CNS remyelination and inflammation: from basic mechanisms to therapeutic opportunities

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Abstract

Remyelination, the myelin regenerative response that follows demyelination, restores saltatory conduction and function and sustains axon health. Its declining efficiency with disease progression in the chronic autoimmune disease multiple sclerosis (MS) contributes to the currently untreatable progressive phase of the disease. While some of the *bona fide* myelin regenerative medicine clinical trials have succeeded in demonstrating proof-of-principle, none of these compounds have yet proceeded towards approval. There therefore remains a need to increase our understanding of the fundamental biology of remyelination so that existing targets can be refined, and new ones discovered. Here we review the role of inflammation, in particular innate immunity, in remyelination, describing its many and complex facets and discussing how our evolving understanding can be harnessed to translational goals.

Introduction

Take any textbook of pathology and you will read that inflammation plays an important role in tissue repair and regeneration. That this central precept of inflammation arrived relatively late to the myelin pathology field is largely a consequence of the most widely occurring disease of myelin, multiple sclerosis (MS), being of autoimmune origin and the focus of attention therefore on how inflammation causes damage and how it can be stopped. Yet, in addition to the loss of myelin in demyelinating disease, there is also the potential for a highly effective regenerative response called remyelination in which new myelin sheaths are restored to demyelinated axons. However, it was not until the beginning of the current century that it became apparent that remyelination, in common with regenerative processes many other tissues, is dependent on inflammation. Remyelination is true regenerative process, restoring tissue architecture and function, and maintaining axonal health. Its declining efficiency in chronic demyelination disease such as MS is a major contributing factor to the currently untreatable progressive phase of the disease, and hence the need to therapeutically promote or sustain failing remyelination has become active of intense current interest. In this review we briefly introduce remyelination, explore in detail how the innate and to a lesser extent the adaptive immune systems contribute to remyelination, and finally how the emerging understanding of the interplay between the inflammation and remyelination might the therapeutically harnessed.

General mechanisms of inflammation in regeneration

The immune system consists of two separate, yet interdependent immune responses. Whereas the adaptive system is antigen specific, requiring gene rearrangement, the inborn innate immune response does not depend upon previous antigen exposure, and is therefore rapidly initiated already within hours. The cells that provide innate immunity include monocytes, tissue macrophages, neutrophil and natural killer cells, which together with antimicrobial molecules, complement and cytokines represent key components of innate immunity. The innate immune system is critical involved in the various steps of the repair program including the remodeling of the injured lesion, the scarring reaction and the restoration of tissue function (Karin and Clevers, 2016; Wynn and Vannella, 2016; Aurora and Olson, 2014; Shechter and Schwartz, 2013). Whether the immune response causes scarring or regrowth of functional tissue is only partially understood. The inverse relationship between immune system complexity and regenerative capacity in evolution and development has initially promoted the concept that immune systems impede the regenerative response (Fukazawa et al., 2009; Mescher and Neff, 2005; Harty et al., 2003). For instances, in anuran species, in particular *Xenopus* larvae, there is a gradual loss of regenerative ability with the development of the immune system. Hindlimbs and tails can regenerate perfectly if amputated early in development. However, after metamorphosis when the immune system has matured, regeneration is much less efficient (King et al., 2012). Likewise, in early fetal periods of mammalian development tissue injury is often followed by complete restoration of scar-less and functional tissue, whereas comparable lesions remain permanently damaged in postnatal stages of development (Colwell et al., 2003). Whilst these data show that regeneration can occur in the absence of a complex immune system, regeneration in adult and higher vertebrates depends on the intricate communication of the immune system with the injured tissue. At the end of the 18th century, Metchnikoff proposed that macrophages played a role in regeneration, when he noted that macrophages were recruited to the site of injured tissue (Gordon, 2016). Likewise, Blinzinger and Kreuzberg suggested a pro-regenerative role for the microglia response that occurred after injury to the facial nerve (Blinzinger and Kreutzberg, 1968). These seminal studies set the stage for research into innate immune biology in regeneration, subsequently demonstrating its central role in all sequential stages of repair (Karin and Clevers, 2016; Wynn and Vannella, 2016; Aurora and Olson, 2014; Shechter and Schwartz, 2013). The early initial stage of wound healing is characterized by a

local activation of the innate immune system resulting in the active recruitment of leucocytes, in which early neutrophil entry is followed by monocyte infiltration. After the injury site has been cleared from debris, the innate immune cells trigger the repair phase, in which proliferative signal instruct the regrowth of new tissue, so called granulation tissue, consisting of new connective tissue, a provisional extracellular matrix (ECM) and blood vessels. Finally, maturation and remodeling of the scaring tissue follows. It is characterized by the proteolytic reorganization of the extracellular matrix, the contraction of the lesion, the regression of granulation tissue, and the transformation of the wound by formation of newly differentiated cells. Even if cells of the innate immune system gradually retreat from the lesions during the final phase of tissue maturation, they play an important role by providing growth factors, proteases, chemokines and cytokines. Thus, the innate system is key to the outcome of the healing process, which is ideally the scar-less regrowth of functional tissue (Figure 1). Nevertheless, prolonged or aberrant innate immune responses are one of the major underlying causes of chronic wound formation or pathological scarring.

What then of the role of the immune system in regenerative processes in the CNS, a tissue notorious for its poor repair capacity? Initially, the immune response was viewed as detrimental, amplifying glial scarring and potentiating injury. However, similar to the healing processes that occur in most peripheral tissue, immune function within CNS lesions is key to the repair process, even if the result, at least in humans, is in most cases the formation of chronic scar tissue comprising largely of hypertrophied but transcriptionally quiescent astrocytes, with significant ECM components if the meninges are disrupted (Doring et al., 2015; Kokaia et al., 2012; Kyritsis et al., 2012; Schwartz, 2010; Kotter et al., 2001). CNS lesions are often accompanied by a disruption of the blood-brain-barrier (BBB), leading to the extravasation of serum components, such as fibrinogen and thrombin, which both can elicit damaging responses within the CNS (Petersen et al., 2018). In addition, circulating antibodies can sometimes enter the brain and exacerbate tissue injury (Hammer et al., 2014). To prevent damage to the CNS, a feed-forward cascade is set in motion, which involves the local activation of microglia and astrocytes, thereby initiating the secretion of inflammatory mediators including cytokine/chemokines, which increase vascular permeability and expression of endothelial adhesion molecules, and thus the recruitment of leukocytes into the CNS. This inflammatory response is necessary to re-establish homeostasis and to protect the CNS from further injury. The selective pressure to rapidly re-seal the injury site and prevent further

damage to the tissue could be one of the reasons for the pronounced scaring response that occurs in the CNS. This response is in large parts carried out by reactive astrocytes with border-forming function that efficiently isolates the lesion area and limit the spread of inflammation and toxic molecules by their hypertrophic dense cellular processes and by the deposition of a network of ECM molecules (Sofroniew, 2015; Sorokin, 2010; Silver and Miller, 2004). Whereas the resulting glial scar is a useful counter-active measure to tissue damage and inflammation, molecules within the scar such as chondroitin sulfate proteoglycan (CSPG), semaphorins and ephrins, have axon growth inhibitory properties. In addition, CNS lesions are often characterized by prolonged inflammatory responses, which in turn can promote further fibrosis. Together, these features contribute to insufficient clearance or maturation of lesions, eventually leading to persistent scars, poor regeneration and loss of functional recovery (Figure 2). Whereas a scaring reaction can have their advantages in areas of the brain with poor regenerative capacity to protect further injury at the expense of regeneration, the situation is different in demyelinating disorders in which axons are partially preserved.

Remyelination in the CNS

Remyelination is the spontaneous regenerative process that follows primary demyelination (the loss of myelin from around an intact axon) and involves reinvesting the denuded axon with a new myelin sheath (Franklin and ffrench-Constant, 2017). This results in restored saltatory conduction and axonal support and recovered function (Smith et al., 1979; Duncan et al., 2009). In most instances, the new myelin sheaths are generated by an oligodendrocyte derived from a widespread population of multipotent CNS adult stem cells called oligodendrocyte progenitor cells (OPCs), that, in response to injury become activated, divide, migrate and finally undergo differentiation (Zawadzka et al., 2010). Over the last few decades, a picture has been pieced together of cell-autonomous and non-cell autonomous machinery, by which this process is regulated. Thus, many of the key pathways and regulators have been identified that cause the OPCs to enter cell cycle during the recruitment phase of remyelination and then exit cell cycle in a timely fashion during the differentiation and maturation phase of remyelination. There are many cellular and non-cellular components of the remyelinating lesion that contribute to the non-cell autonomous cues that control this sequence of events including the extracellular matrix, demyelinated axons itself, reactive astrocytes, pericytes and, as we describe in detail below, inflammatory cells (Ghorbani and

Yong, 2021; Segel et al., 2019; De La Fuente et al., 2017; Gautier et al., 2015). In certain circumstances, notably when there are demyelinated axons within areas that are largely astrocyte deficient, remyelination in the CNS can be mediated by Schwann cells (Chen et al., 2021). Intriguingly, many of these CNS Schwann cells are derived from OPC in an unusual example of transdifferentiation occurring during a naturally occurring regenerative process (Zawadzka et al., 2010). Non-myelinating Schwann cells from the PNS can also contribute (Ma et al., 2018; Itoyama et al., 1983). In recent years, a new form of remyelination has emerged in which oligodendrocyte cell bodies remain intact after loss of the myelin internodes they support and are able to generate new myelin sheaths, albeit with less precision than progenitor-mediated remyelination (Neely et al., 2022; Duncan et al., 2018). It has been suggested that this may be a common form of remyelination in humans, although the difficulties in accurately assessing where remyelination has occurred in human material mean that this question remains unresolved (Yeung et al., 2019; Neumann et al., 2020).

Remyelination can be considered as the default response to demyelination: it is a regenerative process that can work with great efficiency, not only in animal models but also in humans. However, as with all regenerative processes, its efficiency declines with age, an intrinsic, disease-independent feature of remyelination that has a profound impact on the natural history of MS, a chronic disease often of decades duration (Neumann et al., 2019a,b). The failure of remyelination can lead to area of chronic demyelination characterised by a diminishing number of axons surrounded by an astrocyte scar similar to that of traumatic injury in that it comprised a network of hypertrophied astrocytic processes, but a smaller ECM component. In contrast to the scar associated with traumatic injury, the astrocytic scar of chronic demyelination can be viewed as a failure of remyelination rather than its cause.

Pathology of inflammatory demyelinating lesions

Inflammatory demyelinating diseases are a heterogeneous group of disorders, which includes multiple sclerosis (MS), neuromyelitis optica (NMO), acute disseminated encephalomyeltitis (ADAM), and myelin oligodendrocyte glycoprotein (MOG) antibody associated demyelinating disease (MOGAD) (Stadelmann et al., 2019; Popescu and Lucchinetti, 2012). The extent to which demyelinating lesions in these disorder regenerate depends on various factors such as the type and the severity of the inflammatory insult. Among the most damaging lesions are those that occur in NMO, characterized by prominent perivascular immunoglobulin and

complement deposition, extensive macrophage, granulocytes and eosinophils infiltration, which together result in demyelination and necrosis with loss of axons and astrocytes (Lucchinetti et al., 2014). These lesions have only very poor capacity to regenerate and display only sparse remyelination by oligodendrocytes. On the other side of the spectrum are demyelinated lesions with relative preservation of axons. Such lesions are a hallmark of the multifocal areas of demyelination that occur in MS. MS lesions are characterized by a central vein, where the inflammatory reaction arises and leakage of serum component occurs (Reich et al., 2018; Kutzelnigg and Lassmann, 2014). The inflammatory infiltrate of active MS lesions consists mainly of monocyte-derived macrophages and brain-resident proliferating microglia. There is a smaller contribution of lymphocytes, mostly CD8+ T-cells, and some CD4+ T-cells and B-cells (Ingelfinger et al., 2022; Dendrou et al., 2015). Such early MS lesions are densely populated by myeloid cells engaged in myelin debris removal (Mishra and Yong, 2016; Bogie et al., 2014). Neuropathological classification of lesions stages has shown that the most acute lesions comprise myeloid cells filled with lysosomal inclusion of myelin components, such as myelin basic protein (MBP), myelin-associated protein (MAG) and 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) (Bruck et al., 1995). At later stages and after further myelin ingestion, the less abundant proteins, MAG and CNPase, are gradually depleted, while major myelin proteins, such as MBP, remain within the phagocytes, which with time start to display signs of cytoplasmic lipid depositions reactive for Oil red O (Grajchen et al., 2018; Li et al., 1996). Another feature of active lesions is the presence of reactive and hypertrophic astrocytes that contain enlarged cytoplasm and often multiple nuclei. Acute lesions can develop into chronic active lesions with an inactive core and a rim of activated myeloid cells, of which some contain myelin degradation products. Inactive lesions are completely demyelinated and hypocellular, characterized by pronounced loss of axons, very few remaining myeloid cells and T-cells and an expansion of astrocytes that are embedded into a dense fibrillary network of extracellular matrix components. In addition to the white matter, demyelinated lesions also occur in the grey matter, where they are found in perivascular, and also in subpial areas, associated with leptomenigeal inflammation. Such demyelinating lesions, display depletion of oligodendrocytes with relative preservation of axon injury but with less pronounces inflammatory infiltrates, compared to the white matter counterparts.

Despite the unfavorable regenerative environment of the CNS, and in contrast to the limited capacity for neuronal/axonal regeneration, remyelination is possible in MS, giving rise to so-

called shadow plaques, which are sharply demarcated areas, characterized by more sparsely myelinated axonal fibers with thinner myelin sheaths (Patani et al., 2007; Patrikios et al., 2006; Prineas et al., 1993). Approximately one third of chronic lesions show, at the edge, some signs of remyelination, and one fifth of the lesions are fully remyelinated, and therefore classified as shadow plaques at autopsy (Frischer et al., 2015; Patani et al., 2007; Patrikios et al., 2006), although assessing the true extent of remyelination in MS is challenging (Neumann et al., 2020). Remyelinated axons are not only observed in established lesions, but also in the earliest stages of acute demyelination. Remyelination varies between patients but seems to be more active at early stages of the disease. However, inflammatory demyelinating lesions frequently co-exist within remyelinated plaque areas, resulting in repetitive bouts of demyelination of previously remyelinated areas (Bramow et al., 2010; Prineas et al., 1993). Indeed, in a large autopsy study of progressive MS patients, active demyelination correlated with a reduction in remyelination efficacy (Bramow et al., 2010), although experimental evidence suggests that this unlikely to be due to OPC depletion (Penderis et al., 2003). Nevertheless, extensive shadow plaque areas can be observed at autopsy after longer disease duration in some patients, including a subset of patients with progressive disease. However, the major lesion type found at autopsy are chronic demyelinated lesions (Frischer et al., 2015); but it remains unknown why such lesions have failed to regenerate. The presence of OPCs in chronic lesions, even though in small numbers, have led to the notion that a differentiation block, possibly due unfavorable lesion microenvironment, is the basis of remyelination failure, at least in some lesions (Franklin and ffrench-Constant, 2017; Kuhlmann et al., 2008). Among the factors that have been associated with poor differentiation, are inflammation, gliosis, and extracellular matrix components such as hyaluronic acid, CD44, semaphorins and versican (Lau et al., 2013a; Kwok et al., 2011; Piaton et al., 2011; Back et al., 2005). An additional reason could an elevation of chemorepellent molecules that result in insufficient recruitment of OPCs to demyelinated lesions (Rodriguez et al., 2014; Boyd et al., 2013). Moreover, oligodendroglial loss and a hostile tissue environment in mixed active/inactive lesions have been attributed to poor remyelination (Heß et al., 2020). Repair capacity is also dependent on the lesion site, with periventricular lesions showing less, and deep white matter lesions and cortical lesions more remyelination. Apart from disease-specific factors, age-associated decrease in repair capacity will undoubtedly contribute inefficient remyelination in MS (Neumann et al., 2019a; Sim et al., 2002).

Innate immunity and remyelination

Experimental autoimmune encephalomyelitis (EAE) is a commonly used experimental model for the analysis of multifocal inflammatory lesions in the CNS. In EAE, an autoimmune response elicits inflammatory damage with demyelination, axonal loss and gliosis in the CNS (O'Loughlin et al., 2018). The counter-reactive, injury-induced immune responses responsible for the reparative process are difficult to study, because they inter-mix with the auto-inflammatory disease-causing immune activation in EAE. In addition, lesions appear in a relatively random spatial and temporal manner, hampering the analysis of the sequence of reparative events (Lassmann and Bradl, 2017). Some of these shortcomings can be circumvented in the focal cortical EAE model, in which lesions are stereotactically targeted to the cerebral cortex by injection of pro-inflammatory mediators in animals that were subclinically immunized (Merkler et al., 2006). The resulting demyelinated lesions show focal demyelination, infiltration with inflammatory cells, complement deposition, and remyelination occurring within 2-3 weeks. However, the most widely used model to study the biology of remyelination in rodents are toxin-induced models of demyelination, which are based on the focal injection of a toxin into the white matter (McMurran et al., 2019). Demyelination occurs rapidly, followed by a regenerative process that takes a few weeks in young rodents and requires the activity of the immune system. Studies using these models have shown that innate immune system plays a key role in lesion repair (Ronzano et al., 2021; Cunha et al., 2020; Rawji et al., 2020; Lloyd and Miron, 2019; Cantuti-Castelvetri et al., 2018; Lampron et al., 2015; Foote and Blakemore, 2005; Miron et al., 2013; Kotter et al., 2001). The task the innate immune system must accomplish is complex. The injury-induced immune response has to be titrated to the severity of the damage, requiring accurate regulation of the magnitude and the duration the inflammatory reaction (Medzhitov, 2021). If set too low, the reparative process is insufficient, whereas too extensive inflammation can induce collateral tissue damage. In addition, the inflammatory response needs to be adjusted to the nature of tissue damage. For instance, lesions in white matter tracts with high density of myelinated fibers result in the formation of vast amount of lipid-rich myelin debris. In contrast, damage to areas rich in cell bodies, can lead to the release of nucleic acids and cytosolic material. To detect such variances, cells of the innate immune system are equipped with an array of sensors that perceive differences in the damaged structures and mount the appropriate response (Prinz et al., 2021; Butovsky and Weiner, 2018; Colonna and Butovsky, 2017; Wolf et

al., 2017; Okabe and Medzhitov, 2016; Hickman et al., 2013). Because acute damage to the CNS requires rapid immune reaction, and fine-tuned transcriptional responses take time, tailored immune response cannot be an immediate solution to injury. It is therefore likely that the innate immune system employs preemptive defense mechanisms to respond to brain injury. Thus, the innate immune response to CNS injury is possibly an evolving inflammatory reaction, starting with a universal tissue injury program, and continuing with a fine-grained response adjusted to the type of damage that has occurred (Cantuti-Castelvetri et al., 2022). Traditionally, the innate immune response has been regarded as biphasic ("M1" and "M2"), but with the advances in single cell sequencing, it has become clear many more cellular states can be detected (Ransohoff, 2016). In addition, the reactivity of the cells is unlikely to be homogenous (Hammond et al., 2019; Masuda et al., 2019). For instance, cells in the core of a lesion might show different responses and trajectories compared to cells at the lesion edge. One additional challenge is to determine the function of the various cellular states. Successful regeneration requires the coordination of different tasks, which include processes involved in immune activation, cellular recruitment, debris clearance, progenitor cell activation, promotion of differentiation, matrix production and immune resolving functions (Figure 3). It is unlikely that each of these cellular functions occurs within distinctr states, rather cells progress along a ranked order of functional states specialized to some of these tasks.

Role of myeloid cells in remyelination

Activation and recruitment: Pattern-recognition receptors (PRRs) not only sense the presence of microorganisms by recognizing structures conserved among microbial species, which are called pathogen-associated molecular patterns (PAMPs), but also endogenous molecules released from damaged cells, termed damage-associated molecular patterns (DAMPs). They do this by a family of transmembrane protein sensors localized at the cell surface or the endosomal system including Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), as well as the cytoplasmic proteins, RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs) (Takeda et al., 2003). Well known DAMPs include various proteins such as heat shock proteins, high mobility group box protein 1, but also metabolites, for example uric acid and ATP, as well as nucleic acids and lipids. One common structural feature of some of these molecules is their hydrophobic nature and the sudden exposure of such hydrophobicity has been suggested act as a signal that tissue injury has occurred (Seong and Matzinger, 2004). So far, there is little

information on the DAMPs released after myelin disintegration, but myelin lipids together with its hydrophobic proteins, may serve as source for DAMPs when exposed within the extracellular space. Once TLRs or other types of pattern recognition receptors recognize the danger signal, an inflammatory signal pathway activates a response, which consists of the nuclear factor-κB (NF-κB) or an interferon-signaling pathway (Hochreiter-Hufford and Ravichandran, 2013). By analogy to the concept of PAMPs, a neurodegeneration-associated molecular pattern sensing mechanism has recently been put forward in the context of CNS injury (Deczkowska et al., 2018). This system depends on the function of Triggering Receptor Expressed on Myeloid Cells 2 (TREM2), which recognizes anionic lipid released from dying cells such as apoptotic bodies and is present in myelin debris (Keren-Shaul et al., 2017; Krasemann et al., 2017; Ulland et al., 2017). Thus, TLRs together with TREM2 may collectively serve as a sensing and signaling system of damaged myelin, but how both systems act together in time and space is unknown. The activation might occur in distinct cell populations at different sites within the lesion; however, simultaneous or sequential activation in one cell type is also possible. Previous work has shown that both the TLR- and TREM2-dependent pro-inflammatory activation of myeloid cells is essential for remyelination (Bosch-Queralt et al., 2021; Gouna et al., 2021; Cignarella et al., 2020; Cunha et al., 2020; Poliani et al., 2015; Cantoni et al., 2015). Deletion of the myeloid differentiation primary response 88 (MyD88), the canonical adaptor for inflammatory signaling pathways downstream of TLRs, resulted in insufficient microglia/macrophage activation with impaired myelin debris degradation and reduced generation of new myelinating oligodendrocytes (Cunha et al., 2020). Likewise, mice deficient in TREM2 failed to mount an activated microglia/macrophage response, resulting in poor myelin debris clearance and remyelination (Gouna et al., 2021; Cantoni et al., 2015; Poliani et al., 2015). Despite the similarities of both systems in sensing damaged tissue, there are important functional differences in the response. TREM2-activated microglia appear to act primarily cell-autonomously, whereas TLR-activated microglia can communicate with the peripheral immune system by mobilizing neutrophils and monocyte-derived macrophages to the lesion site. There is only limited knowledge of how the underlying cytokine/chemokine response is triggered, but diverse immunocompetent cell types such as microglia/macrophages, astrocytes, endothelial cells and even OPCs are likely to participate. This initial feed-forward inflammatory cascade is necessary to build up a sufficient immune response, of which monocyte-derived macrophages and microglia are key. Because of the

difficulties in distinguishing monocyte-derived macrophages from activated microglia, their role in remyelination is only beginning to be understood. Fate mapping and reporter cell lines have revealed that monocyte-derived macrophages appear early and only in relatively low number in toxin-induced demyelinating lesions and are rapidly outnumbered by resident microglia (Plemel et al., 2020; Lloyd et al., 2019). Nevertheless, monocyte-derived macrophages play an important role, as shown in peripheral ablation experiments using clodronate liposomes, which resulted in poor remyelination (Kotter et al., 2001). Furthermore, heterochronic parabiosis experiments in which young monocytes infiltrate into old lesion provided further evidence for a role of peripherally derived macrophages in remyelination (Ruckh et al., 2012). However, the functional differences of monocyte-derived macrophages compared to microglia remains to be established in remyelination. In EAE, blood-derived macrophages dominate the inflammatory infiltrates, and their numbers correlate with EAE severity, but their role in repair is not well understood. Using reporter mice that convert the pro- or anti-inflammatory polarization of macrophages into distinct fluorescent signals, a recent study revealed that individual blood-derived macrophages can switch to an anti-inflammatory phenotype as lesions move from expansion to resolution (Locatelli et al., 2018). In addition, the death of pro-inflammatory microglia followed by repopulation to a pro-regenerative microglia state has also been described as a mechanism of cell state switch (Lloyd et al., 2019).

Myelin debris clearance: Clearance of damaged myelin is essential to limit tissue injury and to initiate repair. Injured myelin associated with axons is not only non-functional but also damaging to the axons and therefore needs to be rapidly removed. In addition, myelin debris within the extracellular space is growth inhibitory, preventing OPC recruitment and differentiation. The process of myelin clearance is complex and requires multiple steps and transitions. It starts with the recognition of myelin debris by receptors expressed on the surface of microglia/macrophages. These are receptors of the Tyro3, Axl and MerTK (TAM) receptor family, which bind the “eat-me” signal phosphatidylserine on myelin debris using Gas6 and Protein S as bridging ligands (Shen et al., 2021; Lemke and Rothlin, 2008). Furthermore, myelin debris internalization can occur by scavenger, C-type lectin, CD36, Fc or complement receptors after opsonization with antibodies or the complement system (Ren et al., 2021; Smith, 1999). The process of myelin phagocytosis is efficient and redundant, and therefore easily compensated when one of the receptors is deleted (Safaiyan et al., 2021).

However, most of the receptors are present at low levels in non-stimulated, surveying microglia; therefore, microglia activation, is essential to trigger their expression, a process that is reduced in aging. Consequently, myelin debris accumulates in aging lesion, impeding myelin repair (Kotter et al., 2006). Once myelin debris internalization has occurred, microglia/macrophages are confronted with the challenge to degrade and metabolize vast amount of tightly packed myelin whorls. Similar, as after the ingestion of bacteria and apoptotic cells, myelin debris needs to be delivered from phagosomes to lysosomes for degradation. However, these various processes of phagolyosomal maturation show important differences. Whereas bacteria employ a mode of phagosome maturation characterized by enhanced induction of antimicrobial defense systems such as NADPH (nicotinamide adenine dinucleotide phosphate) oxidase (NOX) and nitric oxide synthase, the uptake of apoptotic bodies occurs in more silent and less inflammatory manner (Blander and Medzhitov, 2004). Notably, myelin debris appears to be processed akin to host defense against infection, and not to the system used for degradation of apoptotic cells (Cunha et al., 2020). The reason why myelin debris makes use of the phagocytosis route of microbes could lie in its unique structure, which might require efficient oxidative burst and antimicrobial defense to achieve complete degradation, and in addition induces pro-inflammatory signaling pathways. In agreement with such a notion, there are a number of studies demonstrating that during the first hours after myelin uptake substantial amounts of inflammatory mediators such as IL-1, TNFα, IL-6 and nitric oxide (NO) are released, whereas prolonged uptake of myelin shifts the secretion towards a more anti-inflammatory profile (Starost et al., 2020; Scheffel et al., 2012; van Rossum et al., 2008; Liu et al., 2006; Williams et al., 1994). This biphasic inflammatory response is accompanied by a change in the cellular tasks the phagocytes have to carry out. That is, from degradation to metabolism, of which lipid metabolism is of particular relevance. Due to its high lipid and in particular cholesterol content, several transcriptional modules responsible for lipid metabolism need to be activated (Berghoff et al., 2021; Bogie et al., 2020; Cantuti-Castelvetri et al., 2018; Bogie et al., 2012). Essential receptors for myelin debris clearance are peroxisome proliferator-activated (PPAR), retinoid X (RXR) and liver X (LXR) receptors, which regulate the transcription of genes involved in cholesterol efflux, lipolysis, lipid storage, fatty acid transport and fatty acid β-oxidation (Evans and Mangelsdorf, 2014; Chawla et al., 2001). In particular, cholesterol metabolism needs tight regulation, to prevent accumulation of free cholesterol, which causes endoplasmic reticulum (ER) stress and cell

toxicity (Evans and Mangelsdorf, 2014). Because cholesterol cannot be degraded, it needs to be transferred from late endosomes to the ER, where it is esterified for storage in lipid droplets or coupled to apolipoproteins for secretion into the extracellular space. Both processes, cholesterol efflux and lipid droplet storage, protect phagocytes from the toxic effect of free cholesterol within demyelination lesions (Gouna et al., 2021; Cantuti-Castelvetri et al., 2018). The efflux pathway is under the control of the LXR/RXR transcription factors that clear cells from the accumulating amounts of myelin-debris derived cholesterol, and in addition have anti-inflammatory activity. Ideally, these biphasic inflammatory and metabolic responses are self-limiting, resulting in the resolution of foam cells, thereby fueling the regenerative process. However, myelin debris clearance can be insufficient, in particular in aging, resulting in maladaptive inflammatory responses with prolonged pro-inflammatory signaling. Aged mice fail to induce the LXR/RXR pathway, but re-activation using therapeutic compounds is sufficient to restore myelin debris clearance and remyelination, suggesting that debris clearance, resolution of innate inflammation and myelin repair are mechanistically linked (Penkert et al., 2021; Cantuti-Castelvetri et al., 2018; Huang et al., 2011; Natrajan et al., 2015a).

Interestingly, in human MS with increasing age, there is an increase in the number of chronic active lesions, which can remain inflamed for decades, and are even able to expand. Single nuclear RNA sequencing of the inflammatory cells at the lesion edge, identified them as activated microglia (Absinta et al., 2021). These microglia inflamed in MS came in two states. The first cluster showed an enrichment of genes involved in lipid metabolisms such as foam-cell differentiation and lipid storage, whereas the second cluster is characterized by the upregulation of immune activation pathways and iron-related transcripts. To determine the underlying cause and the pathological consequences of these microglia states, and to define possible connection of metabolisms and inflammation, are important research directions for future studies.

Oligodendrocyte progenitor cell activation: Several factors are known to be released by microglia/macrophages that promote remyelination. Among those are factors that promote survival, recruitment, proliferation and differentiation of OPCs such as insulin-like growth factor 1 (IGF1), activin A (a member of the TGF-β superfamily), IL-1β, TNF-α, neuropilin-1, galectin-3, C-X-C motif chemokine ligand 12 (CXCL12), factor receptor AA (PDGF-AA),

hepatocyte growth-factor (HGF), fibroblast growth factor (FGF2) (Sherafat et al., 2021; Thomas and Pasquini, 2020; Hlavica et al., 2017; Miron et al., 2013; Patel et al., 2010; Pasquini et al., 2011; Lalive et al., 2005; Murtie et al., 2005; Mason et al., 2003; Arnett et al., 2001; Mason et al., 2001).

One important question is how the secretion of these factors is regulated and coordinated to the specific stages of lesion development. The repair process occurs in distinct steps, in which factors involved in proliferation and recruitment need to be released before those that promote differentiation. In addition, these processes might not occur homogenously within the lesion. For example, the lesion core might have different requirements compared to cells at the lesion edge. One solution to this problem could be to coordinate and couple the phases of myelin debris clearance in microglia/macrophages, e.g., degradation and metabolism, to the different stages of OPCs maturation (Figure 2). Indeed, evidence suggest that the production of the proliferation and differentiation promoting factors are linked to microglia states. For instances, the switch from the initial pro-inflammatory (iNOS+) to the subsequent anti-inflammatory (Arg-1+) microglia state, occurs around the same time when proliferating OPCs start to differentiate into oligodendrocytes (Miron et al., 2013). In line with this, factors promoting proliferation are often pro-inflammatory cytokines of which TNF-α is one prominent example (Cunha et al., 2020; Arnett et al., 2001), whereas factor promoting differentiation, such as TGF-β superfamily member activin-A, are released after the switch to the anti-inflammatory state has occurred (Miron et al., 2013). Moreover, the formation and the remodeling of the ECM might undergo a similar mode of regulation. Whereas the formation of the ECM is an early feature of lesion formation, the remodeling and breakdown co-incidences with oligodendrocyte differentiation (Lau et al., 2013b). There is evidence that the secretion of proteases for the degradation of ECM components that are inhibitory to the differentiation of oligodendrocytes is another mechanism of how microglia/macrophage exert control in remyelination (Luo et al., 2018; Lau et al., 2012; Siebert and Osterhout, 2011; Larsen et al., 2003). The model that is emerging can be summarized on the basis of distinct and progressive states in which inflammatory, metabolic and pro-regenerative responses are functionally linked. The tight connection of these various cellular processes by overlapping transcriptional modules ensures the coordination of the various steps in lesion recovery and regeneration.

Role of astrocytes in remyelination

Generally, astrocytes are not regarded as a cellular component of the CNS innate immune system. Yet, much like microglia, they respond to tissue injury, become reactive and convert into states associated with tissue protection (Sofroniew, 2020; Farina et al., 2007). Astrocytes are able to sense tissue damage through TLRs or other types of pattern recognition receptors. They can attract and instruct immune cells by the secretion of immune mediators and can respond to cytokines and chemokines (Rothhammer et al., 2016). In addition, they have shown to be able to phagocytose myelin debris and have a well-known function in lipid metabolism (Camargo et al., 2017). There is also evidence that astrocytes promote OPC proliferation by the secretion of for example PDGF-AA and FGF2, and enhance differentiation by ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF) and IGF-1 (Messersmith et al., 2000; Deverman and Patterson, 2012; Fischer et al., 2014; Ishibashi et al., 2006; Tsai et al., 2002; Hinks and Franklin, 1999). Moreover, there are inhibitory factor released by astrocytes, in particular components of the extracellular matrix, such as CSPG, hyaluran or tenascin, but also growth factors with inhibitory function including endothelin-1 or fibroblast growth factor 9 (Keough et al., 2016; Lindner et al., 2015; Hammond et al., 2014; Back et al., 2005). Astrocytes contribute to the formation of a scar after demyelinating injury, but it is important to consider that we know little about the role of the astrocytes scar and its interaction with microglia in remyelination. As in spinal cord injury, it is likely that the function of the astrocyte scar is complex, because of its dual role in supporting and preventing regeneration (Anderson et al., 2016). Thus, akin to microglia, astrocytes can execute a finely graded response to tissue injury, coordinate immune and repair processes. Yet, despite these similarities, there are important differences. The main function of astrocytes does not appear to be in the execution of inflammation, but rather in the control of the strength of response. To prevent collateral tissue damage, inflammation requires mechanism controlling its duration and magnitude. This occurs by negative feedback and by counter- or anti-inflammatory signals and is of particular relevance to the CNS with its poor capability for regeneration. Reactive astrocytes play an essential role in constraining inflammation, and they do this by the secretion of an extracellular protein meshwork and by their border-forming function. These are essential and beneficial tasks that separate damaged from adjacent viable tissue, and in addition, prevent the spread of the inflammatory response and the diffusion of toxic molecules. Thus, microglia/macrophages and astrocytes must be regarded as partner

that act together in the regulation of the inflammatory response following demyelinating injury (Clark et al., 2021; Rothhammer et al., 2018).

Adaptive immunity and remyelination

Although relatively poorly understood in comparison to the innate immune system, a number of studies have revealed a role for the adaptive immune system in regulating remyelination, similar to the established roles of components of adaptive immunity in the regeneration of other tissues including cardiac muscle, skeletal muscle, intestine and hair follicles (Weirather et al., 2014). A role of both T and B cells was shown first, by the identification of polyreactive IgM autoantibodies that directly promote myelin regeneration (Warrington et al., 2000), and second, the description of impaired remyelination in Rag-/- mice, in which both cell types are missing, following lysolecithin-induced demyelination (Bieber et al., 2003). Subsequent studies have provided more depth into roles of specific cell types, including remyelination enhancing functions for regulatory T cells, through the production of the ECM related protein CCN3, myelin-specific T cells and regulatory B cells, and suppression of remyelination by Th17 CD4+ T cells (Pennati et al., 2020; Dombrowski et al., 2017; Baxi et al., 2015; Hvilsted Nielsen et al., 2011). The complexity of the involvement of adaptive immune cells in remyelination has been revealed in studies in which lymphocytes from healthy donors and MS patients were grafted into demyelinated lesions in nude mice (El Behi et al., 2017). Those from MS patients resulted in an inhibition of the spontaneous remyelination that occurs in this model, either via direct effects on OPCs or indirectly via effects on macrophage phenotype. However, the effects were highly variable, reflecting the variability of remyelination seen between patients.

The role of the adaptive immune response as the principal immunogenic driver of MS is well known and extensively explored both in the disease itself and in EAE. Dissecting apart the distinct contributions different components of the adaptive immune system (and the innate immune system, for that matter) to a complex pathological landscape will be a challenging task. Here, specific models are helpful, the 'sterile' chemical models of demyelination proving useful for studying effects of remyelination in the absence of a disease-inducing adaptive immune response. EAE, which has proven invaluable over several decades in elucidating the mechanisms of CNS autoimmune disease, does not lend itself to studying the mechanisms or remyelination. Aside from the rare and unpredictable nature of remyelination in the various EAE models, it is challenging to distinguish enhancement of remyelination that is due to the

removal of an immune process targeting the oligodendrocyte, thereby allowing the natural tempo of remyelination to occur, from an intervention that is actually enhancing its rate and efficiency.

Translational and therapeutic opportunities

There are multiple approved therapies that target the peripheral immune mechanisms in MS, and consequently reduce relapses. However, we currently lack drugs that efficiently prevent the progressive accumulation of neurological disability and promote repair. There are several therapeutic opportunities for pro-regenerative immune therapies in MS. The most evident are drugs that are able to skew the immune response in such a way that acute inflammatory lesions set off to remyelinate instead of developing into chronic plaques. Such drugs have possibly a narrow therapeutic window, as they are likely to be most effective in lesions at the time of acute inflammatory activity. Clearly, when lesions have turned into a chronic, inactive state, remyelinating therapies that directly target the cells of the oligodendrocyte lineage will be more effective. However, immune-based pro-regenerative therapeutic strategies are not limited to acute inflammatory lesions that form early in the disease in a relapsing-remitting manner. These are of particular importance for progressive MS, when disability becomes permanent, and the pathology develops relatively independently of the peripheral immune cell system and is instead driven by CNS-intrinsic inflammatory activity. These areas of progressive damage develop in the cortex but also in perivascular white matter lesions. Typical features of such regions of injury are their expanding nature and their chronic inflammatory activity. Because most approved disease modifying MS therapies target the peripheral immune system and not CNS-intrinsic inflammation and neurodegeneration, current treatment options have so far only very limited efficacy towards progressive MS. Thus, there is still an unmet need for novel MS therapies that can target myeloid cells, microglia, astrocytes, as well as compartmentalized adaptive immunity in such a way that pro-inflammatory activity resolves and pro-regenerative functions take over.

Pro-regenerative therapies: Such has been the progress in recent years in understanding the basic biology of remyelination that the first steps have been taken towards translating this into clinical practice. The first clinical trials conducted based on pre-clinical data have been completed. One of these was a trial exploring the efficacy of opicinumab, a human monoclonal antibody against LINGO-1, an inhibitor of oligodendrocyte differentiation (Cadavid et al.,

2019). Another was a trial using clemastine, an antagonist of the muscarinic acetylcholine receptor (Mei et al., 2014), and one more based on the RXR agonist, bexarotene (Huang et al., 2011). Neither trial, although some providing evidence of efficacy, generated sufficiently good outcomes to establish them as effective remyelination therapies (in the case of bexarotene this was compounded by significant off target effects) (Brown et al., 2021; Cadavid et al., 2019; Green et al., 2017). The reasons for this are many. For example, these trials are based on the use of drugs that enhance OPC differentiation based on the premise that remyelination in MS fails is due to differentiation block. This is not going to be the case for all lesions or all patients and without a means of stratifying patients to identify likely responders, the danger of underestimating outcome persists. It is also apparent from rodent data that OPCs become unresponsive to inducers of differentiation with increasing age, suggesting that greater efficacy might be achieved by concurrent administration of a cell rejuvenating agent such as metformin (Neumann et al., 2019a). Nevertheless, these early translational steps have been sufficiently encouraging to warrant further intensive development, and there is no shortage of potential targets either ready soon or after further development to enter clinical trials (reviewed elsewhere by Lubetzki et al., 2020; Faissner et al., 2019). Many of the proposed MS regenerative medicines target cells of the oligodendrocyte lineage, and, in particular, the OPCs. There is much to be said for intervention that directly control the function of the cell that will give rise to new myelin sheath forming oligodendrocytes, not least that they bypass any potential inhibitory cues in the lesion environment. However, the biology of the inflammatory involvement in remyelination addressed in this review suggest many possibilities for enhancing remyelination by targeting immune cells, especially microglia/macrophages. For example, a case can be made for drugs that enhance phagocytic clearance of myelin debris, the persistence of which in lesions inhibits OPC differentiation and the clearance of which declines with age. The age-related decline in macrophage clearance of myelin debris can be reversed by niacin (vitamin B3), which increases the expression of the scavenger receptor CD36 (Rawji et al., 2020), by agonists that stimulate the nuclear receptors RXR (some of the positive effects of bexarotene may have been via this mechanism) or PPAR□, or by blocking CD22 (Pluvinage et al., 2019; Natrajan et al., 2015a,b). Alternatively, factors directly regulating OPC differentiation such as macrophage-derived activin-A or Treg-derived CCN3 could potentially be developed as remyelination targets (Dombrowski et al., 2017; Miron et al., 2013). Another potential strategy involves targeting another nuclear receptor, LXR

(Berghoff et al., 2021; Cantuti-Castelvetri et al., 2018). LXR agonists enhance myelin debris clearance and remyelination by promoting cholesterol efflux from microglia/macrophages. In addition, adequate levels of cholesterol are critical for the synthesis of new myelin sheaths. In acute lesions, the microglia/macrophages are possibly the primary source of intra-lesion cholesterol, which they obtain from myelin debris uptake. Upon phagocytosis, they increase the cholesterol precursor, desmosterol, which activates LXR altering the inflammatory state to one that is more favourable to remyelination and increasing the efflux of lipid and cholesterol form lipid-filled microglia/macrophages.

Several emerging therapies with the potential to target CNS inflammation are currently undergoing evaluation in human MS clinical trials. One promising target is Bruton's tyrosine kinase (BTK), a tyrosine kinase that is expressed by haematopoietic cells, in particular B cells and myeloid cells. Some BTK inhibitors are able to cross the blood-brain barrier, and have, thus, the potential to target CNS-compartmentalized and intrinsic inflammation (Owens et al., 2022). Another drug candidate that can possibly target CNS-intrinsic inflammation is Ibudilast, which is a non-selective phosphodiesterase that has already undergone a phase II clinical trial in which it showed efficacy in reducing brain atrophy in progressive MS (Fox et al., 2018). One more promising agent currently being evaluated in progressive MS is α-lipoic acid, an antioxidant that passes the blood-brain barrier that has shown beneficial immunomodulatory effects in animal models (Morini et al., 2004). For a more comprehensive overview of evolving immune therapies for progressive MS, we refer to recent reviews (Oh and Bar-Or, 2022; Yong and Yong, 2022).

Challenges: Because of the complex and multifaceted functions of the innate immune system, especially in the context of autoimmune disease, a major challenge is to target their pro-regenerative properties without exacerbating potentially detrimental functions. Innate immune responses drive the disease by triggering repetitive or sometimes continuous damage to myelin, and at the same time, counter-reactive immune responses are at play and important for the regenerative response. Ideally, immunomodulatory compounds should have a dual function – preventing injury inducing and stimulating reparative inflammation. Several immunomodulatory or immunosuppressive therapies that are efficiently targeting the auto-immune reaction have been approved for the treatment of MS, but we know little about their pro-remyelinating activities in humans. It is likely that these approved disease modifying

drugs need to be combined with brain-permeable therapeutics that enhance pro-regenerative functions of microglia/macrophages. This may be achievable by targeting subsets of cells with prominent regenerative functions such as phagocytosis, the occurrence and identity of which is being revealed in the increasing number of single cell sequencing studies that are emerging from MS and animal models (Kaufman et al. 2022; Mcnair et al. 2022; Absinta et al., 2021; Plemel et al., 2020; Schirmer et al., 2019; Jakel et al., 2019). One example of an experimental pro-remyelinating strategy of how to target microglia to promote clearance and metabolisms of myelin debris is the treatment with TREM2 agonistic antibodies (Bosch-Queralt et al., 2021; Cignarella et al., 2020). Promoting myelin clearance by activating microglia could come with the caveat of pathological phagocytic over-activation, as has been observed for microglia in models of Alzheimer's disease in which increased microglial phagocytic function that clears amyloid can also causes pathological synapse loss (Paolicelli et al., 2017). Other approaches are anti-inflammatory microglia targeting drugs, such as minocycline (Metz et al., 2017) or cell based therapies employing mesenchymal stem cells (Connick et al., 2012) or engineered myeloid cells (Aigrot et al., 2022). One particular promising target are the chronically activated microglia/macrophages present at the lesion rim of chronic active MS lesions. These lesions, found in 15-20% of lesions in progressive MS patients (Frischer et al., 2015), associate with more aggressive disease and ongoing tissue damage, and occur even in individuals treated with effective disease-modifying therapies (Absinta et al., 2019). Chronic inflammation in these lesions may represent non-remitting, ongoing disease activity, possibly due to an imbalance of counter-regulatory anti-inflammatory mechanisms.

Conclusion

Immune cells respond quickly to demyelinating injury by activating inflammatory pathways and mediators, responsible for initiating remyelination. Orchestrating these actions is crucial to optimal recovery, but often and in particular, with increasing age, they become inadequate, resulting in chronic inflammation and scarring. In this review, we explored the contribution of the immune system in coordinating immune cell activation and recruitment, debris clearance, progenitor cell activation, promotion of differentiation, matrix production and immune resolving functions. We propose a model of how the immune cells progress along a hierarchical order of functional states and highlight immune-mediated therapeutic strategies that can possibly be harnessed to improve remyelination. Currently, it is unknown what

determines whether inflammation resolves from MS lesions and how remyelination is initiated. Obviously, the inherent complexity and our incomplete understanding requires further basic research into the biology of remyelination and the role of the immune system in this process. The last twenty years has seen substantial advances in our understanding of the complex and multicellular interactions by which remyelination is orchestrated and how this exquisitely tuned process becomes de-tuned with ageing with the accompanying loss of efficiency. This knowledge, still far from complete, will allow a better understanding of the natural history of myelin diseases, offering new opportunities for therapy and management. Nevertheless, sifting the good from the bad in phenomenon as multifaceted as inflammation, especially in autoimmune disease, remains a pressing challenge for the field.

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Competing interests

The authors declare no competing interests.

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Figures

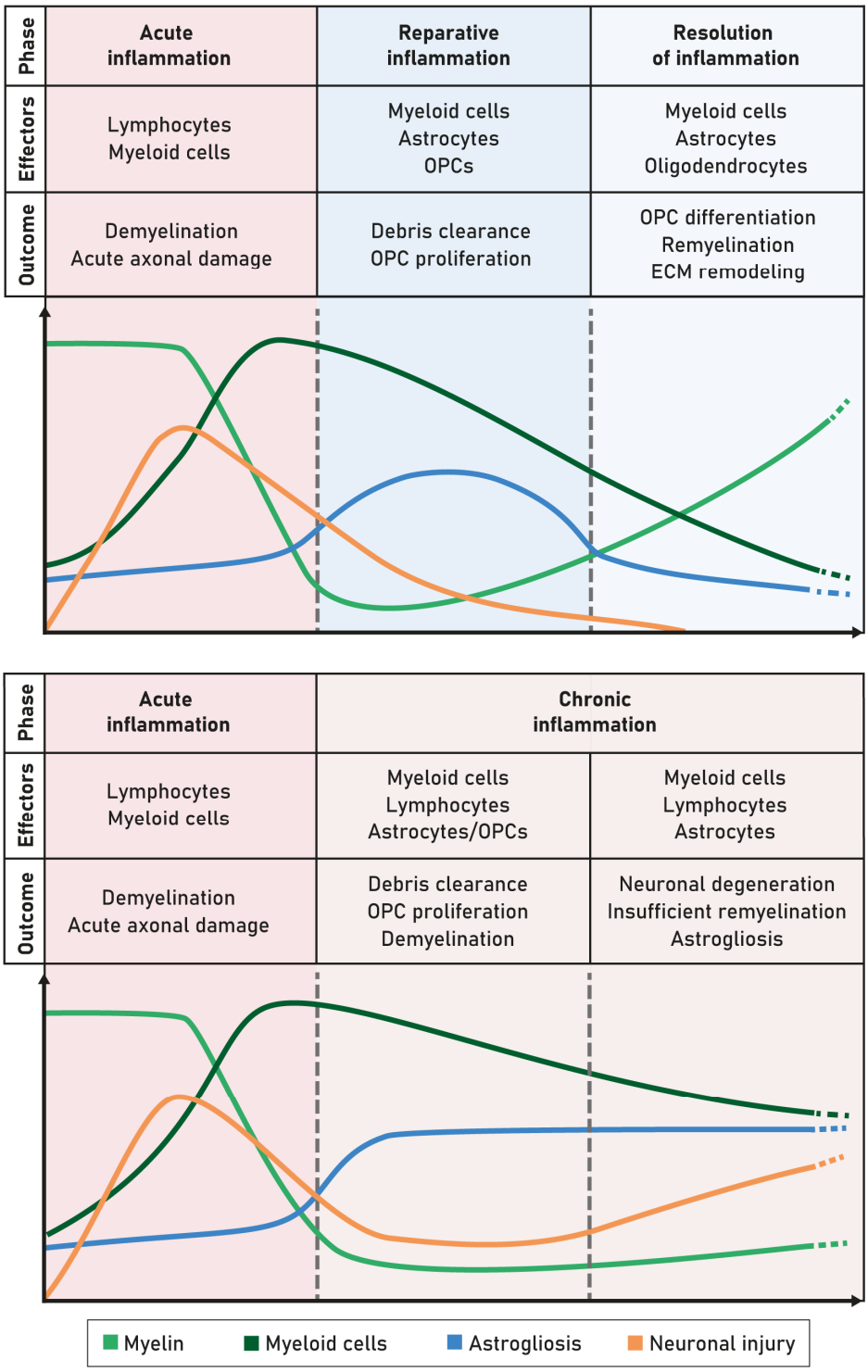

Figure 1: Illustration of the different outcomes following demyelinating injury. The upper panels shows acute demyelinating injury followed by resolving inflammation and remyelination. The lower panels depicts an unresolved chronic inflammatory response that is associated with poor remyelination, astrogliosis and neurodegeneration. Adapted from Schwartz and Baruch, 2014.

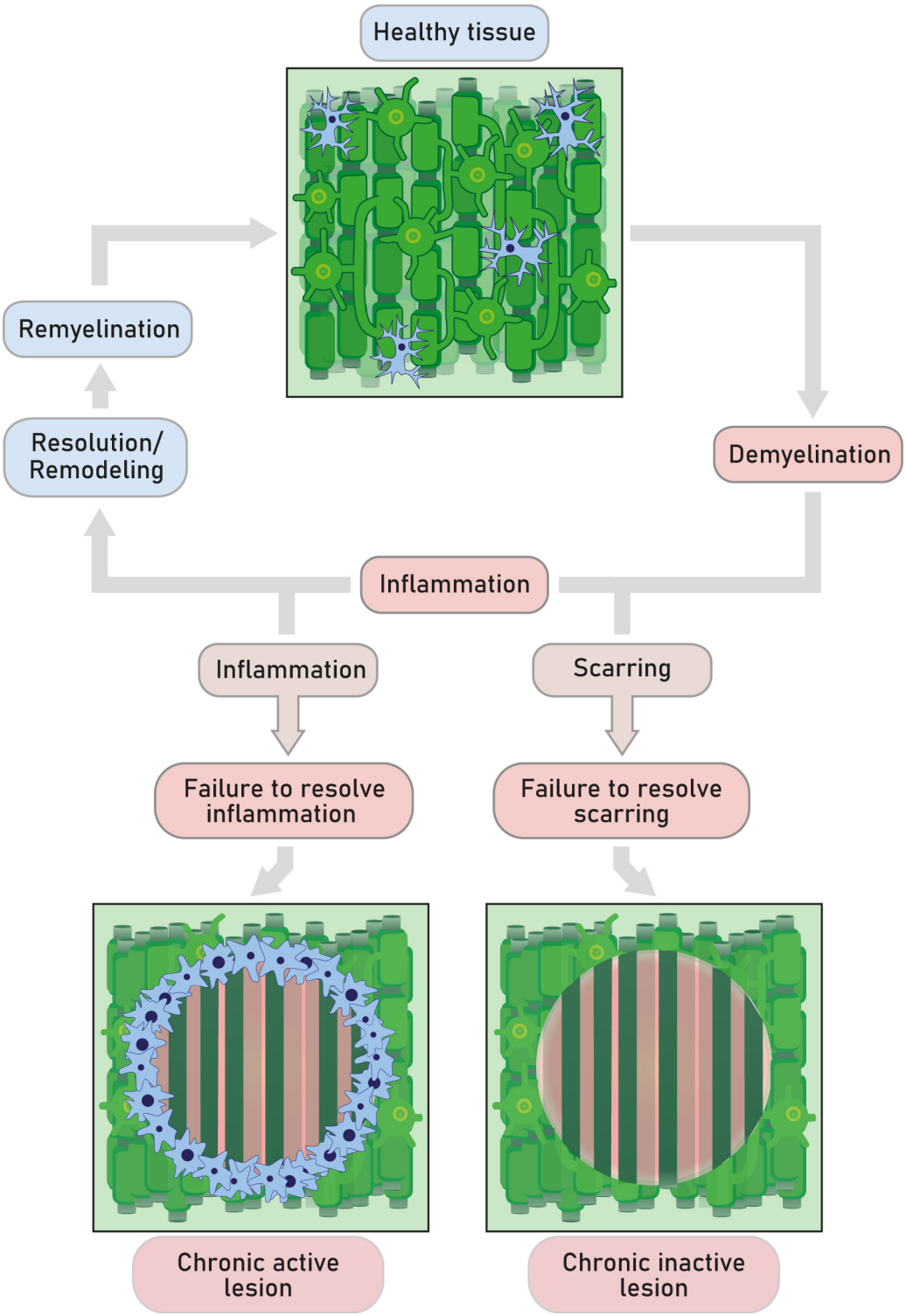

Figure 2: Schematic illustration of the possible recovery pathways from multiple sclerosis lesion pathology. Upon autoinflammatory demyelinating injury, the innate immune cells trigger a repair phase, in which proliferative and growth-promoting signal instruct the generation of new myelinating oligodendrocyetes. This phase requires remodeling of the scaring tissue and resolution of immune cells from the lesion. Prolonged or aberrant innate immune responses can cause the formation of chronic active lesions, which are characterized by a remaining inflammatory rim of activated microglia. Failure to remodel the extracellular

matrix and to initiate the generation of myelin-forming oligodendrocytes results in the chronic inactive lesions.

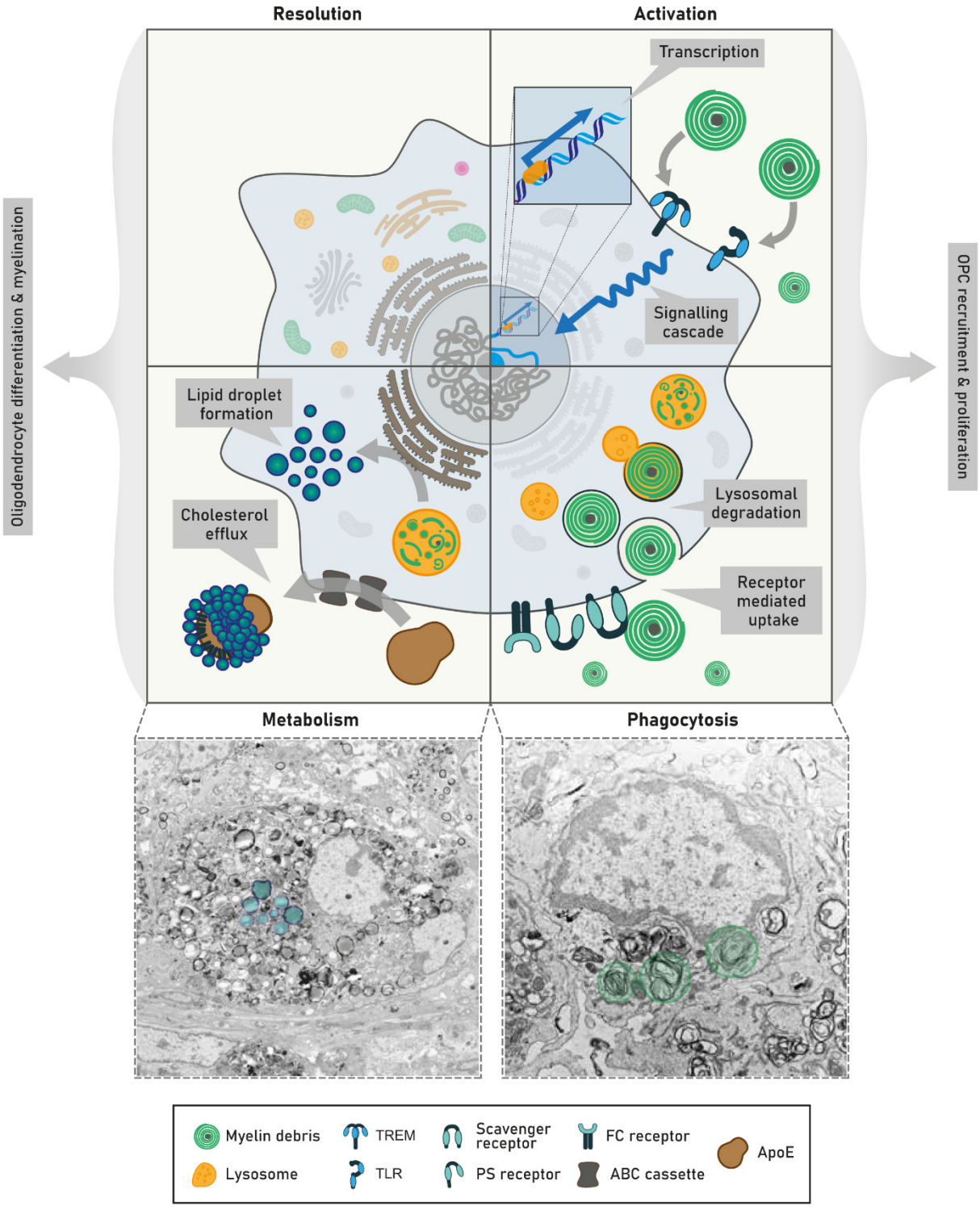

Figure 3: Illustration of the distinct states and functions of microglia and monocyte-derived macrophages in the remyelination. Microglia and monocyte-derived macrophages progress along a hierarchical order of functional states specialized to activation, phagocytosis, metabolisms and resolution. These different steps of debris clearance are possibly linked to the stages of remyelination, which require the secretion of factors for the recruitment and proliferation of OPCs and for the differentiation of oligodendrocytes. Electron microscope images depict microglia phagocytosing and processing lipids. EM images were provided by Martina Schifferer.