Acta Neuropathol (2015) 130:605–618
DOI 10.1007/s00401-015-1482-4

REVIEW

Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury

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Received: 17 August 2015 / Revised: 22 September 2015 / Accepted: 24 September 2015 / Published online: 29 September 2015

Abstract The peripheral nervous system (PNS) has remarkable regenerative abilities after injury. Successful PNS regeneration relies on both injured axons and non-neuronal cells, including Schwann cells and immune cells. Macrophages are the most notable immune cells that play key roles in PNS injury and repair. Upon peripheral nerve injury, a large number of macrophages are accumulated at the injury sites, where they not only contribute to Wallerian degeneration, but also are educated by the local microenvironment and polarized to an anti-inflammatory phenotype (M2), thus contributing to axonal regeneration. Significant progress has been made in understanding how macrophages are educated and polarized in the injured microenvironment as well as how they contribute to axonal regeneration. Following the discussion on the main properties of macrophages and their phenotypes, in this review, we will summarize the current knowledge regarding the mechanisms of macrophage infiltration after PNS injury. Moreover, we will discuss the recent findings elucidating how macrophages are polarized to M2 phenotype in the injured PNS microenvironment, as well as the role and underlying mechanisms of macrophages in peripheral nerve injury, Wallerian degeneration and regeneration. Furthermore, we will highlight the potential application by targeting macrophages in treating peripheral nerve injury and peripheral neuropathies.

Keywords Macrophages · Peripheral nerve · Wallerian degeneration · Peripheral nerve regeneration · Macrophage polarization · Macrophage infiltration

Introduction

Unlike the central nervous system (CNS), where damaged neurons are usually unable to regenerate, axons in the peripheral nervous system (PNS) are capable of regeneration after injury. However, the PNS regeneration is often incomplete, and in turn this can lead to neuropathic conditions [6]. Therefore, there is a great deal of interest in elucidating different factors involved in PNS regeneration, and how they regulate regeneration in injured nerves.

When axons in the PNS are injured, the distal portion progressively degenerates (Wallerian degeneration), a process that is produced by the breakdown of both axons and myelin [80]. The intrinsic degeneration of injured axons has been identified as the key event of Wallerian degeneration [38]. However, PNS is not completely isolated, and the injured axons trigger a complex multi-cellular response that involves multiple components [34, 38]. In addition to cellular responses elicited by injured axons, Wallerian degeneration is accompanied by the de-differentiation of Schwann cells and activation of immune response [34, 80]. Moreover, successful axon regeneration relies on a robust regenerative response of injured axons and the coordinated contribution of non-neuronal cells, including immune cells [38]. Indeed, a large body of evidence demonstrates that immune cells, including innate immune cells (neutrophils, macrophages and dendritic cells) and adaptive cells (T

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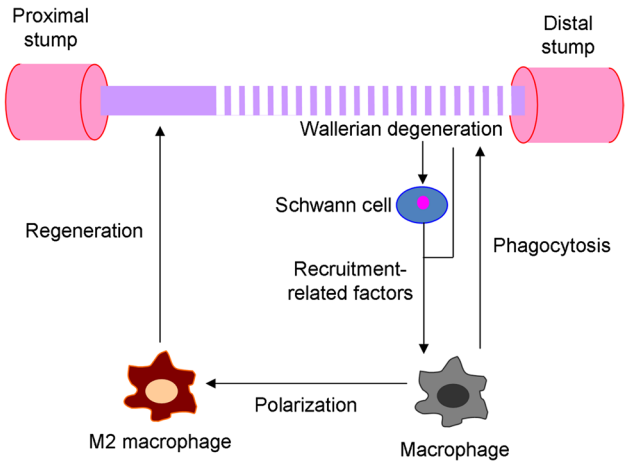

Fig. 1 Schematic diagram summarizing the working model for macrophages in peripheral nerve degeneration and regeneration. Peripheral nerve injury induces the disruption of axon/Schwann cell nerve unit, and then upregulates a variety of chemokines, cytokines and other factors to recruit monocytes/macrophages into injured nerves. The infiltrated macrophages on the one hand contribute to the Wallerian degeneration by removing the debris; on the other hand, they are educated by the local injured microenvironment and are polarized to an anti-inflammatory phenotype (M2), thus promoting peripheral nerve regeneration

and B cells), play a critical role in PNS degeneration and regeneration, and they are recruited into injured sites within hours to days after nerve injury [6]. Among the immune cells operating at the injured sites of peripheral nerves, macrophages are the most notable cell type. A number of studies demonstrated that macrophages not only play a key role for removing myelin debris and modulate the activities of Schwann cells, but also are educated by the local injured microenvironment to promote axonal regeneration by releasing a large number of axonal regeneration-related factors, including extracellular matrix (ECM) proteins, growth factors, cytokines and chemokines [38, 71, 88]. In this review, we first summarize the main properties of macrophages, and then discuss the current knowledge on how monocytes/macrophages are recruited into distal injured sciatic nerves or other nerve tissues as indicated, how they are educated by the injured microenvironment and how they contribute to PNS Wallerian degeneration and axonal regeneration (Fig. 1).

Macrophages and their phenotypes

Macrophages are mononuclear phagocytes that usually originate from progenitor cells residing within the bone marrow [29, 40]. Recently, studies have demonstrated that tissue-resident macrophages, such as microglia, Kupffer cells and alveolar macrophages, originate from yolk-sac-derived myeloid progenitors [39, 91]. The same type of progenitor cells can also differentiate into other cells of the myeloid lineage, such as neutrophils, eosinophils and dendritic cells (DCs), depending on stimuli. The cytokines granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF) are the major stimulus factors for monocyte differentiation from the common myeloid progenitor cells [10]. Upon the emigration from the vasculature into tissues, monocytes can differentiate into distinct populations of macrophages depending on their anatomical location, for example alveolar macrophages in lung, osteoclasts in bone, histiocytes in interstitial connective tissues, Kupffer cells in liver and gut, microglia in brain, and splenic macrophages in spleen (Fig. 2) [10, 19, 40]. Each macrophage population displays a distinct functional profile associated with different gene expression patterns in specific tissue microenvironments [40]. Nonetheless, different populations of macrophages may undertake similar functions upon appropriate stimuli [36]. For example, the specialized phenotype and function of macrophages in gut can be induced from other populations of macrophages following stimulation with intestinal stromal cell products [113]. These findings suggest that macrophages exhibit a remarkable plasticity.

In addition to the plasticity, macrophages are also highly heterogeneous (Fig. 2). It has been shown that macrophages can be divided into "classically activated" pro-inflammatory phenotype (M1) and "alternatively activated" anti-inflammatory phenotype (M2) according to the Th1/Th2 dichotomy (Fig. 2) [10, 19, 40]. In view of recent findings about macrophage phenotypes and functions, a revision of Th1/Th2 dichotomy is needed, because other cytokines and factors, such as interleukin (IL)-10, regulatory T [Treg] products and glucocorticoids, that do not fit clearly with the Th1/Th2 response, but are able to polarize macrophages to specific phenotypes [69]. M1 macrophages are the effector and inducer cells in pro-inflammatory responses, and they are activated by lipopolysaccharide (LPS), interferon gamma (IFN-γ) and/or tumor necrosis factor alpha (TNF-α) [10, 19]. In contrast, M2 macrophages are involved in anti-inflammatory responses and activated by exposure to specific cytokines and factors, including IL-4, IL-13, IL-10, immune complexes, hormones or adenosine A_{2A} receptors ($A_{2A}R$) agonist [10, 19, 32, 33, 35]. These two phenotypes of macrophages have distinct gene and protein expression patterns [19, 35], which have been regarded as the phenotypic markers. These markers include cytokines, chemokines, membrane receptors and enzymes that are involved in distinct functions and processes, thus contributing to the pro- and anti-inflammatory effects of M1 and M2 macrophages, respectively [35]. In general, M1 macrophages are able to kill tumor cells and microorganisms

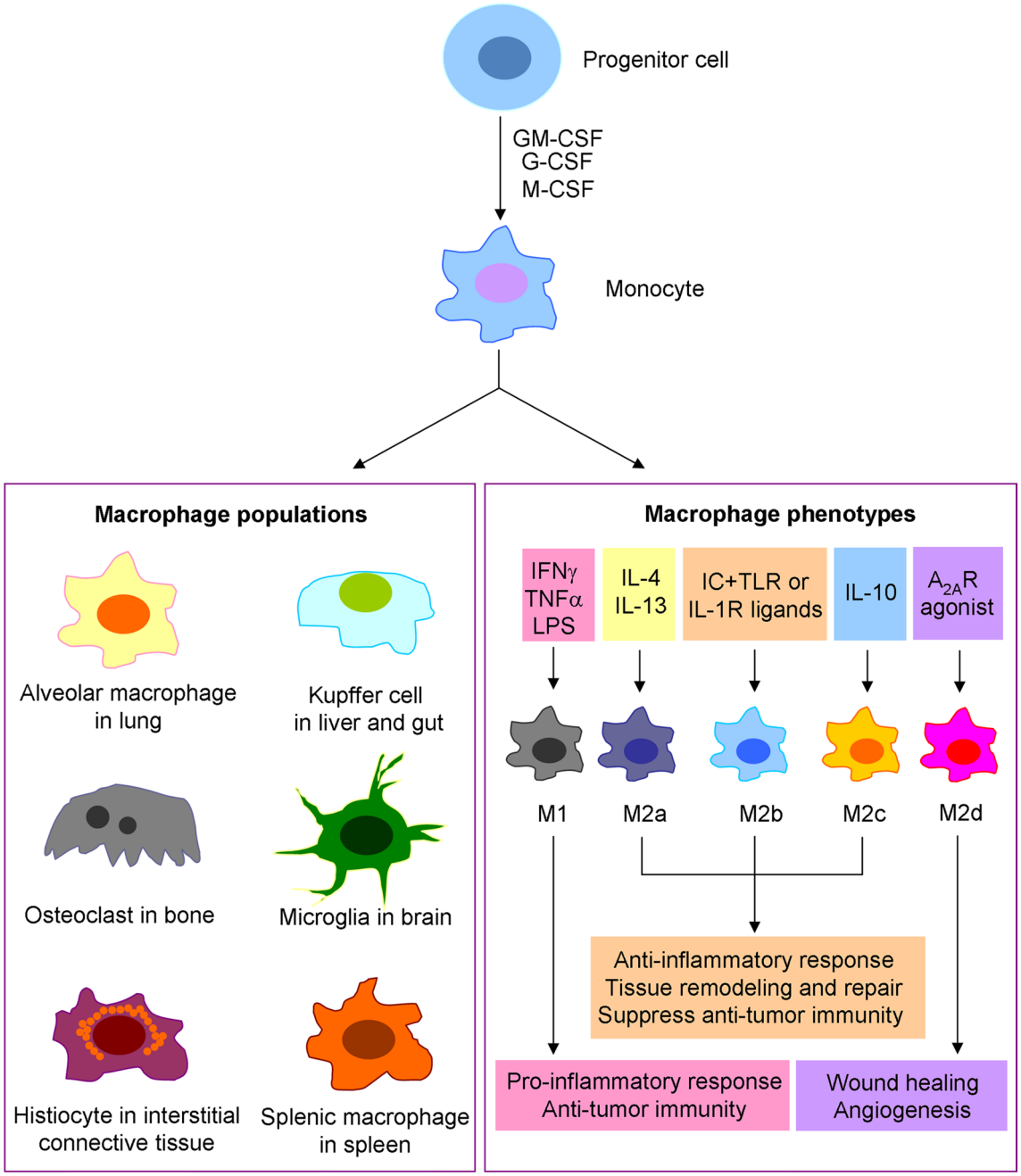

Fig. 2 Schematic diagram summarizing the origin of macrophages, their populations and functional phenotypes. Following the stimulation with granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) or macrophage colony-stimulating factor (M-CSF), progenitor cells differentiate into monocytes, which in turn differentiate into macrophages within different tissues and conditions. Macrophages can be divided into distinct populations based on their anatomical locations (*left panel*) and into different phenotypes based on the distinct functions (*right panel*). Tissue-resident macrophages include alveolar macrophages (lung), osteoclasts (bone), histiocytes (interstitial connective tissue), Kupffer cells (liver and gut), microglia (brain), splenic macrophages (spleen) and others. The molecules that polarize macrophages toward "classically activated" pro-inflammatory phenotype (M1) or "alternatively activated" anti-inflammatory phenotype (M2, including M2a, M2b, M2c and M2d) as indicated (*right panel*) for each phenotype. Accordingly, each phenotype of macrophages has its own functions as indicated (*right panel*). $A_{2A}R$, adenosine A_{2A} receptor; IC, immune complexes; IFN-γ, interferon gamma; IL, interleukin; IL-1R, IL-1 receptor; LPS, lipopolysaccharides; TLR, toll-like receptor; TNF-α, tumor necrosis factor alpha

by activating immune responses, whereas M2 macrophages are immunosuppressive cells that promote tissue remodeling and repair, as well as tumor angiogenesis, growth and progression [19, 68]. Further evidence shows that M2 macrophages can be divided into four distinct subtypes, including M2a, M2b, M2c and M2d (Fig. 2) [32, 35, 67, 70]. Each subtype of M2 macrophages has its own distinct phenotypic inducers and markers, as previously well summarized [35, 67]. M2a, M2b and M2c macrophages are considered as the anti-inflammatory cells, whereas M2d macrophages are derived from M1 pro-inflammatory cells following the activation of $A_{2A}R$ [32, 33, 35] and play an important role in wound healing and angiogenesis (Fig. 2) [32].

As discussed above, different phenotypes of macrophages have distinct functions, and macrophages exhibit an extremely high plasticity. Therefore, it is possible to "educate" macrophages to exhibit a beneficial function by polarizing them toward a specific phenotype. For example, tumor-associated macrophages (TAMs) are usually polarized to M2 phenotype [19, 93], where they promote tumor angiogenesis, growth and metastasis, as well as induce drug resistance [93, 99]. Polarization of TAMs toward M1 phenotype exhibits a promising effect in inhibiting tumor growth and progression, as well as in reversing drug resistance [92, 93, 96, 99]. A growing body of evidence demonstrates that macrophage polarization is regulated by a wide range of signaling pathways, which have been reviewed in depth elsewhere [10, 19, 35, 36, 40, 67, 68, 70]. In this review, we focus on discussing the role and underlying mechanisms of macrophage polarization in peripheral nerve regeneration in the context of nerve injury.

Recruitment of monocytes/macrophages into injury sites

It has been shown that a large number of immune cells, including neutrophils, macrophages and T cells, are recruited into injured sites following nerve injury, where they contribute to the pathogenic processes [63, 72]. The recruitment of neutrophils appears early after nerve injury, whereas the infiltration of macrophages into injured nerves is overtly seen starting from 2 to 3 days and peaking at 7 days post-injury (Fig. 3) [5, 74, 80, 88, 118]. Macrophages are the most abundant immune cells infiltrating into degenerating nerves, and they mainly originate from the circulating hematogenous monocytes [80].

Following nerve injury, the disruption of axon/Schwann cell nerve unit triggers Schwann cell de-differentiation and induces the release of multiple factors, including chemokines, cytokines and other factors, that

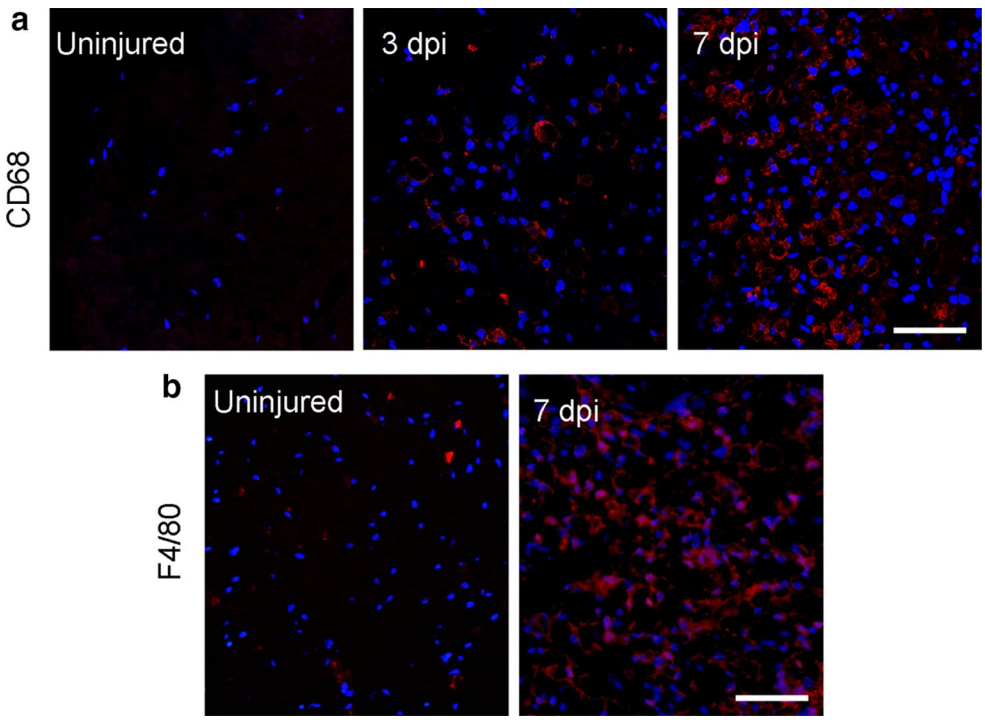

Fig. 3 The infiltration of macrophages into injured nerves. Immunofluorescence for CD68 (**a**) or F4/80 (**b**) in cross sections of distal sciatic nerves from wild-type mice under uninjured conditions, and at 3 and 7 days post-crush injury as indicated. *Scale bar* 50 μm. *dpi* days post-injury

are responsible for macrophage infiltration [80, 81, 121]. Among them, monocyte chemoattractant protein-1 [MCP-1, also known as chemokine (C–C motif) ligand 2, CCL2], leukemia inhibitory factor (LIF), IL-1α, IL-1β and pancreatitis-associated protein III (PAP-III) have been indentified as the major factors regulating monocytes/macrophage recruitment after nerve injury [80, 86, 109, 121, 123]. Both in vitro and in vivo studies have shown that these factors are rapidly produced by Schwann cells after peripheral nerve injury, and then function as the chemoattractants for macrophage infiltration [79, 80, 86, 109, 121]. Additionally, macrophages infiltrated into injured nerves also express and produce several factors, such as CCL2, TNF-α, IL-1α and IL-1β, thus contributing to further recruitment of monocytes/macrophages [52, 109]. These data imply that blockade of these factors would abolish injury-induced macrophage infiltration. However, in vitro evidence shows that addition of MCP-1 neutralizing antibodies at 50 μg/ml to conditional media of Schwann cell culture and peripheral nerve segments is not able to completely block the migration of macrophages [121]. Similar effects are also exhibited in vivo, where blockade of MCP-1 and IL-1β using antibodies does not completely block the macrophage infiltration after peripheral nerve injury [86]. These data suggests that nerve injury may also produce additional factors that contribute to monocyte/macrophage recruitment. Indeed, recent studies provided further evidence showing that some other factors, such as ECM proteins, galectin-1 and the complement system, are also involved in macrophage infiltration after peripheral nerve injury. Collagen VI is a major ECM protein produced by Schwann cells and macrophages in peripheral nerves [20]. Upon sciatic nerve crush injury, the deposition of collagen VI is significantly enhanced in injury sites, where it not only functions as a chemoattractant to recruit monocytes/macrophages (Fig. 3), but also regulates the expression of other chemoattractants, such as MCP-1 and IL-1β [21]. Galectin-1 is 14.5 kDa protein expressed by macrophages, Schwann cells and axons within peripheral nerves, and its expression is significantly elevated after nerve axotomy. The sciatic nerve injury-induced macrophage accumulation is inhibited in galectin-1-deficient mice [37]. The chemotactic function of galectin-1 on macrophages is also supported by the demonstration that injection of oxidized galectin-1 into intact nerve promotes the accumulation of macrophages [37], and galectin-1 enhances the migration of monocytes via p44/42 MAP kinase pathway [66]. Activation of the complement system is a crucial early event upon peripheral nerve injury [26, 94], and the products of activated complement system exhibit a significant role in macrophage migration [8]. In vivo findings show that depletion of complement using cobra venom factor inhibits macrophage infiltration after sciatic nerve injury [25]. Altogether, these findings provide a strong support for the involvement of distinct factors in macrophage infiltration after nerve injury.

By sensing the stimulation provided by distinct factors, monocytes/macrophages are recruited into injured nerves through different signaling pathways. C–C chemokine receptor type 2 (CCR2) is the main receptor of CCL2 in macrophages, and plays a key role for CCL2-induced macrophage migration [19]. Genetic evidence shows that the accumulation of macrophages in injured nerves is significantly inhibited in CCR2-deficient mice, suggesting that CCL2–CCR2 signaling pathway is essential for macrophage infiltration after nerve injury [34, 111]. Toll-like receptors (TLRs) can be activated in injured nerves by endogenous ligands that initiate innate immune response [9]. Ablation of TLR2, TLR4 and MyD88 in mice impairs the production of CCL2 and IL-1β and the infiltration of macrophages, whereas activation of TLR signaling by injection of TLR2 and TLR4 ligands enhances macrophage accumulation in the distal stump of sciatic nerve after injury [9]. Moreover, the production of IL-1β and TNF-α, as well as the infiltration of macrophages in dorsal root ganglion (DRG), is inhibited in TLR2 knockout mice after L5 spinal nerve transection injury [54]. Calcium-binding S100A8/A9 is an endogenous ligand of TLR4 [31, 102], which is important for regulating macrophage function in different pathological conditions [27, 45, 46]. The expression of S100A8 and S100A9 genes is dramatically upregulated in Schwann cells at day 1 post-injury in a sciatic nerve axotomy model [23, 55], where they have the ability to promote macrophage infiltration [23], suggesting the contribution of TLR4-S100A8/A9 signaling pathway in monocyte/macrophage recruitment after nerve injury. These data suggest that TLR and its related signaling pathways are necessary for macrophage infiltration after peripheral nerve injury. P-selectin is a cell adhesion molecule that expresses in the cell surface of macrophages [119] and has an important role in recruiting inflammatory cells into injury sites by binding and interacting with its ligand, P-selectin glycoprotein ligand-1 (PSGL-1) [106, 108]. Genetic evidence demonstrates that the infiltration of macrophages and production of pro-inflammatory cytokines TNF-α and IL-6 are attenuated in P-selectin-deficient mice after partial sciatic nerve ligation [62], suggesting an important effect of P-selectin mediating macrophage infiltration after nerve injury. Intercellular adhesion molecule-1 (ICAM-1) is an inducible surface glycoprotein that is mainly expressed in Schwann cells and nerve blood vein endothelia cells after sciatic nerve injury [126]. It has been shown that ICAM-1 not only involves in inflammation, but also in cell infiltration during the Wallerian degeneration after peripheral nerve injury [12, 17, 101]. Genetic studies showed that ablation of ICAM-1 inhibits macrophage infiltration after sciatic nerve transection injury [125],

suggesting the potential role of ICAM-1 in peripheral nerve injury-induced macrophage infiltration. However, further studies are still needed to confirm this conclusion, because other findings show that blockade of upregulated ICAM-1 does not prevent macrophage infiltration after sciatic nerve injury [2, 11]. Prostacyclin receptor is significant for regulating the effect of prostacyclin in peripheral pain sensation. Peripheral nerve injury upregulates the expression of prostacyclin receptor in IL-1β-containing macrophages, and deficiency of prostacyclin receptor reduces the number of macrophages, whereas local administration of prostacyclin receptor agonist promotes the accumulation of IL-1β- and prostacyclin receptor-expressing cells at injury sites [105]. Serum amyloid A (SAA) is major acute phase reactant that can be released into circulation in response to injury [122]. It modulates immune response by promoting the production of inflammatory chemokines, including CCL2, in monocytes [60, 61]. Sciatic nerve axotomy induces the upregulation of SAA1 and SAA3 from Schwann cells through a mechanism involving modulation of IL-6, which in turn enhances macrophage infiltration by upregulating the production of CCL2 [49], thus suggesting that IL-6-SAA-CCL2 signaling pathway is involved in macrophage accumulation in injured nerves. Activation of AKT and PKA signaling is required for macrophage migration [24, 28], which also mediates collagen VI-regulated macrophage infiltration into injured nerves (Fig. 4) [21]. ERK is a central signaling pathway controlling Schwann cell plasticity, and also exhibits an essential role for macrophage infiltration after sciatic nerve injury [81]. Both genetic and pharmacological findings show that inhibition of Raf/MEK/ERK signaling pathway impairs macrophage infiltration into injured nerves [81]. Erythroid-2-related factor 2 (Nrf2) is a transcription factor that is required for monocyte/macrophage recruitment, as demonstrated by the finding that a lower number of macrophages are accumulated in injured nerves of Nrf2-deficient mice, suggesting a significant contribution of Nrf2 and its related pathways in macrophage infiltration upon nerve injury [128]. Altogether, these findings provide insights into the underlying mechanisms of macrophage infiltration after peripheral nerve injury.

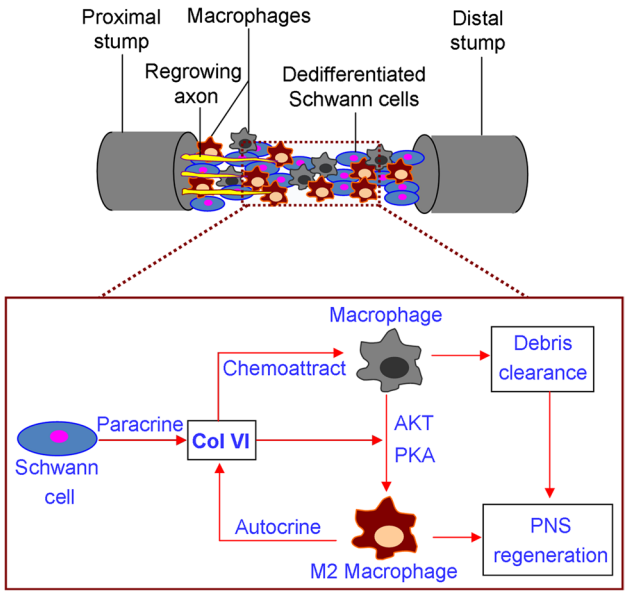

Fig. 4 Collagen VI-regulated macrophage migration and polarization contribute to peripheral nerve regeneration. Nerve injury induces a robust upregulation of collagen VI derived from Schwann cells and macrophages, which in turn recruits macrophages into injured sites in a paracrine and autocrine manner via AKT and PKA signaling pathways. These recruited macrophages on the one hand are involved in the Wallerian degeneration by removing the debris; on the other hand, they are polarized to M2 phenotype by collagen VI through AKT and PKA signaling pathways, thus enhancing peripheral nerve regeneration. *Col VI* collagen VI, *PNS* peripheral nervous system

Role of macrophages in Wallerian degeneration

Wallerian degeneration is a complicated process that is initiated following metabolic or mechanical damage to peripheral nerves and that induces multiple changes including axonal degeneration, myelin breakdown, glial cell proliferation, blood–nerve barrier (BNB) compromise, as well as the infiltration and activation of macrophages [16, 38, 116]. Schwann cells play an important role in the early stage of Wallerian degeneration. Upon injury, Schwann cells begin to de-differentiate in the distal nerve depending on the ubiquitin–proteasome system [59], a process that alters the gene expression profile of Schwann cells and provides an environment for axonal degeneration [38, 59, 76]. Schwann cells are able to remove myelin debris, a component that functions as a barrier to axon regrowth and contains axonal growth inhibitory signals including myelin-associated glycoprotein [38, 48]. Moreover, Schwann cells secrete a large number of trophic factors and ECM molecules promoting axon regeneration [22].

In the later stages of Wallerian degeneration, macrophages are the major cells contributing to remove myelin and axonal debris [30]. It has been demonstrated that the infiltrated macrophages can be seen laden with myelin-derived fat during the process of Wallerian degeneration [88]. Blocking monocytes with silica in mice induces less macrophage infiltration and slower myelin degradation after sciatic nerve injury [7]. Further studies showed that the Wallerian degeneration is delayed in macrophage-depleted animals using distinct pharmacological and genetic approaches [4, 64, 111]. Live cell imaging study also demonstrated that macrophages arrive at the injury sites long before axon fragmentation, and axonal debris can

be engulfed by axon fragmentation-triggered invasion of macrophages in zebrafish model [97]. Taken together, these findings support that macrophages play a key role in Wallerian degeneration.

In addition to the predominant effect of hematogenous macrophages, resident macrophages are also involved in Wallerian degeneration. Resident macrophages account for 2–9 % of total cells in peripheral nerves and are endowed with phagocytic abilities [34, 42]. During the process of Wallerian degeneration, resident macrophages are able to induce inflammation via their expression of TLRs and by producing IL-13 and IL-1β [34, 105, 127], suggesting a potential role of these cells in Wallerian degeneration. The direct experimental evidence from chimeric rats shows that as early as 2 days post-sciatic nerve crush injury and before the influx of hematogenous macrophages, resident macrophages are increased and activated, as well as in a proliferating status [75]. These changes are more pronounced at 3 and 4 days after sciatic nerve injury, and the ED1-positive resident macrophages can be still indentified at 28 days after sciatic nerve injury [75]. More importantly, these resident macrophages are involved in early myelin phagocytosis after sciatic nerve injury [75].

Mechanistic studies demonstrated that the complement system is one of the major factors mediating the effect of macrophages in Wallerian degeneration. Depletion of complement by cobra venom factor suppresses the phagocytic activity of macrophages [25]. In the complement cascade, complement 3 (C3) and its receptor, complement receptor 3 (CR3), are the most prominent players in Wallerian degeneration. Decreased expression of CR3 on macrophages is accompanied by the reduction of macrophage-ingested myelin in vitro [13]. CR3 on the surface of macrophages bounds to the degenerating myelin sheaths to initiate phagocytosis [12], which can be blocked by the presence of CR3 antibodies [15]. Studies on co-cultures of macrophages and nerve segments showed that macrophages are unable to invade degenerating nerves and exhibit phagocytic abilities when the culture is incubated with C3-deficient serum [14]. In vivo evidence suggests that complement is activated by endogenous antibodies after peripheral nerve injury, a process that plays an essential role for macrophage accumulation and is critical for C3b-regulated myelin clearance and macrophage-mediated phagocytosis [124].

In addition to the complement system, many other factors and signaling pathways are also involved in macrophage-mediated Wallerian degeneration, especially for the phagocytosis of debris. It has been shown that IL-1β and TNFα are able to promote the phagocytosis of macrophages in vitro [109]. The phagocytic macrophages in injured nerves are dramatically reduced by the treatment with MCP-1 or IL-1β neutralizing antibodies [86], as well as by ablation of collagen VI [21] or of Nrf2 [128]. Upon peripheral nerve injury, the secreted cytokines and factors released by macrophages affect myelin phagocytosis by stimulating the expression of phospholipase A2 (PLA2) [71]. The binding between lysophosphatidylcholine (LPC, which is generated by PLA2) and C-reactive protein activates the classic complement pathway [43, 71], thus contributing to phagocytosis by macrophages. On the other hand, the expression of LPC on the cell surface can function as an "eat me" signal for macrophages, thus promoting phagocytosis [58, 71]. In addition, BNB plays an important role in Wallerian degeneration after peripheral nerve injury. BNB becomes increasingly leaky during Wallerian degeneration after nerve injury and with a peak at 8 days after crush injury, and then gradually regains its barrier function and becomes intact at 30 days after injury when regeneration is completed [107]. Sciatic nerve transection injury induces a robust upregulation of matrix metalloproteinase 2 (MMP-2) and MMP-9 after axotomy, which correlates with the breakdown of BNB and the accumulation of macrophages [110]. Treatment with nonspecific MMP inhibitor or MMP-9-specific antibody delays Wallerian degeneration of the transected peripheral nerve and attenuates macrophage infiltration [110], indicating a potential role of MMP-mediated macrophages and BNB function in Wallerian degeneration. Further evidence shows that peripheral nerve injury-induced BNB dysfunction is regulated by macrophages, as demonstrated by the evidence that chronic nerve compression injury-induced alterations of BNB are attenuated by macrophages depletion [41]. Taken together, these findings allow shedding light on the underlying mechanisms of macrophages in Wallerian degeneration.

Role of macrophages in peripheral nerve regeneration

Although macrophages were originally highlighted for their potent phagocytic activities, more and more studies using different models and/or approaches have demonstrated that they exhibit a pivotal role in peripheral nerve regeneration. Barrette et al. found that depletion of monocytes and macrophages via continuous local delivery at sciatic nerve injury sites or systemic administration of ganciclovir in CD11b-TK$^{mt\text{-}30}$ mice compromises axonal regeneration and locomotor function recovery [4]. Depletion of macrophages with clodronate inhibits peripheral nerve regeneration of both motor and sensory functions [21], as demonstrated by the finding that clodronate-treated mice exhibit lower sciatic functional index score (Fig. 5a), longer time to initial response to toe pinch (Fig. 5b), and longer time to initial toe extension (Fig. 5c) when compared to the control mice [21]. More specifically in the

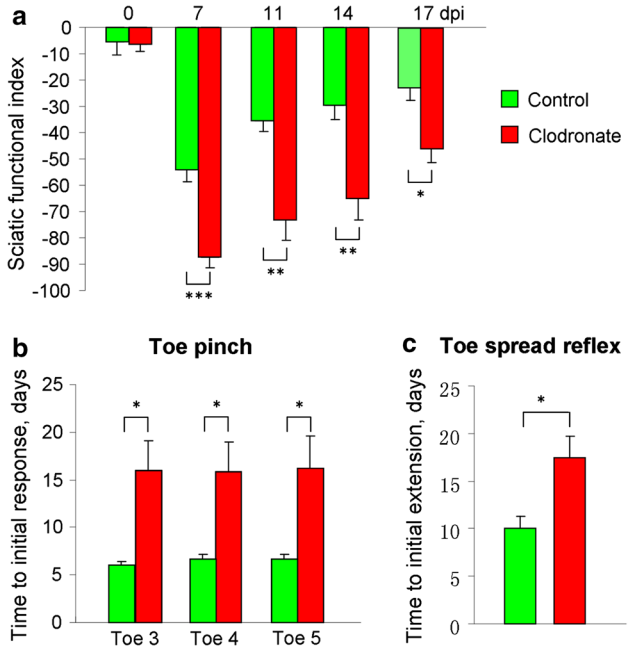

Fig. 5 Depletion of macrophages by clodronate liposome impairs peripheral nerve regeneration. Quantification of the sensory motor function (**a**), sensory function (**b**) and motor function (**c**) of wild-type mice following treatment with PBS liposomes (Control) or clodronate liposomes. The graphs show the sciatic functional index from footprint track before crush (0) and at 7, 11, 14 and 17 days post-crush injury, the initial response time to the pinch using forceps in the digits 3, 4 and 5 after sciatic nerve crush, and the initial extension time to toe spreading reflex, respectively. ($n = 5$–7; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$)

sensory signaling transduction, macrophages play a crucial role in the development of peripheral nerve injury-induced neuropathic pain and in the repair of sensory function [95]. It has been shown that the infiltration of macrophages into injured nerves is delayed in slow Wallerian degeneration mouse (Wld^{s}), which is accompanied by the impairment of thermal hyperalgesia development [77, 115] and peripheral nerve regeneration [83]. Sciatic nerve injury-induced thermal hyperalgesia is alleviated after the depletion of circulating macrophages by clodronate [64]. Taken together, these findings provide strong evidence for an essential role of macrophages during peripheral nerve regeneration and suggest that targeting macrophages is a promising strategy to promote peripheral nerve injury repair and functional recovery.

Several lines of studies using genetic and pharmacological approaches supported the idea that macrophage is a notable target for peripheral nerve regeneration. For example, experimental evidence shows that nicotinamide adenine dinucleotide phosphate oxidase 2 (Nox2)-positive macrophages are infiltrated into DRG after sciatic nerve injury and are involved in neuropathic pain hypersensitivity, which is impaired in Nox2-deficient mice [50], suggesting that Nox2 regulates peripheral nerve sensory function after injury by targeting macrophages. Collagen VI-deficient mice exhibit a delayed peripheral nerve regeneration by impairing macrophage function, which is rescued by transplantation of wild-type bone marrow cells [21]. Although these data strongly suggest that lack of Nox2 or of collagen VI impairs peripheral nerve regeneration via inhibition of macrophage function, these studies were carried out in global knockout mice. Further studies are needed to confirm this conclusion using Nox2 or collagen VI conditional knockout mice or treatment strategies specifically targeting macrophages. In addition, pharmacological treatments also support the concept that macrophage is a promising target for improving PNS regeneration [21, 47, 50]. For example, oxidized galectin-1 (GAL-1/Ox) is produced by Schwann cells and injured axons that specifically binds to and stimulates macrophages to produce axonal growth-promoting factor, thus promoting axonal regeneration, a process that is blocked by galectin-1 neutralizing antibodies [47]. Tacrolimus (FK506) is an immunosuppressant agent exhibiting a potent effect on promoting peripheral nerve regeneration [56] probably by enhancing the accumulation of macrophages in injured nerves [57]. Minocycline is a semisynthetic second-generation tetracycline that exhibits an inhibitory effect on nerve regeneration and decreases macrophage infiltration and activation after sciatic nerve injury [51], suggesting a potential action mechanism of minocycline on peripheral nerve regeneration by targeting macrophages.

Macrophages contribute to peripheral nerve regeneration via distinct mechanisms. After their infiltration, macrophages first contribute to peripheral nerve regeneration by removing the inhibitory regeneration signals from myelin debris and paving the way for axonal regrowth. Moreover, these cells produce a wide range of factors, such as proteases and growth-promoting factors/cytokines, and stimulate ECM remodeling to promote peripheral nerve regeneration [44, 87]. In addition, the infiltrated macrophages also stimulate peripheral nerve regeneration by affecting other cell types or components in injured nerves. It has been well demonstrated that Schwann cells exhibit a crucial role in peripheral nerve regeneration via distinct mechanisms [1, 81, 84, 85]. Interestingly, further studies showed that modulation of macrophage function is able to regulate peripheral nerve regeneration by regulating Schwann cell activities in injured nerves, including mitosis and de-differentiation [87], as well as infiltration and/or migration [47, 73]. Moreover, recent evidence shows that sciatic nerve cut injury induces a hypoxic environment in the bridge, a rejoined structure by two stumps following transection, that is selectively sensed by macrophages. These macrophages secret VEGF-A to polarize vasculatures that help Schwann cells to migrate and cross the wound, thus contributing to

Table 1 Summary of the factors and signaling pathways that regulate macrophage M2 polarization in injured nerves

Factors/signaling pathways	Key findings	References
apoE-p38 apoE-tyrosine kinase	Sciatic nerve crush injury induces a robust upregulation of apoE, which promotes macrophage M2 polarization via activation of p38 and tyrosine kinase signaling pathways	[3, 100, 114]
Collagen VI-AKT Collagen VI-PKA	Collagen VI deposition is dramatically upregulated in injured sciatic nerves, where it stimulates macrophage M2 polarization by activation of AKT and PKA signaling pathways	[21]
CCL2-CCR2	Sciatic nerve ligation injury upregulates the expression of CCL2-CCR2 signal, which in turn polarizes macrophage to M2 phenotype	[52, 103, 112, 117, 120]
GPR84	Ablation of GPR84 induces macrophage M2 polarization after sciatic nerve ligation injury	[82]

nerve regeneration [18]. Besides the infiltration into distal nerve after injury, macrophages also accumulate around axotomized cell bodies [65, 104], where they directly stimulate nerve regeneration [83].

More importantly, recent studies demonstrated that the infiltrated macrophages can be educated by the local microenvironment and are further delineated into distinct activation states. Ydens et al. demonstrated that sciatic nerve transection injury triggers an immunosuppressive response where the negative regulators of pro-inflammatory response and the anti-inflammatory cytokine IL-10 are induced, thus providing a microenvironment favoring macrophage M2 polarization [127]. Indeed, they found that M1 macrophage markers, such as inducible nitric oxide synthase (iNOS), IFN-γ and IL-12 p40, are absent, whereas the M2 macrophage markers, such as arginase-1, IL-13, Ym1 and Trem2, are significantly upregulated upon sciatic nerve transaction injury [127]. Further studies demonstrated that monocyte-derived M1 macrophages are only present at early stages after sciatic nerve ligation injury and they are gone by 3–4 days post-injury, when macrophages at injury sites start to be polarized to M2 phenotype and express high levels of arginase-1 and CD206 [78].

Multiple signaling pathways and molecules have been shown to modulate macrophage polarization after peripheral nerve injury (Table 1). For example, sciatic nerve injury induces a robust upregulation of apolipoprotein E (apoE) [100, 114] and collagen VI (Fig. 4) [21], which have the ability to polarize the infiltrated macrophages toward M2 phenotype by activation of p38 mitogen-activated protein kinase and tyrosine kinase [3], and by activation of AKT and PKA pathways, respectively [21]. In addition, CCL2/CCR2 is an important signal molecule that is enhanced upon peripheral nerve injury [53, 103, 117, 120], and can polarize macrophages toward an M2 phenotype [112]. These findings highlight that the recruited monocytes/macrophages are polarized to M2 phenotype through a mechanism that involves apoE, collagen VI and CCL2/CCR2, and their downstream signaling pathways. These findings are confirmed by genetic studies in knockout mice, which showed that GM-CSF-stimulated macrophages from CCR2-deficient mice display M1 phenotype [112] and that macrophage M2 polarization is dramatically impaired in collagen VI null mice after peripheral nerve injury (Fig. 4) [21]. G-protein-coupled receptor 84 (GPR84) is an orphan receptor that is mainly produced by macrophages during inflammation and upregulated upon sciatic nerve injury [82]. Lack of GPR84 polarizes macrophages toward M2 phenotype and inhibits the development of mechanical or thermal hypersensitivity after sciatic nerve ligation injury [82], suggesting that GPR84 is a negative regulator of macrophage M2 polarization in injured nerves. Taken together, these findings indicate that peripheral nerve injury triggers a microenvironment favoring macrophage M2 polarization via a variety of mechanisms.

As discussed above, M1 macrophages are pro-inflammatory cells that activate immune responses, whereas M2 macrophages are immunosuppressive cells that contribute to tissue remodeling and repair [19, 68]. In the context of PNS, macrophages of different phenotypes also exhibit distinct functions in peripheral nerve regeneration. Indeed, recent studies utilized an approach via local delivery of IFN-γ or IL-4 within polymeric nerve guidance channels to polarize macrophages toward M1 and M2 phenotypes, respectively, and the data demonstrated that polarization of macrophage toward M2 phenotype, but not M1 phenotype, promotes peripheral nerve regeneration [73]. Further evidence shows that sciatic nerve injury enhances the expression of IL-4 receptor α chain (IL-4Rα) in macrophages, which in turn mediates the effect of IL-4 on macrophage M2 polarization and sensory function recovery via STAT6 signaling pathway [53]. These findings highlight that macrophage polarization toward M2 phenotype is a promising strategy for promoting peripheral nerve regeneration after injury. Indeed, recent studies demonstrated that parthenolide, a sesquiterpene lactone occurring in the plant feverfew (*Tanacetum parthenium*), is able to enhance sensory regeneration in sciatic nerve chronic constriction injury model by promoting macrophage polarization to M2 phenotype [89].

As discussed above, IL-10 is a key cytokine that triggers macrophage M2 polarization, suggesting its potential application for promoting peripheral nerve regeneration. However, the therapeutic application of IL-10 has been limited,

due to the fact that the in vivo bioactive half-life of IL-10 or its peptide fragments only remains for minutes to hours [98]. Interestingly, Potas et al. recently found that implantation of IL-10 conjugated electrospun poly(ε-caprolactone) (PCL) nanofibrous scaffolds in sciatic nerve effectively promotes macrophage M2 polarization up to 14 days [90]. These findings shed light for the potential application of IL-10 conjugated PCL nanofibrous scaffolds in promoting peripheral nerve regeneration by stimulating macrophage M2 polarization. However, further studies are needed to confirm this finding in peripheral nerve injury models. Taken together, these data suggest that studies focusing on macrophage M2 polarization will have the opportunity to offer novel and practical therapeutic approaches to improve regenerative outcomes following peripheral nerve injury.

Concluding remarks and future perspectives

Macrophages are one kind of prominent immune cells that play a pivotal role in tissue injury and repair. A number of studies indicated that macrophage infiltration and polarization are extensively regulated upon peripheral nerve injury. Moreover, these findings provide clear evidence supporting that the infiltrated and polarized macrophages exhibit a key role in Wallerian degeneration and PNS regeneration. These collective findings not only point at macrophages as a key type of immune cells involved in peripheral nerve regeneration, but also indicate that modulation of macrophage functions represents a promising strategy for improving PNS regeneration and controlling peripheral neuropathies.

Although our understanding of the role and underlying mechanisms of macrophages in Wallerian degeneration and PNS regeneration has increased recently, multiple questions remain unaddressed or only partially understood regarding how monocytes/macrophages are recruited and modulated after nerve injury, how injury triggers macrophage M2 polarization, how macrophages contribute to Wallerian degeneration and PNS regeneration, as well as how to improve peripheral nerve injury outcomes by targeting macrophages. As we discussed above, nerve injury-induced degeneration is a very complicated process that triggers the recruitment of monocytes/macrophages induced by the release and secretion of many factors from injured axons and Schwann cells, as well as from macrophages themselves. Therefore, it is difficult to prevent monocyte/macrophage recruitment and function after nerve injury by the blockade of a sole factor or multiple factors [86, 121]. Further studies on identifying early drivers and key factors upon peripheral nerve injury will greatly enhance the development of effective strategies to control peripheral nerve regeneration. Although our current knowledge indicates that nerve injury triggers a microenvironment favoring macrophage M2 polarization probably via a mechanism involving upregulation of IL-10 [127] and collagen VI [21], further studies are needed to clarify how these factors are modulated upon nerve injury. Recent studies by Mokarram et al. demonstrated that local delivery of IL-4 within polymeric nerve guidance channels promotes peripheral nerve regeneration by polarizing macrophages toward M2 phenotype [73]. These findings shed light on improving the outcomes after peripheral nerve injury by delivery of factors that are able to promote macrophage M2 polarization, such as IL-10 and collagen VI, within nerve guidance channels or other modified scaffolds. In addition to increasing the understanding of molecular mechanisms and key events of macrophages in peripheral nerve injury and regeneration, prospective findings answering these questions may provide novel therapeutic targets for the treatment of PNS injury and peripheral neuropathies and help to develop new therapeutic strategies by adding macrophage M2 polarization-related factors in scaffolds.

Acknowledgments We apologize to all these authors whose papers we could not cite due to space limitations. This work was supported by the University of Padova and the Italian Ministry of Education. P. Chen was supported by a fellowship from the Cariparo Foundation.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

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