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Review

Functional regeneration beyond the glial scar

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ABSTRACT

Astrocytes react to CNS injury by building a dense wall of filamentous processes around the lesion. Stromal cells quickly take up residence in the lesion core and synthesize connective tissue elements that contribute to fibrosis. Oligodendrocyte precursor cells proliferate within the lesion and entrap dystrophic axon tips. Here we review evidence that this aggregate scar acts as the major barrier to regeneration of axons after injury. We also consider several exciting new interventions that allow axons to regenerate beyond the glial scar, and discuss the implications of this work for the future of regeneration biology.

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Introduction

Traumatic injury to the CNS elicits physical damage to vascular networks and neural circuit architecture. Intense local inflammation is an immediate consequence of injury, and causes progressive cavitation that exacerbates the extent of the primary lesion. In time, resident astrocytes become hypertrophic and form a dense scar that protects intact neural networks from further damage (Bush et al., 1999; Faulkner et al., 2004). For years we have thought of this astrocytic scar as the main impediment for regenerating axons attempting to reach their distal targets. However, we have recently come to recognize the lesion as a complex system of interacting cell types (Barnabé-Heider et al., 2010; Busch et al., 2010; Göritz et al., 2011; Horn et al., 2008; Meletis et al., 2008; Sabelström et al., 2013; Soderblom et al., 2013). These cells react to injury in a stereotyped fashion, forming a mature lesion with two distinct components: The penumbra is composed of hypertrophic astrocytes, whereas the lesion core is composed of NG2 glia/oligodendrocyte precursor cells (OPCs), meningeal and/or vascular derived fibroblasts, pericytes, ependymal cells, and phagocytic macrophages (Fig. 1).

Regenerating axons attempting to navigate the mature lesion ultimately abort their mission and form dystrophic end bulbs that persist indefinitely (Ramón y Cajal, 1928). These dystrophic end bulbs have long been considered a hallmark of regeneration failure (Tom et al., 2004), and have been identified within a human spinal cord lesion 42 years after injury (personal communication with Ruschel, Sliwsinski, Blesch, Weidner, and Bradke). Here we will review the formation of the glial scar as a framework for understanding axonal dystrophy. Additionally, we will consider several active processes within the lesion that

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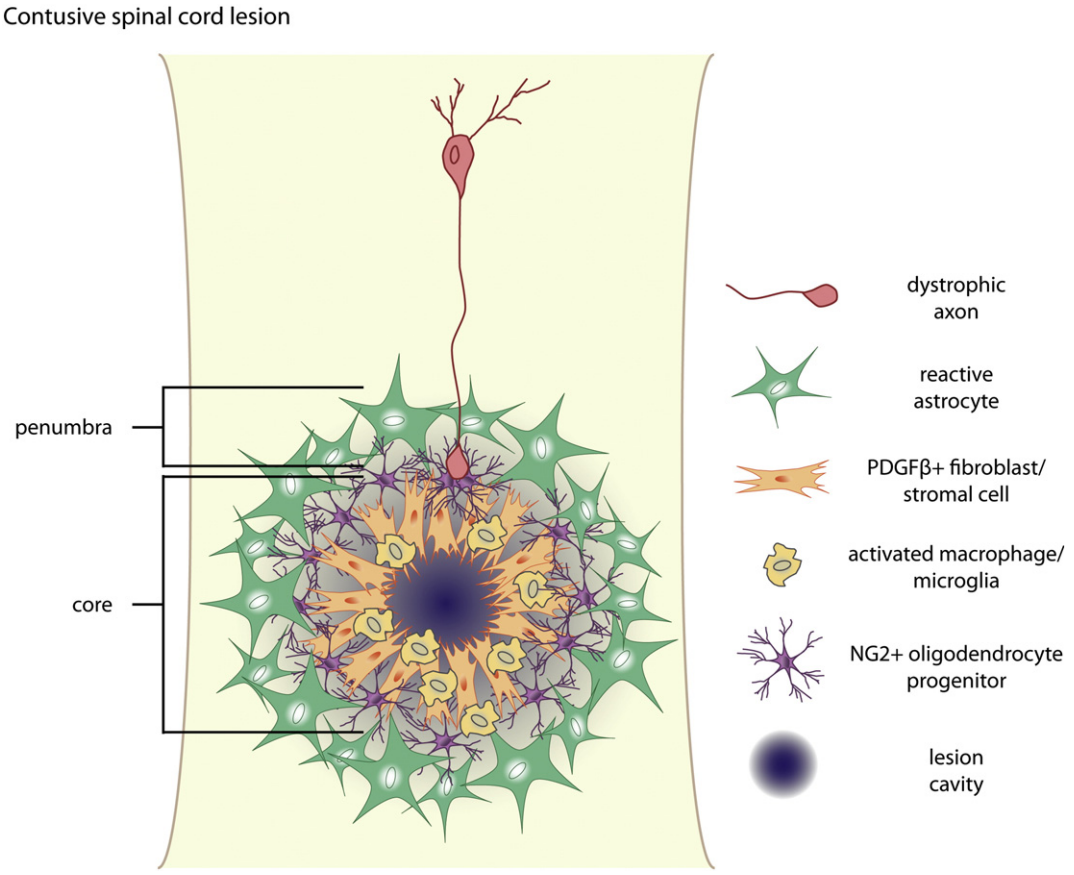

Fig. 1. Anatomy of a contusive spinal cord lesion. Spinal cord lesions have two distinct components—the lesion penumbra is composed of hypertrophic astrocytes, and the lesion core is composed of NG2 + oligodendrocyte precursor cells, PDGFRβ + fibroblasts, and macrophages/microglia. Dystrophic axons become entrapped within the lesion in close association with NG2 glia. The layered architecture of the glial scar is thought to reflect both the dynamic polarization of different populations of cells at distinct times after injury and segregation of distinct populations via chemorepulsion.

underlie regeneration failure—including inhibitory interactions between axons and extracellular matrix, and phagocytic processes that cause axonal dieback.

With our growing understanding of the processes that actively block regeneration of severed axons, strategies have emerged that allow axons to navigate through and beyond injury sites, or to circumvent the lesion altogether. These studies have advanced the idea that long distance regeneration of axons past sites of CNS trauma can restore significant function to denervated targets, providing new hope for translational success.

Glial scar formation after CNS insult

Breakdown of the blood–brain barrier (BBB) and leakage of blood and serum elements into the CNS parenchyma is considered a key event in glial scar formation. As such, several molecules derived from the blood or produced via inflammation have been advanced as potential triggers of scar formation, including interleukin-1 (Giulian et al., 1988), transforming growth factor-β (TGFβ) isoforms (Asher et al., 2000; Lagord et al., 2002; Moon and Fawcett, 2001), and fibrinogen (Schachtrup et al., 2010). Recently, Schachtrup et al. (2010) linked the release of a blood-derived fibrinogen-TGFβ complex directly to astrogliosis. Genetic deletion of fibrinogen reduced the amount of active TGFβ at the lesion, with corresponding decreases in both astrocytic hypertrophy and production of chondroitin sulfate proteoglycans (CSPGs). Active TGFβ acts via a TGFβ-R/Smad2 dependent pathway in astrocytes, presumably turning on an intrinsic transcriptional program responsible for gliosis (Schachtrup et al., 2010). It appears that Smad2 translocation is a critical event in the induction of gliosis—blocking kinesin-dependent Smad2 translocation with the microtubule stabilizing agent taxol has been associated with reduced scarring at the lesion (Hellal et al., 2011).

Perhaps equally as important to the development of the lesion, injury causes leukocyte extravasation and accumulation of inflammatory cells in the lesion core (Figs. 2A,B). Recent work using 3D-imaging combined with genetic labeling of microglia and infiltrating monocyte-derived macrophages (CX3CR1-GFP) has revealed that the density of inflammatory cells increases by 40-fold in lesioned white matter and 9-fold in lesioned gray matter (Ertürk et al., 2011). This is partly due to rapid polarization of microglia toward sites of CNS injury (Davalos et al., 2005; Nimmerjahn et al., 2005), but probably largely reflects the recruitment and accumulation of blood-borne cells (Ajami et al., 2011). Indeed, activated macrophages/microglia markedly increase expression of matrix metalloproteases (MMPs) after injury, and this contributes to vascular permeability and accumulation of more inflammatory cells within the lesion. MMP inhibitors applied in the acute phase of injury enhance functional recovery (Noble et al., 2002). While these pools of activated macrophages/microglia are thought to be important for lesion debridement, they also drive secondary injury through inflammatory processes. Zymosan, a non-toxic but potent inflammatory agent, delivered through microneedles in a manner that causes minimal damage to the CNS parenchyma, induces an inflammatory response that is sufficient for eliciting secondary tissue damage and causes astrocytes to rapidly migrate away from the inflammatory epicenter (Fitch et al., 1999). More recent experiments have led to the surprising finding that activated macrophages are responsible for prolonged dieback of injured axons after injury (Horn et al., 2008) (Fig. 2D). The timing of axonal dieback correlates well with the