Review

Role of Microglia in Ataxias

Austin Ferro[1], **Carrie Sheeler**[1], **Juao-Guilherme Rosa**[1] and **Marija Cvetanovic**[1,2]

*1 - **Department of Neuroscience,** University of Minnesota, Minneapolis, MN 55455, USA*
*2 - **Institute for Translational Neuroscience,** University of Minnesota, Minneapolis, MN 55455, USA*

***Correspondence to Austin Ferro:** ferro029@umn.edu, sheel031@umn.edu, jrosa@umn.edu, mcvetano@umn.edu*
https://doi.org/10.1016/j.jmb.2019.01.016
Edited by Kristine Karla Freude

Abstract

Microglia, the resident macrophages of the central nervous system, critically influence neural function during development and in adulthood. Microglia are also profoundly sensitive to insults to the brain to which they respond with process of activation that includes spectrum of changes in morphology, function, and gene expression. Ataxias are a class of neurodegenerative diseases characterized by motor discoordination and predominant cerebellar involvement. In case of inherited forms of ataxia, mutant proteins are expressed throughout the brain and it is unclear why cerebellum is particularly vulnerable. Recent studies demonstrated that cerebellar microglia have a uniquely hyper-vigilant immune phenotype compared to microglia from other brain regions. These findings may indicate that microglia actively contribute to cerebellar vulnerability in ataxias. Here we review current knowledge about cerebellar microglia, their activation, and their role in the pathogenesis of ataxias. In addition, we briefly review advantages and disadvantages of several experimental approaches available to study microglia.

Introduction

The ataxias are a family of neurological disorders characterized by loss of balance and coordinated motor function [1]. Ataxias are classified as hereditary, sporadic, or acquired to denote what is known of their etiology. Hereditary ataxias are caused by a wide range of known genetic mutations with varying patterns of inheritance [2]. Sporadic ataxias have an unknown cause with potential environmental and genetic components [2]. Acquired ataxias, on the other hand, include disorders with endogenous or exogenous causes that are not genetic in nature, such as those arising from focal cerebellar damage caused by toxic agents, vitamin deficiencies, immune reactions, infection, and injury [3–5].

Autosomal dominant cerebellar ataxias caused by the expansion of CAG trinucleotide repeats, including spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7, and 17, are among the most understood ataxias [1,6]. As CAG encodes for glutamine, these ataxias belong to the group of polyglutamine (polyQ) diseases that includes Huntington's disease, spinal bulbar muscular atrophy, and dentatorubral and pallidoluysian atrophy [2,7]. The clinical manifestation of polyQ ataxias is very similar and often includes progressive cerebellar ataxia, oculomotor abnormalities, dysarthria (difficulties in speech), pigmentary retinopathy, peripheral neuropathy, and cognitive dysfunction [8]. Disruption of cerebellar cortical function accounts for many of the motor and cognitive symptoms in polyQ ataxias [9]. The only sources of output from the cerebellar cortex to deep cerebellar nuclei are axonal projections from Purkinje cells. Cerebellar afferents modulate cerebellar cortical output via transmission to Purkinje cell somas (climbing fibers) and to granule cells (mossy fibers) whose parallel fibers synapse onto the dendritic arbors of several Purkinje cells. PolyQ ataxias are characterized by the predominant neurodegeneration of cerebellar afferents and the Purkinje cells, thereby disrupting the circuitry and function of the cerebellar cortex [10].

Animal models of several CAG repeat diseases have been generated, providing insight into the mechanisms underlying polyQ-induced degeneration of Purkinje

neurons [9,11–16]. However, the contribution of non-neuronal cells, including microglia, astrocytes, and oligodendrocytes to the pathology of ataxia has been much less explored.

Microglia have emerged as key players in age- and disease-associated neurodegeneration, contributing to tissue support and disease progression [17]. Indeed, microglia have been shown to contribute to neurodegeneration through cell autonomous and cell non-autonomous mechanisms [18–20]. These studies provide evidence that microglia may play active roles in diseases traditionally thought to be predominantly neuronal. Here, we will review the importance of microglia in the cerebellum and the evidence for and against their contribution to the pathogenesis of the ataxias and their effect on other non-neuronal cells. Furthermore, we will also review novel techniques that show promise in overcoming current limitations of studying microglia, namely, the use of human-induced pluripotent stem cells (hiPSCs).

Uniqueness of Cerebellar Microglia

Microglia are the resident macrophages of the central nervous system and have a wide variety of functions during development as well as in adulthood. Microglia surveille the local neural state and aid in maintenance of the extracellular space. Moreover, functions that microglia perform are dependent on age, inflammatory state, and location. Unlike other brain cells, microglia originate from the erythromyeloid progenitors that in mice develop around embryonic day 8.5 (E8.5) in the yolk sack. Erythromyeloid progenitors migrate to the brain starting around E9.5 and continue to do so until the blood–brain barrier is formed around E14 [21,22]. During the early development, microglia have lasting effects on the developing neural architecture, including their well-understood role in superfluous cellular and synaptic pruning [23,24]. Recent studies using genome-wide chromatin and gene expression profiling demonstrated that microglia undergo three distinct developmental stages: early microglia (until E14), pre-microglia (from E14 to a few weeks after birth), and adult microglia (from a few weeks after birth). These developmental steps are regulated by distinct transcriptional factors that allow for different roles of microglia at each of these stages of life [25,26]. Importantly, sensome genes, through which microglia sense the environment [27], are expressed even in the early stages of development. This indicates that microglia are environmentally alert at all stages of development and may respond to brain insults. Moreover, evidence suggests that exposure to insults during development may alter the developmental roles of microglia and thus sensitize the brain in ways that may contribute to brain pathology in adulthood [25,26].

Cerebellar development may be particularly sensitive to such changes, as cerebellar maturation occurs over a relatively long developmental time. Human cerebellar development extends from the early embryonic period until the first postnatal years [28]. This protracted period of postnatal development leaves the cerebellum vulnerable to extrinsic and intrinsic injury [29,30]. Early injury may alter the roles of microglia in cerebellar development, causing developmental abnormalities that can have far reaching effects in adulthood. For example, during early postnatal cerebellar development [postnatal day 3 (P3)] in mice, normal apoptosis of Purkinje neurons was strongly reduced by selective elimination of microglia, indicating that microglia eliminate superfluous Purkinje neurons [23]. At P6–7, microglia promote GABA-ergic inhibition on Purkinje cells and prune superfluous synapses [31]. Elimination of immature, functionally redundant synapses during postnatal development is essential for the formation of the functional cerebellar network [32]. Disruption of these developmental processes can lead to cerebellar dysfunction [33,34]. Importantly, studies on mouse models of SCA1 have demonstrated that disruption of these processes may also pre-dispose the cerebellum to age-induced neurodegeneration [35,36].

To maintain adult brain homeostasis, microglia constantly extend and retract their processes to survey the local environment for synaptic activity [37], pathogen presence, and injury [38,39]. Madry *et al.* [40] recently established that constant microglial surveillance depends on the expression of the two-pore domain potassium (K^+) channel (THIK1). They demonstrated that tonic THIK1 activity maintains microglial membrane potential and that blocking THIK1 reduces microglial ramification and surveillance. These results implicate a potential role of extracellular K^+ concentration, arising from local neuronal activity, as a control mechanism for microglial surveillance. While it is unclear whether physiological changes in extracellular K^+ concentration are sufficiently large to alter microglial surveillance, pathological changes in neuronal or astroglial function are likely to do so. For example, it is possible that enhanced neuronal activity [41] and/or reduced clearance by astrocytes [42] may increase extracellular K^+, thereby altering microglial surveillance and morphology through THIK1.

Microglia do seem to be attracted to areas of high physiological neuronal activity, whether drawn by THIK1 signaling to areas of increased extracellular K^+ [40] or by adenosine signaling through the purinergic receptor P2Y12 [38,43]. Li *et al.* [37] demonstrated that microglia briefly (~5 min) contact highly active neurons, subsequently reducing their activity. Intriguingly, during ischemic injury, the duration of these microglia–neuron contacts is prolonged (~1 h) and can lead to synapse removal [41]. Furthermore, Davalos *et al.* [38] demonstrated that increase in ATP causes rapid recruitment of microglia.

Regional heterogeneity of neurons [44] coupled with the intimate, phenotype driving crosstalk between microglia and neurons may suggest microglial

heterogeneity. Indeed, several studies to date have provided evidence of spatial and temporal microglial heterogeneity under steady-state conditions [45–47]. Understanding the regional characteristics of microglia is important to determine their function in healthy brains and their possible contribution to regional susceptibility in neurological diseases.

Heterogeneity may be particularly important for cerebellar diseases, including ataxias, as cerebellar microglia seem to be unique among brain microglia in terms of morphology, tiling density, gene expression, mobility, phagocytosis, bioenergetics, and aging. A study by Lawson *et al.* [47] was one of the first reports indicating that cerebellar microglia may have unique characteristics compared to microglia in other brain regions. Different regions of adult mouse brain, including cerebral cortex, hippocampus, hypothalamus, thalamus, and cerebellum, were analyzed using F4/80 (membrane glycoprotein specific for microglia) immunohistochemistry to assess microglial density and morphology. Lawson *et al.* found that the cerebellum exhibits the lowest microglial density of any part of the brain (~30 cells/mm^2 compared to a total brain mean of 70 cells/mm^2). Also notable was the pattern of microglial density in the cerebellum. While most brain regions contained fewer microglia in white matter than in gray matter, the opposite pattern was found in the cerebellum (e.g., fewer microglia in gray matter of cerebellum than in the white matter) [47].

Further studies demonstrated that microglia in the cerebellum have unique transcriptome. Grabert *et al.* [46] analyzed gene expression of adult mouse microglia isolated from different brain regions. They found enhanced expression of genes regulating immune alertness and energy metabolism in cerebellar microglia compared to microglia in other brain regions, such as the cortex and striatum. It is important to note that the hyper-alert immune state of cerebellar microglia is distinct from the conventional microglial activation associated with injury. It is unclear whether these results indicate that cerebellar microglia may be better equipped to remove foreign and infectious threats. Alternatively, they may need less stimulation to become reactive. In addition, this paper reported that cerebellar microglia exhibited accelerated age-dependent increase in immune related genes compared to the microglia in other brain regions [46]. These results indicate that cerebellar microglia exhibit a more alert immune phenotype throughout life that amplifies with aging. This age-dependent enhancement of microglial hyper-vigilance may contribute to the age-dependent onset of cerebellar ataxias. This is likely even more critical in types of cerebellar ataxias where microglia are altered from the early stages of disease, prior to onset of symptoms of ataxia [48]. As such, it is of utmost importance to understand the molecular mechanisms underlying the unique characteristics of cerebellar microglia so we can better understand their contribution to cerebellar disease.

Several recent studies have uncovered some mechanisms underlying microglial heterogeneity. For example, epigenetic regulator polycomb repressive complex 2 (PRC2) was identified as a factor that plays a role in determining heterogeneous phagocytic potential of microglia, including the enhanced phagocytic phenotype of cerebellar microglia [45]. Using Translating Ribosome Affinity Purification and Chromatin Immune Precipitation followed by sequencing, they have found that PRC2 complex leads to a repression of phagocytic genes in many brain regions, yet it promotes the expression of phagocytic genes in the cerebellar microglia. The authors suggest that the increased phagocytosis of cerebellar microglia may be associated with the more pronounced age-dependent neuronal loss that occurs in the healthy cerebellum compared to other brain regions [49,50]. While the more pronounced loss of Purkinje neurons in the cerebellum starts in adolescence [49], it is unclear whether it is the cause or the consequence of hyper-alert microglia. Gene expression profile comparisons of cerebellar microglia before and after adolescence may be useful in indicating whether enhanced neuronal loss in the cerebellum results from the unique phenotype of cerebellar microglia.

Cerebellar microglia may also contribute to the enhanced age-dependent loss of Purkinje neurons in cerebellum. Previous findings demonstrate that cerebellar microglia contribute to Purkinje neuron loss during early postnatal development in mice [23]. Quantifying neuronal loss in the cerebella of mice in which cerebellar microglia are less phagocytic (e.g., with PRC2 depleted in cerebellar microglia) could provide insight into this possibility.

Thus, critical developmental roles of cerebellar microglia as well as their unique immune-alert phenotype in adults may contribute to the increased vulnerability of cerebellum in ataxia.

Microglia in Neurodegenerative Diseases

While the base knowledge about microglia in the healthy brain is increasing, the role of microglia is best understood in the context of brain disease. Due to their sensory abilities and innate immune function, microglia alter their transcriptomics, proteomics, morphology, and function when confronted with a wide variety of disease states and/or insults to the brain [51,52]. This fundamental change in microglia is commonly considered an “activated state” and seems to be ubiquitously present in many brain diseases and injury conditions. However, the functional outcomes of the activated state of microglia are less clear [53,54].

One of the critical signaling pathways that contributes to microglial activation is the canonical nuclear

factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway [55,56]. The NF-κB pathway is classically initiated when inhibitors of κB are marked for degradation by inhibitors of κB kinases, releasing the NF-κB subunits to translocate to the nucleus where they activate transcription of proinflammatory genes [57]. The NF-κB signaling cascade can be activated in microglia by tumor necrosis factor receptor stimulation or Toll-like receptor stimulation. When initiated, it induces the release of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), extensive morphological changes (ramification), increases in overall microglial density, and increases in the expression of ionized calcium-binding adapter molecule 1 (IBA1) protein [58].

Microglial activation via the NF-κB, or other proinflammatory pathways, can impact the overall neural architecture in several ways: by affecting the neuronal function through direct signaling and released proinflammatory cytokines [59], by regulating the inflammatory state of non-neuronal cells that will in turn cause a variety of secondary effects in neurons [60], through modulation of extracellular proteins and protein aggregates [61–63], and through the direct inappropriate phagocytosis of neural and glial tissues via the classical complement pathway [64–66].

While each of these facets have been characterized in neurodegeneration, divergent theories remain regarding their functional outcomes, namely, whether they are beneficial or harmful [67]. For example, in Alzheimer's disease (AD) evidence shows that microglia could serve to actively reduce pathology [63] as well as to exacerbate intrinsic neural dysfunction [64,65]. Likely, microglia occupy a spectrum between these two extremes, which complicates interpretations when singular functions of microglia are modulated to study their role in disease. While much less studied, similar uncertainty surrounds the role of microglia in the ataxias.

Microglia in the Ataxias

Microglial activation in the cerebellum was found during post-mortem analysis in several types of ataxias and in several rodent models of ataxia, including SCA6 [68], SCA21[69], Friedreich's ataxia [70], ataxia-telangiectasia [71], and multiple system atrophy [72]. Moreover, several animal models have demonstrated microglial activation preceding behavioral symptoms and cerebellar pathology. In the 118Q knock in mouse model ($MPI^{118Q/118Q}$) of SCA6, in which a polyQ expansion in the calcium channel $Ca_v2.1$ causes cerebellar pathology and ataxic behavior, microglial activation precedes apparent Purkinje cell degeneration [68]. This early microglial activation pattern can also be seen in the transmembrane protein 240 (TMEM240) viral expression model of SCA21, with microglial activation preceding the onset of motor deficits [69]. Early activation of cerebellar microglia can also be found in the rat ATM knockout model of ataxia-telangiectasia, where $ATM^{-/-}$ rats had pre-symptomatic and progressive NF-κB-dependent microglial activation. Activation of microglia in the AT rat model correlated with disease severity and preceded Purkinje cell pathology [71]. The prevalence of early microglial activation in these models indicates a mechanism by which microglia actively contribute to pathogenesis and makes them a promising therapeutic target for preventative treatment in the ataxias.

The role of early microglial activation in ataxia is best characterized in SCA1. Early microglial activation can be found in several models of SCA1. Both the Purkinje cell specific transgenic model (*ATXN1[82Q]*)[11] and the knock-in mouse model ($Atxn1^{154Q/2Q}$) [16] show increases in microglial number and TNF-α expression, a possible mechanism for microglia-driven neuroinflammation in disease [48]. Correlative evidence to support this theory was found using the TET-off conditional transgenic mouse model of SCA1, *cATXN1[30Q]-D776* [73]. In a 6-week on 6-week off paradigm, stopping the expression of mutant ATXN1 after the first 6 weeks of life ameliorated ataxia in these mice. This behavioral recovery was found to coincide with a significant decrease in density of cerebellar microglia and in cerebellar TNF-α expression [48,73].

However, pharmacological and genetic approaches to test the role of early microglial activation in SCA1 suggest a limited effect on disease state. Pharmacological depletion of microglia in *ATXN1[82Q]* mice showed only a limited rescue of rotarod performance. Microglia proliferation and survival is dependent on continual activation of colony-stimulating factor 1 (CSF1R) [74]. Qu *et al.* [75] used PLX3397, a novel inhibitor of CSF1R, to deplete microglia by 69% in the cerebella of *ATXN1[82Q]* mice. The depletion of microglia in the *ATXN1[82Q]* mice reduced TNF-α expression, when compared to vehicle treated *ATXN1 [82Q]* mice, yet the effects did not correlate with reduced Purkinje cell neuropathology or astrogliosis, where both progressed equally to that of vehicle treated *ATXN1[82Q]* mice [75]. The limited effect seen after microglial depletion was corroborated through further studies that decreased microglial activation. To avoid off-target effects from non-cerebellar microglial depletion, a genetic approach was taken to selectively reduce the activation of the canonical NF-κB inflammatory pathway in microglia. Reduction of NF-κB signaling, specifically in lysozyme M (LysM) expressing cells, decreased microglial density and TNF-α expression in the cerebellum of SCA1 mice back to control levels. Even so, there was no significant rescue in behavior or cerebellar neuropathology in SCA1 mice with reduced microglial NF-κB signaling [34].

Unexpectedly, Ferro *et al.* [34] found that control mice where NF-κB signaling was reduced exhibited

ataxic like behaviors equal to that of the SCA1 mice. This behavioral effect occurred without apparent Purkinje cell degeneration. These results indicate that this ataxic behavior was possibly due to disrupted pruning of climbing fiber inputs onto Purkinje cell somas [34]. It is well known that microglia are critical for correct neural connectivity via their cellular and synaptic pruning activity throughout the brain ([24,67,76]. Our study implies a functional impact of microglial NF-κB signaling in synaptic pruning during cerebellar cortical development. These findings were recently supported by the study from Hashimoto laboratory where a novel lox-CRE genetic approach was used to deplete microglia [31]. Nakayama *et al*. [31] conditionally knocked out a floxed CSF1R with an IBA1 promoter-driven CRE to deplete microglia (cKO-CSF1R mice) targeting the same downstream process as PLX3397 treatment. Purkinje cells in cKO-CSF1R mice exhibited impaired climbing fiber synapse elimination as well as decreased inhibitory input. These results imply that microglia have profound control over the pruning and development of synaptic inputs to Purkinje cells [31]. Therefore, microglia may be a potential therapeutic target for ataxias in which developmental Purkinje neuron synaptic pruning is effected, such as SCA1, SCA2, SCA14, and multiple system atrophy [73,77–80].

With the advent of pharmacological agents such as PLX3397 as well as potential genetic interventions such as silencing RNAs or antisense oligonucleotides, which have already been used in the context of ataxias, including SCA1, SCA2, and SCA3, there are a variety of means in which the adverse effects of microglia can be targeted in a disease state [81–85]. For example, microglial density could be reduced using PLX3397, which is seemingly well tolerated in a variety of rodent models. With more precise genetic tools, potential microglial disease-modifying proteins could be targeted with antisense oligonucleotides, such as TNFα. However, with either genetic or pharmacological treatment, novel means of increasing spatial and cell specificity would be required to avoid potential off target effects. Furthermore, it is important for microglia treatment design to consider the window of therapeutic efficiency. Especially when considering inherited ataxias, it is possible that the window for therapeutic efficacy should focus on pre-symptomatic stages of disease. However, by doing so, microglia-produced developmental deficits, such as altered climbing fiber synapse elimination and or early proinflammatory response (and their effect on the neurons), may not be reduced.

Crosstalk between Microglia and Astrocytes

There is emerging evidence that communication between microglia and astrocytes is extremely important for defining physiological and pathophysiological functional states of each cell type. For example, astrocyte expression of interleukin 33 during normal development is required for normal circuit formation and microglial synapse engulfment, a precursor to synapse pruning [86]. The communication between microglia and astrocytes during central nervous system insult has severe effects on the neural and glial environment. The neurotoxic astrocytic inflammatory phenotype can be driven by microglial signaling [60]. Likewise, aberrant microglial removal of synapses in AD is regulated by astrocytes [66].

While the unique character of cerebellar microglia has only recently been identified, cerebellar astrocytes and in particular Bergmann glia are well known for their unique interaction with Purkinje cells [87]. Soma of Bergmann glia surround the cell bodies of Purkinje neurons. Their processes extend along Purkinje neuron dendrites, where they enwrap most of the synapses onto Purkinje neurons. This close physical interaction of Bergmann glia and Purkinje neurons is reflected in their functional co-dependence [88]. Perhaps most telling was a study by Al Spada's laboratory, which demonstrated that expression of mutant ataxin-7 in Bergmann glia only was sufficient to cause Purkinje neuron dysfunction and ataxic behavior in mice [89,90]. Little is known about how the interactions between microglia and astrocytes may prime or attenuate the neurodestructive or neuroprotective phenotypes in each other, especially in Bergmann glia in the context of the ataxias.

Kim *et al*. [91] have recently shown that cerebellar astrocytes contribute to the pathogenesis of SCA1 in a bi-phasic disease stage-dependent manner. Reducing inflammatory NF-κB signaling in astrocytes prior to onset of motor symptoms exacerbated Purkinje neuron dysfunction and motor deficits. This indicates that, in early SCA1, astroglial activation may be neuroprotective. Intriguingly, reduced astroglial inflammatory signaling in early SCA1 increased microglial reactivity [91]. This suggests that astrogliosis early in SCA1 may constrain microglial reactivity and that failure to do so results in more reactive microglia and thus greater pathogenesis in Purkinje neurons. Alternatively, it is possible that the enhanced microglial reactivity seen with reduced astrogliosis reflects exacerbated Purkinje neurons pathogenesis. Further work is required to elucidate the causality and timeline of this pathology.

While reduced astrogliosis correlates to increased microglial activity during early stages of SCA1, the reverse has not yet been demonstrated (i.e., modulation of microglial activation has not yet been shown to elicit detectable changes in astroglial reactivity in SCA1) [34,75]. Although this may indicate that microglia do not modulate astroglial phenotypes in SCA1, it is important to consider several restrictions of these studies. Neither pharmacological nor genetic approaches to reducing microglial density and activation

were fully effective in the cerebellum. Pharmacological depletion, which was up to 99% effective in the cerebrum, was only 69% effective in the cerebellum. The remaining 31% of microglia may be sufficient to mediate astrogliosis [60,75]. Likewise, the LysM-Cre genetic approach used to reduce microglial reactivity affected only 10%–15% of cerebellar microglia [34]. It is possible that only a small population of microglia is required to promote astroglial inflammation [34,75]. More profound alterations of cerebellar microglial reactivity are needed to more thoroughly evaluate whether cerebellar microglia can alter astroglial activation in disease.

Challenges of Studying Microglia in Disease: Overcoming Limitations

There are many challenges to studying microglial activation within disease states. Current limitations to the study of brain cell types in disease include the requirement for pharmacological and genetic manipulations that are not endemic to the model system. The challenges present in the field include specifically targeting affected brain regions [92], or activated phenotype of microglia [93]. In addition it is challenging to identify efficient and precise microglia specific promoters [94] and discern activation state in *in situ* microglial cell culture, which is due to a disease process and not culture system [95]. However, even with these issues, we can still glean insights by using convergent methodology while novel tools are developed.

CSFR1 antagonists such as PLX3397 have yielded insights into the function of microglia both activated and in resting state [96–99]. However, there is concern about the suitability of PLX3397 and other CSFR1 receptor antagonists as direct tests of microglial function. Microglia are not the only cell type to express CSFR1, as neurons express CSFR1 as well [100]. This lack of cell type specificity is confounded by the inability to directly target specific brain regions without canalization. This makes targeting and interpretation of microglial depletion difficult, especially when studying the ataxias where pathology is predominantly localized to the cerebellum.

Due to their non-neuronal origins, lysozyme gene *Lyz2* otherwise known as *LysM* is a common methodology for genetically targeting myeloid cells, macrophages, and microglia [101]. Due to the prominent expression of LysM in a variety of progenitor lines, there has and should be some caution in interpretation, due to the off-target labeling of peripheral and neural tissue produced by this promoter sequence [34,102,103]. Specificity issues with LysM can be extrapolated to other targeting sequences such as CSFR1 [103]. To overcome issues regarding specificity and efficiency, promoter sequences such as the C-X3-C motif chemokine receptor 1 (CX3CR1), transmembrane protein 119 (TMEM119), or IBA1 promoter sequence may be used [31,103,104]. Even so, when using genetic approaches to study the ataxias, targeting microglia may not be sufficient, especially with the cerebellar ataxias. Just as with pharmacological depletion of microglia, microglia-specific genetic approaches will modify the function of the entire microglial population, including reactive both beneficial and harmful subtypes of microglia as well as and non-reactive microglia in disease-affected as well as in non-disease affected regions. We stress the importance of well thought-out genetic controls, as there is a substantial probability of observing secondary effects such as those seen through the historic use of the LysM promoter [34,102].

It is important to note that even with these controls in place, species differences in microglial function and reactivity may require the repetition of these studies in human microglia [105].

Beyond Animal Models: iPSCs as a Model of Human Disease

Animal models are excellent tools for studying disease as they provide a natural biological environment to study different facets of disease, including changes in neural circuitry, altered behavioral phenotypes, and the longitudinal effects of disease. Diseases that are not naturally occurring in animal models, including ataxias and other trinucleotide repeat disorders, may be induced or parsed through genetic manipulation, allowing for the study of cell autonomous and non-cell autonomous mechanisms of disease and the assessment of possible treatment options [106]. Much of the basic research on ataxias so far, such as the experiments detailed above, has been achieved using animal and mostly rodent models. However, recent advances in stem cell engineering have introduced hiPSCs as a translational model that can corroborate findings in the mouse to human disease.

hiPSCs are created through the reprogramming of somatic cells donated from human subjects, such as fibroblasts [107], blood mononuclear cells [108], or urine [109]. Transfection of a cocktail of factors including Oct4, Sox2, Klf4, and c-Myc [110] induces donated adult cells to return to an immature, pluripotent state, thus creating hiPSCs. These cells may then be differentiated into numerous cell types including neurons, astrocytes, and microglia following the principles of developmental biology. Differentiated cells may be grown in a monoculture or in a multicellular co-culture. Monoculture serves as an excellent method to ascertain cell-autonomous molecular and morphological phenotypes of disease. Multicellular culture, also known as organoids, better represent cellular function within a three-dimensional system, allow for region-specific co-development of

included cell types, and model the impact of different cell types on disease progression. Recent advances detail methods for the differentiation of microglia from hiPSCs [111–113], which may allow researchers to study the characteristics and function of human microglia in diseases of interest.

As a model for neurological disease mechanisms, hiPSCs have several advantages. iPSCs maintain the genetic blueprint of the cell donor throughout reprogramming and re-differentiation. This provides a clinically relevant *in vitro* model for the cellular pathology of genetic disorders. The use of human cells allows researchers to overcome the potential confounds of species difference in disease modeling and eliminates the requirement for genetic manipulations in order to recapitulate disease [14]. When considering microglia as a specific cell type of interest, there are additional advantages to using hiPSCs. Growing microglia from hiPSCs in culture avoids artificially induced changes in activation state which result from experimental procedures such as isolation from tissue [112]. Furthermore, reported gene expression differences between human and rodent microglia suggest that rodent models may be insufficient to represent the role of microglia in human disease [105]. Creation of microglia from hiPSCs could allow researchers to avoid these species differences to study the cell autonomous effect of given genetic disorders on living human microglia.

Despite these advantages, investigators should be cognizant of the potential confounding factors inherent to the use of hiPSCs in disease modeling. Although the genetic profile of the reprogrammed cells is identical to that of the donor, there is always a potential for genetic variability to arise as the result of cellular mitosis [114]). Furthermore, reprogramming to a pluripotent state resets the epigenetic character of hiPSCs to an immature stage of development, which is problematic when studying neurodegenerative disease where age is a critical factor [115]. There is an inherent degree of variability built into the application of different induction protocols, which may make the replication of published results difficult, particularly when attempting to differentiate cell types, like microglia, which are highly sensitive to their extracellular environment. Even among the few protocols published in the last 2 years, there is variability in the relative expression rates of certain microglia-specific factors [111]. Thus, in order to compare microglia derived from patient or healthy control iPSCs, special attention must be paid to ensure that differentiation is resulting in phenotypically accurate and reproducible microglia-like cells. Finally, while *in vitro* hiPSC culture may be useful to determine unique cellular characteristics of a disease, it does not consider the role of larger biological systems such the inter-regional circuitry so crucial for many brain functions. Thus, when studying disease using hiPSCs, researchers should focus on the molecular, functional, and morphological characteristics of their cell types of interest. Animal models are better for studying the systemic or organotypic effects of disease.

To date, there are several protocols published detailing methods by which hiPSCs may be differentiated into microglia [111–113]. The resulting microglia-like cells demonstrate numerous microglia-specific phenotypes including the ability to phagocytose beads, response to adenosine diphosphate with calcium transients, process extension in response to external secreted factors including interleukin 34 and granulocyte-macrophage colony-stimulating factor, and expression of microglia-specific markers including IBA1, CSF1R, P2RY12, and CX3CR1. In addition to determining the microglia-like phenotype of these differentiated hiPSCs, Muffat *et al.* [112] also found that their induced microglia-like cells expressed neurodegenerative disease-associated factors such as TMEM119, triggering receptor expressed on myeloid cells 2 (TREM2), and apolipoprotein E (APOE). These human microglia-like cells had secreted chemokine and cytokine profiles similar to that of primary microglia and became reactive in response to the lipopolysaccharide, a potent factor that induces the NF-κB inflammatory pathway.

Since the development of these differentiation protocols, there have been several promising studies published using hiPSC-derived microglia-like cells to characterize disease-related phenotypic differences between patients and healthy controls. These techniques have been used in monoculture to report differences in microglia-like cells derived from Rett syndrome donors [112], patients with TREM2 missense mutations [116,117], and APOE4 carriers [118]. Three-dimensional co-culturing of iPSC-derived microglia-like cells with neurons and astrocytes has revealed unique changes in the environmental stress response in *in vitro* models of AD [119].

While the above studies do not directly address the role of microglia in ataxia, they do propose a promising novel method by which disease-specific changes in microglial function may be assessed in ataxia. Given the single-gene and cell-type specific nature of many ataxia variants, there is enormous potential for the use of hiPSCs for drug discovery and basic research into the role of microglia in disease states. Through monoculture, the unique transcriptome of microglia expressing disease-relevant genetic mutation could be assessed in addition to unique morphological and phagocytic characteristics. Long-term co-culture of regionally-specified neurons and astrocytes with human microglia-like cells could reveal some of the mechanisms driving regional cell-type heterogeneity and provide a model to study environmental stress and disease-relevant cell–cell interaction between neurons, astrocytes, and microglia. In short, hiPSC-derived cell culturing has enormous potential for the study of microglia in ataxias.

Conclusions

The ataxias are a large group of neurodegenerative diseases, many of which show microglial activation as a pathological feature of the disease. Hyper-vigilance of cerebellar microglia in combination with early microglial activation detected in many of the ataxias supports microglia a promising therapeutic target. Due to the primary and secondary effects of microglial activation, targeting microglial cytokine release and the microglia-astrocyte communication may have large therapeutic effects, yet more studies need to be done to further characterize the synergy between both glial subtypes inflammatory communication. Lastly, we recommend the adoption of hiPSCs to directly study how microglia in the ataxias may be intrinsically different from isogeneic controls to elucidate further drug targets to curb these debilitating diseases.

Acknowledgements

This work was funded by the Cvetanovic laboratory start-up fund from the Department of Neuroscience and Institute for the Translational Neuroscience.

Received 31 October 2018;
Received in revised form 9 January 2019;
Accepted 11 January 2019
Available online 18 January 2019

Keywords:
Ataxia;
microglia;
Purkinje neurons;
cerebellum;
glia

Abbreviations used:
SCA, spinocerebellar ataxia; polyQ, polyglutamine; hiPSCs, human-induced pluripotent stem cells; PRC2, polycomb repressive complex 2; TNF-α, tumor necrosis factor alpha; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IBA1, ionized calcium-binding adapter molecule 1; AD, Alzheimer's disease; CSF1R, colony-stimulating factor 1; LysM, lysozyme M.

References

[1] Umar Akbar, Tetsuo Ashizawa, Ataxia, Neurol. Clin. 33 (2015) 225–248, https://doi.org/10.1016/j.ncl.2014.09.004.
[2] Helio A.G. Teive, Tetsuo Ashizawa, Primary and secondary ataxias, Curr. Opin. Neurol. 28 (4) (2015) 413–422, https://doi.org/10.1097/WCO.0000000000000227.
[3] Michael Abele, Martina Minnerop, Horst Urbach, Karsten Specht, Thomas Klockgether, Sporadic adult onset ataxia of unknown etiology a clinical, electrophysiological and imaging study, J. Neurol. 254 (2007) 1384–1389, https://doi.org/10.1007/s00415-007-0556-1.
[4] Thomas Klockgether, Ataxias, Parkinsonism Relat. Disord. 13 (2007) (2007) 391–394.
[5] A.G. Teive, Renato Puppi Munhoz, Lineu Cesar Werneck, Acquired cerebellar ataxia due to statin use, Arq. Neuropsiquiatr. 70 (2) (2012) 152.
[6] M. Pandolfo, M. Manto, Cerebellar and afferent ataxias. CONTINUUM: Lifelong Learning in Neurology, 19 (5, Movement Disorders) (2013) 1312–1343.
[7] Huda Y. Zoghbi, Harry T. Orr, Glutamine repeats and neurodegeneration, Annu. Rev. Neurosci. 23 (2000) 217–247.
[8] Y.M. Sun, C. Lu, Z.Y. Wu, Spinocerebellar ataxia: relationship between phenotype and genotype—a review, Clin. Genet. 90 (4) (2016) 305–314, https://doi.org/10.1111/cge.12808.
[9] Ludger Schöls, Peter Bauer, Thorsten Schmidt, Thorsten Schulte, Olaf Riess, Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis, Lancet Neurol. 3 (5) (2004) 291–304, https://doi.org/10.1016/S1474-4422(04)00737-9.
[10] Kay Seidel, Sonny Siswanto, Ewout R.P. Brunt, Wilfred Den Dunnen, Horst Werner Korf, Udo Rüb, Brain pathology of spinocerebellar ataxias, Acta Neuropathol. 124 (1) (2012) 1–21, https://doi.org/10.1007/s00401-012-1000-x.
[11] Eric N. Burright, Brent H. Clark, Antonio Servadio, Toni Matilla, Rodney M. Feddersen, Wael S. Yunis, Lisa Duvick, Huda Y. Zoghbi, Harry T. Orr, SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat, Cell 82 (6) (1995) 937–948, https://doi.org/10.1016/0092-8674(95)90273-2.
[12] Gwenn A. Garden, Randell T. Libby, Ying-hui Fu, Yoshito Kinoshita, Jing Huang, Daniel E. Possin, Annette C. Smith, et al., Polyglutamine-expanded ataxin-7 promotes non-cell-autonomous Purkinje cell degeneration and displays proteolytic cleavage in ataxic transgenic mice, J. Neurosci. 22 (12) (2002) 4897–4905.
[13] Pratap Meera, Stefan M. Pulst, Thomas S. Otis, Cellular and circuit mechanisms underlying spinocerebellar ataxias, J. Physiol. 16 (594) (2016) 4653–4660, https://doi.org/10.1113/JP271897.
[14] Paulson, Henry L, Vikram G Shakkottai, H Brent Clark, and Harry T Orr. 2017. "Polyglutamine spinocerebellar ataxias—from genes to potential treatments." Nat Rev Neurosci 18 (10). Nature Publishing Group: 613–26. https://doi.org/10.1038/nrn.2017.92.
[15] Andreia Teixeira-Castro, Ana Jalles, Sofia Esteves, Soosung Kang, Liliana da Silva Santos, Anabela Silva-Fernandes, Mario F. Neto, et al., Serotonergic signalling suppresses ataxin 3 aggregation and neurotoxicity in animal models of Machado–Joseph disease, Brain 138 (2015) 3221–3237, https://doi.org/10.1093/brain/awv262.
[16] Kei Watase, Edwin J. Weeber, Bisong Xu, Barbara Antalffy, Lisa Yuva-Paylor, Kouichi Hashimoto, Masanobu Kano, et al., A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration, Neuron 34 (6) (2002) 905–919, https://doi.org/10.1016/S0896-6273(02)00733-X.
[17] Michael W. Salter, Beth Stevens, Microglia emerge as central players in brain disease, Nat Med 23 (9) (2017) 1018–1027, https://doi.org/10.1038/nm.4397.

[18] Andrea Crotti, Christopher Benner, Bilal E. Kerman, David Gosselin, Clotilde Lagier-Tourenne, Chiara Zuccato, Elena Cattaneo, Fred H. Gage, Don W. Cleveland, Christopher K. Glass, Mutant huntingtin promotes autonomous microglia activation via myeloid lineage-determining factors, Nat. Neurosci. 17 (4) (2014) 513–521, https://doi.org/10.1038/nn.3668.

[19] Anastasia G. Efthymiou, Alison M. Goate, Late onset alzheimer's disease genetics implicates microglial pathways in disease risk, Mol. Neurodegener. 12 (43) (2017) 1–12, https://doi.org/10.1186/s13024-017-0184-x.

[20] Amanda Sierra, Andres C. Gottfried-blackmore, Bruce S. Mcewen, Karen Bulloch, Microglia derived from aging mice exhibit an altered inflammatory profile, Glia 55 (2007) 412–424, https://doi.org/10.1002/glia.

[21] Florent Ginhoux, Melanie Greter, Marylene Leboeuf, Sayan Nandi, Peter See, Solen Gokhan, Mark F. Mehler, et al., Fate mapping analysis reveals that adult microglia derive from primitive macrophages, Science 330 (November) (2010) 841–846.

[22] Florent Ginhoux, Shawn Lim, Guillaume Hoeffel, Donovan Low, Tara Huber, Origin and differentiation of microglia, Front. Cell. Neurosci. 7 (April) (2013) 45, https://doi.org/10.3389/fncel.2013.00045.

[23] José Luis Marín-Teva, Isabelle Dusart, Catherine Colin, Annie Gervais, Nico Van Rooijen, Michel Mallat, Microglia promote the death of developing Purkinje cells, Neuron 41 (4) (2004) 535–547, https://doi.org/10.1016/S0896-6273(04)00069-8.

[24] Dorothy P. Schafer, Emily K. Lehrman, Amanda G. Kautzman, Ryuta Koyama, Alan R. Mardinly, Ryo Yamasaki, Richard M. Ransohoff, Michael E. Greenberg, Ben A. Barres, Beth Stevens, Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner, Neuron 74 (4) (2012) 691–705, https://doi.org/10.1016/j.neuron.2012.03.026.

[25] Orit Matcovitch-Natan, Devorah Winter, Amir Giladi, Fabiola Zelada González, Pierre Perrin, Hadas Keren-shaul, Meital Gury, et al., Microglia development follows a stepwise program to regulate brain homeostasis, Science 353 (6301) (2016) 789, https://doi.org/10.1126/science.aad8670.

[26] Morgane Sonia Thion, Donovan Low, Aymeric Silvin, Sven Pettersson, Florent Ginhoux, Sonia Garel, Microbiome influences prenatal and adult microglia in a sex-specific manner, Cell 172 (3) (2018) 500–507.e16, https://doi.org/10.1016/j.cell.2017.11.042.

[27] Suzanne E. Hickman, Nathan D. Kingery, Toshiro Ohsumi, Mark Borowsky, Terry K. Means, Joseph El Khoury, The microglial sensome revealed by direct RNA sequencing, Nat. Neurosci. 16 (12) (2013) 1896–1905, https://doi.org/10.1038/nn.3554.

[28] H.J. ten Donkelaar, M. Lammens, P. Wesseling, H.O.M. Thijssen, W.O. Renier, Development and developmental disorders of the human cerebellum, J. Neurol. 250 (2003) 1025–1036, https://doi.org/10.1007/s00415-003-0199-9.

[29] Ketty Leto, Marife Arancillo, Esther B.E. Becker, Annalisa Buffo, Chin Chiang, Baojin Ding, William B. Dobyns, et al., Consensus paper: cerebellar development, Cerebellum 15 (6) (2016) 789–828, https://doi.org/10.1007/s12311-015-0724-2.

[30] Mario U. Manto, Patrice Jissendi, Cerebellum: links between development, developmental disorders and motor learning, Front. Neuroanat. 6 (January) (2012) 1–10, https://doi.org/10.3389/fnana.2012.00001.

[31] Hisako Nakayama, Manabu Abe, Chie Morimoto, Tadatsune Iida, Shigeo Okabe, Kenji Sakimura, Kouichi Hashimoto, Microglia permit climbing fiber elimination by promoting GABAergic inhibition in the developing cerebellum, Nat Commun 9 (2018) 1–2, https://doi.org/10.1038/s41467-018-05100-z.

[32] Kouichi Hashimoto, Ryoichi Ichikawa, Kazuo Kitamura, Masahiko Watanabe, Masanobu Kano, Translocation of a "winner" climbing fiber to the Purkinje cell dendrite and subsequent elimination of "losers" from the soma in developing cerebellum, Neuron 63 (1) (2009) 106–118, https://doi.org/10.1016/j.neuron.2009.06.008.

[33] Chong Chen, Masanobu Kano, Asa Abeliovich, Lu Chen, Shaowen Bao, Jeansok J. Kim, Kouichi Hashimoto, Richard F. Thompson, Susumu Tonegawa, Impaired motor coordination correlates with persistent multiple climbing fiber innervation in PKC gamma mutant mice, Cell 83 (7) (1995) 1233–1242, https://doi.org/10.1016/0092-8674(95)90148-5.

[34] Austin Ferro, Wenhui Qu, Abigail Lukowicz, Daniel Svedberg, Andrea Johnson, Marija Cvetanovic, Inhibition of NF-κB signaling in IKKβ F/F;LysM Cre mice causes motor deficits but does not alter pathogenesis of spinocerebellar ataxia type 1, PLoS One 1 (2018) 1–21.

[35] Heliane G. Serra, Lisa Duvick, Zu Tao, Kerri Carlson, Sam Stevens, Nathan Jorgensen, Alana Lysholm, et al., ROR a-mediated Purkinje cell development determines disease severity in adult SCA1 mice, Cell 127 (2006) 697–708, https://doi.org/10.1016/j.cell.2006.09.036.

[36] Justin A. Barnes, Blake A. Ebner, Lisa A. Duvick, Wangcai Gao, Gang Chen, Harry T. Orr, Timothy J. Ebner, Abnormalities in the climbing fiber–Purkinje cell circuitry contribute to neuronal dysfunction in ATXN1[82Q] mice, J. Neurosci. 31 (36) (2011) 12778–12789, https://doi.org/10.1523/JNEUROSCI.2579-11.2011.

[37] Ying Li, Xu-fei Du, Chang-sheng Liu, Zi-long Wen, Jiu-lin Du, Reciprocal Regulation between Resting Microglial Dynamics and Neuronal Activity In Vivo, Dev Cell 23 (6) (2012) 1189–1202, https://doi.org/10.1016/j.devcel.2012.10.027.

[38] Dimitrios Davalos, Jaime Grutzendler, Guang Yang, Jiyun V. Kim, Yi Zuo, Steffen Jung, Dan R. Littman, Michael L. Dustin, Wen-biao Gan, ATP mediates rapid microglial response to local brain injury in vivo, Nat. Neurosci. 8 (6) (2005) 752–758, https://doi.org/10.1038/nn1472.

[39] Dimitrios Davalos, Jae Kyu Ryu, Mario Merlini, Kim M. Baeten, Natacha Le Moan, Mark A. Petersen, Thomas J. Deerinck, et al., Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation, Nat. Commun. 3 (2012) 1–15, https://doi.org/10.1038/ncomms2230.

[40] Christian Madry, Kyrargyri Vasiliki, I. Lorena Arancibia-ca, Renaud Jolivet, Shinichi Kohsaka, Robert M. Bryan, David Attwell, Microglial ramification, surveillance, and interleukin-1 B release are regulated by the two-pore domain K + channel THIK-1, Neuron 97 (2018) 299–312, https://doi.org/10.1016/j.neuron.2017.12.002.

[41] Hiroaki Wake, Andrew J. Moorhouse, Shozo Jinno, Shinichi Kohsaka, Junichi Nabekura, Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals, J. Neurosci. 29 (13) (2009) 3974–3980, https://doi.org/10.1523/JNEUROSCI.4363-08.2009.

[42] Xiaoping Tong, Yan Ao, Guido C. Faas, Sinifunanya E. Nwaobi, Ji Xu, Martin D. Haustein, Mark A. Anderson, et al., Astrocyte Kir4.1 ion channel deficits contribute to neuronal

dysfunction in Huntington's disease model mice, Nat. Neurosci. 17 (5) (2014) 694–703, https://doi.org/10.1038/nn.3691.
[43] Sharon E. Haynes, Gunther Hollopeter, Guang Yang, Dana Kurpius, Michael E. Dailey, Wen-Biao Gan, David Julius, The P2Y12 receptor regulates microglial activation by extracellular nucleotides, Nat. Neurosci. 9 (12) (2006) 1512–1519, https://doi.org/10.1038/nn1805.
[44] F. Baroni, A. Mazzoni, Heterogeneity of Heterogeneities in Neuronal Networks, Front. Comput. Neurosci. 8 (161) (2014) 1–2, https://doi.org/10.1038/nrn3687.
[45] Pinar Ayata, Ana Badimon, Hayley J. Strasburger, Mary Kaye Duff, Sarah E. Montgomery, Yong-hwee E. Loh, Anja Ebert, et al., Epigenetic regulation of brain region-specific microglia clearance activity, Nat. Neurosci. 21 (2018) 1049–1060, https://doi.org/10.1038/s41593-018-0192-3.
[46] Kathleen Grabert, Tom Michoel, Michail H. Karavolos, Sara Clohisey, J. Kenneth Baillie, Mark P. Stevens, Tom C. Freeman, Kim M. Summers, Barry W. Mccoll, Microglial brain region-dependent diversity and selective regional sensitivities to aging, Nat. Neurosci. 19 (3) (2016) 504, https://doi.org/10.1038/nn.4222.
[47] L.J. Lawson, V.H. Perry, P. Dri, S. Gordon, Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain, Neuroscience 39 (I) (1990) 151–170.
[48] M. Cvetanovic, M. Ingram, H. Orr, P. Opal, Early activation of microglia and astrocytes in mouse models of spinocerebellar ataxia type 1, Neuroscience 289 (2015) 289–299, https://doi.org/10.1016/j.neuroscience.2015.01.003.
[49] Priscilla Mortera, Suzana Herculano-Houzel, Age-related neuronal loss in the rat brain starts at the end of adolescence, Front. Neuroanat. 6 (October) (2012) 1–9, https://doi.org/10.3389/fnana.2012.00045.
[50] Diana S. Woodruff-Pak, Michael R. Foy, Garnik G. Akopian, Ka Hung, Jordan Zach, Kim Phuong, Thi Nguyen, et al., Differential effects and rates of normal aging in cerebellum and hippocampus, PNAS 107 (4) (2010) https://doi.org/10.1073/pnas.0914207107.
[51] Nathalie Cartier, Coral Ann Lewis, Regan Zhang, Fabio M.V. Rossi, The role of microglia in human disease: therapeutic tool or target? Acta Neuropathol. 128 (3) (2014) 363–380, https://doi.org/10.1007/s00401-014-1330-y.
[52] Uwe-Karsten K. Hanisch, Helmut Kettenmann, Microglia: active sensor and versatile effector cells in the normal and pathologic brain, Nat. Neurosci. 10 (11) (2007) 1387–1394, https://doi.org/10.1038/nn1997.
[53] Colm Cunningham, Microglia and neurodegeneration: the role of systemic inflammation, Glia 61 (1) (2013) 71–90, https://doi.org/10.1002/glia.22350.
[54] Richard M. Ransohoff, A polarizing question: do M1 and M2 microglia exist? Nat. Neurosci. 19 (8) (2016) 987–991, https://doi.org/10.1038/nn.4338.
[55] Toby Lawrence, The nuclear factor NF-kappaB pathway in inflammation, Cold Spring Harb. Perspect. Biol. 1 (6) (2009) 1–10, https://doi.org/10.1101/cshperspect.a001651.
[56] S. Mincheva-Tasheva, R.M. Soler, NF-κB signaling pathways: role in nervous system physiology and pathology, Neuroscientist (2012) https://doi.org/10.1177/1073858412444007.
[57] Taiji Matsusaka, Keiko Fujikawa, Yukihiro Nishio, Naofumi Mukaidat, Kouji Matsushimat, Tadamitsu Kishimotot, Shizuo Akira, Transcription factors NF-IL6 and NF-KB synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8 (C/EBP/protein-protein interaction/acute-phase reaction), Proc. Natl. Acad. Sci. U. S. A. 90 (1993).
[58] Flávia V. Santa-Cecília, Benjamin Socias, Mohand O. Ouidja, Julia E. Sepulveda-Diaz, Leonardo Acuña, Rangel L. Silva, Patrick P. Michel, Elaine Del-Bel, Thiago M. Cunha, Rita Raisman-Vozari, Doxycycline suppresses microglial activation by inhibiting the p38 MAPK and NF-kB signaling pathways, Neurotox Res 29 (4) (2016) 447–459, https://doi.org/10.1007/s12640-015-9592-2.
[59] Tony Wyss-Coray, Joseph Rogers, Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature, Cold Spring Harb. Perspect. Biol. (2012) 1–24, https://doi.org/10.1101/cshperspect.a006346.
[60] Shane A. Liddelow, Kevin A. Guttenplan, Laura E. Clarke, Frederick C. Bennett, Christopher J. Bohlen, Lucas Schirmer, Mariko L. Bennett, et al., Neurotoxic reactive astrocytes are induced by activated microglia, Nature 541 (7638) (2017) 481–487, https://doi.org/10.1038/nature21029.
[61] Eva Czirr, Nicholas A. Castello, Kira I. Mosher, Joseph M. Castellano, Izumi V. Hinkson, Kurt M. Lucin, Bernat Baeza-Raja, et al., Microglial complement receptor 3 regulates brain Aβ levels through secreted proteolytic activity, J. Exp. Med. 214 (4) (2017) 1081–1092 http://jem.rupress.org/content/214/4/1081.abstract.
[62] Sakura S. Minami, Sang-Won Min, Grietje Krabbe, Chao Wang, Yungui Zhou, Rustam Asagiv, Yaqiao Li, et al., Progranulin protects against amyloid β deposition and toxicity in Alzheimer's disease mouse models, Nat. Med. 20 (10) (2014) 1157–1164, https://doi.org/10.14440/jbm.2015.54.A.
[63] Peng Yuan, Carlo Condello, C. Dirk Keene, Yaming Wang, Thomas D. Bird, Steven M. Paul, Wenjie Luo, Marco Colonna, David Baddeley, Jaime Grutzendler, TREM2 haplodeficiency in mice and humans impairs the microglia barrier function leading to decreased amyloid compaction and severe axonal dystrophy, Neuron 90 (4) (2016) 724–739, https://doi.org/10.1016/j.neuron.2016.05.003.
[64] S. Hong, V.F. Beja-Glasser, B.M. Nfonoyim, A. Frouin, S. Li, S. Ramakrishnan, K.M. Merry, et al., Complement and microglia mediate early synapse loss in Alzheimer mouse models, Science 352 (6286) (2016) 712–716.
[65] Soyon Hong, Lasse Dissing-Olesen, Beth Stevens, New insights on the role of microglia in synaptic pruning in health and disease, Curr Opin Neurobiol 36 (2016) 128–134, https://doi.org/10.1016/j.conb.2015.12.004.
[66] Hong Lian, Li Yang, Allysa Cole, Lu Sun, Angie C.A. Chiang, Stephanie W. Fowler, David J. Shim, et al., NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's disease, Neuron 85 (1) (2015) 101–116, https://doi.org/10.1016/j.neuron.2014.11.018.
[67] Susanne A. Wolf, H.W.G.M. Boddeke, Helmut Kettenmann, Microglia in physiology and disease, Annu. Rev. Physiol. 79 (1) (2017) 619–643, https://doi.org/10.1146/annurev-physiol-022516-034406.
[68] Tomonori Aikawa, Kaoru Mogushi, Kumiko Iijima-Tsutsui, Kinya Ishikawa, Miyano Sakurai, Hiroshi Tanaka, Hidehiro Mizusawa, Kei Watase, Loss of MyD88 alters neuroinflammatory response and attenuates early Purkinje cell loss in a spinocerebellar ataxia type 6 mouse model, Hum. Mol. Genet. 24 (17) (2015) 4780–4791, https://doi.org/10.1093/hmg/ddv202.
[69] Takahiro Seki, Masahiro Sato, Yuki Kibe, Tomoko Ohta, Mutsumi Oshima, Ayumu Konno, Hirokazu Hirai, Yuki

Kurauchi, Akinori Hisatsune, Hiroshi Katsuki, Neurobiology of disease lysosomal dysfunction and early glial activation are involved in the pathogenesis of spinocerebellar ataxia type 21 caused by mutant transmembrane protein 240, Neurobiol Dis 120 (September) (2018) 34–50, https://doi.org/10.1016/j.nbd.2018.08.022.

[70] Yan Shen, Marissa Z. McMackin, Yuxi Shan, Alan Raetz, Sheila David, Gino Cortopassi, Frataxin deficiency promotes excess microglial DNA damage and inflammation that is rescued by PJ34, PLoS One 11 (3) (2016), e0151026. https://doi.org/10.1371/journal.pone.0151026.

[71] Hazel Quek, John Luff, KaGeen Cheung, Sergei Kozlov, Magtouf Gatei, C. Soon Lee, Mark C. Bellingham, et al., A rat model of ataxia–telangiectasia: evidence for a neurodegenerative phenotype, Hum Mol Genet 26 (1) (2016), ddw371. https://doi.org/10.1093/hmg/ddw371.

[72] Charlotte Havelund Nykjaer, Tomasz Brudek, Lisette Salvesen, Bente Pakkenberg, Changes in the cell population in brain white matter in multiple system atrophy, Mov. Disord. 32 (7) (2017) 1074–1082, https://doi.org/10.1002/mds.26979.

[73] B.A. Ebner, M.A. Ingram, J.A. Barnes, L.A. Duvick, J.L. Frisch, H.B. Clark, H.Y. Zoghbi, T.J. Ebner, H.T. Orr, Purkinje cell ataxin-1 modulates climbing fiber synaptic input in developing and adult mouse cerebellum, J. Neurosci. 33 (13) (2013) 5806–5820, https://doi.org/10.1523/JNEUROSCI.6311-11.2013.

[74] Monica Renee, Pittman Elmore, Allison Rachel Najafi, Maya Allegra Koike, Nabil Nazih, Elizabeth Erin Spangenberg, Rachel Anne Rice, et al., CSF1 receptor signaling is necessary for microglia viability, which unmasks a cell that rapidly repopulates the microglia-depleted adult brain, Neuron 82 (2) (2015) 380–397, https://doi.org/10.1016/j.neuron.2014.02.040.CSF1.

[75] Wenhui Qu, Andrea Johnson, Joo Hyun Kim, Abigail Lukowicz, Daniel Svedberg, Marija Cvetanovic, Inhibition of Colony-Stimulating Factor 1 Receptor Early in Disease Ameliorates Motor Deficits in SCA1 Mice, Journal of Neuroinflammation, 14, 2017 1–11, https://doi.org/10.1186/s12974-017-0880-z.

[76] G. Jean Harry, Microglia during development and aging, Pharmacol. Ther. 139 (3) (2013) 313–326, https://doi.org/10.1016/j.pharmthera.2013.04.013 Pope.

[77] Polina A. Egorova, Alexandra V. Gavrilova, Ilya B. Bezprozvanny, In vivo analysis of the climbing fiber–Purkinje cell circuit in SCA2–58Q transgenic mouse model, Cerebellum 17 (5) (2018) 590–600, https://doi.org/10.1007/s12311-018-0951-4.

[78] Mohamed F. Ibrahim, Emmet M. Power, Kay Potapov, Ruth M. Empson, Motor and cerebellar architectural abnormalities during the early progression of ataxia in a mouse model of SCA1 and how early prevention leads to a better outcome later in life, Front. Cell. Neurosci. 11 (September) (2017) 292, https://doi.org/10.3389/fncel.2017.00292.

[79] Sheng-Han Kuo, Chi-Ying Lin, Jie Wang, Peter A. Sims, Ming-Kai Pan, Jyun-you Liou, Danielle Lee, et al., Climbing fiber–Purkinje cell synaptic pathology in tremor and cerebellar degenerative diseases, Acta Neuropathol. 133 (1) (2017) 121–138, https://doi.org/10.1007/s00401-016-1626-1.

[80] A.N. Shuvaev, H. Horiuchi, T. Seki, H. Goenawan, T. Irie, A. Iizuka, N. Sakai, H. Hirai, Mutant PKC in spinocerebellar ataxia type 14 disrupts synapse elimination and long-term depression in Purkinje cells in vivo, J. Neurosci. 31 (40) (2011) 14324–14334, https://doi.org/10.1523/JNEUROSCI.5530-10.2011.

[81] Jillian Friedrich, Christine Henzler, Harry T. Orr, Jillian Friedrich, Holly B. Kordasiewicz, Brennon O. Callaghan, Hillary P. Handler, Harry T. Orr, Antisense oligonucleotide–mediated ataxin-1 reduction prolongs survival in SCA1 mice and reveals disease-associated transcriptome profiles, J. Clin. Investig. 3 (21) (2018).

[82] Megan S. Keiser, Ryan L. Boudreau, Beverly L. Davidson, Broad therapeutic benefit after RNAi expression vector delivery to deep cerebellar nuclei: implications for spinocerebellar ataxia type 1 therapy, Mol. Ther. 22 (3) (2014) 588–595, https://doi.org/10.1038/mt.2013.279.

[83] Megan S. Keiser, Holly B. Kordasiewicz, Jodi L. McBride, Gene suppression strategies for dominantly inherited neurodegenerative diseases: lessons from Huntington's disease and spinocerebellar ataxia, Hum. Mol. Genet. (2016) https://doi.org/10.1093/hmg/ddv442.

[84] Hayley S. Mcloughlin, Lauren R. Moore, Ravi Chopra, Robert Komlo, Megan Mckenzie, Kate G. Blumenstein, Hien Zhao, Holly B. Kordasiewicz, Vikram G. Shakkottai, Henry L. Paulson, Oligonucleotide therapy mitigates disease in spinocerebellar ataxia type 3 mice, Ann. Clin. Transl. Neurol. 84 (1) (2018) 64–77, https://doi.org/10.1002/ana.25264.

[85] Daniel R. Soles, Pratap Meera, Matthew D. Schneider, Sharan Paul, Warunee Dansithong, Karla P. Figueroa, Gene Hung, et al., Antisense oligonucleotide therapy for spinocerebellar ataxia type 2, Nature 544 (7650) (2017) 362–366, https://doi.org/10.1038/nature22044.

[86] Ilia D. Vainchtein, Gregory Chin, Frances S. Cho, Kevin W. Kelley, John G. Miller, Elliott C. Chien, Shane A. Liddelow, et al., Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development, Science 359 (6381) (2018) 1269–1273, https://doi.org/10.1126/science.aal3589.

[87] Tomas C. Bellamy, Interactions between Purkinje neurones and Bergmann glia, Cerebellum 5 (2006) 116–126, https://doi.org/10.1080/14734220600724569.

[88] Masae Iino, Kaori Goto, Wataru Kakegawa, Haruo Okado, Makoto Sudo, Shogo Ishiuchi, Akiko Miwa, et al., Glia-synapse interaction through Ca-permeable AMPA receptors in Bergmann glia, Science 292 (5518) (2001) 923–926, https://doi.org/10.1126/science.1059162.

[89] Sara K. Custer, Gwenn A. Garden, Nishi Gill, Udo Rueb, Randell T. Libby, Christian Schultz, Stephan J. Guyenet, et al., Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport, Nat. Neurosci. 9 (10) (2006) 1302–1311, https://doi.org/10.1038/nn1750.

[90] S.A. Furrer, M.S. Mohanachandran, S.M. Waldherr, C. Chang, V.A. Damian, B.L. Sopher, G.A. Garden, A.R. La Spada, Spinocerebellar ataxia type 7 cerebellar disease requires the coordinated action of mutant ataxin-7 in neurons and glia, and displays non-cell-autonomous Bergmann glia degeneration, J. Neurosci. 31 (45) (2011) 16269–16278, https://doi.org/10.1523/JNEUROSCI.4000-11.2011.

[91] Joo Hyun Kim, A. Lukowicz, W. Qu, A. Johnson, M. Cvetanovic, Astroglia contribute to the pathogenesis of spinocerebellar ataxia type 1 (SCA1) in a biphasic, stage-of-disease specific manner, Glia (2018) 1–16, https://doi.org/10.1002/glia.23451.

[92] Aymeric Silvin, Florent Ginhoux, Microglia heterogeneity along a spatio-temporal axis: more questions than answers, Glia (2018) 1–13, https://doi.org/10.1002/glia.23458.

[93] Adriano Aguzzi, Ben A. Barres, Mariko L. Bennett, Microglia: scapegoat, saboteur, or something else?

Science 339 (6116) (2013) 156–161, https://doi.org/10.1126/science.1227901.
[94] Clare L. Abram, Gray L. Roberge, Yongmei Hu, Clifford A. Lowell, Comparative analysis of the efficiency and specificity of myeloid-Cre deleting strains using ROSA-EYFP reporter mice, J. Immunol. Methods 408 (2015) 89–100, https://doi.org/10.1016/j.jim.2014.05.009.Comparative.
[95] Christoph D. Schmid, Benoit Melchior, Kokoechat Masek, Shweta S. Puntambekar, Patria E. Danielson, David D. Lo, J. Gregor Sutcliffe, Monica J. Carson, Differential gene expression in LPS/IFNy activated microglia and macrophages: in vitro versus in vivo, J. Neurochem. 109 (Suppl. 1) (2009) 117–125, https://doi.org/10.1111/j.1471-4159.2009.05984.x.
[96] Monica R.P. Elmore, Rafael J. Lee, Brian L. West, Kim N. Green, Characterizing newly repopulated microglia in the adult mouse: impacts on animal behavior, cell morphology, and neuroinflammation, PLoS One 10 (4) (2015), e0122912. https://doi.org/10.1371/journal.pone.0122912.
[97] R.A. Rice, E.E. Spangenberg, H. Yamate-Morgan, R.J. Lee, R.P.S. Arora, M.X. Hernandez, A.J. Tenner, B.L. West, K.N. Green, Elimination of microglia improves functional outcomes following extensive neuronal loss in the hippocampus, J. Neurosci. 35 (27) (2015) 9977–9989, https://doi.org/10.1523/JNEUROSCI.0336-15.2015.
[98] Justyna Sosna, Stephan Philipp, Ricardo Albay, Jorge Mauricio Reyes-Ruiz, David Baglietto-Vargas, Frank M. LaFerla, Charles G. Glabe, Early long-term administration of the CSF1R inhibitor PLX3397 ablates microglia and reduces accumulation of intraneuronal amyloid, neuritic plaque deposition and pre-fibrillar oligomers in 5XFAD mouse model of Alzheimer's disease, Mol. Neurodegener. 13 (1) (2018) 11, https://doi.org/10.1186/s13024-018-0244-x.
[99] Krista J. Spiller, Clark R. Restrepo, Tahiyana Khan, Myrna A. Dominique, Terry C. Fang, Rebecca G. Canter, Christopher J. Roberts, et al., Microglia-mediated recovery from ALS-relevant motor neuron degeneration in a mouse model of TDP-43 Proteinopathy, Nat. Neurosci. 21 (3) (2018) 329–340, https://doi.org/10.1038/s41593-018-0083-7.
[100] Jian Luo, Fiona Elwood, Markus Britschgi, Saul Villeda, Hui Zhang, Zhaoqing Ding, Liyin Zhu, et al., Colony-stimulating factor 1 receptor (CSF1R) signaling in injured neurons facilitates protection and survival, J Exp Med 210 (1) (2013) 157–172, https://doi.org/10.1084/jem.20120412.
[101] B.E. Clausen, C. Burkhardt, W. Reith, R. Renkawitz, I. Förster, Conditional gene targeting in macrophages and granulocytes using LysMcre mice, Transgenic Res. 8 (4) (1999) 265–277, https://doi.org/10.1023/A:1008942828960.
[102] Johannes Orthgiess, Martin Gericke, Kerstin Immig, Angela Schulz, Johannes Hirrlinger, Ingo Bechmann, Jens Eilers, Neurons exhibit Lyz2 promoter activity in vivo: implications for using LysM-Cre mice in myeloid cell research, Eur. J. Immunol. 46 (6) (2016) 1529–1532, https://doi.org/10.1002/eji.201546108.
[103] Peter Wieghofer, Klaus-Peter Knobeloch, Marco Prinz, Genetic targeting of microglia, Glia 63 (1) (2015) 1–22, https://doi.org/10.1002/glia.22727.
[104] Bennett, Mariko L, F Chris Bennett, Shane A Liddelow, Bahareh Ajami, Jennifer L Zamanian, Nathaniel B Fernhoff, Sara B Mulinyawe, et al. 2016. "New tools for studying microglia in the mouse and human CNS." Proc. Natl. Acad. Sci. U. S. A. 113 (12). National Academy of Sciences: E1738-46. https://doi.org/10.1073/pnas.1525528113.
[105] Amy M. Smith, Mike Dragunow, The human side of microglia, Trends Neurosci 37 (3) (2014) 125–135, https://doi.org/10.1016/j.tins.2013.12.001.
[106] Florian T. Merkle, Kevin Eggan, Perspective modeling human disease with pluripotent stem cells: from genome association to function, Stem Cell 12 (6) (2013) 656–668, https://doi.org/10.1016/j.stem.2013.05.016.
[107] Kazutoshi Takahashi, Koji Tanabe, Mari Ohnuki, Megumi Narita, Tomoko Ichisaka, Kiichiro Tomoda, Shinya Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872, https://doi.org/10.1016/j.cell.2007.11.019.
[108] Judith Staerk, Meelad M. Dawlaty, Qing Gao, Dorothea Maetzel, Jacob Hanna, Cesar A. Sommer, Gustavo Mostoslavsky, Rudolf Jaenisch, Reprogramming of human peripheral blood cells to induced pluripotent stem cells, Stem Cell 7 (1) (2010) 20–24, https://doi.org/10.1016/j.stem.2010.06.002.
[109] Ting Zhou, Christina Benda, Sarah Dunzinger, Yinghua Huang, Jenny Cy Ho, Jiayin Yang, Yu Wang, et al., Generation of human induced pluripotent stem cells from urine samples, Nat. Protoc. 7 (12) (2012) 2080–2083, https://doi.org/10.1038/nprot.2012.115.
[110] Kazutoshi Takahashi, Shinya Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676, https://doi.org/10.1016/j.cell.2006.07.024.
[111] Douvaras, Panagiotis, Bruce Sun, Minghui Wang, Ilya Kruglikov, Gregory Lallos, Matthew Zimmer, Cecile Terrenoire, et al. 2017. "Directed differentiation of human pluripotent stem cells to microglia." Stem Cell Rep 8 (6). ElsevierCompany.: 1516–24. https://doi.org/10.1016/j.stemcr.2017.04.023.
[112] Julien Muffat, Yun Li, Bingbing Yuan, Maisam Mitalipova, Attya Omer, Sean Corcoran, Grisilda Bakiasi, et al., Efficient derivation of microglia-like cells from human pluripotent stem cells, Nat. Med. 22 (11) (2016) 1358–1367, https://doi.org/10.1038/nm.4189.Efficient.
[113] Hetal Pandya, Michael J. Shen, David M. Ichikawa, Andrea B. Sedlock, Yong Choi, R. Johnson, Gloria Kim, et al., Differentiation of human and murine induced pluripotent stem cells to microglia-like cells, Nat. Neurosci. 20 (5) (2017) 753–759, https://doi.org/10.1038/nn.4534.Differentiation.
[114] Dongwei Li, Jing Liu, Xuejie Yang, Andrew P. Hutchins, Jiekai Chen, Dongwei Li, Jing Liu, et al., Chromatin accessibility dynamics during iPSC reprogramming, Stem Cell 21 (6) (2017) 819–833, https://doi.org/10.1016/j.stem.2017.10.012.
[115] K. Brennand, J.N. Savas, Y. Kim, N. Tran, A. Simone, K.G. Beaumont, H.J. Kim, A. Topol, I. Ladran, Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia, Mol. Psychiatry 20 (3) (2015) 361–368, https://doi.org/10.1038/mp.2014.22.
[116] Philip W. Brownjohn, James Smith, Ravi Solanki, Ebba Lohmann, Henry Houlden, John Hardy, Sabine Dietmann, Frederick J. Livesey, Functional studies of missense TREM2 mutations in human stem cell-derived microglia, Stem Cell Rep 10 (4) (2018) 1294–1307, https://doi.org/10.1016/j.stemcr.2018.03.003.
[117] Pablo Garcia-Reitboeck, Alexandra Phillips, Thomas M. Piers, Henry Houlden, John Hardy, Jennifer M. Pocock, Human induced pluripotent stem cell-derived microglia-like cells harboring TREM2 missense mutations show specific deficits in phagocytosis, Cell Rep. 24 (9) (2018) 2300–2311, https://doi.org/10.1016/j.celrep.2018.07.094.

[118] Yuan-Ta Lin, Jinsoo Seo, Fan Gao, Tak Ko, Bruce A. Yankner, Li-Huei Tsai, APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types, Neuron 98 (6) (2018) 1141–1154.e7, https://doi.org/10.1016/j.neuron.2018.05.008.

[119] Edsel M. Abud, Ricardo N. Ramirez, Eric S. Martinez, Monica J. Carson, Wayne W. Poon, Mathew Blurton-jones, iPSC-derived human microglia-like cells to study neurological diseases, Neuron 94 (2) (2017) 278-293.e9, https://doi.org/10.1016/j.neuron.2017.03.042.