers for innate immune cells. In addition, pericytes are able to interact with leukocytes mediated by adhesion molecules as well as chemokines and are involved from the development of leukocytes in the bone marrow to their migration to injured or infected areas in target tissues. During disease processes pericytes regulate blood flow, vascular permeability, and leukocyte recruitment, but also contribute to maladaptive tissue responses, such as fibrosis making pericytes an attractive therapeutic target.

2. INTRODUCTION

Although it is believed that myeloid leukocytes are first responders to infectious and sterile inflammatory processes, it is increasingly recognized that not only leukocytes participate in immune responses, but that there is also a significant contribution of stromal cells and extracellular matrix in shaping and perpetuating this process (1–3). The quantity and phenotype of innate immune cells within the blood and tissue is tightly regulated. Yet, the mechanisms regulating these

processes are largely unknown. Recent publications provide strong evidence supporting a critical importance of pericytes in regulating leukocyte function, positioning, and development.

Pericytes form together with endothelial cells the vessel wall of the microcirculation. They are embedded within the basement membrane and are wrapped around the endothelial layer from the abluminal side. This key position between the interstitial space and the vasculature makes them ideal candidates to integrate information from both sides. Therefore, pericytes are not only involved in physiological and pathological processes affecting the microcirculation, but they also influence compartments beyond the vasculature. Their natural interaction partners are endothelial cells, but this interplay is highly dynamic and organ-specific. So far, pericytes have mainly been implicated in supporting vessel formation during angiogenesis, where they stabilize the developing endothelial tube, which is a mandatory step to allow vessel maturation (4). In addition, pericytes are essential for development and maintenance of the blood brain barrier (BBB) (5–8) and influence cerebral blood flow both during physiologic as well as pathologic conditions (9). Pericytes have also been considered to have mesenchymal stem cell potential, because they are able to differentiate into muscle, bone, cartilage, and adipose tissue (10, 11). However, there is growing evidence that pericytes – occupying a strategic position between the blood stream and the

interstitial space – are actively involved in inflammatory processes (12, 13). We have recently shown that arteriolar NG2⁺ (neuron-glia antigen 2) pericytes regulate interstitial leukocyte migration during sterile inflammation in the skin *in vivo* (14). Pericytes are able to sense sterile inflammatory processes through pattern recognition receptors (PRRs) and react by secreting chemokines and by exposing adhesion molecules on their surfaces (14–18). This triggers direct interactions of extravasated myeloid leukocytes with arteriolar NG2⁺ pericytes, mediated by the adhesion molecule ICAM-1 (intercellular adhesion molecule 1) and the chemoattractant MIF (macrophage migration inhibitory factor). The physical contact with pericytes has a significant impact on the phenotype and function of these leukocytes, increasing their immunosurveillance as well as effector functions (14). This review summarizes the contribution of pericytes to the different steps of leukocyte recruitment as well as their role in disease processes.

3. THE TISSUE-SPECIFIC HETEROGENEITY OF PERICYTES

Pericytes are identified by their anatomic location around the microvascular endothelium and expression of markers, such as α -SMA (smooth muscle actin), NG2, and PDGFR β (platelet-derived growth factor receptor β). However, they are a heterogeneous and highly plastic population of cells with different properties depending on their localization along the vascular tree, organ, and disease processes (see Table 1) (5, 19). Thus, a marker combination in addition to anatomic and morphological information is needed to identify pericytes, but due to the high variability of marker expression, unambiguous identification is difficult.

Arteriolar pericytes are densely packed in a circular array around the endothelium and express NG2 as well as α -SMA. On capillaries, the pericytes coverage is much looser while marker expression is preserved, but their morphology is different: their elongated, spindle-shaped cell bodies are orientated parallel to the vessel and they form multiple extensions contacting endothelial cells. In postcapillary venules their conformation is denser again and they have a stellate-like morphology. In addition, they lose NG2 as marker, while they can still be identified by α -SMA (20). During microvascular remodeling, however, this classical marker expression pattern can change, and venular pericytes can express NG2 as well (21). Depending on their function, the density of pericytes is highly variable and highest in the brain, but relatively low in muscle tissue (22, 23). This is not surprising, since brain pericytes are a very specialized population of mural cells, due to the fact that they are a key component of the blood brain barrier (8).

In addition to differences along the vascular tree, there are organ-specific differences of pericytes which also involve their immunological properties. Recently, a new role of bone marrow pericytes has been described, showing that they regulate the quiescence and activation of HSCs (hematopoietic stem cells) (24, 25): using *ex vivo* whole-mount imaging it was demonstrated that quiescent HSCs are preferentially located around NG2⁺ arteriolar pericytes and that activation of HSCs resulted in their relocation close to perisinusoidal LEPR⁺ (leptin receptor) pericytes. In turn, depletion of NG2⁺ pericytes induced activation of HSCs. Hence, arteriolar pericytes form a niche within the bone marrow maintaining HSC quiescence. In contrast, the perisinusoidal compartment dominated by LEPR⁺ pericytes seems to constitute a proliferative niche. Of note, arteriolar NG2⁺ pericytes show high expression of CXCL12 and are the main source of this chemokine in the bone marrow, whose signaling through CXCR4 is responsible for the maintenance of HSCs (26). In line with that, by secreting CXCL12 NG2⁺ pericytes regulate the retention as well as maintenance of HSCs in the bone marrow, and targeted deletion CXCL12 in NG2⁺ cell results in a marked reduction of HSCs. Perisinusoidal LepR⁺ pericytes are a source of Scf (stem cell factor), which is also involved in HSC maintenance (27). However, it is unclear if pericytes also regulate systemic trafficking of mature myeloid leukocyte populations (see Figure 1). Notably, in the thymus neural-crest derived pericytes promote the egress of mature T cells through sphingosine-1-phosphate (28). Mobilization from and retention within the bone marrow are both critical processes allowing an adequate response to inflammatory stimuli depending on their severity while avoiding potentially harmful overwhelming immune reactions. For monocytes, egress from the bone marrow has been shown to be dependent on CCL2 derived from a poorly described bone marrow mesenchymal cell population in the setting of bacterial infection (29–31). In addition, CCL7 has been implicated in regulating the exit of monocytes from the bone marrow into the blood stream (32). However, it is not known to date whether there are active retention signals that keep monocytes in the BM. In contrast, it has been shown that neutrophils are retained by CXCL12-CXCR4 signaling and that they are mobilized in response to CXCR2 ligation by CXCL2 after G-CSF (granulocyte-colony stimulating factor) stimulation, yet the factors involved in egress during inflammation are also largely unknown (33).

The function of pericytes in immunosurveillance is best investigated in the brain, where pericytes form the backbone of the neurovascular unit. They are gatekeepers to the brain and tightly regulate blood flow, leukocyte infiltration, as well as the entry of circulating mediators and metabolites (34). In response to inflammation of the central nervous

Table 1. Markers for pericytes in different vascular beds and tissues

Marker	Location	Function	References
NG2 (CSPG4)	Arteriolar and capillary pericytes; expression is lost in postcapillary venules	Pericyte/endothelial cell interaction in tumor blood vessel formation; chondroitin sulfate proteoglycan	(102–104)
PDGFR-β	Especially useful as a marker for brain pericytes	Recruitment of pericytes during embryogenic blood vessel formation; knock-out leads to micro-aneurysm formation/rupture	(105–107)
αSMA	Expressed throughout arterioles, pre- and postcapillary segments and venules	Regulation of blood flow via contraction/relaxation	(105, 108)
RGS5	Marker for brain pericytes in mouse embryos amongst other pericyte populations, predominantly described in embryogenic development	Possible role of RGS5 in tumor and embryogenic angiogenesis	(109, 110)
LEPR	Bone marrow; perisinusoidal cells	Important source of Stem cell factor; LEPR ⁺ cell dominated compartment represents a proliferative niche	(26, 27)
Nestin	Bone marrow; bright NG2 ⁺ periarteriolar and dim LepR ⁺ perisinusoidal population	Periarteriolar subpopulation regulates HSC quiescence; sinusoidal subpopulation associated with HSC cycling	(24)
FOXD1 ⁺ - progeny	Described in lung and kidney; perivascular fibroblasts or pericytes, respectively	Major contributor to the myofibroblast pool in kidney fibrosis and pulmonary fibrosis	(41, 60, 69)
Gli1 ⁺ - progeny	Perivascular MSC-like cells in multiple organs	Major contributor to the myofibroblast pool in kidney, lung, liver, heart;	(66)
ADAM12 ⁺ - progeny	Described in adult skeletal muscle and skin; Mesenchymal perivascular cell	Reactivation of ADAM12 drives profibrotic fate and function of the ADAM12 ⁺ lineage; genetic ablation of ADAM12 ameliorates fibrosis	(61)

system, pericytes integrate this environmental change and increase blood flow to the affected region, allowing the increased delivery of leukocytes to the endothelium (35). Once leukocytes adhere to the endothelium and start to transmigrate, they encounter the dense network of pericytes, which usually limits further infiltration into the brain parenchyma. However, in the setting of inflammation, pericytes increase the permeability of the blood brain barrier not only regarding macromolecules, but also for immune cells (17, 36, 37).

Another organ where the function of pericytes has been studied extensively is the lung. Due to its primary task of gas exchange, the lung combines an extensive surface exposed to the environment with a dense vascular network (38). This is reflected by a high vessel coverage with pericytes, which occupy a position between the pulmonary epithelium and the blood stream. Due to the high abundance of lung pericytes and the relatively easy isolation, much of our knowledge regarding pericyte function in this organ is derived from *in vitro* experiments, whereas the data for an *in vivo* relevance of pulmonary pericytes is just emerging. In this organ, pericytes are specifically dedicated to detect inflammatory processes, because breaching of the epithelial lining indicates imminent threat to the host (39). Equipped with the machinery to detect DAMPs (damage associated molecular pattern) and PAMPs (pathogen associated molecular pattern), pericytes react to binding of LPS (lipopolysaccharide)

to TLR4 (toll-like receptor) with the secretion of IL-1β (interleukin 1β), IL-6, and TNFα (tumor necrosis factor α) (16, 40). In the setting of chronic inflammation, pericytes have additional functions beyond propagating the inflammatory response: due to their high phenotypic variability they can contribute to lung fibrosis by adapting myofibroblast properties (41, 42). In summary, different pericyte populations contribute to immunosurveillance in an organ-specific manner.

4. VENULAR PERICYTES SERVE AS GATEKEEPERS FOR LEUKOCYTE TRANSMIGRATION

After adhesion to the endothelium of postcapillary venules and passing the endothelial layer on their way into the interstitial space, leukocytes face the basement membrane with the embedded pericytes (12). By intravital confocal microscopy, regions of preferential leukocyte transmigration could be identified: these show a low coverage with the matrix proteins laminin-511 as well as type IV collagen and are therefore called low-expression regions (LER) (43, 44). Interestingly, these LERs are associated with gaps between venular pericytes and are highly dynamic in the setting of inflammation, where their size increases opening the gates for the influx of leukocytes into the tissue (43). This is not only due to neutrophils which diminish the laminin

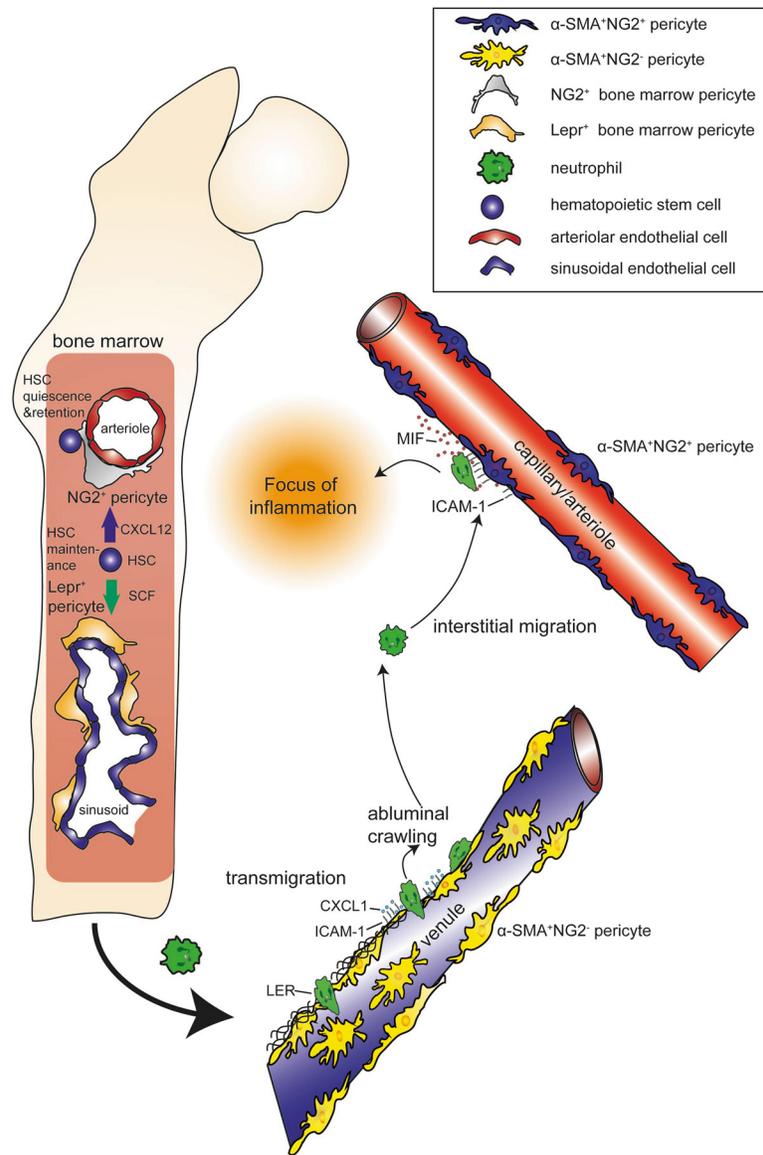


Figure 1. Pericytes subpopulations influence leukocyte development and trafficking. In the bone marrow arteriolar NG2⁺ pericyte maintain the quiescence and retention of hematopoietic stem cells (HSC) by secreting CXCL12. Perisinusoidal Lepr⁺ pericytes secrete stem cell factor (SCF) acting on HSC maintenance. In postcapillary venules NG2⁺ α -SMA⁺ pericytes promote neutrophil transmigration at LERs and abluminal crawling through ICAM-1 and CXCL1. In the interstitial space NG2⁺ α -SMA⁺ pericytes along capillaries and arterioles guide myeloid leukocytes to a focus of inflammation mediated by ICAM-1 and MIF. Top right: legend of depicted cells.

content of LERs, but also mediated by shape changes of adjacent pericytes by binding of TNF α and IL1 β to TNFRI/II (tumor necrosis factor receptor I/II) or IL1-RI (interleukin 1 receptor, type I), resulting in an increased size of LERs (18, 43). The predominant sources of TNF α responsible for the change in venular morphology in the setting of inflammation are neutrophils. They rapidly release TNF α in response to CXCL1 (KC), LTB₄ (leukotriene B4), or C5a (complement component 5a) (45). Once leukocytes have passed this structural border, they engage in intensive contacts with α -SMA⁺NG2⁺ pericytes:

myeloid leukocytes crawl along pericyte processes within the venous vessel wall. This interplay is mediated by the interaction of ICAM-1 with LFA-1 (lymphocyte function-associated antigen 1)/Mac-1 (Macrophage-1 antigen) as well as the chemokine CXCL1, which induces neutrophil migration and activation by binding to CXCR2 (46, 47). Both factors – ICAM-1 and CXCL1 – are preferentially located at the cell body of pericytes (18) (see Figure 1). In addition, in the setting of staphylococcus aureus neutrophils face another cell type after passing the pericyte layer: perivascular macrophages secrete high

amount of chemokines, and neutrophils preferentially transmigrate adjacent to this macrophage population (48). However, potential interactions between pericytes and perivascular macrophages have not been investigated, and the differential contribution for neutrophils transmigration of these cell populations might depend on the disease setting and tissue.

Within the interstitial space, innate immune cells migrate to the focus of inflammation to clear necrotic areas or to limit the dissemination of pathogens. However, neutrophils can also perform reverse transmigration through LTB₄ and neutrophil elastase mediated loss of endothelial JAM-C (junctional adhesion molecule C), and reenter the systemic circulation to systemically disseminate inflammation (49, 50). However, the contribution of pericytes to the process of reverse transmigration is unclear. In addition, it is not known if pericytes are also involved in regulating the migration of other cell types across the vessel wall or the dissemination and organ infiltration of pathogens. In addition to regulating the extravasation of immune cells, pericytes are also involved in the regulation of vascular permeability during systemic inflammation. In sepsis, maladaptive effects in the microcirculation influence the systemic host reaction as well as clinical outcomes. In this circulatory network, vascular stability is regulated by the ANG1 (Angiopoietin 1)-TIE2 system, which is destabilized during systemic inflammation by the antagonistic effect of ANG2 resulting in systemic hypotension and pericyte loss (51, 52). Targeting the ANG2 (Angiopoietin 2)-TIE2 interaction in sepsis reduced systemic hemodynamic changes as well as mortality in mice (53). In conclusion, venular pericytes have gatekeeper functions, actively regulating the transmigration of neutrophils and vascular permeability during inflammation.

5. NG2⁺ PERICYTES SHAPE THE INTERSTITIAL IMMUNE RESPONSE

While these studies underscore the contribution of pericytes to leukocyte recruitment from the circulation and in microvascular permeability, the importance of pericytes for leukocyte trafficking does not end with transmigration: arteriolar NG2⁺ pericytes support effective interstitial leukocyte migration by increasing their immunosurveillance and effector function (14). Within the interstitial space, leukocytes are exposed to numerous new mediators, but eventually they have to navigate to the focus of inflammation to fight infections or to clear dead cells (13). In lymph nodes, a scaffold of fibroblastic reticular cells guides interstitial migration of adaptive immune cells (54, 55).

Pericytes are able to sense inflammatory processes through pattern recognition receptors

(PRRs); stimulation of these receptors results in secretion of chemokines (MIF, CCL2, CXCL8) and exposure of the adhesion molecule ICAM-1 on the pericyte surface (14, 16, 18). Using intravital 2-photon microscopy in the skin it could be shown that extravasated neutrophils and monocytes are attracted by arteriolar NG2⁺ pericytes, resulting in direct interactions mediated by MIF. These interactions markedly influence the phenotype of myeloid leukocytes *in vitro* as well as *in vivo*. Specifically, after contacting pericytes myeloid leukocytes show increased expression of integrins, matrix metalloproteinases (MMPs), and PRRs *in vitro*. This translates into an altered migration phenotype *in vivo* – the interaction with pericytes leads to an increased velocity and more directed migration. In turn, this allows neutrophils and monocytes to effectively navigate to a focus of sterile inflammation as well as to cover larger areas searching for an infection or a necrosis in the absence of a clear focus, i.e. diffuse inflammation without a gradient guiding leukocytes in a specific direction (see Figure 1). In summary, this work could identify a new regulatory role of arteriolar NG2⁺ pericytes for interstitial leukocyte migration in non-lymphoid organs (14). This implicates that pericytes – which are not considered to be immune cells – tightly regulate inflammatory processes and positioning of myeloid leukocytes within the interstitial space. However, it is unclear whether pericytes only control myeloid leukocyte trafficking locally within inflamed tissue or rather also tune the supply of new myeloid leukocytes systemically.

6. THE ROLE OF PERICYTES IN DISEASE MODELS

The ubiquitous presence and the involvement of pericytes in multiple microvascular functions including microvascular homeostasis, angiogenesis, inflammation, and maintenance of blood-tissue barriers is in line with the accumulating evidence of their participation in various diseases. Their tissue resident location predisposes them to take over a previously underestimated role in acute as well as chronic conditions to which they contribute in an organ specific manner.

6.1. Pericytes contribute to tissue fibrosis.

Pericytes can influence the outcome of numerous inflammatory diseases, including the maladaptive endpoint of fibrosis. This process is characterized by the deposition of extracellular matrix and a permanent scarring of tissue, resulting in an impairment of normal organ function. Fibrosis is orchestrated predominantly by myofibroblasts as key cellular mediators (56–58). In this context pericytes have recently been implicated as an important source

of myofibroblast formation in multiple disease models and organs, including the kidney (59, 60), lung (41, 42), skin, muscle (61), spinal cord (62), and the liver (63, 64). However, the origin of myofibroblasts in fibrosis is still under debate. Newer fate mapping studies (65, 66) are in contrast to earlier ones, which had proposed that most myofibroblasts are derived from endothelial cells (67, 68). The TIE2 receptor used in these studies is not restricted to endothelial cells and is expressed also by the myeloid lineage, FOXD1 (Forkhead box D1) lineage and other cell types (69).

Numerous fibrosis studies have been performed on the murine kidney, where the FOXD1 lineage has been identified as an important contributor to fibrosis. FOXD1 is a forkhead transcription factor, activated in some progenitors of the MSC (mesenchymal stem cell) lineage during embryonic development (41). Fate tracing studies in the kidney marked the progeny of FOXD1 positive cells in an inducible Cre recombinase dependent manner (60). The interstitial cells derived from the FOXD1 lineage were positive for PDGFR β , and this marker was restricted to cells that had expressed FOXD1 developmentally. A unilateral urethral obstruction (UJO) model and an ischemia-reperfusion injury (IRI) model both suggested that the predominant cell type responsible for accumulation of interstitial myofibroblasts are pericytes or perivascular fibroblasts, respectively. Endothelial cells did not seem to pertinently contribute to the myofibroblast pool (60). These findings have been questioned by a fate mapping study to unravel the main origins of myofibroblasts in the kidney, employing multiple transgenic mouse strains (70). With the same UJO disease model, LeBleu *et al.* yielded opposite results to Humphreys *et al.*, stating that most of the myofibroblasts are derived either from resident tissue fibroblasts or from bone marrow-derived mesenchymal stem cells. Accordingly, NG2⁺ and PDGFR β ⁺ cells did not seem to contribute in a notable manner to either fibrosis or to myofibroblast counts (60, 70). A NG2 and a PDGFR β promoter driven model was used instead of FOXD1 to investigate the pericyte contribution, which could explain the discrepant results. Leaf *et al.* found that Myd88 (myeloid differentiation primary response gene 88) and IRAK4 (interleukin-1 receptor-associated kinase 4), critical factors in the intracellular signaling of multiple TLRs, control inflammatory and fibrotic tissue responses in pericytes during kidney injury (71). Pericytes obtained from biopsies of patients with acute kidney injury showed activation of multiple pathways involved in innate immune responses, as was determined by gene ontology analysis. Fibrotic murine kidneys from an IRI model were analyzed by translating ribosome affinity purification, which likewise unraveled several innate immune pathways, including TLR and Nod-like Receptor (NLR) signaling. Ablation of Myd88 signaling by pharmacological

IRAK4 inhibition protected against kidney injury and fibrosis, respectively, by attenuating the appearance of myofibroblasts, reducing the transcription of several cytokines (including IL6, IL1 β and IL18), as well as reducing neutrophil recruitment.

The involvement of lung pericytes in pulmonary fibrosis is similarly contradictory. One of the studies, which has been performed using a bleomycin lung injury model, showed that up to 68% of α -SMA⁺ cells in fibrosis are pericyte derived (41). In contrast, Rock *et al.* could not determine a large population of pericytes contributing to myofibroblasts, although they described an increased expression of pericyte markers (PDGFR- β , NG2 and desmin) in fibrotic areas (42). This inconsistency might have been due to an inefficient recombination of the reporter by the Cre recombinase under the NG2 promoter, thereby underestimating the pericyte contribution to myofibroblasts (39). One of the clinically promising approaches targeting pericytes is the PDGFR signaling pathway, which has been described to be important for MSC amplification and pathological matrix accumulation (38). Nintedanib is a multiple tyrosine kinase inhibitor which targets the intracellular signaling of PDGFR α/β , VEGFR2 (vascular endothelial growth factor receptor 2), and the FGFR (fibroblast growth factor receptor) and is given to patients with idiopathic pulmonary fibrosis (72–75). The synopsis of the above mentioned studies and the fact that PDGFR- β is particularly common on pericytes highlights the importance of pericytes as a target of nintedanib. Two replicate randomized controlled phase 3 trials involving 1066 patients have shown a significant slowdown of disease progress, measured by annual decrease in FVC after administering 150 mg of nintedanib twice daily for 52 weeks (-114.7. ml with nintedanib versus -239.9. ml with placebo in INPULSIS-1 and -113.6. ml with nintedanib versus -207.3. ml with placebo in INPULSIS-2; P<0.0.01) (74). Henderson *et al.* set up a comprehensive study including multiple organs and using the widely accepted pericyte promoter PDGFR- β for Cre-mediated GFP expression (65). In a CCl₄-induced liver fibrosis model, all of the α -SMA⁺ coexpressed the reporter. Deleting the α v integrin subunit under the same promoter markedly attenuated fibrosis in the liver, lung and kidney. CWHM 12, a small molecule inhibitor of α v integrins, protected mice from liver and lung fibrosis. In summary, the role of pericytes in fibrosis is still highly controversial and organ dependent.

6.2. Multiple roles of pericytes in brain injury

Their main microvascular functions by which pericytes may contribute to brain injury include the control of capillary perfusion, angiogenesis, maintenance of the BBB, release of neuroprotective agents, scar formation, and debris clearance (76, 77). Bearing in mind the versatile capabilities of cerebral pericytes,

their contribution to brain injury is accordingly diverse and can be beneficial or detrimental. Representing an acute inflammatory setting, brain ischemia/reperfusion injury (IRI) or stroke are conditions in which pericytes have been extensively studied.

The endothelium-pericyte interplay is a central factor for post-ischemia neuroprotection. PDGF-B seems to be an important neuroprotective agent inducing cell growth and anti-apoptotic responses in pericytes and the expression of nerve growth factor (NGF) and neurotrophin-3 (NT-3) *in vitro*. Correspondingly, PDGFR β is upregulated in pericytes and PDGF-B expression induced in endothelial cells in peri-infarct areas (78). Vice versa, endothelial cell survival is promoted by brain pericytes through VEGF-A signaling as well (79). Moreover, PDGF-B and VEGF, together with transforming growth factor- β , angiopoietin and other receptor-ligand systems, are candidates to facilitate pericyte driven formation of new blood vessels (76, 80–82). Notably, prophylactic VEGF-A administration promotes pericytes coverage and improves blood flow in subsequently induced ischemia (28). However, it simultaneously increases capillary leakage by disturbing the BBB (83). VEGFR-2, the VEGF-B receptor, has a much higher expression level in pericytes than VEGFR-1, the VEGF-A receptor. Administration of VEGF-B after ischemic stroke induced neurovascular repair without triggering microvascular instability, making VEGF-B a more promising target for potential clinical use (84). In addition, stimulation of PDGFR- β has neuroprotective effects: conditional PDGFR- β knock-outs show increased infarct volumes in mice after middle cerebral artery occlusion (85). Besides regulating the BBB, pericytes are also capable of phagocytosis (86). Scavenging this cell detritus may mitigate the overall inflammatory response and thereby tissue damage (77).

Concerning the detrimental functions of pericytes, it has been shown that they influence microvascular blood flow after stroke (9, 77, 87). Recent studies show elegantly that pericytes constrict in an ischemic microenvironment and die in a constricted state, making the development of small molecule inhibitors of pericyte death a particularly appealing concept for acute stroke treatment (9). However, a subsequent, microvascular entrapment of erythrocytes impedes the microvascular blood flow, even after revascularization (87). Controversially, Hill *et al.* recently provided evidence that questions the role of pericytes in impeding microvascular flow and indicates arteriolar smooth muscle cells to mainly regulate regional blood flow (88).

6.3. Pericytes in myocardial infarction

Pericytes have lately been recognized as a potential clinical target in this setting. The fact that

pericytes have high phenotypic and functional plasticity and occupy a central role in angiogenesis predisposes them to an involvement in the post-infarct healing processes. The Notch3 receptor plays a central role in pericyte recruitment and vascular maturation in the murine heart. Hence, a Notch3 mutation indeed leads to a decrease in pericyte counts, microvascular rarefaction and, presumably thereby, to a worse post-MI recovery of the heart. Similar to other organs, the Notch3 signaling pathway seems to be entangled in a pathway with ANG1 and VEGF; Notch3 knock-out mice showed a reduction of these latter signaling pathways (89). Microvascular damage after MI is promoted by cardiomyocyte derived proNGF, targeting p75NTR (neurotrophin receptor p75) in pericytes. p75NTR deficiency limited infarct size after myocardial ischemia-reperfusion injury, providing momentum for clinical proNGF or p75NTR targeting (90). Still elusive but likely is the contribution of cardiac pericytes to the failure of coronary reflow after MI treatment, complementary to the mechanisms Hall *et al.* have delineated in stroke (9, 91).

As described earlier, pericytes can differentiate to cells of the MSC lineage, including chondrocytes, adipocytes, osteocytes, smooth muscle cells, and can also regenerate skeletal muscle (11, 92–97). Human saphenous vein-derived pericyte progenitor cells (SVPs) transplanted into the peri-infarct zone of immunodeficient and immunocompetent mice showed improved ejection fraction, less left ventricular dilatation, scar formation, interstitial fibrosis and cardiomyocyte apoptosis, improved myocardial blood flow, neovascularisation and simultaneously mitigated vascular leakage. These findings can be broken down to the potential support of a beneficial microenvironment by human SVPs. A paracrine, pericyte derived VEGF-B, ANG1, and chemokine signaling was found to evoke angiogenesis in the host. Moreover, miR-132 secretion by SVPs stimulated endothelial tube formation and myofibroblast formation *in vitro* (98). Vice versa, miR-132 (microRNA-132) inhibition *in vivo* alleviated the positive impact of SVPs to post-myocardial infarction cardiac recovery. Chen *et al.* confirmed these findings and could add that only minor direct cell-cell interactions are responsible for the beneficial outcome of human pericyte transplantation. A small fraction of the pericytes differentiated into endothelial cells, smooth muscle cells and cardiomyocytes (99). Hence, minding the beneficial effects but still poor capabilities of human pericytes to form new cardiomyocytes, Avolio *et al.* used complementary cell types (saphenous vein-derived pericytes and cardiac stem cells) for a post MI-treatment trial and found a favorable multiplier effect in this combined approach in mice (100, 101). In contrast to these potential beneficial effects, Kramann *et al.* found a small population of perivascular Gli1⁺

progenitors expressing PDGFR α , Nestin, and 3G5. Ablation of Gli1⁺ cells ameliorated cardiac fibrosis and rescued left ventricular function in the setting of a heart failure model (66). However, a more detailed understanding of the function of cardiac pericytes is necessary to uncover beneficial or detrimental effects under physiologic and pathologic conditions.

7. CONCLUSIONS

An understanding of the inflammatory functions of pericytes is just beginning to emerge: this cell population might be a novel therapeutic target in various diseases ranging from neuroinflammation and sepsis to tissue fibrosis. In addition, they regulate leukocyte trafficking and development, beginning in the bone marrow and thymus until their transmigration in postcapillary venules as well as effector function in the interstitial space. A critical issue is the unambiguous identification of pericytes, which is complicated by their high phenotypic and functional variability as well as organ-specificity. In addition, more *in vivo* studies are needed to establish a detailed understanding of pericyte-leukocyte interactions in disease models. In conclusion, pericytes are critical components of the immune response, which is why specifically targeting this cell population might be a promising approach in various diseases.

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