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Reparative inflammation in multiple sclerosis

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Summary

Multiple sclerosis is a chronic inflammatory disease, in which repeated episodes of acute auto-inflammatory attacks trigger demyelinating injury in the central nervous system. Whereas our understanding of the disease-causing immune activation is constantly increasing, much less is known about the injury-induced reparative innate immune responses. Here, we discuss the essential function of microglia and monocyte-derived macrophages in orchestrating debris clearance and regeneration. Dampening reparative inflammation can result in insufficient clearance and, thus, to persisting damage, with the consequences of prolonged inflammation. Thus, an understanding of the entire spectrum of inflammatory responses is essential for the prevention of injury-inducing and the stimulation of repair promoting functions of the immune system.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), which causes multifocal lesions with confluent foci of myelin loss and relative preservation of axons [1,2]. The underlying reasons for the formation of inflammatory demyelinating lesions remain unknown, but the prevailing view is that they are caused by an autoimmune mechanism. Once formed, acute or active MS lesions can develop into chronic inactive plaques consisting of a glial scar, or they progress into shadow plaques with areas of remyelination. Lesions can arise anywhere in the CNS, including both white and gray matter areas. Predilection sites are the optic nerve, spinal cord, brain stem, periventricular white matter, and the gray matter adjacent to the subarachnoid space. One pathological hallmark of white matter lesions is demyelination encircled by a central vein, the site where the inflammatory reaction is thought to arise, and from where the demyelinating injury appear to expand into the brain. In the cortex, lesions can be perivascular, but there are also abundant demyelinated areas, so called subpial lesions, lacking a central vein and instead associated with leptomenigeal inflammation. The inflammatory cells infiltrating active MS lesions comprising mainly of $CD8^+$ T-cells, with a smaller contribution of $CD4^+$ T-cells and B-cells, which are localized primarily in the perivascular or meningeal space [3]. The vast majority of immune cells in active MS lesions are myeloid cells, both monocyte-derived macrophages that have migrated into the brain and brain-resident activated microglia [4,5]. Myeloid cells together with soluble factors produced by lymphocytes are thought to induce the demyelinating injury [2]. Consequently, numerous immunomodulatory or immunosuppressive targeting this auto-inflammatory reaction have proven beneficial for the treatment of MS by reducing new lesion formation [6,7]. Whereas auto-inflammation drives the disease by triggering repetitive episodes of demyelination, the resulting lesions will eventually elicit counter-reactive, injury-induced immune responses. The immune activation that occurs upon tissue damage attempts to restore homeostasis by orchestrating regenerative mechanisms. Reparative inflammation is triggered when molecules that are normally sequestered in the cell interior are exposed to the external environment, where they interact with pattern recognition receptors [8,9]. Signaling downstream of pattern recognition receptors initiates an inflammatory response with the aim of eliminating the perturbing stimuli [10]. Thus, inflammation within MS lesions constitutes a spectrum of functions, and in this review, we will focus on the biology of injury-induced reparative innate immune responses.

2. Quantitative control of reparative inflammation

The magnitude and the duration of the reparative inflammation require precise regulation [9]. In general, the extent of tissue injury determines the strength of the inflammatory response. Regeneration requires strong inflammatory responses, but overshooting inflammation

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must be avoided, as it comes with the cost of inducing collateral tissue damage. Therefore, the response requires careful titration, which occurs by negative feedback and by anti-inflammatory signals. The price of collateral damage is particularly high for the CNS with its poor capacity to regenerate. Thus, inflammation that occurs upon brain injury activates typically potent anti-inflammatory responses with the aim to antagonize the mounting inflammation. Such counter inflammatory tasks are also carried out by glial cells that are responsible for the generation of a physical barrier, consisting of a fibrous scar [11–13]. Glial scar generation occurs by astrocytes and oligodendrocyte progenitor cells (OPCs), but also meningeal fibroblasts and pericytes, which together deposit extracellular matrix proteins components, including chondroitin sulfate glycosaminoglycans (CSPGs), which forms an extracellular proteineous meshwork around hyaluronic acid [14,15]. The CSPG family comprises a number of proteins, such as neurocan, phosphacan, brevican, versican, aggrecan, which normally play important roles in forming boundaries for axonal outgrowth during development. The main purpose of the glial scar is to generate a limiting border for the inflammatory response. Thus, CNS anti-inflammatory responses that control the strength and the spread of the inflammatory response are one reason for poor regeneration of the CNS. In addition, there are immune-intrinsic mechanisms that modulate the magnitude and the duration of the inflammatory response. Negative feedback loops within the immune signaling pathways are at work to prevent the over-activation and to protect non-injured brain tissue. These include, for example, the A20 deubiquitinase, a critical negative regulator of NF-κB, SOCS (suppressor of cytokine signalling) proteins, negative-feedback inhibitors of Janus kinase (JAK)-mediated signaling, and cleavage of Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) by metalloproteases, which terminate downstream signaling [16,17] [18]. By initiating such counter inflammatory measures, the brain limits collateral injury, possibly at the cost of dampened reparative inflammation. Reparative inflammation must not only be regarded as a specific inflammatory state of the immune cells, but rather as a dynamic process. It requires a robust pro-inflammatory response that is necessary to remove the perturbing inflammatory stimuli, which is ideally followed by the resolution of inflammation [19]. Dampening the inflammatory response can result in insufficient clearance with the possible consequences of prolonged inflammation. Thus, chronic inflammation after acute tissue injury can also be a result of insufficient immune activation with insufficient resolution of inflammatory triggers.

3. Qualitative regulation of reparative inflammation

In addition, to quantitative control, reparative inflammation requires qualitative regulation. The inflammatory response needs to be tailored to the type of damage that has occurred, which will vary in the amount and composition of the cellular debris, depending on where the lesions formed. For example, in the case of MS, inflammatory lesions can occur in the gray or white matter with dramatic differences in the density of myelinated fibers. Ideally, reparative inflammatory should sense the type of injured cellular structure to orchestrate the appropriate restorative response. It is difficult to envision how inflammation can be of sufficient speed, and at the same time tailored to the specific injury. One solution is that tissue damage induces a hierarchical inflammatory reaction. Microglia are among be the first responders, which react upon tissue injury with an immediate induction of a universal tissue injury program, setting the stage for a more fine-tuned response that incorporates information on the type of ingested material. Microglia are equipped with a wide range of sensors present at the cell surface but also in the cytosol and nucleus, which detect deviations in homeostatic variables [20–24]. Such sensors can detect various molecules and conditions including nucleic acids, lipids, amino acids, hypoxia, osmolarity and extracellular matrix components [25]. Ideally, sensed deviations are transformed into a specific transcriptional response with the aim to restore homeostasis. For example, cholesterol or fatty acid overload is sensed by lipid sensing nuclear receptors that trigger degradation, storage, metabolisms, or export of lipids [26,27]. Low oxygen levels are identified by hypoxia-inducible transcription factor, HIF-1α, which can induce the generation of VEGF-A to promote angiogenesis. When microglia responsiveness is overwhelmed, and the functional requirements cannot be met, inflammatory signal are released to promote proliferation and recruitment of additional microglia, monocyte-derived macrophages and other immune cells. The first tissue damage responding microglia may send out signals to recruit additional cells. Microglia are also an important source of growth factors for other cell types, and can have regenerative functions in supporting neurogenesis and myelin repair.

4. Reparative inflammation in model systems

Experimental autoimmune encephalomyelitis (EAE) is a useful experimental model to study MS-related autoimmunity that causes multifocal inflammatory lesions in the CNS. The counter-regulatory inflammatory mechanisms responsible for the reparative process are more difficult to study in EAE, because lesions are rarely primary demyelinating and occur in an unpredictable spatial and temporal manner [28]. Instead, toxin-induced models of demyelination are frequently employed to study the myelin repair process [29]. The focal injection of the toxin into the white matter of the spinal cord, corpus callosum or the cerebellum induces focal demyelinating lesions, which can be studied at different time points after lesion induction [30]. Demyelination occurs within days and is followed by a repair process that takes a few weeks and requires rapid clearance of damaged myelin by microglia and few monocyte-derived macrophages [31,32]. Studies using these models have shown that microglia/macrophages play a key role in lesion repair [33–39]. Instead of summarizing these studies, we will try to put these studies into a conceptual framework that integrates the various functions of microglia/macrophages in remyelination. The initial framework to describe distinct microglia/macrophages functional states was to categorize them into M1, as an umbrella term for their pro-inflammatory, and into M2 for their anti-inflammatory function. Whereas this categorization has served as a useful starting point for our understanding of microglia/macrophages in demyelinating lesions, they are too broad too accurately reflect their functional states in vivo. Recent single-cell RNA sequencing of microglia in models of demyelination have identified multiple states of injury-responsive microglia with downregulated expression of the canonical microglia markers *P2ry12* and *Cx3cr1*, and upregulation of a common set of core genes associated with the injury response [31,40] [41]. In addition, a number of unique genes such as Cxcl10, Ccl3, and Ccl4 characterize the different subsets of injury-responsive microglia states in demyelinating lesions [41].

Future work need to relate the distinct transcriptional programs to the various microglial functions. Signaling pathways, including TREM2, interferon signaling, and signals promoting proliferation and homeostatic functions control such transcriptional modules. These signals may alter microglia/macrophage states differently based on their epigenetic memories, which are transcriptionally silent under physiological conditions. Upon injury, the epigenetic state could be critical, as it will allow microglia/macrophage to respond faster to certain stimuli such as neuronal injury or myelin degeneration. For example, proliferative-region associated microglia (PAM) reside in the white matter during early developmental periods, but the transcriptional PAM identity is not detected during adult [42]. PAM microglia may maintain an epigenetic memory in adult white matter to respond more effectively to myelin damage. In the non-diseased brain microglia are found in a surveying state, constantly sensing and sampling their environment, and responding to changes [43]. Once the damage is sensed, microglia are rapidly converted into an injury-induced state, which may represent a highly plastic condition that allows microglia to respond to the various possible threats they can encounter. We hypothesize that injury will shift microglia state depending on the combination of signals received and

their epigenetic state. When the perturbing stimuli are cleared away, microglia will convert back again into their original transcriptional state. The model proposes a hierarchical arrangement and temporal sequence of microglia trajectories in a disease condition that depend on the epigenetic memory of microglia. The initial injury-induced response could be triggered by signals released by the damage that are sensed by pattern recognition or purinergic receptors. One outcome of this process could be the migration microglia towards the source of injury, and the expansion of the pioneering population of microglia by inducing proliferation and recruitment of monocyte-derived macrophages. From here on, microglia may convert to the disease-associated microglia, or DAM, state that represents a universal tissue injury program responsible for the upregulation of genes involved in lysosomal, phagocytic, and lipid metabolism pathways [44,45]. TREM2 is the key sensor that is responsible for setting the DAM response in motion [46]. TREM2 senses a wide range of molecules present on and released from dying cells such as apoptotic bodies, myelin debris and anionic lipid [45,47–51]. In analogy to the concept of pathogen sensing by pattern recognition receptors, the TREM2-dependent microglia activation represents a sensing mechanism related to neurodegeneration (and demyelination), and has therefore been termed neurodegeneration-associated molecular pattern sensing [52]. One remarkable feature of the DAM state is that it is induced across different neurological diseases entities [53]. One possibility to explain the DAM response in such a wide range of brain disorders is that it induces a program in which the induced proteins have a universal function in handling consequences of any brain injury. Another possibility is that it constitutes, in part, a preemptive defense response preparing microglia for the various consequences of brain injury. Once the microglia has had sufficient time to sense the damage, for example, myelin debris versus apoptotic bodies, the response is fine-tuned by activating additional transcriptional modules and inactivating others. Indeed injury-responsive in demyelinating lesions share a common transcriptional DAM signature, and in addition express a number of unique genes [41]. To deal with myelin debris that is released in large quantities in demyelinating lesions, microglia have to undergo multiple transitions. First, myelin debris needs to be rapidly removed from the extracellular space where it is inhibitory to the regeneration process [54,55]. This is carried out by large number of cellular receptors, some of them such as Tyro3/Axl/Mer (TAM), are part of the DAM program [56]. Phosphatidylserine becomes exposed on the surface of myelin debris, and consequently myelin debris phagocytosis is dependent on uptake by phophatidylserine receptors including TAM and TREM2 [57]. Furthermore, myelin debris phagocytosis relies on scavenger and C-type lectin receptors and on Fc or complement receptors when complexed with immunoglobulins or complement proteins [58]. Upon myelin debris phagocytosis, microglia are faced with a major challenge, namely to break down myelin, rich in lipids and composed of multilamellar and tightly compacted membrane [36,59] [60] [61] [62]. While lysosomal enzymes are responsible for the degradation of most myelin components, cholesterol cannot be degraded, and is instead transferred from late endosomes to the endoplasmic reticulum (ER). In the ER it is either esterified by the activity of the acyl-CoA: cholesterol acyltransferase (*soat1/Acat*) for storage in lipid droplets or complexed with apolipoproteins for secretion into the extracellular space [36,49]. In addition, microglia need to neutralize oxidized phosphatidylcholines that accumulate within demyelinating lesions over time [63]. In order to cope with lipid overload and toxicity that microglia are facing after myelin debris phagocytosis, they engage a system of nuclear lipid receptors [27]. These receptors function as lipid sensors that respond to cellular lipid levels and elicit gene expression changes to protect cells from lipid overload. Part of this system is the liver X receptor (LXR) and peroxisome proliferator-activated receptor (PPAR), which regulate the transcription of distinct gene sets in lipid metabolism, ranging from cholesterol export genes, lipolysis, lipid storage, fatty acid transport, fatty acid binding, and peroxisomal and mitochondrial fatty acid β-oxidation. Thus, transcriptional activation of the liver X receptor (LXR) and peroxisome proliferator-activated receptor activated gene programs can be regarded as the adaption to the functional demand arising from phagocytosis of myelin debris in demyelinating lesions. Perhaps the order of expression of these transcription factors follows a temporal pattern that is determined by the functional requirements. The model we propose is that microglia reactivity is determined by a hierarchical order of functional states, starting with a general and highly plastic injury response, continuing with precisely sensed on-demand programs, and ending with resolution once the injury and its resulting consequences are eliminated. Microglia reactivity within demyelinating lesions is unlikely to be homogenous [41,64], therefore the response must adaptto the localization of the cells within the lesions. It is likely that the environment within the core differs from the lesion edge, which may affect the magnitude and the duration of the response. Responses may not only differ quantitatively but also qualitatively. It is conceivable that subpopulations of microglia/macrophages follow completely distinct, non-overlapping trajectories. For example, a subpopulation may not respond to DAM signals, and may instead be exposed to different signals, such as RNA or CpG motifs, to activate Toll-like receptors, which initiates the JAK-STAT signaling pathway driving the transcription of a large panel of interferon-stimulated genes [65]. Recently, using a live imaging approach based on reporter mice that translate the pro- or anti-inflammatory polarization of phagocytes into distinct fluorescent signals, the evolution of individual phagocyte phenotypes was followed in a model of MS [66]. This study showed that the initial pro-inflammatory polarization of phagocytes is established after CNS entry; subsequently individual phagocytes switch to a more anti-inflammatory phenotype as lesions move from expansion to resolution [66]. Interestingly, death of pro-inflammatory microglia followed by repopulation to a pro-regenerative state is another mechanism of how the switch occurs [32]. The switch in phenotype is necessary for microglia to fulfill its pro-regenerative functions, not only by generating a permissive environment by removing the inhibitory myelin debris, but also by actively secreting pro-regenerative factors [34,67] [68]. If remyelination is successful, microglia/macrophages are gradually replaced by myelin-generating oligodendrocytes within lesions. Resolution of the immune infiltrate is an active, tightly coordinated process, which reverses initial inflammatory reactivity by generating anti-inflammatory mediators such as specialized pro-resolving fatty acids and cytokines [69,70]. In addition, the evolving microglia/macrophage secretome orchestrates recruitment, proliferation and differentiation of OPC into myelin-generating oligodendrocytes. Intriguingly, distinct microglia/macrophage states have been implicated in various aspects of remyelination [33,71]. Lesion recovery is characterized by a gradual switch of microglia/macrophage state, from an initial more pro-inflammatory activated microglia phenotype ($iNOS^+/CD68^+$) to a $Arg\text{-}1^+/CD68^+$ state. This transition occurs at around the time when OPCs that have been recruited into the lesion and start to differentiate into oligodendrocytes. The switch in microglia/macrophage state was necessary for the regenerative response, and, at least in part, mediated by secretion of the TGF-β superfamily member activin-A [33]. Strikingly, pro-regenerative microglia/macrophages-derived factors that have been identified are often pro-inflammatory cytokines [35,72,73]. TNF-α is one example of a pro-inflammatory factor that drives the expansion of OPC and pre-myelinating oligodendrocytes [35,74]. Thus, the initial pro-inflammatory response may be required to activate OPC proliferation and survival, while polarization of microglia/macrophages to a less inflammatory state could be important to generate the factors for OPC differentiation. One outstanding question is whether the loss of myelin is actively sensed by microglia/macrophages to generate a response aimed at regenerating myelin or whether the pro-remyelinating responses occur by default. If remyelination is a default pathway, any brain injury should lead to recruitment, proliferation and differentiation to OPC. However, in the absence of permissive axons, oligodendrocytes may not survive, thereby terminating the pro-remyelinating response.

5. Effect of aging and diet on reparative inflammation

Although repair of MS lesions can occur, magnetic resonance and positron emission tomography imaging indicate that lesion recovery becomes less efficient in the progressive phase of the disease. Aging is one key factor contributing to the failure of remyelination in MS [68, 75]. With increasing age, the capacity of OPCs to become activated and to differentiate into myelin-forming oligodendrocytes declines [68, 76–78]. In addition, aging alters the lesion environment in such a way that it becomes non-conducive for oligodendrocytes to carry out the regenerative process. There are a number of cells contributing to the generation of the non-permissive environment, but one of the most upstream changes are those occurring in microglia/macrophages. Heterochronic parabiosis experiments in which aged mice subjected to demyelinating injury were paired with young mice, have shown that monocytes-derived from young mice were sufficient to restore remyelination in the aged mice [79]. These proof-of-principle experiments provide evidence that macrophages are among the key cell types that are affected by age. How does aging affect microglia/macrophages? One feature of aging is the accumulation of deleterious molecules such as free radicals from oxidative stress, which are recognized by a system of damage sensing receptors that initiate a debris clearance inducing immune reaction. With time when damage accumulates and clearance mechanisms are overwhelmed the immune activation can become chronic, inducing low-grade inflammation in various organs, sometimes called 'inflammaging' [80]. Age-associated inflammation increases also in the brain, characterized mainly by elevated expression of inflammatory markers in a subpopulation of microglia. Microglia, in particular, within the white matter become particular responsive, as they have to deal with the accumulating amounts of age-related myelin degeneration [57,81–83]. Because of their elevated inflammatory profile, microglia in the aging brain are often termed 'primed', implying that they are more sensitized towards secondary stimuli [84]. One this basis one might assume that microglia become more responsive upon demyelinating injury. However, when a demyelinating injury is induced in the aging white matter, microglia reaction is initially impaired resulting in an insufficient pro-inflammatory reaction [35,36]. One consequence of the aberrant activation is that microglia/macrophages do not appear to reach a state at which they are able to initiate transcriptional modules necessary for myelin debris clearance. Various functions of microglia/macrophage are impaired within lesions of aged rodents (Fig. 1). Not only the phagocytosis of myelin debris is reduced, but also its lysosomal degradation and subsequent metabolism is impaired. In particular, myelin-debris derived cholesterol poses a challenge to phagocytes. Free cholesterol is toxic to cells; it therefore needs to be stored in lipid droplets and to be transferred from the microglia/macrophages to the extracellular space onto lipoprotein particles. Cholesterol efflux is under the control of the LXR transcription factor, which form heterodimers with the obligate partner retinoid X receptor (RXR), and together they enhance the expression of *Apoe*, *Abca1* and *Abcg1* transcripts that are necessary to clear microglia/macrophages from the accumulating amounts of cholesterol. Aged mice fail to induce the LXR/RXR pathway, and consequently foamy microglia/macrophages with signs of cholesterol overloading accumulate in

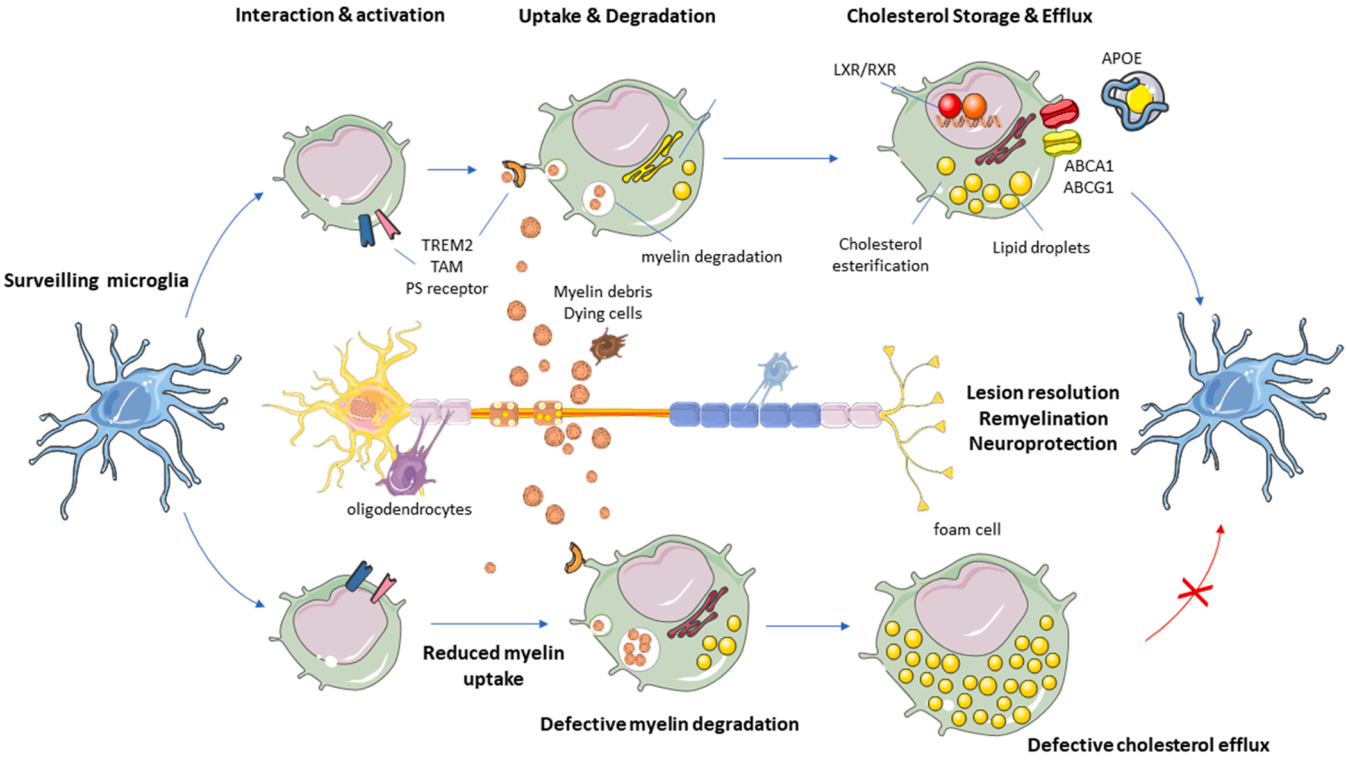

Fig. 1. This figure summarizes the steps in the recruitment and activation of microglia during demyelination. On top, surveilling microglia are activated upon sensing myelin and cell damage. Pattern recognition receptors and phosphatidylserine receptors recognize specific moieties on degenerating myelin, myelin debris and apoptotic cells, triggering the activation of microglia ("Interaction & activation"), and mediate the uptake of myelin debris, followed by its degradation in the lysosomes ("Uptake & degradation"). Oxysterols and desmosterol trigger the activation of liver X receptor (LXR), leading to the upregulation of genes involved in lipid efflux ("Cholesterol storage & efflux"). Free cholesterol is delivered to the endoplasmic reticulum for esterification. Upregulation of the cholesterol transporters ABCA1 and ABCG1 allows microglia to export and load cholesterol onto lipoproteins. Once the cholesterol overload is cleared, the inflammatory response gradually subsides, and the microglia returns to its surveilling state. On the bottom, the steps of maladaptive inflammation during aging are shown. Reduced myelin uptake results in the accumulation of myelin debris in the extracellular space, where they interfere with remyelination ("Reduced myelin uptake"). Foamy microglia, characterized by numerous lipid droplets, are formed as the reverse cholesterol transport is impaired ("Defective cholesterol efflux"). Foam cells persist, delaying the resolution of the inflammation and remyelination.

lesions. Activation of LXR or RXR by small molecules is sufficient to fully restore the reparative inflammatory response necessary form myelin debris clearance and remyelination [36,55]. Interesting, LXR and RXR agonists have also direct pro-regenerative effects on oligodendrocytes [55,85]. These results show that it is in principle possible to revert the effects of aging on microglia/macrophages by stimulating selected transcriptional modules. Why microglia become locked into an unresponsive state in the aging brain is unknown, but one possibility could be that anti-inflammatory molecules are building up in the aging brain to suppress 'inflammaging'. Thus, unleashing microglia/macrophage activation could possibly represent a strategy to produce potent pro-regenerative responses in the aging brain. Indeed, by applying TREM2-enhancing monoclonal antibodies, which stimulates microglia/macrophage reactivity promotes myelin debris clearance and cholesterol efflux [48,50]. Apart from age, unhealthy nutrition is another repair-limiting factor. Strikingly, feeding mice with a Western-type of diet (WD) induced an aging-related, dysfunctional metabolic response that is associated with impaired myelin-debris clearance in microglia. The underlying reason was an upregulation of transforming growth factor beta (TGF-β) signaling in demyelinating lesions, which suppressed the activation of the LXR pathway. The reason for enhanced TGFβ signaling in WD-fed mice is not known, but could possibly arise as a compensating mechanism, which limits the mounting pro-inflammatory microglia activation induced by the diet. One consequence, of enhanced TGFβ signaling, could be poor microglia responsiveness, thereby limiting the required activation of LXR-regulated genes involved in myelin debris clearance and cholesterol efflux. Intriguingly, fasting and the fasting mimetic metformin rejuvenate poor remyelination in aged rodents [77]. Although not all consequences of impaired myelin debris clearance have been resolved, it is clear that myelin debris remaining within the extracellular environment generates a non-permissive condition for the generation of myelin-forming oligodendrocytes. In addition, the myelin debris components that accumulate intracellularly can induce maladaptive inflammatory responses causing chronic inflammation. Thus, the failure of microglia/macrophages to phagocytose, to degrade and to metabolize myelin debris has profound impact on the regenerative process, and may eventually increases the likelihood of scarring reactions.

6. Chronic inflammation in MS

Whereas the pathophysiological mechanisms underlying the conversion of relapsing-remitting MS (RRMS) into progressive MS are unknown, there are indications that the changing inflammatory pattern could contribute [86]. RRMS is characterized by the formation of acute inflammatory demyelinating lesions, which can be seen and followed by MRI. Lesions build up and resolve relatively rapidly with the acute inflammatory edema disappearing within weeks and the inflammatory activity within months. These relapsing-remitting type of inflammatory episodes become less frequent and disappear completely with advancing and progressive disease stages. In progressive MS, chronic, non-resolving inflammation dominates [86]. These are leptomeningeal lymphoid tissue, sometimes associated with demyelinating lesions located in the subpial layers of the cortex [87,88]. In addition, there are diffuse inflammatory infiltrates in the so-called normal-appearing white and gray matter [89]. They often consist of clusters of reactive microglia and of perivascular lymphocytes. Whereas most T cells in MS lesions are thought to be short-lived, a small subpopulation of long-lived tissue-resident memory T cells persist in the brains of patients with progressive MS [90]. Furthermore, patients with progressive MS have more often chronic active (or also called smoldering or mixed active-inactive lesions). In autopsy series, chronic active lesions constitute over one-third of the total lesions in progressive MS [91]. Chronic active lesions are characterized by two areas of mixed inflammatory activity, a central region from which inflammation has resolved and a remaining inflammatory rim of activated microglia. Longitudinal imaging with high-resolution, ultrafield MRI have revealed that lesions remain inflamed for years, and have sometimes even expand [92,93]. A recent MRI-informed single nuclear RNA sequencing study determined the transcriptional profile of cells within the edge of chronic active lesions [94]. The study confirmed that inflammatory cells at the leading edge where mainly reactive microglia, and found that these microglia inflamed in MS ('MIMS') cluster into two different states. The first cluster was named MIMS-foamy, because of their enrichment of regulated transcripts involved in lipid metabolisms, in particular foam-cell differentiation and lipid storage. The second cluster, MIMS-iron, was characterized by the upregulation of genes encoding ribosomal proteins, inflammatory activation, and iron-related genes. Both populations shared around one third of genes that are regulated by interferon-γ. Because these data clearly show that microglia at the lesion edge, appear to remain in a reactive, chronic inflammatory state, it will be important to determine what defines whether inflammation resolves or persists. It is possible that the inflammatory process that with time becomes trapped behind a closed brain-barrier drives and maintains microglia reactivity at the lesion edge in progressive MS. Because of the enrichment of genes involved in lipid metabolism in one of the clusters of reactive microglia, it is tempting to speculate that insufficient lipid clearance is another factor keeping microglia within a reactive state. At present, it is unknown, how these two subpopulations of microglia are formed and whether they are functionally connected. It is certainly possible that MIMS-iron and MIMS-foamy are formed along separate trajectories, but it also conceivable that MIMS-iron represent a later stage of microglia reactivity. Myelin is not only rich in lipids but also in iron, and it therefore possible that MIMS-foamy and MIMS-iron represent distinct and possibly arrested stages in myelin debris clearance.

7. Conclusion

In this review, we argue that inflammation that occurs in MS must be considered in its entire spectrum. Whereas the auto-inflammatory adaptive immune activation has been a key subject of research in the past, much less is known about brain-intrinsic reparative innate inflammation that is triggered after demyelinating injury. Here, we have highlighted the important function of reparative innate inflammation in MS, which can be seen as a universal reaction to tissue injury that is required for debris clearance and regeneration. It builds of rapidly after tissue injury, and subsequently resolves. Failure to form or to resolve could be one of the underlying reasons of chronic inflammation in progressive MS. While auto-inflammatory and reparative inflammation have so far been studied in isolation, a key challenge for future research will be to understand how both interact, and influence each other. Insights into this cross talk is likely to yield new important therapeutic avenues of how to treat chronic inflammation in MS.

Declaration of interest

The authors declare no competing financial interests.

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