



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

## Microglia: origins, homeostasis, and roles in myelin repair

**Citation for published version:**

Lloyd, A, Davies, C & Miron, V 2017, 'Microglia: origins, homeostasis, and roles in myelin repair', *Current Opinion in Neurobiology*. <https://doi.org/10.1016/j.conb.2017.10.001>

**Digital Object Identifier (DOI):**

[10.1016/j.conb.2017.10.001](https://doi.org/10.1016/j.conb.2017.10.001)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Current Opinion in Neurobiology

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



## Manuscript Details

|                          |   |
|--------------------------|---|
| <b>Manuscript number</b> | CONEUR_2017_101_R1  |
| <b>Title</b>             | Microglia: origins, homeostasis, and roles in myelin repair |
| <b>Short title</b>       | Microglia and remyelination                                 |
| <b>Article type</b>      | Review Article  |

### Abstract

Microglia are the resident macrophages of the central nervous system (CNS), implicated in developmental processes, homeostasis, and responses to injury. Derived from the yolk sac during development, microglia self-renew, self-regulate their numbers during homeostatic conditions, and show a robust proliferative capacity even in adulthood. Together with monocyte-derived macrophages (MDM), microglia coordinate the regeneration of CNS myelin around axons, termed remyelination. Gene expression analyses and experimental modelling have identified pro-remyelination roles for microglia/MDM in clearance of myelin debris, secretion of growth factors, and remodelling of the extracellular matrix. Further investigations into the molecular mechanisms controlling these regenerative functions will reveal novel therapeutic strategies to enhance remyelination, via harnessing the beneficial effects of the innate immune response to injury.

|   |   |
|---|---|
| <b>Keywords</b>                           | microglia; macrophage; remyelination; regeneration; repair; myelin; oligodendrocyte |
| <b>Corresponding Author</b>               | Veronique Miron   |
| <b>Corresponding Author's Institution</b> | The University of Edinburgh   |
| <b>Order of Authors</b>                   | Amy Lloyd, Claire L. Davies, Veronique Miron  |
| <b>Suggested reviewers</b>                | Wee Yong, Robin Franklin  |

## Submission Files Included in this PDF

### File Name [File Type]

Cover letter revision.docx [Cover Letter]

Current Opinion in Neurobiology highlights.docx [Highlights]

Lloyd Davies Miron Current Opinion in Neurobiology \_R1.docx [Manuscript File]

Figure R1.tif [Figure]

Current Opinion in Neurobiology COI.docx [Conflict of Interest]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Dr. Veronique E. Miron, Ph.D.  
The Queen's Medical Research Institute  
Edinburgh, United Kingdom  
Email: vmiron@staffmail.ed.ac.uk



THE UNIVERSITY  
of EDINBURGH

Dear Prof. Stevens,

Herein is our revised version of the review article CONEUR\_2017\_101 'Microglia: origins, homeostasis, and roles in myelin repair'. We appreciate the reviewer's comment that the review is well written, concise, and timely, and have addressed all the suggestions which have been highlighted in yellow in the text, and described below:

**At least some mention could be made of the potential "dark side" of microglia/MDMs in demyelinating disease, even if only to put the current focus on their beneficial roles into a historical perspective (e.g., contribution to oxidative stress, inflammatory cytokine production...)**

We have added the following to the introduction: 'As such, microglia are early responders to CNS damage or abnormality and are thus placed to quickly coordinate subsequent responses. These can be damaging, for instance in contributing to neurodegeneration<sup>3</sup>, dysregulated synaptic pruning<sup>4</sup>, and associated with dementia<sup>5</sup>, reviewed in detail elsewhere<sup>6</sup>.' We chose not to elaborate on the damaging aspects of microglia reactions further due to existing review articles on this topic, and our focus on the regenerative properties. The damaging effects of MDMs are outlined in main text, as described in answer to the reviewer's second comment, below.

**The discussion of the differing transcriptional profiles of resident microglia and MDMs during demyelination is interesting but would be complemented by greater focus on the few studies that have shown functional differences between the two populations. The description of the recent TNFR2 ablation study by Gao et al. (2017) is a good start but could be complemented by Yamasaki et al. (2014)'s findings that MDMs appear to show greater association with nodes of Ranvier in EAE, actively removing myelin segments. Ajami et al., 2011 (Nature Neuroscience 14, 1142-1149) could also be used as evidence for functionally distinct roles between the two.**

In addition to the description of distinct microglia and MDM functions in EAE from Yamasaki and Lewis papers, we have now added the following to the main text: 'Other studies have demonstrated the requirement for MDM in initiating EAE disease and progression<sup>51</sup> via CCR2 signalling<sup>52,53</sup>; accordingly, Yamasaki et al. showed that MDMs in close association with axoglial units have myelin inclusions<sup>49</sup>, suggesting that these cells may directly induce demyelination', referencing the above studies and some additional ones we found were relevant.

**Figure: Consider including OPCs/OLs in the diagram and indicate where some of the microglia functions are thought to act on the lineage.**

We have updated the figure to include the oligodendroglial lineage cell responses.

**"Microglia are the resident tissue macrophages in the central nervous system (CNS), comprising up to 12-16 % of total neural cells in mouse and human". These figures seem to be the upper estimates, with figures for most CNS regions being somewhat below these percentages. The sentence could be revised to reflect more typical estimates**

We have updated the sentence to read: 'Microglia are the resident tissue macrophages in the central nervous system (CNS), comprising 5-12 % of total neural cells in mouse brain and 0.5-16% in human brain<sup>1,2</sup>'

Best regards

*Veronique Miron*

**Current Opinion in Neurobiology**

**Invited review special issue: Glial Biology**

**Title: Microglia: origins, homeostasis, and roles in myelin repair**

**Authors:** Amy F. Lloyd\*<sup>1</sup>, Claire L. Davies\*<sup>1</sup>, Veronique E. Miron<sup>1</sup>

**Highlights:**

- Microglia origins differ in development versus during postnatal homeostasis
- Microglia gene expression profiles during remyelination reveal roles in tissue homeostasis and regulation of oligodendroglial lineage cell recruitment/differentiation
- Microglia and monocyte-derived macrophages coordinate remyelination via phagocytosis of myelin debris, remodelling of the extracellular matrix, and secretion of growth factors that drive oligodendrocyte lineage cell responses.

## Current Opinion in Neurobiology

### Invited review special issue: Glial Biology

#### Title: Microglia: origins, homeostasis, and roles in myelin repair

**Authors:** Amy F. Lloyd\*<sup>1</sup>, Claire L. Davies\*<sup>1</sup>, Veronique E. Miron<sup>1</sup>

\*These authors contributed equally to this work

#### Affiliation:

<sup>1</sup>Medical Research Council Centre for Reproductive Health, The Queen's Medical Research Institute, The University of Edinburgh, 47 Little France Crescent, Edinburgh, United Kingdom, EH16 4TJ.

#### Corresponding author:

Veronique E. Miron, [vmiron@staffmail.ed.ac.uk](mailto:vmiron@staffmail.ed.ac.uk), +44 (0)131 242 6642

#### Highlights:

- Microglia origins differ in development versus during postnatal homeostasis
- Microglia gene expression profiles during remyelination reveal roles in tissue homeostasis and regulation of oligodendroglial lineage cell recruitment/differentiation
- Microglia and monocyte-derived macrophages coordinate remyelination via phagocytosis of myelin debris, remodelling of the extracellular matrix, and secretion of growth factors that drive oligodendrocyte lineage cell responses.

## **Abstract**

Microglia are the resident macrophages of the central nervous system (CNS), implicated in developmental processes, homeostasis, and responses to injury. Derived from the yolk sac during development, microglia self-renew, self-regulate their numbers during homeostatic conditions, and show a robust proliferative capacity even in adulthood. Together with monocyte-derived macrophages (MDM), microglia coordinate the regeneration of CNS myelin around axons, termed remyelination. Gene expression analyses and experimental modelling have identified pro-remyelination roles for microglia/ MDM in clearance of myelin debris, secretion of growth factors, and remodelling of the extracellular matrix. Further investigations into the molecular mechanisms controlling these regenerative functions will reveal novel therapeutic strategies to enhance remyelination, by harnessing the beneficial effects of the innate immune response to injury.

## Introduction

Microglia are the resident tissue macrophages in the central nervous system (CNS), comprising 5-12 % of total neural cells in mouse brain and 0.5-16% in human brain<sup>1,2</sup>. As members of the innate immune system, a major function of microglia is to survey the microenvironment for signs of injury, such as damage-associated molecular patterns (DAMPs) released from dying or stressed cells. As such, microglia are early responders to CNS damage or abnormality and are thus placed to quickly coordinate subsequent responses. These can be damaging, for instance in contributing to neurodegeneration<sup>3</sup>, dysregulated synaptic pruning<sup>4</sup>, and associated with dementia<sup>5</sup>, reviewed in detail elsewhere<sup>6</sup>. However, although less studied, microglial post-injury responses can also be regenerative; indeed, studies over the past 16 years have revealed the supportive role of macrophages (i.e. microglia and monocyte-derived macrophages (MDMs)) in the regeneration of myelin, termed remyelination<sup>7-15</sup>.

Remyelination reinstates and preserves axonal function and health<sup>16-20</sup>. This involves recruitment and proliferation of oligodendrocyte progenitor cells (OPCs), their differentiation into mature oligodendrocytes, and ensheathment of axons with new myelin. The failure of remyelination in various neurological contexts, such as with ageing and in progressive multiple sclerosis (MS), is considered to contribute to the axonal loss which correlates to decline in motor, sensory, and cognitive functions. Failed remyelination with ageing has been associated with dysregulated macrophage responses in experimental models<sup>10,11,21</sup>, highlighting a key pro-regenerative therapeutic target. Although clinical trials aimed at enhancing remyelination by directly stimulating oligodendrocyte lineage cells are currently ongoing for the first time, whether these drugs will be successful, and whether they will work efficiently in all patients, remains to be seen. Given the plethora of evidence

implicating macrophages in remyelination in both rodent and human pathological studies, further investigations into microglia biology will reveal novel therapeutic strategies based on manipulating the innate immune response. Here, we summarize current knowledge on microglia development, homeostasis, and gene expression/ functions during remyelination.

### **Origins, maintenance, & repopulation of microglia during homeostasis**

Microglia regulate developmental processes, including but not limited to oligodendrocyte differentiation<sup>22</sup>, neural precursor migration<sup>23</sup>, and synaptic pruning<sup>24</sup>. Accordingly, microglia arise early on in development, populating the murine neuroepithelium by embryonic day 9.5 (E9.5)<sup>25</sup>, and subsequently being closed off from the periphery by the formation of the blood brain barrier by E13.5. Between E10.5 and birth, microglia rapidly proliferate to colonise the CNS, maturing into ramified microglia<sup>26</sup>. This early CNS colonisation of microglia is conserved across vertebrates<sup>27</sup>. Indeed, in humans, Iba-1<sup>+</sup> CD68<sup>+</sup> CD45<sup>+</sup> MHCII<sup>+</sup> amoeboid microglia penetrate the cerebral cortex by gestational week (gw) 4.5<sup>28,29</sup> and migrate to the immature white matter via the ventricular lumen and leptomeninges<sup>28</sup>. A second wave of infiltration of microglia at 12-13 gw then seeds the embryonic brain via the vasculature<sup>29</sup>. By 22 gw, microglia take on a ramified morphology, becoming fully mature by 35 gw.

Microglia originate from erythromyeloid progenitors (EMPs) in the yolk sac<sup>25</sup>, elegantly shown using fate mapping where progenitor cells expressing runt-related transcription factor 1 (Runx1) express yellow fluorescent protein (YFP) after tamoxifen-induced Cre recombination. Specific labelling of yolk sac EMPs in embryos, achieved by injection of 4-hydroxytamoxifen (4-OHT) into pregnant females prior to foetal liver haematopoiesis (at E7.5), allowed tracing of YFP<sup>+</sup> cells to the rudimental brain<sup>25</sup>. This migratory process is



circulation-dependant, as *Ncx-1*<sup>-/-</sup> mice devoid of a functioning blood circulation lack microglia<sup>30</sup> despite undergoing normal haematopoiesis<sup>25</sup>. Although yolk sac EMP-derived primitive macrophages can colonise the whole embryo by E9.5, these cells are committed to a microglia fate by downregulation of *Timd4* and *Cd206* and upregulation of *Sall1* and *Sall3*<sup>31</sup>. In addition, interferon regulatory factor 8 (*Irf8*), working in a heterodimeric partnership with *Pu1*, is a critical survival factor expressed during early microgliogenesis, essential for microglia development and specification<sup>26</sup>. Furthermore, RNAseq analysis revealed that the precise coordination of gene expression directs differentiation of early progenitors (expressing *Mcm5*, *Dab2*, *Lyz2* and *Pf4*) into post-natal microglia (upregulating *Csf1R* and *Cxcr2*) and finally into mature microglia (expressing *Cd14* and *Mafb*)<sup>32</sup>. Yolk sac-derived microglia can persist until adulthood<sup>25,33,34</sup>, yet it cannot be dismissed that a small proportion of microglia may be replaced by haematopoietic stem cells (HSCs) since in zebrafish, adult microglia originate from the ventral wall of the dorsal aorta, a source of definitive haematopoiesis<sup>33</sup>.

After birth, microglial numbers initially increase in the first two weeks of life, followed by a decrease to a steady homeostatic level via apoptosis and reduced proliferation<sup>35,36</sup>. Under homeostatic conditions, yolk-sac derived microglia were historically considered to be long-lived cells with low turnover. However, recent evidence has shown that the microglia population in the mouse brain is dynamically regulated at the individual cell level via coupling of apoptosis and proliferation, with a minimum of 1.38% of microglia estimated to be proliferating (as shown by BrdU incorporation) at any given time and 1.23% of microglia dying every 24 hours<sup>37</sup>. Altogether, it is therefore estimated that a whole microglial population in the murine CNS can turn over in 96 days. In human brain, slightly higher rates

of proliferation were observed (Ki67<sup>+</sup> Iba-1<sup>+</sup> cells)<sup>37</sup>, but rates of death are unknown. Dynamic regulation of the microglia population in the adult mouse brain has also been observed by repopulation from CNS-endogenous cells following global depletion of microglia<sup>38-41</sup>. These studies involved adult mice, thereby revealing that repopulation of microglia occurs in a mode distinct from the original seeding in development. There are currently 2 proposed models of microglia repopulation. The first study, using a *Cx3cr1-CreERT2*;iDTR mouse model, ablated a minimum of 80% of microglia in the cortex, cerebellum, and spinal cord by 3 days post-diphtheria toxin<sup>40</sup>. A subsequent rapid repopulation was observed at 7 days post-diphtheria toxin, mediated by self-renewing proliferating microglia (expressing Nestin), with numbers returning back to control levels 1 week later<sup>40</sup>. The second model, where >90% of microglia were depleted using a CSF1R inhibitor, observed repopulation from a CX3CR1-negative CNS-resident cell<sup>39</sup>. BrdU labelling showed that 70% of labelled cells were positive for Nestin but negative for microglia markers such as Iba-1. When repopulation was complete, almost all BrdU labelled cells were positive for microglial markers, leading to the conclusion that the Nestin<sup>+</sup> cells differentiated into microglia<sup>39</sup>. These repopulating cells heterogeneously expressed a range of markers for hematopoietic stem cells or neural stem cells including Nestin, CD34, and c-kit, as well as lectin-IB4, CD45, and Ki67<sup>39</sup>. Further investigations showed that the repopulated microglia have similar inflammatory gene expression and functional responses to lipopolysaccharide compared to pre-depletion microglia<sup>39</sup>, however the identity and origin of these cells remains unclear and is hotly debated. Definitive lineage tracing and RNA-sequencing of Nestin<sup>+</sup> cells should ultimately uncover their identity and role in the CNS. Differences in between these two studies may reflect different levels of depletion (>99%<sup>39</sup> compared to 80%<sup>40</sup>), and only the DTR-driven depletion leading to a robust pro-

inflammatory response and astrogliosis<sup>40</sup>. Although there is uncertainty as to the role of Nestin in microglia repopulation, be it re-expression by residual microglia<sup>40</sup> or cells potentially capable of differentiating into microglia<sup>39</sup>, it is clear that cells expressing Nestin are nonetheless integral to the rapid repopulation of microglia following depletion. Determining whether the dynamic regulation of microglial turnover/ repopulation is dysregulated in the context of failed remyelination may be critical in understanding how to therapeutically target microglia.

### **Transcriptional profiles of microglia and monocyte-derived macrophages during central nervous system remyelination**

The importance of inflammation and microglia/ MDM in remyelination was initially suggested by transcriptomic studies of whole remyelinating lesions. For example, a microarray gene expression analysis of whole mouse brain tissue following cuprizone toxin-induced demyelination revealed regulation of genes associated with inflammation and/or the recruitment/stimulation of macrophages. For instance, lysozyme M (*LyzM*) and leukocyte common antigen/CD45 (*Ptprc*), and genes associated with recruitment/ activation of macrophages (*Ccl2* (MCP-1), *Ccl9* (MIP $\gamma$ ), *Ccr5*, *Mmp12*), were upregulated at the time of concomitant demyelination and initiation of remyelination (6 weeks after initiation of cuprizone treatment) and subsequently downregulated during the later stages of remyelination (6 weeks recovery on normal diet)<sup>42</sup>. Downregulation of these genes during late remyelination coincided with the upregulation of myelin-associated genes (such as proteolipid protein 1 (*Plp*), myelin associated glycoprotein (*Mag*), myelin oligodendrocyte glycoprotein (*Mog*)), as well as synectin (*Gipc1*), BCL tumour suppressor 7C (*Bcl7c*), lysophosphatidic acid receptor 1 (*Lpar1*; important for Schwann cell myelination<sup>43</sup>), kinesin

family member 5A (*Kif5a*; required for transport of neurofilament), and NK6 transcription factor related (*Gtx*; expressed during myelination and remyelination<sup>44</sup>). To further identify genes involved in remyelination, Arnett, et al. <sup>45</sup> analysed whole brains isolated from *Tnfa*<sup>-/-</sup> mice, which do not remyelinate efficiently after cuprizone-induced demyelination. In comparison to wildtype mice, microarray analysis identified differential expression of genes during remyelination (6 weeks recovery on normal diet after 6 weeks of cuprizone diet) associated with the immune response (downregulation of *Mhc-II*, *Ccr6*, *Cd19*, *Cd105*; upregulation of *Ifn*), as well as the cell cycle (upregulation of *Cyclin-e*, *Polg*, *Cdk5r2*), development (downregulation of *Crygd*, *Crybb2*, *Igf1/2*; upregulation of *Fzd*, *Sema5b*) and regulation of transcription (upregulation of *Myt1*)<sup>45</sup>.

Importantly, these studies support that the immune response is a critical component of remyelination; however, these results do not reflect transcriptional profiles of individual cell populations. Indeed, Srinivasan, et al. <sup>46</sup> showed that the expression profiles of cells extracted from tissues affected by neurodegenerative disease are influenced by cell composition, and whole tissue RNA analysis may obscure significant gene changes in microglia and, thus, could be misleading. Similarly, some studies have combined microglia and MDM together for analysis, as a result of the challenges of distinguishing and isolating microglia from MDM<sup>47</sup>. To overcome this, Lewis, et al. <sup>48</sup> isolated microglia from MDM by fluorescence-activated cell sorting (FACS) using differential expression of the surface marker CD44, and investigated the transcriptomes of these cell populations in the pathogenesis of experimental autoimmune encephalomyelitis (EAE). Microglia and MDM were isolated from naive mouse brains when clinical symptoms were absent (at 7 days post immunisation; disease score of 0) and when partial hind-limb paralysis was observed (at 14 days post

immunisation; disease score of 3), and transcriptomes were determined using RNA sequencing. Principal component analysis revealed that the two cell populations remained distinct at the transcriptome level during the course of EAE. The distinct microglia and MDM expression profiles during EAE were confirmed in a separate study<sup>49</sup>, which identified that MDM were more likely than microglia to express genes related to effector functions, including secreted factors and surface molecules. Furthermore, it was recently shown that differential expression of genes by microglia and MDM can lead to differing functions in EAE pathogenesis. For instance, microglial expression of tumour necrosis factor receptor 2 (*Tnfr2*) is protective against EAE, whilst MDM expression of *Tnfr2* drives immune cell activation and initiation of EAE<sup>50</sup>. Other studies demonstrated the requirement for MDM in initiating EAE disease and progression<sup>51</sup> via CCR2 signalling<sup>52,53</sup>; accordingly, Yamasaki et al. showed that MDMs in close association with axoglial units have myelin inclusions<sup>49</sup>, suggesting that these cells may directly induce demyelination. These studies highlight the differential functions of microglia and macrophages during myelin injury, and point to the requirement to analyse these populations separately to accurately assess their roles during remyelination.

Using this approach, Olah et al. performed microarray analysis of microglia isolated from cuprizone-treated mice and identified 6200 genes expressed during homeostasis, demyelination and remyelination<sup>13</sup>. The major patterns observed were i) downregulation of metabolic processes and acute inflammatory responses during demyelination and remyelination; ii) upregulation of cell cycle during demyelination only; and iii) upregulation of immune response, phagocytosis and antigen processing/presentation during demyelination and remyelination<sup>13</sup>. However, the microglial transcriptome was largely

similar between demyelination/ early remyelination (5 weeks cuprizone treatment) and late remyelination (5 weeks cuprizone treatment then 2 weeks recovery). At both these time points, microglia upregulated expression of toll-like receptor 4 (*Tlr4*), lysozyme 1 and 2 (*Lyz1*, *Lyz2*), tumour necrosis factor (*Tnf*), interleukin 1 $\beta$  (*Il1b*), interleukin receptor 4 $\alpha$  (*Il4ra*), genes associated with MHC-II (*Cd74*, *H2Aa*), whilst common microglial markers such as Iba1 (*Aif1*), F4/80 (*Emr1*), and Pu.1 (*Spi1*) were stably expressed. The authors proposed numerous microglial effector functions during demyelination and remyelination, including phagocytosis of debris/ apoptotic cells, salvage of myelin constituents, recruitment of OPCs and trophic support, and tissue remodelling. Overall, this study highlighted that the overarching role of microglia is to maintain tissue homeostasis and to provide an environment supportive of regeneration, which was even evident during demyelination.

A significant function associated with microglia is the clearance of myelin debris, which is required for remyelination to occur. Impaired removal of myelin debris is one factor contributing to poor remyelination following cuprizone treatment of *Trem2*<sup>-/-</sup> mice<sup>54</sup>. This is underpinned by impaired upregulation of genes associated with phagocytosis of myelin debris (*Axl*) and molecules central to lipid transport and metabolism (apolipoprotein-E (*ApoE*), apolipoprotein C1 (*Apoc1*), lipoprotein lipase (*Lpl*), cholesterol 25-hydroxylase (*Ch25h*)) during demyelination and remyelination. Furthermore, microglia provide trophic support to newly recruited OPCs, and a growth factor critical for OPC differentiation (insulin-like growth factor 1; *Igf1*) was found to be downregulated in *Trem2*<sup>-/-</sup> mice after cuprizone-induced demyelination. Together, these results suggest that a central role of microglia in responding to demyelination is to carry out myelin clearance and promote OPC maturation for remyelination to occur efficiently. These studies have elucidated microglia gene

expression profiles during demyelination and remyelination, however subsequent unbiased investigations into expression profiles of microglia versus MDM during remyelination may reveal cell-specific functions during regeneration.

### **Functional contributions of microglia and monocyte-derived macrophages to central nervous system remyelination**

Activated macrophages are a component of remyelinating lesions in both experimental models and human disease (e.g. multiple sclerosis, spinal cord injury, Alzheimer's disease)<sup>55</sup>. For instance, the density of macrophages (HLA-DR+) at the border of MS lesions coincides with areas of robust remyelination<sup>56</sup>. In contrast to experimental models with concurrent demyelination and remyelination (such as EAE and the cuprizone model), focal toxin-induced lesion models with temporally distinct damage and regeneration are the most appropriate to specifically associate cellular responses/ gene expression profiles/ molecules with the regenerative response to damage. Indeed, a recent study identified that remyelination is limited in EAE and occurs only at very late stages<sup>20</sup>. Conversely, focal models show robust remyelination (in young animals) following injection of demyelinating agents (such as lysolecithin, ethidium bromide, or lysophosphatidylserine (LPS)) into white matter tracts such as the spinal cord, corpus callosum, or caudal cerebellar peduncles. These models have been imperative in linking the beneficial effects of the immune response to the regulation of OPC responses during remyelination<sup>7-11,57</sup>. More specifically, this allowed for the discovery that macrophages are required for efficient remyelination, given that blocking the macrophage response soon after demyelination, either via depletion using clodronate-liposomes<sup>7,10</sup> or by administration of minocycline<sup>58</sup>, inhibits remyelination. Furthermore, inducing macrophage activation is sufficient to enhance myelination or remyelination, for

example via stimulation with the Toll-like receptor (TLR)-4 agonist LPS<sup>59</sup>, TLR-2 agonist zymosan<sup>60</sup>, or a combination of the anti-fungal agent amphotericin B with macrophage colony stimulating factor (M-CSF)<sup>14</sup>.

Our recent work has identified that efficient remyelination requires the dynamic regulation of functional macrophage phenotypes<sup>10</sup>. More specifically, soon after demyelination macrophages adopt expression of inducible nitric oxide synthase (iNOS), CD16/32, and tumor necrosis alpha (TNF- $\alpha$ ), and support the proliferation of OPCs, yet these pro-inflammatory macrophages are not required for remyelination to proceed<sup>10</sup>. Importantly, the rate limiting step in remyelination efficiency is the transition to a pro-regenerative macrophage phenotype expressing arginase-1, mannose receptor (CD206), and insulin-like growth factor-1 (IGF-1) which drives oligodendrocyte differentiation to initiate remyelination<sup>10</sup>. Lineage tracing identified that both microglia and MDM contributed to both phenotypes<sup>10</sup>. Additionally, using *Ccr2* knockout mice in which monocytes cannot extravasate from bone marrow and are thus excluded from lesions, we observed that microglia could undergo this transition in activation even in the absence of MDM<sup>10</sup>. Although the molecular mechanisms underpinning this critical transition in macrophage activation are unknown, microglia and MDM gene expression/ activation can be manipulated by electrical nerve stimulation<sup>61</sup>, antipsychotic drug/ calcium suppression<sup>62</sup>, inhibition of Rho Kinase<sup>63</sup>, modulation of signalling through NF $\kappa$ B (reviewed by Lloyd and Miron<sup>64</sup>), and histone deacetylase (HDAC) inhibition<sup>65</sup>.

The aforementioned phagocytosis of myelin debris by macrophages is required for remyelination to proceed, as this debris is otherwise inhibitory for the recruitment/ differentiation of oligodendrocyte lineage cells<sup>9</sup> and potentially for new myelin sheath



extension<sup>66</sup>. Indeed, in MS lesions, the density of oligodendrocyte precursor cells (O4<sup>+</sup>) is proportional to the density of macrophages which have phagocytosed debris<sup>67</sup>. Microglia and MDM may have differential phagocytic potential for engulfment of myelin debris depending on the context, being greater *in vitro* in human primary microglia versus MDM<sup>68,69</sup> yet relatively repressed in microglia at onset of EAE<sup>49</sup>. Nonetheless, several receptors have been implicated in regulating the phagocytosis of myelin debris: MerTK<sup>68</sup>, TLR4<sup>66</sup>, CX3CR1<sup>15</sup>, TREM2<sup>54</sup>, and RXR $\alpha$ <sup>21</sup>. For instance, myelin debris clearance is impaired following spinal cord injury in TLR4 knockout mice<sup>70</sup> and accelerated following TLR4 stimulation after peripheral nerve injury<sup>66</sup>. Additionally, stimulation of MS patient-derived monocytes with the RXR $\alpha$  agonist bexarotene enhances their phagocytic capacity<sup>21</sup>. Healy *et al.* found that TGF- $\beta$  treatment of primary human brain-derived microglia, which renders *in vitro* microglia more like *in vivo* microglia<sup>71</sup>, increased myelin ingestion via upregulation of MerTK, whilst blocking MerTK decreased phagocytic potential<sup>68</sup>. Stimulation of human microglia and MDMs also influences uptake of myelin *in vitro*, with highest efficiency seen following treatment with M-CSF, interleukin (IL)-13 and IL-4<sup>69</sup>. An additional beneficial role of microglia and MDM during remyelination is the release of factors that support oligodendrocyte lineage cell responses. These include activin-A, endothelin-2, IGF-1, TNF $\alpha$ , IL-1 $\beta$ , platelet-derived growth factor (PDGF)-AA, fibroblast growth factor (FGF)-2, galectin-3, osteopontin-M, CXCL12, semaphorin-3F, and iron (reviewed in <sup>72</sup>). Thus, it is conceivable that these factors synergistically induce coordinated and temporally controlled signalling in OPCs which drives their responses during remyelination. Furthermore, macrophages play important roles in remodelling the extracellular matrix to support remyelination, for instance by degrading chondroitin sulfate proteoglycans<sup>73-75</sup>. Microglia and MDMs may also coordinate a pro-remyelination environment via interaction with other cell types. For

instance, a recent study identified that regulatory T cells drive remyelination<sup>57</sup>, and one may speculate that macrophages and regulatory T cells may cooperate to support regeneration. Indeed, a recent study found that a secreted phenylalanine oxidase termed interleukin-4-induced-1 (IL4I1), which is highly enriched in microglia and upregulated during remyelination, regulates both macrophage activation during remyelination and reduces T cell expansion in EAE, leading to less dystrophic axons<sup>76</sup>. Using cultures of primary human neural cells, another study found that microglia release cytokines which modulate astrocytes, which in turn influence OPC responses<sup>77</sup>.

Importantly, the failure of remyelination seen with ageing is associated with a reduced recruitment of macrophages, reduced activation to a pro-regenerative macrophage phenotype<sup>10,78</sup>, and impaired phagocytic potential<sup>11,21,78</sup>. Altogether, these findings point towards the importance of regulating macrophages following demyelination for efficient remyelination to take place, and the need for macrophage-specific targeting in developing effective therapeutic strategies for the enhancement of remyelination.

## **Conclusions**

As sensors of damage in the central nervous system, microglia are early responders to injury, such as demyelination, and have roles in supporting the regenerative process of remyelination. Transcriptomics and functional assays have identified these to include the phagocytosis of myelin debris, remodelling of the extracellular matrix, and secretion of factors influencing oligodendrocyte lineage cell responses (such as differentiation into myelinating oligodendrocytes). Although microglia are likely supported by MDMs in many of these functions, whether there are distinct roles of the endogenous and exogenously-derived macrophages during remyelination, as observed during myelin injury, is unknown. It

also remains to be determined whether the dynamic turnover of microglia observed during homeostasis is altered during injury and remyelination. Nonetheless, the numerous studies implicating macrophages in supporting remyelination highlight that these cells are important therapeutic targets for promoting remyelination in various neurological disorders characterized by poor myelin integrity.

## **Acknowledgements**

A.F.L. is supported by a studentship from the Biotechnology and Biological Sciences Research Council and GlaxoSmithKline, V.E.M. and C.L.D. are supported by a career development award from the Medical Research Council and the United Kingdom Multiple Sclerosis Society (to V.E.M.).

## Figure legend

In development, erythromyeloid progenitors (EMPs) derived from the yolk sac colonize the embryonic brain (at embryonic day 9.5 in mice, and gestational week (gw) 4.5 and 12-13 in human). Mouse studies showed these cells will express *Runx1*, *Pu.1*, *Irf8*, *Sall1*, and *Sall3*. These cells reach full maturation between E10.5 and birth in the mouse, and gw 22-35 in the human. In mouse, this involves expression of *Csfr1*, *Cxcr2*, *Cd14*, and *Mafb*. In adulthood, the microglia population turns over regularly by coupled apoptosis (via *Bad*) and proliferation (expression of *Mad211*, *Mdm2*, *Cdca3*, *Cdk1*, *Cdc20*, *Cdc20b*). In the cuprizone model of demyelination, injury is associated with microglial expression of *LyzM*, *Ccl2*, *Ccl19*, *Ccr5*, and *Mmp12*. The regeneration of myelin that ensues (remyelination) is coordinated by microglia and monocyte-derived macrophages (MDM) which phagocytose myelin debris, remodel tissue, and secrete regenerative factors that altogether affect the recruitment of oligodendrocyte progenitor cells (OPCs) via chemoattraction, their proliferation, and differentiation into mature myelin-forming oligodendrocytes. Gene expression profiling of microglia identified pathways associated with decreased metabolic processing, increased immune response, antigen presentation and processing, and expression of *Tlr4*, *Lyz1*, *Lyz2*, *Tnf*, *Il1b*, *Il4ra*, *Cd74*, *H2Aa*, *Axl*, *Apoe*, *Apoc1*, *Lpl*, and *Ch25h*. The remyelination-associated gene expression profile in MDM is currently unknown.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as \* of special interest and \*\* of outstanding interest.

- 1 Lawson, L. J., Perry, V. H., Dri, P. & Gordon, S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**, 151-170 (1990).
  - 2 Mittelbronn, M., Dietz, K., Schluesener, H. J. & Meyermann, R. Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta neuropathologica* **101**, 249-255 (2001).
  - 3 Yamanaka, K. *et al.* Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nature neuroscience* **11**, 251-253, doi:10.1038/nn2047 (2008).
  - 4 Hong, S. *et al.* Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* **352**, 712-716, doi:10.1126/science.aad8373 (2016).
  - 5 Verloes, A. *et al.* Nasu-Hakola syndrome: polycystic lipomembranous osteodysplasia with sclerosing leucoencephalopathy and presenile dementia. *Journal of medical genetics* **34**, 753-757 (1997).
  - 6 Masuda, T. & Prinz, M. Microglia: A Unique Versatile Cell in the Central Nervous System. *ACS chemical neuroscience* **7**, 428-434, doi:10.1021/acschemneuro.5b00317 (2016).
  - 7 Kotter, M. R., Setzu, A., Sim, F. J., Van Rooijen, N. & Franklin, R. J. Macrophage depletion impairs oligodendrocyte remyelination following lysolecithin-induced demyelination. *Glia* **35**, 204-212 (2001).
  - 8 Kotter, M. R., Zhao, C., van Rooijen, N. & Franklin, R. J. Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. *Neurobiology of disease* **18**, 166-175, doi:10.1016/j.nbd.2004.09.019 (2005).
  - 9 Kotter, M. R., Li, W. W., Zhao, C. & Franklin, R. J. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 328-332, doi:10.1523/JNEUROSCI.2615-05.2006 (2006).
  - 10 Miron, V. E. *et al.* M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nature neuroscience* **16**, 1211-1218, doi:10.1038/nn.3469 (2013).
  - 11 Ruckh, J. M. *et al.* Rejuvenation of regeneration in the aging central nervous system. *Cell stem cell* **10**, 96-103, doi:10.1016/j.stem.2011.11.019 (2012).
  - 12 Voss, E. V. *et al.* Characterisation of microglia during de- and remyelination: can they create a repair promoting environment? *Neurobiology of disease* **45**, 519-528, doi:10.1016/j.nbd.2011.09.008 (2012).
  - 13 Olah, M. *et al.* Identification of a microglia phenotype supportive of remyelination. *Glia* **60**, 306-321, doi:10.1002/glia.21266 (2012).
  - 14 \*\*Doring, A. *et al.* Stimulation of monocytes, macrophages, and microglia by amphotericin B and macrophage colony-stimulating factor promotes remyelination. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **35**, 1136-1148, doi:10.1523/JNEUROSCI.1797-14.2015 (2015).
- This paper showed that drug targeting of macrophages, using amphotericin B and M-CSF, enhances the macrophage response and consequently improves remyelination.**
- 15 Lampron, A. *et al.* Inefficient clearance of myelin debris by microglia impairs remyelinating processes. *The Journal of experimental medicine* **212**, 481-495, doi:10.1084/jem.20141656 (2015).
  - 16 Kornek, B. *et al.* Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions.

- The American journal of pathology* **157**, 267-276, doi:10.1016/S0002-9440(10)64537-3 (2000).
- 17 Irvine, K. A. & Blakemore, W. F. Remyelination protects axons from demyelination-associated axon degeneration. *Brain : a journal of neurology* **131**, 1464-1477, doi:10.1093/brain/awn080 (2008).
- 18 Murray, P. D., McGavern, D. B., Sathornsumetee, S. & Rodriguez, M. Spontaneous remyelination following extensive demyelination is associated with improved neurological function in a viral model of multiple sclerosis. *Brain : a journal of neurology* **124**, 1403-1416 (2001).
- 19 Duncan, I. D., Brower, A., Kondo, Y., Curlee, J. F., Jr. & Schultz, R. D. Extensive remyelination of the CNS leads to functional recovery. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 6832-6836, doi:10.1073/pnas.0812500106 (2009).
- 20 \*Mei, F. *et al.* Accelerated remyelination during inflammatory demyelination prevents axonal loss and improves functional recovery. *eLife* **5**, doi:10.7554/eLife.18246 (2016).
- Using an autoimmune demyelination model, experimental autoimmune encephalomyelitis (EAE), this paper showed that new myelin remains stable at the height of inflammation but is very limited overall. This paper also showed that remyelination can be enhanced in a pro-inflammatory environment and this supports axon preservation.**
- 21 \*\*Natrajan, M. S. *et al.* Retinoid X receptor activation reverses age-related deficiencies in myelin debris phagocytosis and remyelination. *Brain : a journal of neurology* **138**, 3581-3597, doi:10.1093/brain/awv289 (2015).
- This paper showed that the decreased phagocytosis of myelin debris seen with ageing results from RXR pathway expression changes in macrophages. The impaired myelin debris phagocytosis observed in multiple sclerosis patient-derived macrophages was rescued with an RXR agonist, bexarotene, showing that targeting macrophages could be an effective therapeutic strategy to enhance remyelination.**
- 22 Erbllich, B., Zhu, L., Etgen, A., Dobrenis, K. & Pollard, J. Absence of Colony Stimulation Factor-1 Receptor Results in Loss of Microglia, Disrupted Brain Development and Olfactory Deficits. *PLOS one* doi:https://doi.org/10.1371/journal.pone.0026317 (2011).
- 23 Aarum, J., Sandberg, K., Budd Haeberlein, S. & Persson, M. Migration and differentiation of neural precursor cells can be directed by microglia. *PNAS* **100**, 15983-15998 (2003).
- 24 Schafer, D. *et al.* Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron* **74**, 691-705 (2012).
- 25 Ginhoux, F. *et al.* Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages. *Science* **330**, 841-845 (2010).
- 26 Kierdorf, K. *et al.* Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nature Neuroscience* **16**, 273-280 (2013).
- 27 Ginhoux, F. & Prinz, M. Origin of Microglia: Current Concepts and Past Controversies. *Cold Spring Harbor Perspectives in Biology*, doi:10.1101/cshperspect.a020537 (2015).
- 28 Monier, A. *et al.* Entry and Distribution of Microglial Cells in Human Embryonic and Fetal Cerebral Cortex *Journal of Neuropathology and Experimental Neurology* **66**, 372-382 (2007).
- 29 Verney, C., Monier, A., Fallet-Bianco, C. & Gressens, P. Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. *Journal of Anatomy* **217**, 436-448 (2010).
- 30 Koushik, S. *et al.* Targeted inactivation of the sodium-calcium exchanger (Ncx1) results in the lack of a heartbeat and abnormal myofibrillar organization. *The FASEB journal* **15** (2001).
- 31 Mass, E. *et al.* Specification of tissue-resident macrophages during organogenesis. *Science* **353** (2016).
- 32 Matcovitch-Natan, O. *et al.* Microglia development follows a stepwise program to regulate brain homeostasis. *Science* **353** (2016).

- 33 Xu, J. *et al.* Temporal-Spatial Resolution Fate Mapping Reveals Distinct Origins for Embryonic and Adult Microglia in Zebrafish. *Developmental Cell* **34**, 632-641 (2015).
- 34 Sheng, J., Ruedl, C. & Karjalainen, K. Most Tissue-Resident Macrophages Except Microglia Are Derived from Fetal Hematopoietic Stem Cells. *Immunity* **43**, 382-393 (2015).
- 35 Alliot, F., Godin, I. & Pessac, B. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Developmental Brain Research* **117**, 145-152 (1999).
- 36 Nikodemova, M. *et al.* Microglial numbers attain adult levels after undergoing a rapid decrease in cell number in the third postnatal week. *Journal of Neuroimmunology* **278**, 280-288 (2015).
- 37 \*Askew, K. *et al.* Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain. *Cell Reports* **18**, 391-405 (2017).

**This paper showed that microglia turnover in the brain is faster than previously considered, with the whole population being dynamically regulated at the cellular level by coupled apoptosis and proliferation.**

- 38 Asai, H. *et al.* Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nature Neuroscience* **18**, 1584-1593 (2015).
- 39 Elmore, M. *et al.* Colony-Stimulating Factor 1 Receptor Signaling Is Necessary for Microglia Viability, Unmasking a Microglia Progenitor Cell in the Adult Brain. *Neuron* **82**, 380-397 (2014).
- 40 \*\*Bruttger, J. *et al.* Genetic Cell Ablation Reveals Clusters of Local Self-Renewing Microglia in the Mammalian Central Nervous System. *Immunity* **43**, 96-106 (2015).

**This paper showed that following global depletion of microglia in adults using a Cx3cr1-CreERT2-iDTR model, microglia robustly proliferate to repopulate the entire brain.**

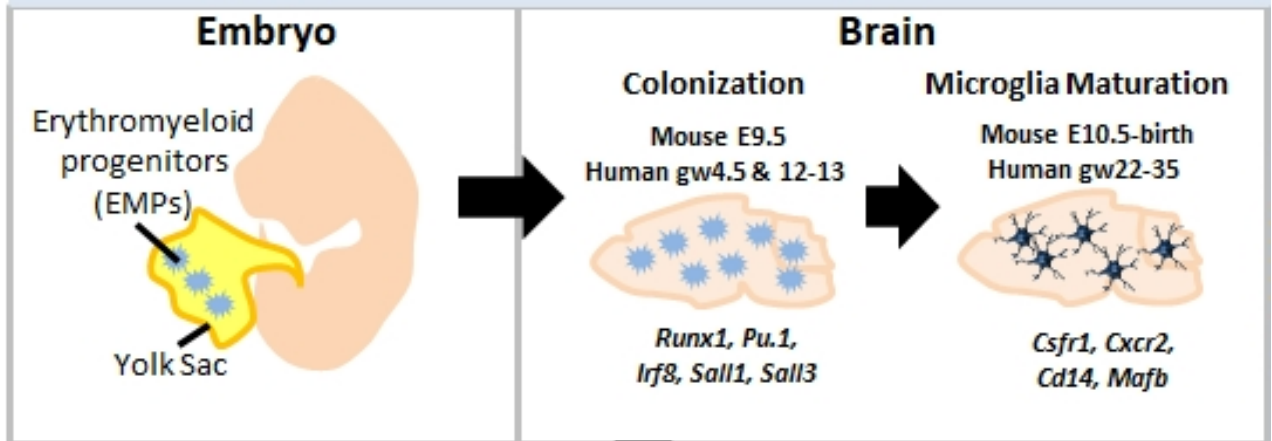
- 41 Parkhurst, C. *et al.* Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* **155**, 1596-1609 (2013).
- 42 Jurevics, H. *et al.* Alterations in metabolism and gene expression in brain regions during cuprizone-induced demyelination and remyelination. *Journal of neurochemistry* **82**, 126-136 (2002).
- 43 Anliker, B. *et al.* Lysophosphatidic acid (LPA) and its receptor, LPA1, influence embryonic schwann cell migration, myelination, and cell-to-axon segregation. *Glia* **61**, 2009-2022, doi:10.1002/glia.22572 (2013).
- 44 Sim, F. J., Hinks, G. L. & Franklin, R. J. The re-expression of the homeodomain transcription factor Gtx during remyelination of experimentally induced demyelinating lesions in young and old rat brain. *Neuroscience* **100**, 131-139 (2000).
- 45 Arnett, H. A., Wang, Y., Matsushima, G. K., Suzuki, K. & Ting, J. P. Functional genomic analysis of remyelination reveals importance of inflammation in oligodendrocyte regeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **23**, 9824-9832 (2003).
- 46 Srinivasan, K. *et al.* Untangling the brain's neuroinflammatory and neurodegenerative transcriptional responses. *Nature communications* **7**, 11295, doi:10.1038/ncomms11295 (2016).
- 47 Ransohoff, R. M. & Cardona, A. E. The myeloid cells of the central nervous system parenchyma. *Nature* **468**, 253-262, doi:10.1038/nature09615 (2010).
- 48 Lewis, N. D., Hill, J. D., Juchem, K. W., Stefanopoulos, D. E. & Modis, L. K. RNA sequencing of microglia and monocyte-derived macrophages from mice with experimental autoimmune encephalomyelitis illustrates a changing phenotype with disease course. *Journal of neuroimmunology* **277**, 26-38, doi:10.1016/j.jneuroim.2014.09.014 (2014).
- 49 Yamasaki, R. *et al.* Differential roles of microglia and monocytes in the inflamed central nervous system. *The Journal of experimental medicine* **211**, 1533-1549, doi:10.1084/jem.20132477 (2014).



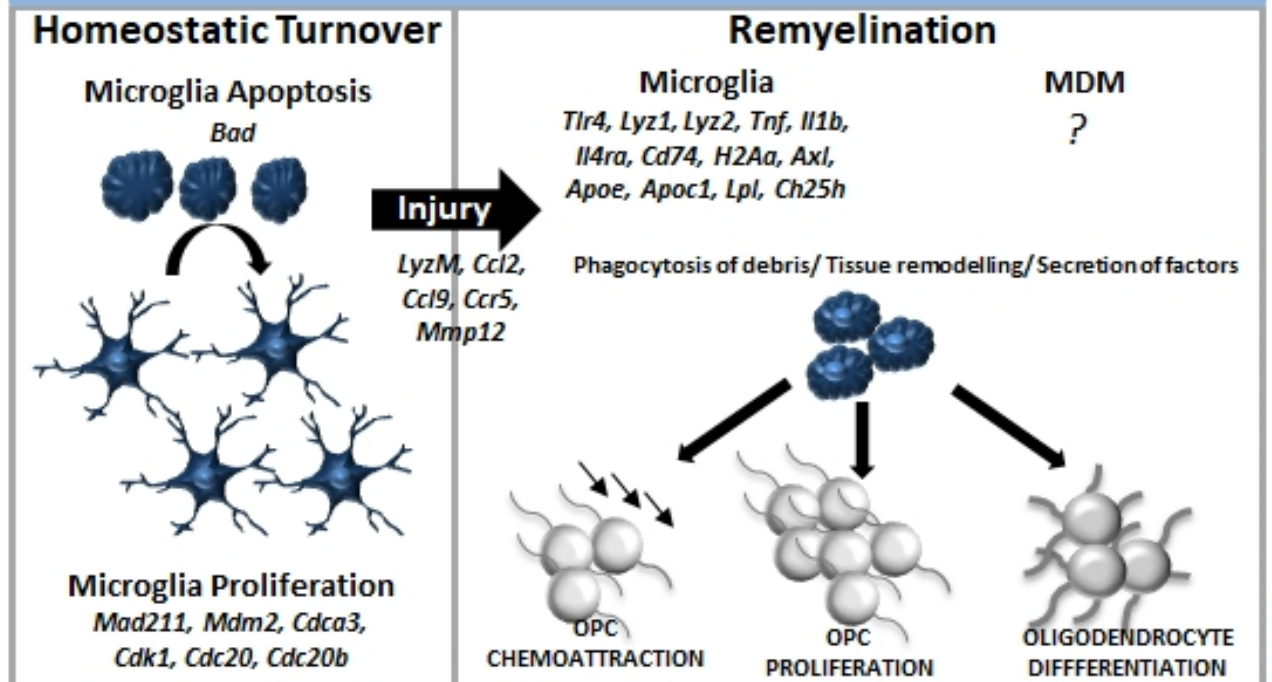
- 50 Gao, H. *et al.* Opposing Functions of Microglial and Macrophagic TNFR2 in the Pathogenesis of Experimental Autoimmune Encephalomyelitis. *Cell reports* **18**, 198-212, doi:10.1016/j.celrep.2016.11.083 (2017).
- 51 Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M. & Rossi, F. M. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nature neuroscience* **14**, 1142-1149, doi:10.1038/nn.2887 (2011).
- 52 Saederup, N. *et al.* Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PloS one* **5**, e13693, doi:10.1371/journal.pone.0013693 (2010).
- 53 Mildner, A. *et al.* CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system. *Brain : a journal of neurology* **132**, 2487-2500, doi:10.1093/brain/awp144 (2009).
- 54 Poliani, P. L. *et al.* TREM2 sustains microglial expansion during aging and response to demyelination. *The Journal of clinical investigation* **125**, 2161-2170, doi:10.1172/JCI77983 (2015).
- 55 Shechter, R. & Schwartz, M. Harnessing monocyte-derived macrophages to control central nervous system pathologies: no longer 'if' but 'how'. *The Journal of pathology* **229**, 332-346, doi:10.1002/path.4106 (2013).
- 56 Patani, R., Balaratnam, M., Vora, A. & Reynolds, R. Remyelination can be extensive in multiple sclerosis despite a long disease course. *Neuropathology and applied neurobiology* **33**, 277-287, doi:10.1111/j.1365-2990.2007.00805.x (2007).
- 57 Dombrowski, Y. *et al.* Regulatory T cells promote myelin regeneration in the central nervous system. *Nature neuroscience*, doi:10.1038/nn.4528 (2017).
- 58 Li, W. W., Setzu, A., Zhao, C. & Franklin, R. J. Minocycline-mediated inhibition of microglia activation impairs oligodendrocyte progenitor cell responses and remyelination in a non-immune model of demyelination. *Journal of neuroimmunology* **158**, 58-66, doi:10.1016/j.jneuroim.2004.08.011 (2005).
- 59 Glezer, I., Lapointe, A. & Rivest, S. Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 750-752, doi:10.1096/fj.05-5234fje (2006).
- 60 Setzu, A. *et al.* Inflammation stimulates myelination by transplanted oligodendrocyte precursor cells. *Glia* **54**, 297-303, doi:10.1002/glia.20371 (2006).
- 61 McLean, N. A. & Verge, V. M. Dynamic impact of brief electrical nerve stimulation on the neural immune axis-polarization of macrophages toward a pro-repair phenotype in demyelinated peripheral nerve. *Glia* **64**, 1546-1561, doi:10.1002/glia.23021 (2016).
- 62 Wang, H. *et al.* Quetiapine Inhibits Microglial Activation by Neutralizing Abnormal STIM1-Mediated Intercellular Calcium Homeostasis and Promotes Myelin Repair in a Cuprizone-Induced Mouse Model of Demyelination. *Frontiers in cellular neuroscience* **9**, 492, doi:10.3389/fncel.2015.00492 (2015).
- 63 Li, Y. H. *et al.* FSD-C10, a Fasudil derivative, promotes neuroregeneration through indirect and direct mechanisms. *Scientific reports* **7**, 41227, doi:10.1038/srep41227 (2017).
- 64 Lloyd, A. F. & Miron, V. E. Cellular and Molecular Mechanisms Underpinning Macrophage Activation during Remyelination. *Frontiers in cell and developmental biology* **4**, 60, doi:10.3389/fcell.2016.00060 (2016).
- 65 Wang, G. *et al.* HDAC inhibition prevents white matter injury by modulating microglia/macrophage polarization through the GSK3beta/PTEN/Akt axis. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 2853-2858, doi:10.1073/pnas.1501441112 (2015).

- 66 Church, J. S., Milich, L. M., Lerch, J. K., Popovich, P. G. & McTigue, D. M. E6020, a synthetic TLR4 agonist, accelerates myelin debris clearance, Schwann cell infiltration, and remyelination in the rat spinal cord. *Glia* **65**, 883-899, doi:10.1002/glia.23132 (2017).
- 67 Wolswijk, G. Oligodendrocyte precursor cells in the demyelinated multiple sclerosis spinal cord. *Brain : a journal of neurology* **125**, 338-349 (2002).
- 68 Healy, L. M. *et al.* Differential transcriptional response profiles in human myeloid cell populations. *Clinical immunology*, doi:10.1016/j.clim.2016.04.006 (2016).
- 69 Durafourt, B. A. *et al.* Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia* **60**, 717-727, doi:10.1002/glia.22298 (2012).
- 70 Church, J. S., Kigerl, K. A., Lerch, J. K., Popovich, P. G. & McTigue, D. M. TLR4 Deficiency Impairs Oligodendrocyte Formation in the Injured Spinal Cord. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **36**, 6352-6364, doi:10.1523/JNEUROSCI.0353-16.2016 (2016).
- 71 Butovsky, O. *et al.* Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. *Nature neuroscience* **17**, 131-143, doi:10.1038/nn.3599 (2014).
- 72 Miron, V. E. Microglia-driven regulation of oligodendrocyte lineage cells, myelination, and remyelination. *Journal of leukocyte biology*, doi:10.1189/jlb.3RI1116-494R (2017).
- 73 Larsen, P. H., Wells, J. E., Stallcup, W. B., Opdenakker, G. & Yong, V. W. Matrix metalloproteinase-9 facilitates remyelination in part by processing the inhibitory NG2 proteoglycan. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **23**, 11127-11135 (2003).
- 74 Lau, L. W. *et al.* Chondroitin sulfate proteoglycans in demyelinated lesions impair remyelination. *Annals of neurology* **72**, 419-432, doi:10.1002/ana.23599 (2012).
- 75 Keough, M. B. *et al.* An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. *Nature communications* **7**, 11312, doi:10.1038/ncomms11312 (2016).
- 76 Psachoulia, K. *et al.* IL4I1 augments CNS remyelination and axonal protection by modulating T cell driven inflammation. *Brain : a journal of neurology* **139**, 3121-3136, doi:10.1093/brain/aww254 (2016).
- 77 Moore, C. S. *et al.* Direct and indirect effects of immune and central nervous system-resident cells on human oligodendrocyte progenitor cell differentiation. *Journal of immunology* **194**, 761-772, doi:10.4049/jimmunol.1401156 (2015).
- 78 Rawji, K. S., Mishra, M. K. & Yong, V. W. Regenerative Capacity of Macrophages for Remyelination. *Frontiers in cell and developmental biology* **4**, 47, doi:10.3389/fcell.2016.00047 (2016).

## Development



## Adulthood



**Current Opinion in Neurobiology**

**Invited review special issue: Glial Biology**

**Title: Microglia: origins, homeostasis, and roles in myelin repair**

**Authors:** Amy F. Lloyd\*<sup>1</sup>, Claire L. Davies\*<sup>1</sup>, Veronique E. Miron<sup>1</sup>

**Conflict of interest**

V.E.M. has received grant funding from Biogen Idec, GlaxoSmithKline, and Clene Nanomedicine related to the role of microglia and macrophages in remyelination. A.F.L. and C.L.D. have nothing to declare.