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The Origin, Fate, and Contribution of Macrophages to Spinal Cord Injury Pathology

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Abstract

Virtually all phases of spinal cord injury pathogenesis, including inflammation, cell proliferation and differentiation, as well as tissue remodeling are mediated in part by infiltrating monocyte-derived macrophages. It is now clear these infiltrating macrophages have distinct functions from resident microglia, and are capable of mediating both harmful and beneficial effects after injury. These divergent effects have been largely attributed to environmental cues, such as specific cytokines, that influence the macrophage polarization state. In this review, we also consider the possibility that different macrophage origins, including the spleen, bone marrow, and local self-renewal, may also affect macrophage fate, and ultimately their function that contribute to the complex pathobiology of spinal cord injury.

Keywords

Leukocytes; inflammation; myeloid cells; wound healing; glial and fibrotic scar; regeneration; peripheral; systemic

Origin of monocyte-derived macrophages in the injured spinal cord

Splenic versus bone marrow-derived macrophages

Circulating monocytes are blood mononuclear phagocytes with the potential to differentiate into macrophages and/or dendritic cells upon infiltration into the injured CNS. Injured tissue releases cytokines and chemokines into circulation that serve as chemoattractants for circulating monocytes. Monocytes migrate to the injury site and differentiate into macrophages in a multiphasic manner, where they perform multiple functions involved in the wound healing process. The first wave starts around 3 days after injury and reaches a peak at around 7 days. After a slight decline, the second wave begins at 14 days, peaks again at 60 days, and is maintained level until at least 180 days after injury [2, 18]. Humans follow

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a similar temporal pattern acutely, but it is not known whether humans also display a second wave of macrophages [21].

Mouse monocytes can be generally classified into Ly6C^{hi} and Ly6C^{lo} subtypes, although Ly6C^{middle} is also used. Ly6C^{hi} monocytes are also Cx3Cr1^{lo} and CCR2^{hi}, and considered to be phagocytic and pro-inflammatory. Ly6C^{lo} monocytes are Cx3Cr1^{hi} and CCR2^{lo}, and considered to be anti-inflammatory [24, 102]. Previous SCI studies on Cx3Cr1^{GFP} mice have demonstrated the presence of both Cx3Cr1^{lo} and Cx3Cr1^{hi} macrophages at the injury site, indicating that both pro-inflammatory Ly6C^{hi}, and anti-inflammatory Ly6C^{lo} monocytes contribute to the macrophage population after SCI (discussed in more detail below) [17, 107]. Alternatively, either the Ly6C^{hi} or the Ly6C^{lo} monocytes could give rise to both pro- and anti-inflammatory macrophages at the injury site.

While the bone marrow was once thought to be the only source of these monocytes, the spleen is now recognized as a major monocyte reservoir during the acute injury phase in many organ systems. Using a mouse model of myocardial infarction, Swirsky et al [94] calculated that the number of circulating monocytes derived from bone marrow could not fully account for the number of macrophages at the injury site. Their investigation into alternative sources of monocytes led to the identification of a population of monocytes residing within the subcapsular red pulp of the spleen [94]. These splenic monocytes had both ly6C^{hi} and ly6C^{lo} subtypes that were similar in proportion to those in blood. After splenectomy, the number of pro-inflammatory ly6C^{hi} monocytes at the site of myocardium infarct decreased by about 75% by 1 day after injury, while the number of anti-inflammatory ly6C^{lo} monocytes remained unchanged, implicating the spleen as the major source of the pro-inflammatory subtype. These results give the impression that the pro-inflammatory characteristic is intrinsic to splenic macrophages, however, it should be noted that tumor associated macrophages, which are pro-regenerative, also arise from the spleen [11], indicating a complex interplay between splenic macrophages and the tissue microenvironment that finally determines the phenotype.

Similar results have been reported in many tissues, including the spinal cord. After splenectomy, there is a significant decrease in the number of macrophages present at the injury site during the first week after SCI [3]. Whether these macrophages are derived from the pro-inflammatory ly6C^{hi} monocyte subtype is not known, but splenectomized mice displayed improved locomotor recovery chronically after SCI. Pharmacological depletion of monocytes using liposome-encapsulated clodronate has shown similar results in multiple independent studies [36, 47, 73]. However, it should be noted that liposome-encapsulated clodronate also affects monocytes in the bone marrow [100], so its effects cannot be isolated to the spleen. An interesting and potentially important observation is that even after a splenectomy, macrophages start to reappear at the injury site, and over time the number of macrophages are similar to nonsplenectomized animals [3]. This indicates another source of macrophages, such as the bone marrow, and raises the interesting possibility that the benefits observed after splenectomy or other methods of acute macrophage depletion could be due to repopulation of the injury site by anti-inflammatory, pro-regenerative macrophages from the bone marrow, in addition to depletion of pro-inflammatory macrophages from the spleen. Accordingly, under resting conditions, primary splenic macrophages produce higher levels

of pro-inflammatory cytokines, such as TNF, IL-6 and IL-12, than bone marrow-derived macrophages [97].

The observation of macrophage repopulation after splenectomy is consistent with previous findings of macrophage infiltration occurring in a multiphasic manner (in rodents) as described above. Interestingly, preventing the late phase of macrophage infiltration via C5a receptor inhibition worsens locomotor recovery, suggesting that this later macrophage population is beneficial to functional recovery [2]. The source of this late wave of macrophages is unknown, but could be of same origin as macrophages that repopulate the injury site after splenectomy as mentioned above. Whether this population originates from the bone marrow remains to be tested, but the different contributions of macrophages from different origins could provide important insight into identification of novel therapeutic interventions that target distinct macrophages populations.

Self-renewal of macrophages

Macrophages persist chronically at the injury site in both rodents and humans [21]. This long timespan raises the question of their turnover rate and origin at various stages of injury progression. Although circulating monocytes have a lifespan of 1–3 days, monocyte-derived macrophages typically have a lifespan of several weeks [37], which means that macrophages must be somehow replenished in disorders such as SCI in which macrophages persist for years. The traditional view holds that since circulating monocytes terminally differentiate into macrophages, turnover of macrophages must depend on new monocyte influx. However, accumulating evidence indicates that local proliferation of macrophages at the injury site may also significantly contribute to the total population. In a model of peritonitis, proliferation of both resident and monocyte-derived macrophages contribute to macrophage expansion [14]. Local proliferation, rather than monocyte recruitment, also seems to be the major mechanism of accumulation of tumor-associated macrophages in a mouse model of spontaneous mammary carcinogenesis [96]. Similarly, continuous administration of BrdU labels nearly all macrophages in four weeks in a mouse model of atherosclerosis, and this turnover kinetics is not altered after monocyte depletion or parabiosis, suggesting that monocyte recruitment cannot fully account for the number of macrophages in an atherosclerotic lesion [81].

Although local macrophage proliferation needs to be experimentally confirmed in spinal cord injury in the future, several independent reports in multiple disease models discussed above make it highly probable that it also contributes to the maintained presence of macrophages chronically after spinal cord injury. This raises the possibility that the repopulation of macrophages after splenectomy and/or the second wave of macrophage accumulation mentioned above could have significant contributions from local proliferation rather than, or in addition to, infiltration of monocytes from peripheral organs. If local proliferation, rather than monocyte infiltration, is the predominant mechanism behind macrophage renewal at the injury site, this could have important implications on how macrophage-targeting therapeutics are delivered after spinal cord injury.

Macrophage fate in the injured spinal cord

Temporospatial distribution of macrophages

Resolving the spatiotemporal dynamics of macrophage infiltration is complicated by the presence of microglia. Due to the phenotypic and antigenic similarities between microglia and macrophages, it is difficult to differentiate between these two populations. Flow cytometry can distinguish between $CD45^{hi}/Cd11b^{+}$ macrophages and $CD45^{lo}/CD11b^{+}$ microglia on a population level, but this method does not provide information on spatial distribution. Many studies have used expression levels of certain antigens to experimentally differentiate between both populations. For example, it has been reported that resident microglia express the chemokine receptor Cx3Cr1 to a much higher degree than macrophages. In addition, macrophages have a higher expression level of CD45 and Mac-2 (Galectin-3) than microglia [26]. In addition, lysM-eGFP and Ccr2-RFP mutant mice specifically label macrophages rather than microglia [57, 84].

One of the first studies to address the spatial distribution of macrophages used bone marrow chimeric mice with a $Cx3Cr1^{GFP}$ donor and wildtype host, resulting in a model in which GFP is expressed in bone marrow-derived myeloid cells. The authors demonstrated that GFP^{+} cells were present mostly surrounding the GFAP-negative fibrotic injury center, thus concluding that the $CD11b^{+}/GFP^{-}$ cells in the injury center were microglia, whereas the GFP^{+} cells in the surrounding region were macrophages [82, 90]. However, these studies failed to account for the fact pro-inflammatory $Ly6C^{hi}$ macrophages express low levels of Cx3Cr1. Later studies using multiple chimeric models addressed this issue and demonstrated that macrophages occupy both the fibrotic lesion center as well as the surrounding glial scar regions, whereas microglia are present almost exclusively in the glial scar region [17, 99, 107] (Figure 1). Interestingly, the $Cx3Cr1^{GFP}$ chimera studies clearly show the presence of both $Cx3Cr1^{hi}$ and $Cx3Cr1^{lo}$ macrophages at the injury site with different spatial distribution; $Cx3Cr1^{hi}$ macrophages are present in neural tissue that is characterized by the glial scar, whereas $Cx3Cr1^{lo}$ macrophages are present in the non-neural tissue characterized by the fibrotic scar. Whether these two populations represent anti-inflammatory M2-like macrophages (i.e. $Ly6C^{lo}/Cx3Cr1^{hi}$) and pro-inflammatory M1-like macrophages (i.e. $Ly6C^{hi}/Cx3Cr1^{lo}$) remains a possibility to be tested. The fact that $Cx3Cr1^{lo}$ and $Cx3Cr1^{hi}$ macrophages occupy distinct regions of the injury site (fibrotic and glial scar regions, respectively) suggests that the local environment could be driving the macrophage phenotype, or that these macrophage subtypes drive distinct physiological processes (i.e. fibrosis vs gliosis). Alternatively, these two types of macrophages could have differential attraction to the glial and fibrotic scar regions.

Macrophage polarization spectrum

The M1/M2 macrophage classification system was originally defined in a seminal study by Mills et al [58] where the authors investigated fundamental differences in activation of macrophages from different strain of common inbred mice. Using primary cultures of resident peritoneal macrophages from mice that typically display a Th1 response (C57BL/6 and B10D2 mice) or a Th2 response (Balb/C and DBA/2 mice) in response to infection, the authors demonstrated that macrophages from Th1 strains, but not Th2 strains, were readily

activated by IFNγ or LPS. Activation of Th2 strain macrophages required both IFNγ and LPS. Macrophage activation was measured by metabolism of arginine into nitric oxide, which is iNOS-dependent, or ornithine, which is arginase-dependent. Thus, macrophages from Th1 strains were labeled M1 macrophages characterized by iNOS activity, and those from Th2 strains were labeled M2 macrophages characterized by arginase activity. This M1/M2 nomenclature was soon adopted by the scientific community as a binary classification in a large wave of ensuing publications. However, it is now recognized that macrophage polarization state is more of a continuum between M1 and M2 [56], but it should be noted that Mills et al explicitly stated in their original manuscript that "there may be a continuum of phenotypes between M1 and M2 macrophages."

The observation that M1-like macrophages can kill nearby cells and prevent cellular proliferation, whereas M2-like macrophages can promote cell proliferation and tissue growth [59] led to the hypothesis that promoting M2-like polarization would be beneficial in CNS injuries, such as SCI, where cellular regeneration is limited. The first study to define M1/M2 macrophages in the injured spinal cord was by Kigerl et al [38], which reported that, other than a momentary increase of M2 macrophages at 7 days, the SCI site is comprised predominantly of M1 macrophages. In addition, the authors used *in vitro* BMDM (bone marrow-derived macrophages) assays to show that compared to M1 macrophage-conditioned media, M2 macrophage-conditioned media enhance neurite outgrowth even on inhibitory substrates. The persistence of M1 macrophages after SCI is in contrast to what happens in typical wound healing process, which is associated with predominantly M2 macrophages [43]. Thus, this gave rise to the possibility that the persistence of M1 macrophages after SCI contributes to a chronic inflammatory state that impedes cellular regeneration.

An important issue that remains to be addressed is the origin of M1/M2-like macrophages. One possibility is that that the injury environment is the major determinant of macrophage polarization. As monocytes enter the injury site, the injured environment could be favorable to an M1-like polarization. The fact that most macrophages persist chronically in this state rather than an M2-like state associated with wound resolution (as mentioned above) indicates that the injured spinal cord environment is unique compared to other injured tissue. For example, due to the abundance of myelin debris in the injured spinal cord, macrophages become lipid-laden foam cells that are characterized as pro-inflammatory M1-like cells [99] (discussed in more detail below). If M1-like polarization is mainly due to the injury environment, then altering this environment would be predicted to shift macrophage polarization state. Accordingly, degradation of chondroitin sulfate proteoglycans (CSPG) by chondroitinase ABC promotes a more M2-like macrophage phenotype [15].

An alternative possibility is that macrophage polarization states are cell autonomously pre-determined, which is supported by the original M1/M2 macrophage study described above where macrophages from Th1 and Th2 mouse strains display distinct activation response. Accordingly, a previous study comparing multiple mouse strains with different susceptibility to EAE (experimental autoimmune encephalomyelitis) found that C57BL/6 mice (EAE-susceptible Th1 strain) display a much stronger inflammatory response than Balb/C mice (EAE-resistant Th2 strain), including increased macrophage activation, neutrophil and T-cell

infiltration, and greater fibrotic response [39]. However, despite the significantly less inflammatory response, Balb/C mice displayed similar lesion morphometry and locomotor recovery as C57Bl/6 mice [1, 39]. Other studies have also reported such disconnection between the inflammatory response and behavioral recovery. Although 129X1/SvJ mice show decreased macrophage infiltration and greater axonal growth into the lesion, their lesion size and locomotor recovery are similar to C57BL/6 mice [54]. In fact, in MRL/MpJ mice, which display scarless skin regeneration, attenuated macrophage infiltration is associated with worse locomotor recovery and greater lesion size [41]. Taken together, these studies suggest that there are beneficial and detrimental aspects of the macrophage inflammatory response that must be considered when targeting inflammation after spinal cord injury.

Transcriptional regulation of macrophage polarization

Cellular changes in phenotype and function often require significant alterations in gene expression, which is regulated by transcription factors. Transcription factors that affect macrophage polarization have been an intense area of investigation in various disorders, and are reviewed in detail elsewhere [20, 75, 98]. Thus, we will focus on a few in the context of SCI. IFNγ and LPS are the classical activating ligands for inducing M1 polarization. IFNγ binds to the IFNγ receptor, which signals primarily through STAT1 activation. However, since IFNγ is not highly expressed at the SCI site [30], it is not clear whether this signaling pathway plays a major role in macrophage polarization after SCI. Although LPS stimulation is not very representative of sterile inflammatory mechanisms that occur after SCI, its receptor, TLR4, is one of the major receptors for damage associated molecular patterns (DAMPs), such as HMGB1, that are widespread at the injury site [16] and present in higher levels in plasma samples from humans with SCI [70]. TLR4 receptors signal through activation of NF-κB, which is a classic transcription factor for many pro-inflammatory cytokines, including TNF, IL-1β, COX2, and IL-6, and a potent inducer of M1-like polarization [reviewed by 52].

TNF and IL-1β are often used to model sterile inflammation that induces M1 polarization in vitro, and both cytokines are expressed highly after SCI [72]. The peak of TNF mRNA expression is within the first hour after injury, followed by peak of IL-1β mRNA expression at around 12 hours after injury [72]. TNF exists in soluble (solTNF) and transmembrane (tmTNF) forms, and bind preferentially to receptors TNFR1 and TNFR2, respectively [28], both of which are expressed acutely after SCI [101]. Most of the inflammatory effects attributed to TNF is through activation of TNFR1 by solTNF, which eventually leads to NF-κB activation and M1-like macrophage polarization as described above. Importantly, pharmacological inhibition of solTNF signaling has been reported to improve function after SCI, although it is not clear whether the effects were mediated by effects on macrophage polarization [61, 68]. IL-1β also activates NF-κB by binding to the Type I IL-1 receptor (IL-1RI), thereby leading to M1-like polarization. Administration of recombinant IL-1β exacerbates histopathology and loss of function, whereas genetic deletion of IL-1β has a beneficial effect on these outcome measures [4]. However, it is not clear whether either the detrimental or the beneficial effects are mediated by changes in macrophage polarization.

IL-4 and IL10 are the classic M2 polarizing ligands. IL-4 binds to IL-4Ra, which activates STAT6 that functions as an important mediator of the M2-like phenotype. STAT6 stimulation leads to activation of several other transcription factors that are important for M2-like polarization, including PPARγ, PPARδ, and KLF4 [reviewed by 75]. PPARγ activation leads to expression of classical M2-associated genes such as Arg1 and MMR (CD206) [12, 69]. IL-10 binds to IL-10R, which activates the JAK1/STAT3 pathway that indirectly suppresses expression of pro-inflammatory cytokines through expression of several effector genes [reviewed by 64]. However, STAT3 is also activated by the pro-inflammatory cytokine IL-6. A potential explanation for this paradox is that IL-6 results in a more transient STAT3 activation, whereas IL-10 results in a more sustained activation [8], perhaps due to negative feedback from SOCS family members after IL-6 activation but not IL-10 [103]. Interestingly, expression of IL-4 and IL-10, along with most other anti-inflammatory cytokines, exhibit transient and acute expression after SCI [22, 46, 63], which may contribute to the prevalence of M1-like macrophages that persist chronically at the injury site. Accordingly, exogenous administration of IL-4 can promote M2-like polarization that is associated with improved histopathology and behavioral outcomes [22].

Formation of foamy macrophages after SCI

Macrophages are most often defined in terms of the M1/M2 spectrum nomenclature, which is conceptually helpful, but difficult to apply in practice, especially in vivo. The classic M1/M2 markers of iNOS and Arg1 are no longer considered to be sufficient as definitive polarization markers, and although most studies now use several markers, there is no clear consensus on which combinations correspond to which region along the polarization spectrum, and these markers often do not provide functional insight. Instead of using individual markers, an alternative approach is to assess the entire macrophage transcriptional profile, which provides a more comprehensive and unbiased method of comparing cellular phenotypes.

By using the Ribotag method [85] to obtain the first macrophage-specific transcriptome from the injured spinal cord, Zhu et al [106] used single-sample Gene Set Enrichment Analysis (ssGSEA) to compare SCI macrophages to many other macrophage phenotypes from both in vitro and in vivo samples. This analysis revealed that the transcriptome of SCI macrophages was most similar to that of lipid laden “foamy” macrophages rather than M1- or M2-like macrophages. Interestingly, pathway analysis of differentially expressed genes revealed molecular mechanisms that were very similar to those described in the atherosclerosis literature [62], suggesting overlapping pathological mechanisms, and therapeutic targets between the two disorders.

One of the first reports of foamy macrophages after SCI was in human post-mortem tissue where these lipid-laden macrophages were present in the lesion even at one year after injury [21]. We have only recently started to gain mechanistic understanding of how foamy macrophages contribute to SCI pathology. Foamy macrophages display a pro-inflammatory, M1-like phenotype in vivo [99], and genetic deletion of the classic lipoprotein scavenger receptor, CD36, leads to decreased macrophage lipid content, smaller lesion size, and improved locomotor recovery [106] (myelin debris receptors are discussed in more detail

below). These results are consistent with the pathological contribution of foamy macrophages in atherosclerosis [62], but contradict the anti-inflammatory roles of foamy macrophages proposed in multiple sclerosis [5, 7, reviewed by 40]. The exact mechanisms governing macrophage phagocytosis after SCI, including foam cell formation, have yet to be fully elucidated, although existing studies suggest they play a central role in striking the balance between inflammatory aggravation and resolution after SCI. Furthermore, conflicting data on this topic suggest that macrophage phagocytosis is influenced by an array of microenvironmental cues that comprise the complex physiological landscape of the lesion site.

Macrophage phagocytosis mechanisms

Cellular debris clearance by macrophages

In the immediate aftermath of SCI, peripheral neutrophils are recruited to the lesion and enter the CNS through the mechanically compromised blood brain barrier, beginning in the first hours following injury and peaking at 24 hours post injury [67]. Subsequent infiltration is further assisted by the secretion of matrix metalloproteases (MMP's), which disrupt the integrity of the neurovasculature and enhance neutrophil, and later monocyte, transendothelial migration [67]. This early population of infiltrating myeloid cells are responsible for the initial phase of debris clearance in the injury parenchyma, stimulating pro-inflammatory mechanisms through their secretion of cytokines such as TNF [65], proteases including MMP's, as well as reactive oxygen and nitrogen species [2, 31, 79]. In fact, recent evidence suggests that after a peripheral nerve lesion, clearance of the distal nerve stump during Wallerian degeneration is primarily by neutrophils rather than macrophages [51]. As neutrophils begin to undergo apoptosis, monocytes are subsequently recruited to the site via chemoattractive signals such as MCP-1 (Ccl2). Accordingly, its receptor Ccr2 has been shown to be essential to monocyte mobilization [45, 55, 74]. Other attractants include the CC chemokines, such as MIP-1a (Ccl3), and IL-1β, which have also been shown in models of neurodegeneration to effectively recruit peripheral macrophages, although these have not been specifically implicated in SCI [71].

Once at the injury site, monocytes differentiate into macrophages in response to cytokines and chemokines present in the lesion environment and begin to remove dying neutrophils through a process called efferocytosis [32, 38]. The specific molecular mechanisms underlying macrophage recognition and internalization of apoptotic neutrophils as they occur in SCI have not been fully delineated. However, *in vitro* work has shown a dependence of macrophage phagocytosis of apoptotic neutrophils on the interaction between the scavenger receptor CD36 with the vitronectin receptor and thrombospondin [86]. Other molecular cues of apoptotic cells, such as phosphatidylserine from the exposed inner leaflet or ATP, recognized by receptors Mertk and P2Py, respectively, may also drive these interactions [49].

Receptors mediating macrophage phagocytosis

In addition to their role in neutrophil clearance, macrophages also participate in general tissue debris clearance once they arrive at the injury site, expressing a heterogeneous profile

of surface receptors to mediate these processes. A large portion of the cellular debris after SCI is derived from myelin, and myelin phagocytosis has been largely attributed to CR3 (Mac-1, CD11b), SR-AI/II (Msr1), and FcR [reviewed by 13, 78, 83]. However, since antibodies to myelin are absent after SCI, FcR can be most likely ruled out as a potential mechanism, and Msr1 has not been investigated in SCI. Injection of purified myelin into the mouse spinal cord triggers a large influx of neutrophils and macrophages that causes increased expression of many pro-inflammatory cytokines, and these effects are significantly mitigated in CR3 knockout mice, implicating CR3 as an important mechanism of myelin phagocytosis in the spinal cord [93]. However, whether CR3 knockout mice display decreased levels of foamy macrophages that leads to reduced inflammation and improved functional outcomes is not known.

As mentioned above, class B scavenger receptor, CD36 (SR-B2), has also been shown to be involved in myelin phagocytosis after SCI [106]. First identified by their ability to bind oxidized lipoproteins [9, 10], scavenger receptors are multifaceted and promiscuous in ligand interactions and functional capacities. CD36 shows a wide range of interacting partners, including proteins such as integrins, thrombospondins and fibronectin as well as lipid metabolic byproducts such as fatty acids and modified lipoproteins [76]. Additionally, CD36 can form multi-receptor complexes with receptors like Toll-like receptors to further modulate the macrophage inflammatory phenotype. This can occur either by endocytosis of the CD36-TLR complex or induction of specific intracellular signaling cascades, including PPAR transcriptional upregulation [92]. It is important to note that, despite leading to improved histopathology and locomotor recovery, genetic deletion of CD36 only led to a modest decrease in lipid droplet content in macrophages at the injury site [106]. This underscores the importance of investigating the contribution of other receptors in macrophage function and spinal cord pathobiology in hopes that even further benefits can be achieved.

In addition to these classical myelin receptors, many others have been recently implicated. Genetic knockdown of collectin placenta 1 (CL-P1), a class A scavenger receptor (SCARA4) that is upregulated in MS lesions, as well as pharmacological inhibition of MerTK, tyrosine kinase phagocytic receptor of the TAM family, reduced myelin uptake in vitro [6, 33]. Inhibition of low-density lipoprotein-related protein 1 (Lrp1) also reduced myelin phagocytosis in primary oligodendrocytes, microglia, and astrocytes [23]. Induction of the PPAR/LXRα/ABCA1 pathway through ligands such as adiponectin has been show to attenuate myelin foam cell formation in vitro through enhanced lipid efflux and decreased production of pro-inflammatory cytokines, as well as restore normal macrophage function, such as phagocytosis of apoptotic neutrophils [105]. Similar results were found in vivo where administration of AdipoRon, an adiponection-receptor agonist, decreased macrophage recruitment and myelin-lipid accumulation at the injury site, and significant improvements in locomotor recovery [105]. Thus, there is a wide range of receptors classes that can mediate myelin phagocytosis, but most of these studies have been performed using in vitro assays, and the contribution of these receptors in animal models of CNS injury remains poorly understood.

Macrophage regulation of cellular regeneration

There is ample evidence of infiltrating immune cells having beneficial contributions to tissue repair, a concept central to the theory of "protective autoimmunity" [87–89]. This school of thought posits that the immune response to CNS injury or disease is not harmful but in fact essential to counteracting CNS tissue damage generated by this insult [104]. Following this logic, the notion of the CNS as an "immune privileged" site would underlie the devastating effects of secondary damage and its poor intrinsic regenerative capacity. Therefore, facilitating this neuroimmune axis would confer protective effects and promote restoration of tissue in response to pathological conditions. Although this theory encompasses T cell-mediated effects, macrophages are also included as important players in this protective immune response. For example, injection of macrophages pre-exposed to sciatic nerve segments promotes axon regeneration and restoration of electrophysiological function, as well as hindlimb locomotor recovery in the injured rat spinal cord [77]. Similar results were found where enhancing monocyte numbers and their recruitment to the injury site through either vaccination or adoptive transfer improved recovery, and depleting this monocyte pool through cell-specific ablation worsened histological and functional outcomes after SCI [90]. Furthermore, depletion of microglia and peripherally derived macrophages has been shown to impair remyelination and alter OPC differentiation and signaling processes [42, 50, 60], which may represent another avenue of macrophage modulation of neural regeneration.

Immune activation via mild inflammation is another proposed means of promoting regeneration. This phenomenon was demonstrated in a recent study where systemic LPS injection was paired with rehabilitative training, resulting in improved forelimb function than compared to training alone in rats after SCI [95]. The notion of a "conditioning lesion," where a peripheral nerve lesion results in enhanced regeneration of the associated central ascending dorsal column sensory axons [80], is also consistent with the idea that priming the immune system can promote regeneration and repair. This conditioning lesion phenomenon has traditionally been thought to result from changes in intrinsic growth mechanisms, and has been commonly used to investigate intrinsic regenerative capacity of neurons. However, recent studies implicate macrophages as the major mediator of the axon regenerative response. Hervera et al [34] recently demonstrated that NOX2-containing endosomes released by macrophages at the peripheral nerve lesion site are taken up by severed axons and then retrogradely transported to the ganglion cell body. Here, NOX2 inactivates PTEN, stimulating regeneration of the central DRG (dorsal root ganglion) axons. It is not yet known why such pro-regenerative mechanism exists only after lesion of peripheral but not central axons. In addition to effects at the peripheral nerve lesion site, macrophages also affect DRG neurons at the cell body region. Conditioning lesion of the peripheral nerve is typically associated with accumulation of macrophages in the corresponding DRG [53], and the conditioning effect is diminished upon genetic deletion of CCR2, or mimicked upon overexpression of CCL2 both in vitro and in vivo [44, 66, 108].

Macrophages may also elicit a beneficial effect through interactions with CNS resident microglia by modulating the phagocytic and inflammatory response. This is supported by the observation that the onset of macrophage infiltration to the injury site is associated with a decrease in microglia phagocytosis [27]. Additionally, co-culturing macrophages and

microglia isolated from the injury site resulted in a decrease in microglial expression of inflammatory cytokines such as IL-1β and reduced phagocytic capacity, potentially due to prostaglandin E2 signaling via EP2 receptor activity [27]. These effects were further probed in vivo using CCR2-deficient mice incapable of effectively recruiting monocytes to the injury site, which resulted in enhanced microglial activation and impaired motor recovery after SCI, providing further evidence of anti-inflammatory consequences of macrophage recruitment after SCI [27]. However, these results are in contrast to macrophage depletion studies mentioned above, where mostly beneficial effects were observed. One possibility that remains to be tested in the future is that macrophage depletion (using splenectomy of clodronate liposomes) and CCR2 genetic deletion target different macrophage populations with distinct effects on SCI pathology.

Macrophages may also promote spinal cord repair by mediating tissue remodeling. The injection of a novel polyphosphazene-based hydrogel into the injury site virtually eliminated cavitation, significantly improved tissue sparing, and increased axonal growth as well as locomotor recovery after SCI in rats [35]. Interestingly, these beneficial effects were mediated by infiltration of M2-like macrophages into the hydrogel implant, which promoted infiltration of perivascular fibroblasts and extracellular matrix remodeling. Elimination of macrophage infiltration via minocycline administration, or elimination of fibrosis via taxol administration both led to cavitation at the injury site. These responses are similar to proper wound healing processes that are mediated by M2-like macrophages in peripheral tissue [43].

There is also abundant evidence indicating that macrophages are deleterious to regeneration and spinal cord repair. Numerous experiments utilizing models of macrophage depletion/ablation, such as liposome clodronate administration, have reported improved functional and histological outcomes, suggesting that macrophages are neurotoxic and impede recovery [29, 48, 73, 107]. Macrophages may mediate these harmful effects through direct interactions, as in vivo two-photon imaging of GFP^+ bone marrow chimeras showed that blood-born macrophages, and not resident microglia, interact directly with axons during secondary dieback after SCI [19]. Alternatively, it could be the more indirect effects of macrophage depletion, such as reduced fibrotic scarring, as the predominant mechanism underlying this functional improvement in animals subjected macrophage depletion after SCI [107].

These positive and negative effects of macrophages on cellular regeneration have been attributed mainly to their different polarization states (i.e. M1-like or M2-like). In addition to macrophage origin as mentioned above, their mode of entry into the CNS upon injury may also influence the polarization state. Reparative and neuroprotective macrophages were found to originate from the choroid plexus and travel to the injury site via the central canal whereas more inflammatory macrophages of hematopoietic origin entered through the meningeal barrier [91]. However, these divergent effects are not necessarily the byproduct of distinct populations. For example, zymosan-activated macrophages have been shown to elicit both deleterious and pro-regenerative processes in the spinal cord, suggesting that these responses can co-occur in the same macrophage subtype [25]. The crux of the issue, therefore, is not completely abolishing inflammation, but ensuring the efficient and proper

synchronization of the transition to a more pro-regenerative phenotype at the appropriate time point, a resolving phase characteristic of the classical wound healing model and observed in PNS, but not CNS, injury disease models.

Concluding remarks and future directions

It is now abundantly clear that macrophages play a central role in the wound healing process after SCI. However, there are fundamental issues that remain to be addressed. The acute origin of macrophages seems to be from a monocyte reservoir in the spleen, but there seems to be an alternative source during the chronic time points (Figure 2). Is there an endogenous pool that self-renews, or is there a constant influx of bone marrow-derived monocytes from the circulation? What are the functional similarities and differences between these temporally separated population of macrophages? In addition, although the M1/M2 classification of macrophages is useful conceptually, these polarization states remain elusive in vivo. In fact, macrophage-specific transcriptomic analysis suggests that SCI macrophages are best characterized as foam cells, and future studies at the single cell level will bring us closer to understanding the true identity and heterogeneity of macrophages in the injured CNS. A better understanding of the origin of macrophages may provide important insight into their fate and function after spinal cord injury, which can help identify novel therapeutic targets.

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References

1. Basso DM, Fisher LC, Anderson AJ, Jakeman LB, McTigue DM, Popovich PG (2006) Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. J Neurotrauma 23: 635–659 Doi 10.1089/neu.2006.23.635 [PubMed: 16689667]
2. Beck KD, Nguyen HX, Galvan MD, Salazar DL, Woodruff TM, Anderson AJ (2010) Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. Brain 133: 433–447 Doi 10.1093/brain/awp322 [PubMed: 20085927]
3. Blomster LV, Brennan FH, Lao HW, Harle DW, Harvey AR, Ruitenberg MJ (2013) Mobilisation of the splenic monocyte reservoir and peripheral CX(3)CR1 deficiency adversely affects recovery from spinal cord injury. Exp Neurol 247: 226–240 Doi 10.1016/j.expneurol.2013.05.002 [PubMed: 23664962]
4. Boato F, Rosenberger K, Nelissen S, Geboes L, Peters EM, Nitsch R, Hendrix S (2013) Absence of IL-1beta positively affects neurological outcome, lesion development and axonal plasticity after spinal cord injury. J Neuroinflammation 10: 6 Doi 10.1186/1742-2094-10-6 [PubMed: 23317037]
5. Bogie JF, Jorissen W, Mailleux J, Nijland PG, Zelcer N, Vanmierlo T, Van Horssen J, Stinissen P, Hellings N, Hendriks JJ (2013) Myelin alters the inflammatory phenotype of macrophages by activating PPARs. Acta Neuropathol Commun 1: 43 Doi 10.1186/2051-5960-1-43 [PubMed: 24252308]
6. Bogie JF, Mailleux J, Wouters E, Jorissen W, Grajchen E, Vanmol J, Wouters K, Hellings N, Van Horsen J, Vanmierlo T et al. (2017) Scavenger receptor collectin placenta 1 is a novel receptor involved in the uptake of myelin by phagocytes. Sci Rep 7: 44794 Doi 10.1038/srep44794 [PubMed: 28317919]

7. Boven LA, Van Meurs M, Van Zwam M, Wierenga-Wolf A, Hintzen RQ, Boot RG, Aerts JM, Amor S, Nieuwenhuis EE, Laman JD (2006) Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. Brain 129: 517–526 Doi 10.1093/brain/awh707 [PubMed: 16364958]
8. Braun DA, Fribourg M, Sealfon SC (2013) Cytokine Response Is Determined by Duration of Receptor and Signal Transducers and Activators of Transcription 3 (STAT3) Activation. Journal of Biological Chemistry 288: 2986–2993 Doi 10.1074/jbc.M112.386573 [PubMed: 23166328]
9. Brown MS, Basu SK, Falck JR, Ho YK, Goldstein JL (1980) The scavenger cell pathway for lipoprotein degradation: specificity of the binding site that mediates the uptake of negatively-charged LDL by macrophages. J Supramol Struct 13: 67–81 Doi 10.1002/jss.400130107 [PubMed: 6255257]
10. Brown MS, Goldstein JL (1979) Receptor-mediated endocytosis: insights from the lipoprotein receptor system. Proc Natl Acad Sci U S A 76: 3330–3337 [PubMed: 226968]
11. Cortez-Retamozo V, Etzrodt M, Newton A, Rauch PJ, Chudnovskiy A, Berger C, Ryan RJ, Iwamoto Y, Marinelli B, Gorbatov R et al. (2012) Origins of tumor-associated macrophages and neutrophils. Proc Natl Acad Sci U S A 109: 2491–2496 Doi 10.1073/pnas.1113744109 [PubMed: 22308361]
12. Coste A, Dubourdeau M, Linas MD, Cassaing S, Lepert JC, Balard P, Chalmeton S, Bernad J, Orfila C, Seguela JP et al. (2003) PPAR gamma promotes mannose receptor gene expression in murine macrophages and contributes to the induction of this receptor by IL-13. Immunity 19: 329–339 Doi Doi 10.1016/S1074-7613(03)00229-2 [PubMed: 14499109]
13. da Costa CC, van der Laan LJ, Dijkstra CD, Bruck W (1997) The role of the mouse macrophage scavenger receptor in myelin phagocytosis. Eur J Neurosci 9: 2650–2657 [PubMed: 9517470]
14. Davies LC, Rosas M, Jenkins SJ, Liao CT, Scurr MJ, Brombacher F, Fraser DJ, Allen JE, Jones SA, Taylor PR (2013) Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. Nat Commun 4: 1886 Doi 10.1038/ncomms2877 [PubMed: 23695680]
15. Didangelos A, Iberl M, Vinsland E, Bartus K, Bradbury EJ (2014) Regulation of IL-10 by chondroitinase ABC promotes a distinct immune response following spinal cord injury. J Neurosci 34: 16424–16432 Doi 10.1523/JNEUROSCI.2927-14.2014 [PubMed: 25471580]
16. Didangelos A, Puglia M, Iberl M, Sanchez-Bellot C, Roschitzki B, Bradbury EJ (2016) High-throughput proteomics reveal alarmins as amplifiers of tissue pathology and inflammation after spinal cord injury. Sci Rep 6: 21607 Doi 10.1038/srep21607 [PubMed: 26899371]
17. Donnelly DJ, Longbrake EE, Shawler TM, Kigerl KA, Lai W, Tovar CA, Ransohoff RM, Popovich PG (2011) Deficient CX3CR1 signaling promotes recovery after mouse spinal cord injury by limiting the recruitment and activation of Ly6Clo/iNOS+ macrophages. J Neurosci 31: 9910–9922 Doi 10.1523/JNEUROSCI.2114-11.2011 [PubMed: 21734283]
18. Donnelly DJ, Popovich PG (2008) Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp Neurol 209: 378–388 Doi 10.1016/j.expneurol.2007.06.009 [PubMed: 17662717]
19. Evans TA, Barkauskas DS, Myers JT, Hare EG, You JQ, Ransohoff RM, Huang AY, Silver J (2014) High-resolution intravital imaging reveals that blood-derived macrophages but not resident microglia facilitate secondary axonal dieback in traumatic spinal cord injury. Exp Neurol 254: 109–120 Doi 10.1016/j.expneurol.2014.01.013 [PubMed: 24468477]
20. Fang P, Li X, Dai J, Cole L, Camacho JA, Zhang Y, Ji Y, Wang J, Yang XF, Wang H (2018) Immune cell subset differentiation and tissue inflammation. J Hematol Oncol 11: 97 Doi 10.1186/s13045-018-0637-x [PubMed: 30064449]
21. Fleming JC, Norenberg MD, Ramsay DA, Dekaban GA, Marcillo AE, Saenz AD, Pasquale-Styles M, Dietrich WD, Weaver LC (2006) The cellular inflammatory response in human spinal cords after injury. Brain 129: 3249–3269 Doi 10.1093/brain/awl296 [PubMed: 17071951]
22. Francos-Quijorna I, Amo-Aparicio J, Martinez-Muriana A, Lopez-Vales R (2016) IL-4 Drives Microglia and Macrophages Toward a Phenotype Conducive for Tissue Repair and Functional Recovery After Spinal Cord Injury. Glia 64: 2079–2092 Doi 10.1002/glia.23041 [PubMed: 27470986]

23. Gaultier A, Wu XH, Le Moan N, Takimoto S, Mukandala G, Akassoglou K, Campana WM, Gonias SL (2009) Low-density lipoprotein receptor-related protein 1 is an essential receptor for myelin phagocytosis. J Cell Sci 122: 1155–1162 Doi 10.1242/jcs.040717 [PubMed: 19299462]
24. Geissmann F, Jung S, Littman DR (2003) Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 19: 71–82 [PubMed: 12871640]
25. Gensel JC, Nakamura S, Guan Z, van Rooijen N, Ankeny DP, Popovich PG (2009) Macrophages promote axon regeneration with concurrent neurotoxicity. J Neurosci 29: 3956–3968 Doi 10.1523/JNEUROSCI.3992-08.2009 [PubMed: 19321792]
26. Ginhoux F, Prinz M (2015) Origin of microglia: current concepts and past controversies. Cold Spring Harb Perspect Biol 7: a020537 Doi 10.1101/cshperspect.a020537 [PubMed: 26134003]
27. Greenhalgh AD, Zarruk JG, Healy LM, Baskar Jesudasan SJ, Jhelum P, Salmon CK, Formanek A, Russo MV, Antel JP, McGavern DB et al. (2018) Peripherally derived macrophages modulate microglial function to reduce inflammation after CNS injury. PLoS Biol 16: e2005264 Doi 10.1371/journal.pbio.2005264 [PubMed: 30332405]
28. Grell M, Douni E, Wajant H, Lohden M, Clauss M, Maxeiner B, Georgopoulos S, Lesslauer W, Kollias G, Pfizenmaier K et al. (1995) The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell 83: 793–802 [PubMed: 8521496]
29. Gris D, Marsh DR, Oatway MA, Chen Y, Hamilton EF, Dekaban GA, Weaver LC (2004) Transient blockade of the CD11d/CD18 integrin reduces secondary damage after spinal cord injury, improving sensory, autonomic, and motor function. J Neurosci 24: 4043–4051 Doi 10.1523/JNEUROSCI.5343-03.2004 [PubMed: 15102919]
30. Guerrero AR, Uchida K, Nakajima H, Watanabe S, Nakamura M, Johnson WE, Baba H (2012) Blockade of interleukin-6 signaling inhibits the classic pathway and promotes an alternative pathway of macrophage activation after spinal cord injury in mice. J Neuroinflammation 9: 40 Doi 10.1186/1742-2094-9-40 [PubMed: 22369693]
31. Guo L, Rolfe AJ, Wang X, Tai W, Cheng Z, Cao K, Chen X, Xu Y, Sun D, Li J et al. (2016) Rescuing macrophage normal function in spinal cord injury with embryonic stem cell conditioned media. Mol Brain 9: 48 Doi 10.1186/s13041-016-0233-3 [PubMed: 27153974]
32. Hawthorne AL, Popovich PG (2011) Emerging concepts in myeloid cell biology after spinal cord injury. Neurotherapeutics 8: 252–261 Doi 10.1007/s13311-011-0032-6 [PubMed: 21400005]
33. Healy LM, Perron G, Won SY, Michell-Robinson MA, Rezk A, Ludwin SK, Moore CS, Hall JA, Bar-Or A, Antel JP (2016) MerTK Is a Functional Regulator of Myelin Phagocytosis by Human Myeloid Cells. J Immunol 196: 3375–3384 Doi 10.4049/jimmunol.1502562 [PubMed: 26962228]
34. Hervera A, De Virgiliis F, Palmisano I, Zhou L, Tantardini E, Kong G, Hutson T, Danzi MC, Perry RB, Santos CXC et al. (2018) Reactive oxygen species regulate axonal regeneration through the release of exosomal NADPH oxidase 2 complexes into injured axons. Nat Cell Biol 20: 307–319 Doi 10.1038/s41556-018-0039-x [PubMed: 29434374]
35. Hong LTA, Kim YM, Park HH, Hwang DH, Cui Y, Lee EM, Yahn S, Lee JK, Song SC, Kim BG (2017) An injectable hydrogel enhances tissue repair after spinal cord injury by promoting extracellular matrix remodeling. Nat Commun 8: 533 Doi 10.1038/s41467-017-00583-8 [PubMed: 28912446]
36. Iannotti CA, Clark M, Horn KP, van Rooijen N, Silver J, Steinmetz MP (2011) A combination immunomodulatory treatment promotes neuroprotection and locomotor recovery after contusion SCI. Exp Neurol 230: 3–15 Doi 10.1016/j.expneurol.2010.03.010 [PubMed: 20338167]
37. Kezic J, McMenamin PG (2008) Differential turnover rates of monocyte-derived cells in varied ocular tissue microenvironments. J Leukoc Biol 84: 721–729 Doi 10.1189/jlb.0308166 [PubMed: 18577714]
38. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci 29: 13435–13444 Doi 10.1523/JNEUROSCI.3257-09.2009 [PubMed: 19864556]

39. Kigerl KA, McGaughy VM, Popovich PG (2006) Comparative analysis of lesion development and intraspinal inflammation in four strains of mice following spinal contusion injury. J Comp Neurol 494: 578–594 Doi 10.1002/cne.20827 [PubMed: 16374800]
40. Kopper TJ, Gensel JC (2018) Myelin as an inflammatory mediator: Myelin interactions with complement, macrophages, and microglia in spinal cord injury. J Neurosci Res 96: 969–977 Doi 10.1002/jnr.24114 [PubMed: 28696010]
41. Kostyk SK, Popovich PG, Stokes BT, Wei P, Jakeman LB (2008) Robust axonal growth and a blunted macrophage response are associated with impaired functional recovery after spinal cord injury in the MRL/MpJ mouse. Neuroscience 156: 498–514 Doi 10.1016/j.neuroscience.2008.08.013 [PubMed: 18786615]
42. Kotter MR, Zhao C, van Rooijen N, Franklin RJ (2005) Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. Neurobiol Dis 18: 166–175 Doi 10.1016/j.nbd.2004.09.019 [PubMed: 15649707]
43. Krzyszczyk P, Schloss R, Palmer A, Berthiaume F (2018) The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes. Front Physiol 9: 419 Doi 10.3389/fphys.2018.00419 [PubMed: 29765329]
44. Kwon MJ, Shin HY, Cui Y, Kim H, Thi AH, Choi JY, Kim EY, Hwang DH, Kim BG (2015) CCL2 Mediates Neuron-Macrophage Interactions to Drive Proregenerative Macrophage Activation Following Preconditioning Injury. J Neurosci 35: 15934–15947 Doi 10.1523/JNEUROSCI.1924-15.2015 [PubMed: 26631474]
45. Landsman L, Bar-On L, Zernecke A, Kim KW, Krauthgamer R, Shagdarsuren E, Lira SA, Weissman IL, Weber C, Jung S (2009) CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. Blood 113: 963–972 Doi 10.1182/blood-2008-07-170787 [PubMed: 18971423]
46. Lee SI, Jeong SR, Kan YM, Han DH, Jin BK, Namgung U, Kim BG (2010) Endogenous Expression of Interleukin-4 Regulates Macrophage Activation and Confines Cavity Formation After Traumatic Spinal Cord Injury. Journal of Neuroscience Research 88: 2409–2419 Doi 10.1002/jnr.22411 [PubMed: 20623539]
47. Lee SM, Rosen S, Weinstein P, van Rooijen N, Noble-Haeusslein LJ (2011) Prevention of both neutrophil and monocyte recruitment promotes recovery after spinal cord injury. J Neurotrauma 28: 1893–1907 Doi 10.1089/neu.2011.1860 [PubMed: 21657851]
48. Lee YS, Funk LH, Lee JK, Bunge MB (2018) Macrophage depletion and Schwann cell transplantation reduce cyst size after rat contusive spinal cord injury. Neural Regen Res 13: 684–691 Doi 10.4103/1673-5374.230295 [PubMed: 29722321]
49. Ley K, Pramod AB, Croft M, Ravichandran KS, Ting JP (2016) How Mouse Macrophages Sense What Is Going On. Front Immunol 7: 204 Doi 10.3389/fimmu.2016.00204 [PubMed: 27313577]
50. Li WW, Setzu A, Zhao C, Franklin RJ (2005) Minocycline-mediated inhibition of microglia activation impairs oligodendrocyte progenitor cell responses and remyelination in a non-immune model of demyelination. J Neuroimmunol 158: 58–66 Doi 10.1016/j.jneuroim.2004.08.011 [PubMed: 15589038]
51. Lindborg JA, Mack M, Zigmond RE (2017) Neutrophils Are Critical for Myelin Removal in a Peripheral Nerve Injury Model of Wallerian Degeneration. J Neurosci 37: 10258–10277 Doi 10.1523/JNEUROSCI.2085-17.2017 [PubMed: 28912156]
52. Liu T, Zhang L, Joo D, Sun SC (2017) NF-kappaB signaling in inflammation. Signal Transduct Target Ther 2: Doi 10.1038/sigtrans.2017.23
53. Lu X, Richardson PM (1993) Responses of macrophages in rat dorsal root ganglia following peripheral nerve injury. J Neurocytol 22: 334–341 [PubMed: 8315414]
54. Ma M, Wei P, Wei T, Ransohoff RM, Jakeman LB (2004) Enhanced axonal growth into a spinal cord contusion injury site in a strain of mouse (129X1/SvJ) with a diminished inflammatory response. J Comp Neurol 474: 469–486 Doi 10.1002/cne.20149 [PubMed: 15174067]
55. Ma M, Wei T, Boring L, Charo IF, Ransohoff RM, Jakeman LB (2002) Monocyte recruitment and myelin removal are delayed following spinal cord injury in mice with CCR2 chemokine receptor deletion. J Neurosci Res 68: 691–702 Doi 10.1002/jnr.10269 [PubMed: 12111830]

56. Martinez FO, Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6: 13 Doi 10.12703/P6-13 [PubMed: 24669294]
57. Mawhinney LA, Thawer SG, Lu WY, Rooijen N, Weaver LC, Brown A, Dekaban GA (2012) Differential detection and distribution of microglial and hematogenous macrophage populations in the injured spinal cord of lys-EGFP-ki transgenic mice. J Neuropathol Exp Neurol 71: 180–197 Doi 10.1097/NEN.0b013e3182479b41 [PubMed: 22318123]
58. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164: 6166–6173 [PubMed: 10843666]
59. Mills CD, Shearer J, Evans R, Caldwell MD (1992) Macrophage arginine metabolism and the inhibition or stimulation of cancer. J Immunol 149: 2709–2714 [PubMed: 1401910]
60. Miron VE, Boyd A, Zhao JW, Yuen TJ, Ruckh JM, Shadrach JL, van Wijngaarden P, Wagers AJ, Williams A, Franklin RJM et al. (2013) M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat Neurosci 16: 1211–1218 Doi 10.1038/nn.3469 [PubMed: 23872599]
61. Mironets E, Osei-Owusu P, Bracchi-Ricard V, Fischer R, Owens EA, Ricard J, Wu D, Saltos T, Collyer E, Hou S et al. (2018) Soluble TNFalpha Signaling within the Spinal Cord Contributes to the Development of Autonomic Dysreflexia and Ensuing Vascular and Immune Dysfunction after Spinal Cord Injury. J Neurosci 38: 4146–4162 Doi 10.1523/JNEUROSCI.2376-17.2018 [PubMed: 29610439]
62. Moore KJ, Sheedy FJ, Fisher EA (2013) Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 13: 709–721 Doi 10.1038/nri3520 [PubMed: 23995626]
63. Mukhamedshina YO, Akhmetzyanova ER, Martynova EV, Khaiboullina SF, Galieva LR, Rizvanov AA (2017) Systemic and Local Cytokine Profile following Spinal Cord Injury in Rats: A Multiplex Analysis. Front Neurol 8: Doi ARTN 581 10.3389/fneur.2017.00581
64. Murray PJ (2006) Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. Curr Opin Pharmacol 6: 379–386 Doi 10.1016/j.coph.2006.01.010 [PubMed: 16713356]
65. Nguyen HX, O'Barr TJ, Anderson AJ (2007) Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. J Neurochem 102: 900–912 Doi 10.1111/j.1471-4159.2007.04643.x [PubMed: 17561941]
66. Niemi JP, DeFrancesco-Lisowitz A, Cregg JM, Howarth M, Zigmond RE (2016) Overexpression of the monocyte chemokine CCL2 in dorsal root ganglion neurons causes a conditioning-like increase in neurite outgrowth and does so via a STAT3 dependent mechanism. Exp Neurol 275 Pt 1: 25–37 Doi 10.1016/j.expneurol.2015.09.018 [PubMed: 26431741]
67. Noble LJ, Donovan F, Igarashi T, Goussev S, Werb Z (2002) Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. J Neurosci 22: 7526–7535 [PubMed: 12196576]
68. Novrup HG, Bracchi-Ricard V, Ellman DG, Ricard J, Jain A, Runko E, Lyck L, Yli-Karjanmaa M, Szymkowski DE, Pearse DD et al. (2014) Central but not systemic administration of XPro1595 is therapeutic following moderate spinal cord injury in mice. J Neuroinflammation 11: 159 Doi 10.1186/s12974-014-0159-6 [PubMed: 25204558]
69. Odegaard JI, Ricardo-Gonzalez RR, Eagle AR, Vats D, Morel CR, Goforth MH, Subramanian V, Mukundan L, Ferrante AW, Chawla A (2008) Alternative M2 activation of Kupffer cells by PPAR delta ameliorates obesity-induced insulin resistance. Cell Metab 7: 496–507 Doi 10.1016/j.cmet.2008.04.003 [PubMed: 18522831]
70. Papatheodorou A, Stein A, Bank M, Sison CP, Gibbs K, Davies P, Bloom O (2017) High-Mobility Group Box 1 (HMGB1) Is Elevated Systemically in Persons with Acute or Chronic Traumatic Spinal Cord Injury. J Neurotrauma 34: 746–754 Doi 10.1089/neu.2016.4596 [PubMed: 27673428]
71. Perrin FE, Lacroix S, Aviles-Trigueros M, David S (2005) Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1alpha and interleukin-1beta in Wallerian degeneration. Brain 128: 854–866 Doi 10.1093/brain/awh407 [PubMed: 15689362]
72. Pineau I, Lacroix S (2007) Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500: 267–285 Doi 10.1002/cne.21149 [PubMed: 17111361]

73. Popovich PG, Guan Z, Wei P, Huitinga I, van Rooijen N, Stokes BT (1999) Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. Exp Neurol 158: 351–365 Doi 10.1006/exnr.1999.7118 [PubMed: 10415142]
74. Popovich PG, Hickey WF (2001) Bone marrow chimeric rats reveal the unique distribution of resident and recruited macrophages in the contused rat spinal cord. J Neuropathol Exp Neurol 60: 676–685 [PubMed: 11444796]
75. Porta C, Riboldi E, Ippolito A, Sica A (2015) Molecular and epigenetic basis of macrophage polarized activation. Semin Immunol 27: 237–248 Doi 10.1016/j.smim.2015.10.003 [PubMed: 26561250]
76. PrabhuDas MR, Baldwin CL, Bollyky PL, Bowdish DME, Drickamer K, Febbraio M, Herz J, Kobzik L, Krieger M, Loike J et al. (2017) A Consensus Definitive Classification of Scavenger Receptors and Their Roles in Health and Disease. J Immunol 198: 3775–3789 Doi 10.4049/jimmunol.1700373 [PubMed: 28483986]
77. Rapalino O, Lazarov-Spiegler O, Agranov E, Velan GJ, Yoles E, Fraidakis M, Solomon A, Gepstein R, Katz A, Belkin M et al. (1998) Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. Nat Med 4: 814–821 [PubMed: 9662373]
78. Reichert F, Rotshenker S (2003) Complement-receptor-3 and scavenger-receptor-AI/II mediated myelin phagocytosis in microglia and macrophages. Neurobiol Dis 12: 65–72 [PubMed: 12609490]
79. Ren Y, Stuart L, Lindberg FP, Rosenkranz AR, Chen Y, Mayadas TN, Savill J (2001) Nonphlogistic clearance of late apoptotic neutrophils by macrophages: efficient phagocytosis independent of beta 2 integrins. J Immunol 166: 4743–4750 [PubMed: 11254736]
80. Richardson PM, Issa VM (1984) Peripheral injury enhances central regeneration of primary sensory neurones. Nature 309: 791–793 [PubMed: 6204205]
81. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, Gorbatov R, Sukhova GK, Gerhardt LM, Smyth D et al. (2013) Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med 19: 1166–1172 Doi 10.1038/nm.3258 [PubMed: 23933982]
82. Rolls A, Shechter R, London A, Segev Y, Jacob-Hirsch J, Amariglio N, Rechavi G, Schwartz M (2008) Two faces of chondroitin sulfate proteoglycan in spinal cord repair: a role in microglia/macrophage activation. PLoS Med 5: e171 Doi 10.1371/journal.pmed.0050171 [PubMed: 18715114]
83. Rotshenker S (2003) Microglia and macrophage activation and the regulation of complement-receptor-3 (CR3/MAC-1)-mediated myelin phagocytosis in injury and disease. J Mol Neurosci 21: 65–72 Doi 10.1385/JMN:21:1:65 [PubMed: 14500997]
84. Saederup N, Cardona AE, Croft K, Mizutani M, Cotleur AC, Tsou CL, Ransohoff RM, Charo IF (2010) Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One 5: e13693 Doi 10.1371/journal.pone.0013693 [PubMed: 21060874]
85. Sanz E, Yang L, Su T, Morris DR, McKnight GS, Amieux PS (2009) Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. Proc Natl Acad Sci U S A 106: 13939–13944 Doi 10.1073/pnas.0907143106 [PubMed: 19666516]
86. Savill J, Hogg N, Ren Y, Haslett C (1992) Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. J Clin Invest 90: 1513–1522 Doi 10.1172/JCI116019 [PubMed: 1383273]
87. Schwartz M (2003) Macrophages and microglia in central nervous system injury: are they helpful or harmful? J Cereb Blood Flow Metab 23: 385–394 Doi 10.1097/01.WCB.0000061881.75234.5E [PubMed: 12679714]
88. Schwartz M, Raposo C (2014) Protective Autoimmunity: A Unifying Model for the Immune Network Involved in CNS Repair. Neuroscientist 20: 343–358 Doi 10.1177/1073858413516799 [PubMed: 24395337]
89. Schwartz M, Yoles E (2005) Macrophages and dendritic cells treatment of spinal cord injury: from the bench to the clinic. Acta Neurochir Suppl 93: 147–150 [PubMed: 15986745]

90. Shechter R, London A, Varol C, Raposo C, Cusimano M, Yovel G, Rolls A, Mack M, Pluchino S, Martino G et al. (2009) Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med 6: e1000113 Doi 10.1371/journal.pmed.1000113 [PubMed: 19636355]
91. Shechter R, Miller O, Yovel G, Rosenzweig N, London A, Ruckh J, Kim KW, Klein E, Kalchenko V, Bendel P et al. (2013) Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. Immunity 38: 555–569 Doi 10.1016/j.immuni.2013.02.012 [PubMed: 23477737]
92. Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, Halle A, Rayner KJ, Boyer L, Zhong R, Frazier WA et al. (2010) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nat Immunol 11: 155–161 Doi 10.1038/ni.1836 [PubMed: 20037584]
93. Sun X, Wang X, Chen T, Li T, Cao K, Lu A, Chen Y, Sun D, Luo J, Fan J et al. (2010) Myelin activates FAK/Akt/NF-kappaB pathways and provokes CR3-dependent inflammatory response in murine system. PLoS One 5: e9380 Doi 10.1371/journal.pone.0009380 [PubMed: 20186338]
94. Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, Figueiredo JL, Kohler RH, Chudnovskiy A, Waterman P et al. (2009) Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 325: 612–616 Doi 10.1126/science.1175202 [PubMed: 19644120]
95. Torres-Espin A, Forero J, Fenrich KK, Lucas-Osma AM, Krajacic A, Schmidt E, Vavrek R, Raposo P, Bennett DJ, Popovich PG et al. (2018) Eliciting inflammation enables successful rehabilitative training in chronic spinal cord injury. Brain 141: 1946–1962 Doi 10.1093/brain/awy128 [PubMed: 29860396]
96. Tymoszuk P, Evens H, Marzola V, Wachowicz K, Wasmer MH, Datta S, Muller-Holzner E, Fiegl H, Bock G, van Rooijen N et al. (2014) In situ proliferation contributes to accumulation of tumor-associated macrophages in spontaneous mammary tumors. Eur J Immunol 44: 2247–2262 Doi 10.1002/eji.201344304 [PubMed: 24796276]
97. Wang C, Yu X, Cao Q, Wang Y, Zheng G, Tan TK, Zhao H, Zhao Y, Wang Y, Harris D (2013) Characterization of murine macrophages from bone marrow, spleen and peritoneum. BMC Immunol 14: 6 Doi 10.1186/1471-2172-14-6 [PubMed: 23384230]
98. Wang N, Liang H, Zen K (2014) Molecular mechanisms that influence the macrophage m1–m2 polarization balance. Front Immunol 5: 614 Doi 10.3389/fimmu.2014.00614 [PubMed: 25506346]
99. Wang X, Cao K, Sun X, Chen Y, Duan Z, Sun L, Guo L, Bai P, Sun D, Fan J et al. (2015) Macrophages in spinal cord injury: phenotypic and functional change from exposure to myelin debris. Glia 63: 635–651 Doi 10.1002/glia.22774 [PubMed: 25452166]
100. Winkler IG, Sims NA, Pettit AR, Barbier V, Nowlan B, Helwani F, Poulton IJ, van Rooijen N, Alexander KA, Raggatt LJ et al. (2010) Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood 116: 4815–4828 Doi 10.1182/blood-2009-11-253534 [PubMed: 20713966]
101. Yan P, Liu N, Kim GM, Xu J, Xu J, Li Q, Hsu CY, Xu XM (2003) Expression of the type 1 and type 2 receptors for tumor necrosis factor after traumatic spinal cord injury in adult rats. Exp Neurol 183: 286–297 [PubMed: 14552870]
102. Yang J, Zhang L, Yu C, Yang XF, Wang H (2014) Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark Res 2: 1 Doi 10.1186/2050-7771-2-1 [PubMed: 24398220]
103. Yasukawa H, Ohishi M, Mori H, Murakami M, Chinen T, Aki D, Hanada T, Takeda K, Akira S, Hoshijima M et al. (2003) IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. Nature Immunology 4: 551–556 Doi 10.1038/ni938 [PubMed: 12754507]
104. Yoles E, Hauben E, Palgi O, Agranov E, Gothilf A, Cohen A, Kuchroo V, Cohen IR, Weiner H, Schwartz M (2001) Protective autoimmunity is a physiological response to CNS trauma. J Neurosci 21: 3740–3748 [PubMed: 11356861]
105. Zhou Q, Xiang H, Li A, Lin W, Huang Z, Guo J, Wang P, Chi Y, Xiang K, Xu Y et al. (2018) Activating Adiponectin Signaling with Exogenous AdipoRon Reduces Myelin Lipid Accumulation and Suppresses Macrophage Recruitment after Spinal Cord Injury. J Neurotrauma: Doi 10.1089/neu.2018.5783

106. Zhu Y, Lyapichev K, Lee DH, Motti D, Ferraro NM, Zhang Y, Yahn S, Soderblom C, Zha J, Bethea JR et al. (2017) Macrophage Transcriptional Profile Identifies Lipid Catabolic Pathways That Can Be Therapeutically Targeted after Spinal Cord Injury. J Neurosci 37: 2362–2376 Doi 10.1523/JNEUROSCI.2751-16.2017 [PubMed: 28130359]

107. Zhu Y, Soderblom C, Krishnan V, Ashbaugh J, Bethea JR, Lee JK (2015) Hematogenous macrophage depletion reduces the fibrotic scar and increases axonal growth after spinal cord injury. Neurobiol Dis 74: 114–125 Doi 10.1016/j.nbd.2014.10.024 [PubMed: 25461258]

108. Zigmond RE, Echevarria FD (2019) Macrophage biology in the peripheral nervous system after injury. Prog Neurobiol 173: 102–121 Doi 10.1016/j.pneurobio.2018.12.001 [PubMed: 30579784]

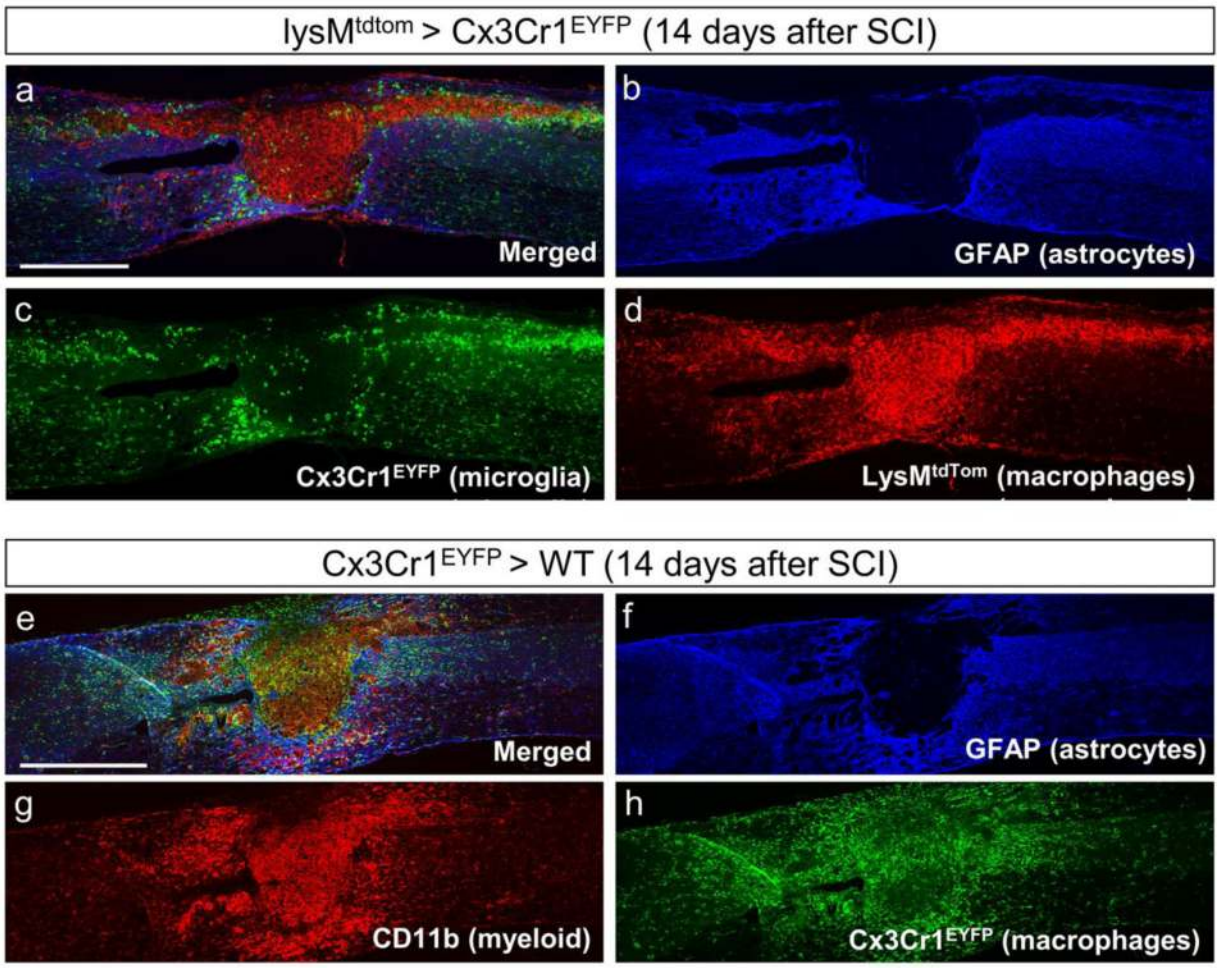

Figure 1. Bone marrow chimeras reveal distinct spatial distribution of macrophages and microglia at the spinal cord injury site.

(a-d) LysM-Cre mice were bred to Rosa26-tdTomato mice to generate $LysM^{tdTom}$ reporter mice in which myeloid cells are labeled with tdTomato. Bone marrow from $LysM^{tdTom}$ donor mice were transplanted into irradiated $Cx3Cr1^{EYFP}$ recipient mice to generate $LysM^{tdTom}$ > $Cx3Cr1^{EYFP}$ chimera mice in which microglia are labeled with EYFP and hematogenous macrophages are labeled with tdTomato. At 14 days after SCI, microglia (c) are distributed mostly around in the lesions site in the $GFAP^+$ astroglial scar region (b), whereas macrophages are located both within the $GFAP^-$ lesion site and in the surrounding astroglial scar (d). (e-h) Bone marrow from $Cx3Cr1^{EYFP}$ donor mice were transplanted into irradiated wildtype recipient mice to generate $Cx3Cr1^{EYFP}$ > WT chimera mice in which only macrophages are labeled with EYFP. In theory, this should result in a similar distribution pattern as $lysM^{tdTom}$ macrophages shown in (d). However, large areas of the $GFAP^-$ region (f) are devoid of EYFP signal (h) even though large number of $CD11b^+$ myeloid cells can be observed immunohistochemically (g). This leads to the false impression that macrophages are located in the surrounding astroglial scar region, and that the $CD11b^+/EYFP^-$ cells in the $GFAP^-$ region are microglia. Scale bar in A, E = 500μm. Unpublished images from [107].

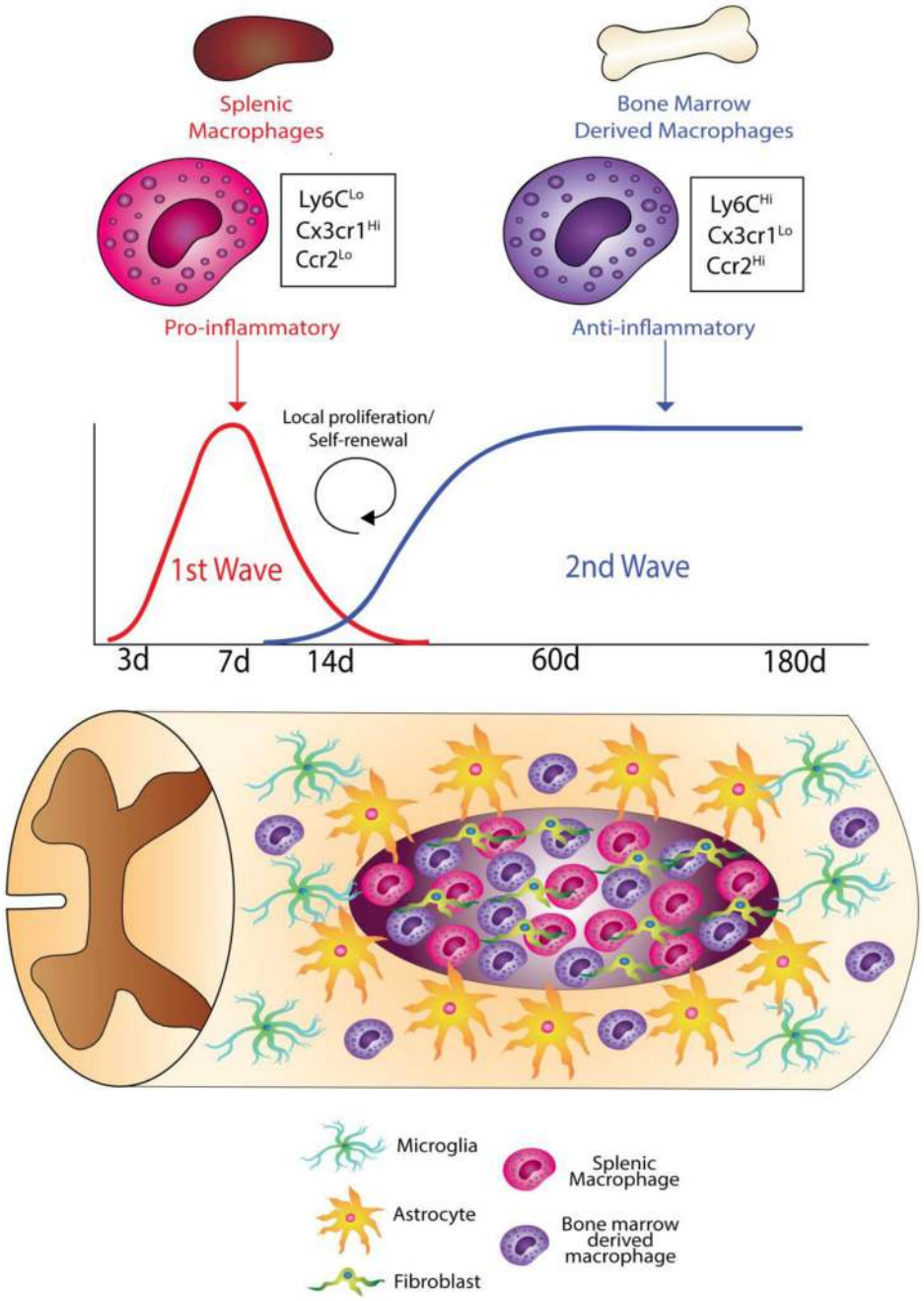

Figure 2: Schematic illustration of putative sources of macrophages after spinal cord injury.

Pro-inflammatory splenic macrophages comprise the major source of the initial wave of macrophage influx. A second wave of anti-inflammatory macrophages could be from either the bone marrow or from a self-renewing source at the injury site. The spinal cord injury site is comprised of a fibrotic core comprised of non-neural cells such as fibroblasts and macrophages surrounded by neural cells such as astrocytes and microglia. Bone marrow chimera studies (Figure 1) demonstrate the presence of Cx3cr1^{lo} macrophages in the fibrotic region, and Cx3cr1^{hi} macrophages in both the fibrotic and surrounding neural tissue. These

two types of macrophages could correspond to the pro-inflammatory and anti-inflammatory subtypes from the spleen and bone marrow, respectively.