degeneration in SBMA? Will amelioration of the disease by targeting muscle uncover other CNS manifestations? Answering these questions will help in translating this potential treatment option into effective treatment in patients.

In summary, the reports by Cortes et al. (2014) and Lieberman et al. (2014) emphasize the role of skeletal muscle as an important contributor to SBMA pathogenesis and underscore the importance of exploring approaches targeting peripheral tissues for treatment.

Clearly, in this disease the muscle matters.

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Hidden Progenitors Replace Microglia in the Adult Brain

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Microglia are highly dynamic components of the innate immune system. In this issue of *Neuron*, Elmore et al. (2014) report that global depletion of microglia triggers mobilization of latent microglial progenitors throughout the CNS, resulting in rapid repopulation.

There is a common misconception that the adult CNS has a limited capacity for repair and regeneration. While this is true for most neuronal populations, many types of glial cells exhibit remarkable homeostasis and strive to maintain a constant density in response to internal or external perturbations. Understanding how this process of cellular maintenance is regulated, and identifying the progenitors responsible for replenishing distinct classes of glia, could lead to new strategies for speeding recovery from injury and limiting the abnormal cellular proliferation that leads to brain cancer. In this issue of *Neuron*, Elmore et al. (2014) describe a new method to deplete microglia from the adult CNS and use this approach to investigate how homeostasis of microglia is achieved and the broader roles of these ubiquitous glial cells in controlling behavior.

Unlike other glial cells (astroglia, oligodendroglia), microglia are formed outside the developing CNS from hematopoietic progenitors in the yolk sac and migrate into the CNS before the blood-brain barrier (BBB) is formed (Kierdorf et al., 2013). There they expand through proliferation to establish a grid-like distribution throughout the CNS. Microglia are crucial for the innate immune response and secrete numerous cytokines and chemokines to regulate tissue repair and can transform into highly migratory cells that engulf dead cells and debris, playing a crucial role in CNS development, recovery from injury, and progression of disease. Recent studies suggest that these glial

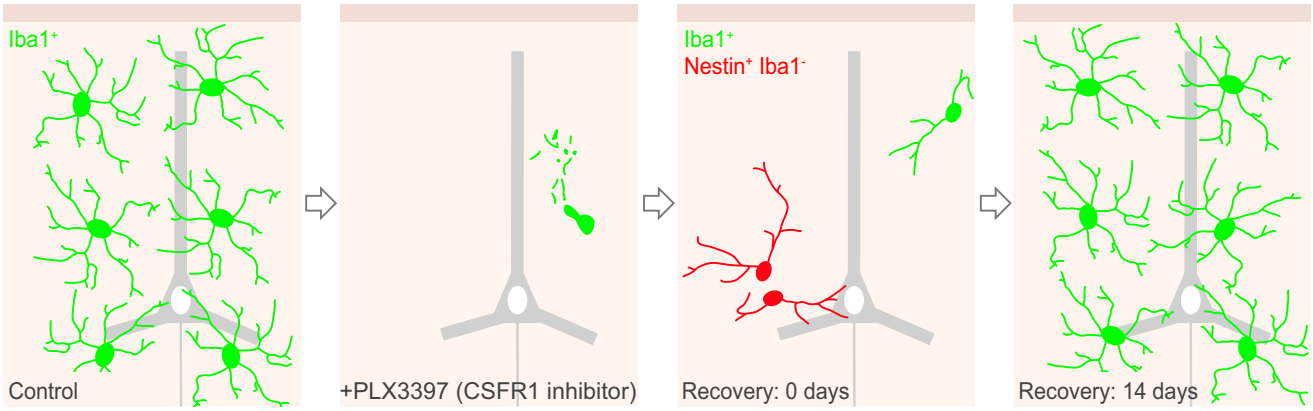

Figure 1. Inhibition of CSFR1 Induces Death of Microglia in the Adult CNS that Are Repopulated through Recruitment of Latent Progenitors

cells also engage in local control of excitatory synapses, by pruning synapses and controlling dendritic spine density (Paolicelli et al., 2011; Schafer et al., 2012), suggesting that they play an important role in remodeling neural circuits.

Microglia are known to require activation of colony-stimulating factor receptor 1 (CSFR1), a receptor tyrosine kinase, for proliferation and survival during early development; mice that lack this receptor do not form microglia and rarely survive to adulthood (Erblich et al., 2011; Ginhoux et al., 2010). However, this receptor continues to be expressed by microglia in the adult CNS, raising the possibility that persistent activation of CSFR1 is required to sustain their population. To explore how signaling through this receptor controls the inflammatory response of microglia, Elmore et al. (2014) administered the CSFR1 inhibitor PLX3397 (Plexxikon) to adult mice. Remarkably, exposure to this compound led to a rapid and pronounced reduction in expression of IBA1, a microglia-specific protein in the brain, below that seen in untreated controls; after 3 weeks of PLX3397 exposure, brains were largely devoid of IBA1$^+$ microglia (Figure 1). Although this might have resulted from an acute reduction in IBA1 expression rather than the disappearance of microglia, when experiments were performed in transgenic mice in which microglia express GFP (CX3CR1-GFP$^{+/-}$ mice), CSFR1 inhibition induced a similar depletion of GFP$^+$ cells. Moreover, brains from these mice exhibited reductions in microglia-enriched mRNAs, such as AIF1 (which encodes IBA1), CSF1R, CX3CR1, FCGR1, ITGAM, and TREM2. Microglia continue to divide at a low rate in the adult CNS; however, this drug-induced depletion resulted primarily from accelerated death, rather than reduced proliferation, as there was an increase in activated caspase-3$^+$ and propidium iodide$^+$ microglia after exposure to PLX3397. These findings indicate that microglia require CSFR1 signaling for survival in the adult CNS.

Despite the widespread death of microglia in this condition, the brain appeared remarkably unperturbed: brain volume was unchanged, the blood-brain barrier remained intact, and there was no global increase in inflammation. The exception was a marked increase in S100β and GFAP expression, a characteristic of reactive astrocytes, although other features of astrocytosis, such as hypertrophy and enhanced proliferation, were surprisingly absent. This suggests that death of microglia alone, as occurs after CNS injury, is not sufficient to provoke an inflammatory response. Similar observations were reported in a recent study by Parkhurst et al. (2013), in which microglia were ablated through transgenic expression of the diphtheria toxin receptor.

The ability to deplete specific cell classes using chemical agents or genetic means (i.e., exposure to saporin-linked ligands or expression of diphtheria toxin), has helped to define their roles in vivo and explore the response of remaining cells. Here, Elmore et al. (2014) subjected mice depleted of microglia with PLX3397 to a battery of behavioral tests. Remarkably, short- (7 days) or long-term (2 months) depletion of microglia did not result in overt behavioral abnormalities or reduced performance on standard tests of anxiety (elevated plus maze and open field), locomotion (accelerating rotarod), or learning and memory tasks (Barnes maze). Adult mice depleted of microglia actually performed slightly better on the learning and memory task. Recent studies show that mice exhibiting a transient reduction of microglia during development due to the loss of the fractalkine receptor (Cx3cr1) display reduced synaptic connectivity and deficits in social behavior (Zhan et al., 2014), raising the possibility that microglia play different roles in the developing and mature brain. The findings of Elmore et al. (2014) run also counter to those of Parkhurst et al. (2013), in which adult microglia-deficient mice performed worse on multiple learning tasks, including rotarod, and exhibited reduced synapse formation after motor learning. However, the deficits in motor performance observed by Parkhurst et al. (2013) were observed with much more intensive training, raising the possibility that the consequences of microglia depletion on motor learning only manifest in response to more challenging protocols. Additionally, CSFR1 inhibition triggers loss of both CNS microglia and peripheral macrophages, whereas the approach used by Parkhurst et al. (2013) enabled selective depletion of microglia. This added manipulation of peripheral cells could have masked a CNS phenotype. In light of the modest behavioral changes observed in mice treated with PLX3397, it will be important to determine whether they

exhibit deficits in dendritic spine remodeling similar to those observed after genetic ablation of microglia.

Although microglia remained depleted in mice maintained on PLX3397 for several months, cessation of CSFR1 inhibition resulted in rapid repopulation of the brain, and within 14 days microglia were restored to their normal density (Figure 1). How are microglia repopulated so rapidly? Previous studies using a model of microglia depletion induced by thymidine kinase + gancyclovir revealed that peripheral macrophages can masquerade as microglia after invading the CNS when the BBB is disrupted (Varvel et al., 2012). However, CSFR1 inhibition did not disrupt the BBB, macrophage-specific mRNAs were not increased, and $CCR2^+$ or $Cd11b^+$ macrophages were not detected during repopulation. Moreover, in mice engineered to express RFP in macrophages (*CCR2-RFP* mice), no RFP^+ cells were observed in the brain after repopulation, suggesting that repopulation occurs from within the brain.

As shown by Parkhurst et al. (2013), microglia, like oligodendrocyte progenitors (Hughes et al., 2013), exhibit homeostasis and are stimulated to divide and reestablish their normal density if depleted. Thus, repopulation could simply reflect proliferation of the microglia that survive CSFR1 inhibition. However, Elmore et al. (2014) find that proliferating cells present 3 days after cessation of PLX3397 exhibited less complex morphologies than microglia, were $IBA1^-$, and expressed markers of stem/progenitor cells, including nestin and CD45. These results raise the possibility that this remarkable homeostatic response is catalyzed, in part, by the mobilization of a latent pool of hematopoietic progenitors that are widely distributed throughout the CNS. This widespread appearance of microglial progenitors suggests that they may reside in a perivascular niche. This regeneration of microglia recapitulates some aspects of normal development, as microglia progenitors transiently express nestin. A detailed profiling of gene expression by these regenerated cells may yield further clues about their origin.

What if CSRF1inhibition simply induces dedifferentiation of microglia? This could explain the rapid "loss" and reemergence of microglia when CSFR1 inhibition is removed and the corresponding changes in microglia-specific gene expression. However, this hypothesis is inconsistent with the increase in apoptotic microglia in the presence of PLX3397 and the increase in proliferating $nestin^+$ cells, unless more complex scenarios occur, involving both death of microglia and dedifferentiation of survivors. One way to define the lineage of microglia that appears during repopulation is to perform in vivo fate tracing. Here, Elmore et al. (2014) labeled microglia by allowing repopulation to occur in the presence of BrdU, a thymidine analog that is permanently incorporated during DNA replication. Subsequent readministration of PLX3397 resulted in the loss of $BrdU^+$ cells, suggesting that death rather than dedifferentiation accounts for the disappearance of microglia.

Homeostasis is a critical feature of dynamic cells, which must be maintained at an optimum density to ensure proper organ and tissue function. This rapid recruitment of microglial progenitors throughout the brain was unexpected, given the myeloid origin of microglia and the intrinsic ability of resident microglia to proliferate. Parallels can again be drawn with oligodendrocyte progenitors, which exhibit their own homeostatic proliferation after depletion (Hughes et al., 2013; Robins et al., 2013) but can also be replaced through recruitment of latent progenitors within the SVZ after injury or demyelination (Menn et al., 2006). The different modes of repopulation observed in different models of microglia depletion, involving infiltration of macrophages, proliferation of endogenous microglia, or recruitment of latent progenitors, highlight that there are redundant mechanisms to ensure that their functions are preserved. It remains to be determined whether the $nestin^+$ progenitors that emerge in this depletion model also contribute to the normal homeostasis of microglia in the adult CNS and whether pathological changes in CSFR1 ligands CSF or IL-34 alter the ability of microglia to participate in repair. Defining the mechanisms responsible for this cellular homeostasis would be further aided by genetic lineage-tracing studies and continuous monitoring of microglia in vivo using two-photon imaging. This discovery that CSFR1 inhibition by PLX3397 depletes microglia in the adult CNS provides a valuable new tool to help define the role of these dynamic glial cells in both CNS repair and cognitive function.

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