MINI-SYMPOSIUM: Pericytes, the Neurovascular Unit and Neurodegeneration

The Fibrotic Scar in Neurological Disorders

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Abstract

Tissue fibrosis, or scar formation, is a common response to damage in most organs of the body. The central nervous system (CNS) is special in that fibrogenic cells are restricted to vascular and meningeal niches. However, disruption of the blood–brain barrier and inflammation can unleash stromal cells and trigger scar formation. Astroglia segregate from the inflammatory lesion core, and the so-called "glial scar" composed of hypertrophic astrocytes seals off the intact neural tissue from damage. In the lesion core, a second type of "fibrotic scar" develops, which is sensitive to inflammatory mediators. Genetic fate mapping studies suggest that pericytes and perivascular fibroblasts are activated, but other precursor cells may also be involved in generating a transient fibrous extracellular matrix in the CNS. The stromal cells sense inflammation and attract immune cells, which in turn drive myofibroblast transdifferentiation. We believe that the fibrotic scar represents a major barrier to CNS regeneration. Targeting of fibrosis may therefore prove to be a valuable therapeutic strategy for neurological disorders such as stroke, spinal cord injury and multiple sclerosis.

INTRODUCTION

A scar can be defined as a mark left (as on the skin) by the healing of damaged tissue. Scars are the consequence of failed tissue regeneration and represent a repair mechanism that replaces the normal tissue with extracellular matrix (ECM) consisting predominantly of fibronectin and type I collagen (24). The key effector cells in tissue remodeling and fibrosis are myofibroblasts that express α-smooth muscle actin (SMA) and produce pathological ECM proteins (19). Recent genetic fate mapping studies revealed that resident fibroblasts and pericytes as well as circulating fibrocytes are the predominant sources of myofibroblasts in peripheral organs (32, 47, 74). In a mouse model of kidney fibrosis, 50% of myofibroblasts arise from local resident fibroblasts through proliferation, and the remaining nonproliferating myofibroblasts derive through differentiation from bone marrow (35%), endothelial-to-mesenchymal transition (10%) or epithelial-to-mesenchymal transition (5%) (46).

The central nervous system (CNS) is different in that resident astroglia have been regarded as the key effector cells in forming a dense scar in response to injury. The astroglial scar localizes at the lesion penumbra (Figure 1), confines inflammation to the lesion core and protects the intact neural tissue (22, 57). At the same time, the glial scar serves as a major barrier to regenerating axons by secreting growth-inhibitory chondroitin sulfate proteoglycans (51). Interestingly, the glial scar is compartmentalized with resident astrocytes forming the periphery of the scar, and ependymal neural stem cell-derived astrocytes forming the central part that provides an important source of neurotrophic factors (72). In addition, glial cells expressing chondroitin sulfate proteoglycan 4 (NG2) migrate toward the lesion and stabilize the regenerating front of dystrophic axons in the inhibitory environment of the glial scar (10).

In the lesion core, inflammatory cells interact with fibroblasts that form a dense fibrotic scar (Figure 1) composed of fibronectin, collagen and laminin (75). The fibroblasts segregate from astrocytes via bidirectional signaling between ephrin-B2 on reactive astrocytes and EphB2 on meningeal fibroblasts (9). The fibrotic response is perhaps more detrimental in the CNS compared to other tissues because the brain's ECM normally contains relatively small amounts of fibrous proteins and adhesive glycoproteins (45). Instead, the neural interstitial matrix comprises a network of proteoglycans, hyaluronan, tenascins and link proteins. In addition, dense ECM aggregates form so-called "perineuronal nets" during development and periods of synaptic maturation that are important regulators of CNS plasticity (44). Rather than purely providing mechanical support to the tissue, the ECM of the highly specialized CNS tissue serves as a substrate for the compartmentalization of the extracellular space, concentrating soluble and membrane-bound molecules at synaptic sites, and functioning as a scaffold during development or adult neurogenesis (16). In this regard, a stiff fibrous ECM might impede the anatomical plasticity of the neural network that is required for learning and memory throughout life (102). Indeed, neural cells sense the stiffness of the microenvironment and adjust their differentiation accordingly, for example, "softer" microenvironments promote neuronal differentiation (36). There are more indications to suggest that the CNS parenchyma might represent a "fibro-privileged" site. For instance, overexpression of transforming growth factor (TGF)-β1 by astrocytes does not lead to parenchymal deposition of ECM molecules such as collagens, laminin or
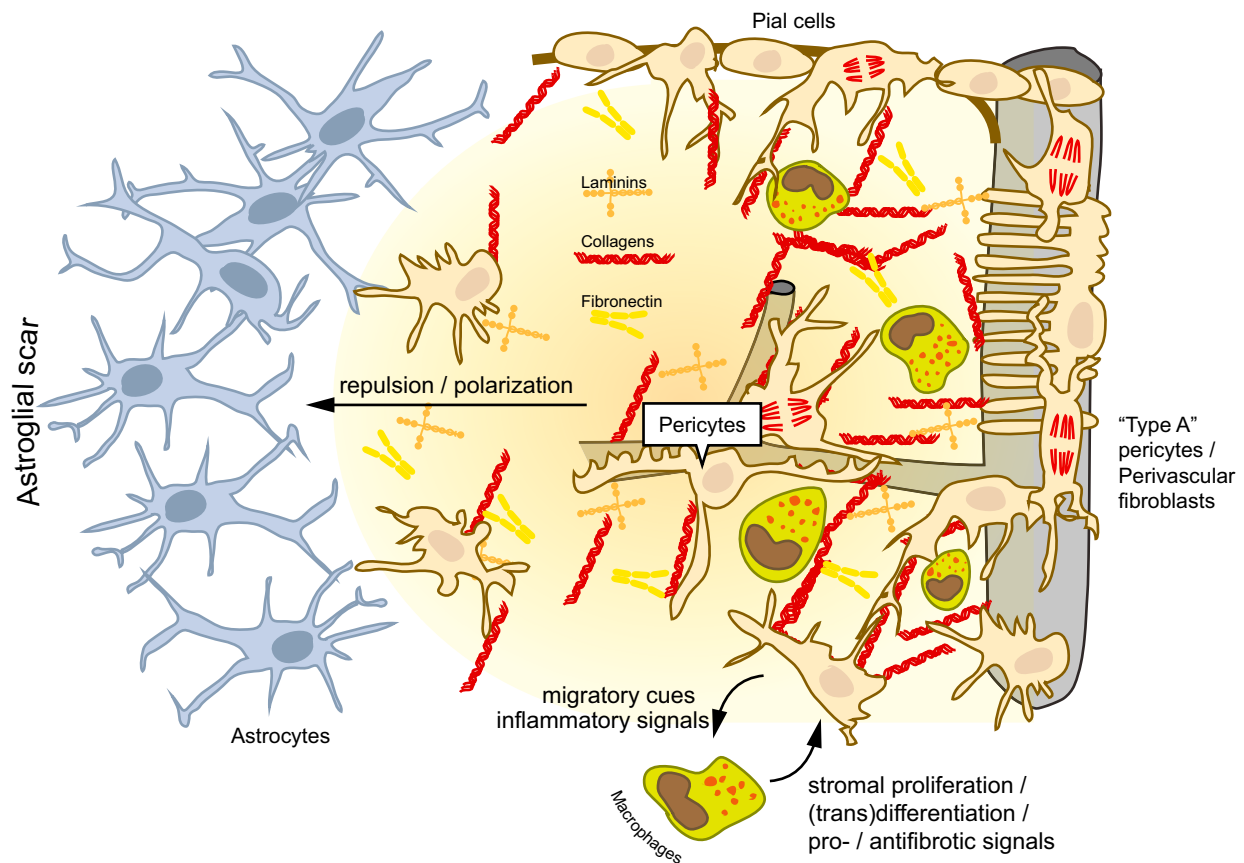
Figure 1. Cellular and molecular interactions in central nervous system (CNS) scar formation. The fibrotic scar is characterized by the deposition of extracellular matrix molecules that are otherwise scarcely expressed in the neural parenchyma such as collagens, laminins and fibronectin. These molecules are generated by stromal cells (myofibroblasts), which are normally absent from the CNS parenchyma. The stromal cells may originate from meningeal precursors (eg, pial cells of the leptomeningeal lining), perivascular fibroblasts or pericytes ("type A pericytes"). Blood-borne macrophages and microglia contribute to the proliferation and differentiation of stromal cells by producing profibrotic mediators, and they are also involved in the resolution of the fibrous scar. Conversely, stromal cells modulate neuroinflammation by producing cytokines, chemokines and adhesion molecules. The astroglial scar is neatly separated from the fibrotic scar. Cellular and molecular constituents of the fibrotic scar induce the repulsion and polarization of astrocytes, and a new glia limitans is generated at the interphase of both cell populations.

fibronectin, but the meninges and vascular vessel walls are thickened (100). In contrast, overexpression of TGF-β1 or platelet-derived growth factor (PDGF)-B by hepatic cells causes increased deposition of fibrillar ECM molecules around hepatocytes as well as fibrosis in extrahepatic organs (13, 73). These results suggest that fibroblasts are absent from the normal neural parenchyma and are restricted to vascular and meningeal niches. Importantly, the basement membranes lining the cerebral microvasculature are composed of collagen IV, laminin, fibronectin and heparan sulfate proteoglycans. Three recent studies have underscored the importance of perivascular fibroblasts and pericytes, the mural cells that are embedded within the vascular basement membrane, in generating the fibrotic scar in the CNS (25, 27, 85).

Pericytes

First identified by Eberth and Rouget in the 19th century, pericytes are today considered to be a heterogenous population of mural cells. Because of the lack of specific markers and their plasticity in response to pathological conditions, the identification and fate mapping of pericytes have been challenging. We like to refer the reader to two excellent reviews on this topic (3, 42). Pericytes populate all segments of the brain vasculature with a continuum of phenotypes (Figure 2). Notably, the CNS has the highest density of pericytes in the body with a 3-1:1 ratio between pericytes and endothelial cells (81). Pericytes in precapillary arterioles and capillaries have a round protruding cell body (~10 to 15 μm) and extend longitudinal processes that trace the capillary endothelium. The longitudinal processes are not randomly placed but follow the endothelial cleft containing tight junctions, which underscores the important role of pericytes in the regulation of endothelial permeability (4, 76). In larger vessels, the vascular basement membrane and the basement membrane of the astrocytic glia limitans delimitate the perivascular (Virchow–Robin) space. These basement membranes are fused into a single structure in capillaries and fully enclose the pericyte (42). Besides pericytes, vascular smooth
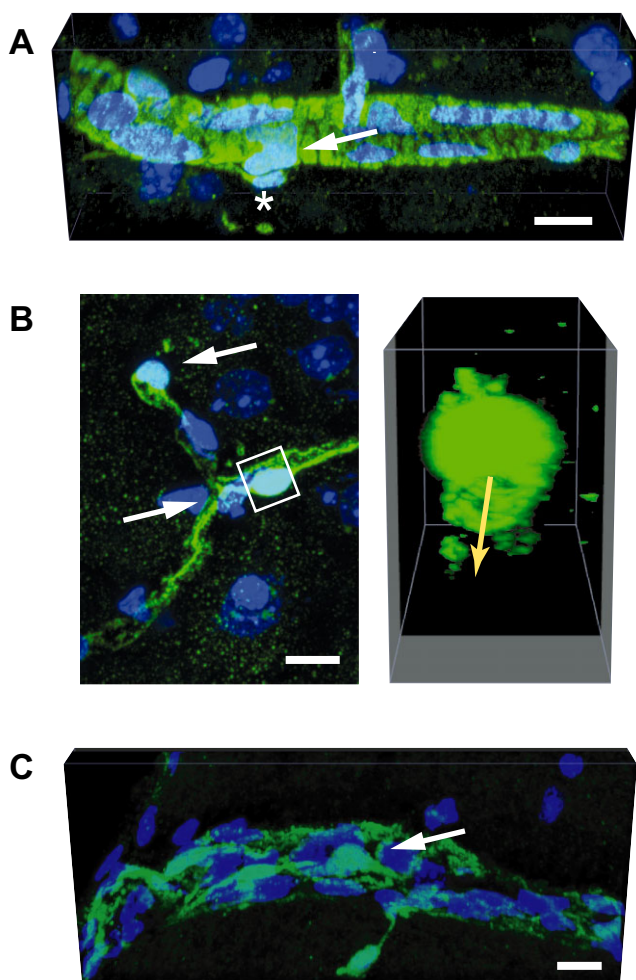
Figure 2. Mural cells of the central nervous system (CNS) vasculature. This figure demonstrates the different phenotypes of mural cells along different segments of the CNS vasculature. The mural cells can be identified based on their expression of the green fluorescent protein (GFP) in a mouse line where the pericyte-specific *rgs5* gene has been replaced with a GFP reporter (56). The images represent maximal projections or three-dimensional (3D) renderings of confocal stacks obtained from GFP-expressing cells in the brains of *rgs5*GFP/GFP mutant mice. Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Scale bars: 10 μm. **A.** In a precapillary arteriole, mural cells (arrow) exhibit robust and densely packed circumferential processes, suggesting a contractile phenotype. An abluminal GFP-expressing cell is marked by an asterisk. **B.** Pericytes investing capillaries (white arrows in the left panel) show a typical protruding fusiform cell body with long primary processes running in the length of the capillary. The primary processes give rise to perpendicular secondary processes. The 3D reconstruction (right panel) of the volume marked by the white box demonstrates circumferential processes underneath the pericyte cell body that surround the capillary lumen (indicated by the yellow arrow). **C.** Mural cells (arrow) in a postcapillary venule show a flattened, stellate morphology and loosely cover the length of the vessel.

muscle cells (vSMCs) are part of the pre- and postcapillary vascular wall. Although there is no single marker to distinguish vSMCs from pericytes, there are important functional differences among vSMCs in arteries, the capillary pericytes, and pericytes/SMCs in venules. Obviously, different segments of the vasculature exert different functions, and the mural cells adapt to these particularities. For instance, venular pericytes lack expression of NG2 (86), a proteoglycan involved in regulating endothelial permeability and adhesion of pericytes to endothelial cells (104). Currently, antibodies against platelet-derived growth factor receptor (PDGFR)-β, alanyl aminopeptidase (CD13), NG2, desmin and α-SMA are used to stain pericytes in histological CNS samples (3). However, none of these markers are specific for pericytes, and the issue is complicated after CNS lesions when marker expression is dynamically regulated or shifts to other cell populations (eg, PDGFR-β in fibroblasts, CD13 in macrophages, NG2 in glia and macrophages). Notably, ectoderm-derived neural crest normally gives rise to pericytes and vSMCs in the CNS and thymus (21, 55), whereas mural cells in peripheral organs originate from mesoderm-derived mesothelium (5, 71, 98). After cerebral ischemia, bone marrow-derived cells have been reported to generate desmin-positive pericytes in the brain by cell fusion (38, 66), but these findings remain controversial (25).

Other perivascular cells

In addition to these established cell types, other stromal cell populations have been identified in the perivascular spaces of the CNS. Using a mouse genetically engineered to express Cre recombinase under the control of the *Glast* promoter and originally generated to target astrocytes (84), Göritz *et al* discovered a perivascular cell type in the spinal cord that resides abluminal to other mural cells and gives rise to scar-forming fibrotic cells (27). They termed these cells "type A pericytes" in order to distinguish them from the more abundant desmin-expressing "type B pericytes" in capillaries that were not targeted in their animal model. Recently, another transgenic mouse line that expresses the green fluorescent protein (GFP) under the control of the *collagen 1α1* promoter (103) was used to reveal a perivascular cell population that is associated with larger CNS blood vessels and also participates in the fibrotic response to spinal cord injury (85). It is unclear to what extent this population overlaps with "type A pericytes." The perivascular cells described in these two publications share some markers of pericytes (PDGFR-β, CD13) but lack others such as NG2, desmin or α-SMA (27, 85). Pericytes in many organs, including the brain, have also been identified as multipotent mesenchymal stromal cells (MSCs) that can differentiate into various tissues such as muscle, bone, cartilage and adipose tissue (12). Moreover, MSCs isolated from the adult human brain express pericyte markers such as PDGFR-β, CD13, NG2 or RGS5 (62). Because the identification of MSCs relies on the *in vitro* assessment of their differentiation potential, it is not clear whether all pericytes or just a subset are multipotent. Hence, the mural and perivascular cell pool in the brain comprises heterogeneous cell populations, both in terms of marker expression and function, for which the broad term "pericyte" may be an oversimplification. Future work using genetic and imaging approaches to label and fate map distinct pericyte and perivascular cell populations will shed light on the diversity.

The fibrotic scar in the CNS

Scarring occurs after injury to any tissue in the body when regeneration fails. The evolutionary forces have likely shaped wound-healing mechanisms to prevent infection, wall off foreign bodies and rapidly restitute missing tissue at the expense of optimal functioning (24). This can be exemplified in a number of neuropathological conditions where ectopic fibrous or laminar ECM is deposited.

Infection and parasite infestations

Bacteria, parasites and fungal infections of the CNS induce a robust and conserved fibrotic response that encapsulates affected tissue and may function to limit the spread of the pathogen. Bacterial abscesses, caused mainly by streptococcal strains and *Staphylococcus aureus*, are associated with rapid fibrotic wall formation within days after their seed, organized as a capsule containing myofibroblasts that secrete collagen I and fibronectin. These cells originate from bone marrow-derived fibrocyte-like cells and local precursors (2). Interestingly, the size of the capsule is reduced over time, which suggests tissue remodeling by myofibroblasts. A similar fibrotic wall formation occurs during infestation with parasites. For instance, larval stages of the *Taenia solium* tapeworm are rapidly encapsulated, generate little inflammatory reaction in the CNS and may therefore remain asymptomatic for years (26).

Spinal cord injury

Following the trauma, a large population of fibroblasts migrates to the lesion core, and forms a fibrotic scar with accessory glia limitans (78). It has long been assumed that fibroblasts would only enter the neural parenchyma after penetrating CNS injuries with disruption of the meningeal lining. However, this view has been challenged recently with the identification of collagen 1α1-expressing PDGFR-β$^+$ perivascular fibroblasts that are distinct from pericytes and fibrocytes (negative for NG2 and CD11b), and give rise to the fibrotic scar after contusive, non-penetrating spinal cord injury (85). Moreover, "type A pericytes" were identified in the CNS vasculature that proliferate locally, transform into myofibroblasts, and generate the fibrotic scar in response to dorsal funiculus incision or dorsal hemisection in mice (27). Notably, blocking the generation of progeny by this cell type results in failure to seal the injured CNS tissue, underscoring the importance of scar formation in rapidly replacing missing tissue.

Stroke

Recent findings from our laboratory suggest that PDGFR-β$^+$ stromal cells infiltrate the ischemic brain, and form a fibrotic scar surrounded by reactive astrocytes (Figures 3 and 4). Importantly, no fibrosis is detected in Alzheimer's disease brains despite substantial neurodegeneration and vascular pathology (Figure 4). Pericytes are rapidly lost after cerebral ischemia in both experimental and human stroke (25). Coincident with the pericyte loss is a massive proliferation of resident RGS5-expressing PDGFR-β$^+$ CD13$^+$ stromal cells that originate from the neurovascular unit, transform into α-SMA$^+$ CD105$^+$ myofibroblasts and deposit fibrous ECM in the ischemic brain. No contribution of bone marrow-derived cells is detected (25). In analogy to the constricting fibrotic capsules of brain abscesses or the healing of spinal cord lesions, the stroke-induced fibrotic scar shrinks over time, resulting in the generation of cysts and the enlargement of the subarachnoid space.

Scar-free healing in the developing CNS

In contrast to the fibrotic scarring described previously, embryonic tissue is able to regenerate without scar formation (24). Only few adult vertebrates like primitive amphibians have retained this capacity, and even in these animals some tissues heal with a scar. It is not clear which factors determine the conversion to the scarring phenotype, but embryonic skin wounds that heal without a scar have very low levels of TGFβ1 and TGFβ2, whereas adult wounds contain predominantly TGFβ1 and PDGF, which are released by platelets and inflammatory cells (70, 97). In the rodent CNS, the change to mature scarring occurs around postnatal days 8–12 and is characterized by the deposition of collagen I–IV, astrocyte end-feet alignment over a glia limitans, and the permanent presence of fibroblasts and macrophages in the core of the lesion (7, 50). Interestingly, fibrous scarring can be inhibited in the adult CNS by the transplantation of fetal tissue (43). The scarless bridging of traumatic tissue boundaries in the developing CNS is reminiscent of organisms like fish that do not develop any scarring in the CNS into adulthood (15).

Stromal cell/astrocyte interactions

When a fibrotic scar is generated in the CNS, a sharp boundary between the stromal tissue and the astroglial scar develops. Two compartments are generated: a fibrotic/inflammatory lesion core devoid of astrocytes, and an astroglial scar at the lesion penumbra (23, 35, 37, 83). This compartmentalization can also be reproduced *in vitro* (79). The astrocytes in the glial scar, particularly those bordering the fibrous scar, display a marked upregulation of the glial fibrillary acidic protein (GFAP) and form a mesh of highly overlapping hypertrophic cellular processes (95). Most of the astrocytes in the glial scar appear to proliferate locally and first adopt a radial or bipolar morphology oriented perpendicularly toward lesion (95). Selective deletion of STAT3 from astrocytes leads to fuzzy lesion borders, enhanced infiltration of inflammatory cells and increased neuronal loss (95). Astroglial scarring thus limits the spread of inflammation and marks the boundary of savaged neural tissue. The astroglial response appears to reproduce the development of a polarized neuroepithelial lining which culminates in the generation of the glia limitans. Notably, a glia limitans forms wherever astrocytes and meningeal cells come into contact (1). Along these lines, coculture of astrocytes with either inflammatory or fibromeningeal cells induces corralling of the meningeal cells into segregated clusters in a STAT3-dependent manner (95). It is not clear how stromal and inflammatory cells contribute to the segregation of the fibrotic and glial scars *in vivo*. Interestingly, loss of the dystroglycan molecule on astrocyte foot processes at the glia limitans disturbs the binding of laminin and leads to basal lamina abnormalities as well as aberrant neuronal migration (53). Thus, ECM proteins in the fibrotic scar might contribute to restricting the expansion of neural cells into the
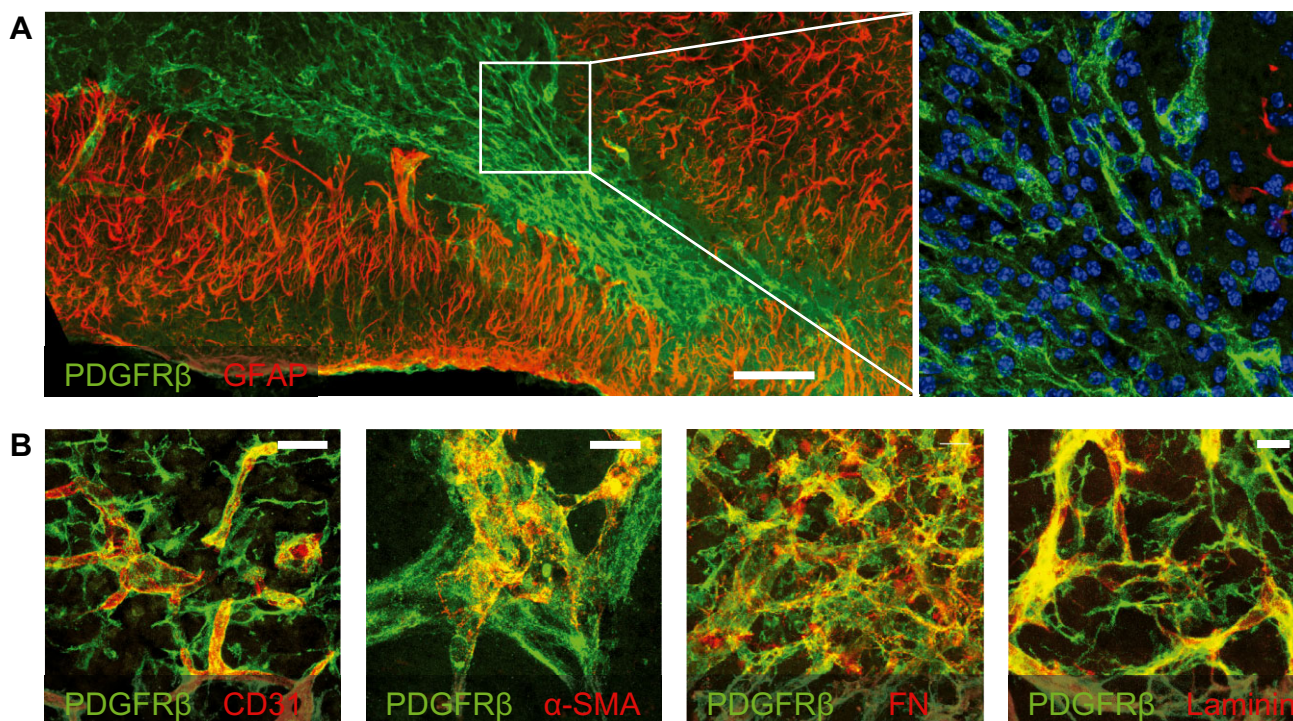
Figure 3. Fibrotic and glial scar after experimental stroke. Laser confocal microscopic images of immunostained mouse brain sections at 7 days after transient middle cerebral artery occlusion (25). Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Scale bars: (a) 100 μm, (b) 20 μm. **A.** The infarct core exhibits an abundant infiltrate of platelet-derived growth factor receptor (PDGFR)-β-immunoreactive cells (green), which is surrounded by the glial fibrillary acidic protein (GFAP)- immunoreactive astroglial scar (red). A high-power magnification of the area marked by the white square is shown on the right and shows the inflammatory cell infiltrate. **B.** PDGFR-β-immunoreactive cells (green) detach from CD31-immunoreactive endothelial cells, express α-smooth muscle actin (α-SMA) and fibronectin (FN), and juxtapose laminin-immunoreactive extracellular matrix (ECM) deposits (all in red).

lesion. Among the molecules involved in the confinement of astrocytes and the generation of a glia limitans, integrins play a major role as the most abundant membrane-bound ECM receptors. Thus, deficiency of the integrin β1 subunit in astrocytes results in diffuse borders of the glial scar *in vitro* (64). Moreover, the ECM molecules fibronectin and laminin enhance the interleukin (IL)-1β-induced activation of astrocytes in an integrin-dependent manner (88). Osteopontin is another ECM-associated protein that is expressed by microglia and macrophages after ischemic or traumatic brain injury, and contributes to the formation of the gliotic barrier via binding to α(v)β3 integrin on astrocytes (20, 28). Last, cell contact-mediated bidirectional signaling between ephrin-B2 on reactive astrocytes and EphB2 on meningeal fibroblasts is an early event in the development of the glial scar and the exclusion of meningeal fibroblasts from the neural tissue (9).

Overall, it seems that fibroblasts in the lesion core together with inflammatory cells cooperate to shape the formation of the astroglial scar, partly by co-opting molecular mechanisms of the developing CNS (48). It is important to note that pericytes in the normal CNS also seem to influence the structure of the glia limitans as their absence distorts the polarization of astrocytes in the CNS parenchyma (4). Hence, the assembly of the glia limitans might be a conserved function among CNS mural cells and their fibrogenic progeny in the injured brain.

Implications of fibrotic scarring in the CNS

In general, scar formation is considered to be a detrimental event after CNS injury because it poses a barrier for axon regrowth (23, 83). Indeed, stromal cells in the fibrotic core of brain and spinal cord lesions secrete the chemorepellent semaphorin III that exerts long-lasting inhibitory effects on the outgrowth of injured CNS neurites (60, 61). Interestingly, vigorous regrowth of injured axons is observed in the absence of semaphorin III following early neonatal lesions in rats (61). After experimental stroke, Sema3A is induced in the ischemic core, but not in the glial scar, and impairs stroke recovery (63).

Some attempts have been made at inhibiting tissue fibrosis in the CNS. Thus, pharmacological inhibition of collagen IV production after lesion of the postcommissural fornix or spinal cord significantly improves axonal regeneration (37, 87). Moreover, inhibition of "type A pericyte" proliferation after spinal cord hemisection results in reduced ECM deposition, increased axonal regrowth across the scar tissue and improved functional recovery (27). However, this comes at the cost of failing to seal the injured tissue resulting in a large dehiscent wound (27).

In conclusion, more studies are needed to evaluate the functions of fibrotic scarring in the CNS, which are likely to be time and context dependent.
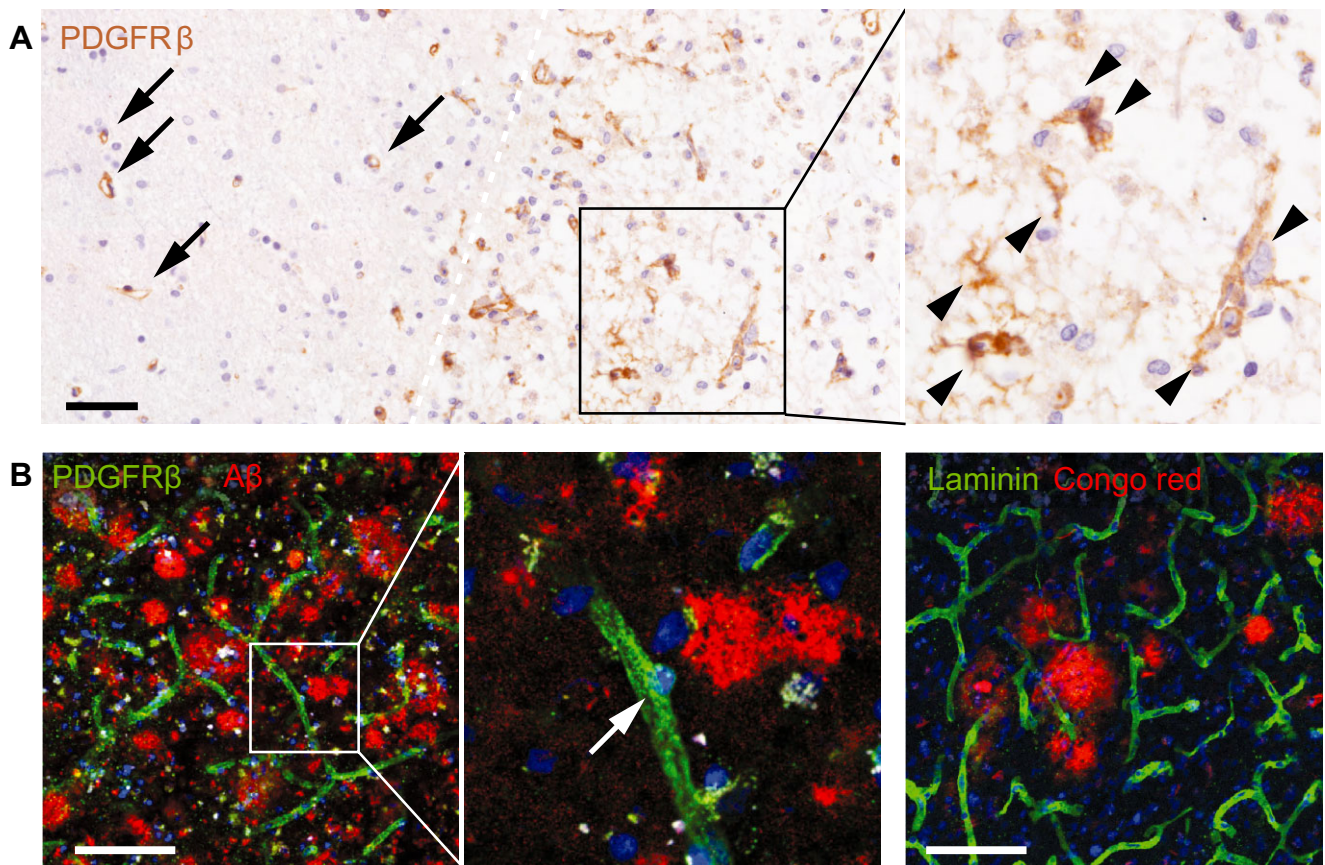
Figure 4. Fibrosis in human neurological disorders. Conventional and laser confocal microscopic images of immunostained human post-mortem brain sections. Nuclei are counterstained with hematoxylin or 4′,6-diamidino-2-phenylindole (DAPI). Scale bars: 100 μm. **A.** Platelet-derived growth factor receptor (PDGFR)-β-immunoreactive cells (brown) in a subacute stroke lesion. PDGFR-β-immunoreactive cells are associated with vessels in the non-ischemic tissue (arrows), but detach from the vasculature and spread into the parenchyma (arrowheads) in the ischemic tissue (right of the white dashed line). **B.** PDGFR-β-immunoreactive cells in Alzheimer's disease (left and middle panels). PDGFR-β-immunoreactive cells (green) remain at perivascular sites. There is no apparent proliferation of PDGFR-β-immunoreactive cells around amyloid plaques (red). In addition, no ectopic deposition of laminin (green) can be detected around plaques (red) in the Alzheimer's disease brain (right panel).

Factors mediating fibrosis in the CNS

In most organs, fibrosis is considered to be part of the inflammatory response. Protracted inflammation due to ongoing infectious or toxic tissue destruction causes excessive amounts of ECM deposition. Similarly, chronic inflammation in autoimmune diseases such as systemic sclerosis results in fibrosis. Hence, neuroinflammation needs to be considered as a potential driving force of fibrotic scarring in the CNS. Indeed, cytokines such as IL-1β, IL-6 and tumor necrosis factor (TNF)-α that aggravate fibrosis in peripheral tissues (19) are also expressed by microglia or infiltrating inflammatory cells in the CNS. Macrophages in nonneural tissues adapt their function in response to pathogens, tissue damage and lymphocyte interactions. This process, termed polarization, enables the adaptive responses of innate immunity to take place (8). The appearance of alternatively activated macrophages that secrete anti-inflammatory and profibrotic factors such as TGF-β1 and PDGF has been associated with the accumu-lation of (myo)fibroblasts and the deposition of fibrillar ECM (54). At the same time, macrophages are also critical for the resolution of fibrosis (18), which is associated with the expression of IL-13 receptor α2, IL-10 and arginase (65, 99). Hence, different functional subsets of macrophages orchestrate different phases of fibrosis. The relative contribution of resident microglia and peripherally derived myeloid cells in this process remains to be explored (68).

Among the cytokines that act directly on fibroblasts or their precursors to induce a pro-fibrotic phenotype are IL-1β (34) and TGF-β1. We have already mentioned the importance of members of the TGF-β family for the conversion of embryonic scar-free healing to fibrosis in the adult. TGF-β1 is normally present at low levels in the adult CNS, with a constitutive expression in menin-geal cells and some neuronal populations (89, 91). Chronic overexpression of TGF-β1 by astrocytes only affects the meningeal lining and the microvascular compartment in transgenic mice, resulting in increased basement membrane deposition as well as

degeneration of endothelial cells and pericytes (101). In cell culture, TGF-β1 is produced at much higher levels by microglia than by astrocytes, and its production is regulated by vitronectin (96). Microglia, infiltrating monocytes/macrophages and reactive astrocytes are the major cellular sources of increased TGF-β1 levels in CNS disorders such as ischemia, trauma and multiple sclerosis (17). After spinal cord injury, TGF-β1 drives fibrotic scarring as demonstrated by the reduced deposition of fibrous scar tissue and attenuated limiting glial membrane formation after the administration of neutralizing TGF-β1 antibodies (48). Type I and type II TGF-β receptors are barely expressed in the intact brain, but gene expression is strongly induced in invading meningeal fibroblasts and much less in reactive astrocytes after penetrating injury to the striatum (39). The results suggest that fibroblasts, not reactive astrocytes, are a major target of TGF-β1 that is upregulated after CNS injury. Pericytes also express type I and type II TGF-β receptors, and TGF-β1 induces ECM production and α-SMA expression in cultured pericytes compatible with a myofibroblast phenotype (82, 90). Moreover, TGF-β1 protects myofibroblasts from apoptosis (105), and acts as a chemoattractant for fibroblasts *in vitro* (67).

PDGF was originally recognized as a growth factor for glial cells, fibroblasts and vSMCs (29, 30). The family comprises four isoforms, namely PDGF-A, -B, -C and -D, and two receptor chains, PDGFR-α and -β (59). Pericytes and other mesenchymal cells in the vasculature and meninges express both PDGFR-α and PDGFR-β, as do proliferating stromal cells after CNS injury (25, 27). The interaction of ECM-bound PDGF-BB with PDGFR-β is necessary for the proper attachment of pericytes to CNS blood vessels (4, 6, 14). During fibrosis, PDGFs act as potent prolifera-tive, chemotactic and differentiation cues for myofibroblasts (59). Other important profibrotic growth factors such as connective tissue growth factor (CTGF) or fibroblast growth factor (FGF)-2 are also induced in the injured CNS (31, 77). There is extensive cross talk with proinflammatory cytokines. For instance, TGFβ-1 induces PDGFR-α expression in fibroblasts (52). The cytokine IL-1β also induces the expression of PDGFR-α in fibroblasts, and IL-13 promotes the autocrine release of PDGF-AA (34). Impor-tantly, PDGFR signaling is involved in pericyte activation, prolif-eration and differentiation into myofibroblasts during progressive kidney injury in mice (11). *Pdgfb* mRNA is induced in neurons and macrophages after experimental stroke (33), and *Pdgfa* mRNA is increased after lysolecithin-induced spinal cord demyelination (31). Notably, *Pdgfrb* knockout mice exhibit larger infarct volumes, increased vascular leakage and reduced astroglial scar formation after cerebral ischemia (80).

Do stromal cells modulate neuroinflammation?

We have already stressed the importance of inflammation for driving fibrotic scarring in the CNS. Conversely, it is increasingly recognized that stromal cells play an active role during inflamma-tion. Pericytes, arguably the most abundant stromal cell population in the quiescent CNS, might contribute to different stages of neuroinflammation.

Pericytes form a loose mesh in CNS venules, the main site for the diapedesis of inflammatory cells. In the inflamed cremaster muscle, leukocytes migrate along pericyte processes in the subendothelial space and later invade the parenchyma at specific locations dependent on pericyte-expressed intercellular adhesion molecule (ICAM)-1 and the leukocyte integrin ligands, Mac-1 and LFA-1 (69). The transmigration sites coincide with regions of loose ECM in the basement membrane of venules (92, 94). The contraction of pericytes may influence the patency of these exit points for emigrating leukocytes (93). In the skin, capillary and arteriolar pericytes enhance the ability of extravasated neutrophils and macrophages to screen the interstitial space for damaged tissue and execute their effector functions by expressing ICAM-1, toll-like receptors and macrophage migration-inhibitory factor (MIF) (86). The available data on CNS pericytes are more scarce, but it appears that cultured brain microvascular pericytes respond to lipopolysaccharide stimulation by producing a vast array of cytokines and chemokines (40). Moreover, pericytes activate a battery of immune response genes that initiate immune cell recruitment in the brains of conditional knock-in mice with acti-vating mutations at the *Pdgfrb* locus (58). Pericytes also give rise to (PDGFR-β-negative) follicular dendritic cells in ectopic tertiary lymphoid tissue in the context of chronic inflammation (41). Inter-estingly, chronic neuroinflammatory conditions such as some types of multiple sclerosis have been associated with the formation of tertiary lymphoid tissue at pial and perivascular sites (49). Altogether, it is clear that pericytes participate actively in inflam-matory responses. Their specific impact on CNS disorders remains to be further explored.

CONCLUSION

The CNS shares more repair mechanisms with peripheral tissues than previously recognized. We believe that scar formation (fibro-sis) is a conserved healing mechanism that has not received enough attention by neuroscientists in the past. Fibrosis is driven by neuroinflammation, and originates from resident stromal cell populations that have yet to be characterized in more detail. Our article leaves many questions unanswered: Is there a common origin of PDGFR-β+ cells in different CNS pathologies? Is the fibrotic reaction necessary for the development of the astroglial scar? Do resident and peripherally derived inflammatory cells play different roles in fibrosis? What are the cues that provoke resolu-tion and regression of the scar? Given the importance of fibrosis in many neurological disorders and the availability of novel antifibrotic therapies, the quest for answers to these issues appears very rewarding.

ACKNOWLEDGMENTS

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