Microglia's heretical self-renewal

The dogma that self-renewal is a defining characteristic of stem cells, which stemmed from studies of the hematopoietic hierarchy and quickly spread by analogy to all tissues, has been shattered by scientists pointing a microscope at the hematopoietic system itself. A microglial cell is clearly fully differentiated, and yet it self-renews.

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It has been ten years since it became clear that adult microglia are independent of hematopoietic stem cells for their maintenance, yet the notion that clearly differentiated cells may have self-renewal ability has not been easy to accept. The search for a precursor from which adult microglia originates has continued, moving from the bone marrow to the CNS itself. Huang et al.[1] directly address a previous report[2] stating that adult microglia can originate from a non-microglial precursor and convincingly show that this is not the case, hopefully putting that question to rest.

The conclusion that a non-microglial precursor capable of regenerating these cells exists within the CNS stemmed from a report by Elmore et al.[2] showing that, after pharmacological ablation of microglia, these cells rapidly return to normal numbers. The key finding in this paper was that maintenance of the microglial population was dependent on continuous signaling by CSFR1, with pharmacological inhibition of the CSFR1 receptor leading to the virtual disappearance of these cells from the CNS. The authors, however, went on to conclude that, given the extensive depletion and the impressive speed with which microglia returned, repopulation could not possibly be sustained by the rare (<1%) microglia that survived CSFR1 inhibition.

This prompted Elmore et al.[2] to postulate the existence of a non-microglial origin for the returning cells, a conclusion supported by evidence that the newly generated microglia expressed nestin. Nestin, an intermediate filament protein found in neuroectodermal progenitors, as well as in a large variety of other cell types, is expressed by the proliferating, non-microglial CNS cells observed after release of CSFR1 inhibition. However, it is not expressed in microglia at steady state, and this was taken as a suggestion that the new microglia came from a progenitor of neuroectodermal origin.

Huang et al.[1] investigated this proposed alternative origin in depth. They started by using parabiosis to exclude a contribution to the recovered microglia from circulating precursors. While it had been shown extensively that such contribution does not take place during maintenance or in neurodegenerative conditions, it can occur in neuroinflammatory conditions that lead to disruption of the blood–brain barrier[3,4]. Thus, it was important to show that the wave of cell death that leads to the disappearance of microglia does not create an inflammatory response capable of inducing blood–brain barrier breakdown.

Next, they turned their attention to the CNS. They used an inducible Cre recombinase under the control of the nestin (*Nes*) promoter to express an inheritable marker in nestin-expressing cells before microglia ablation and recovery. None of the new microglial cells were labeled, conclusively showing that they originated from a cell that did not express nestin at steady state. Thus, nestin expression had to be a response to either CSFR1 inhibition or the activation of the cells upon its cessation.

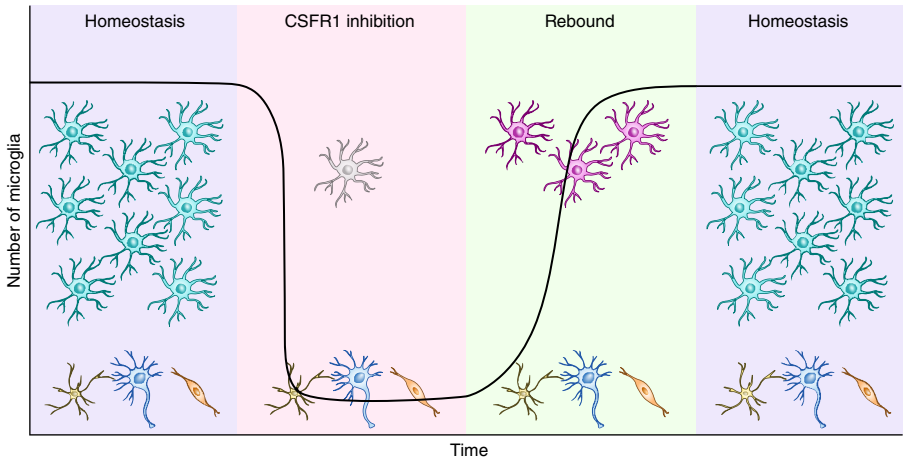

Fig. 1 | Microglial repopulation. In homeostasis, microglia are dependent on constitutive CSFR1 activity. When that is inhibited, a wave of cell death leads to the loss of most, but not all, microglial cells. Some cells remain dormant but alive (gray). Upon cessation of CSFR1 inhibition, these cells enter the cell cycle and activate the expression of nestin (pink), which is now a recognized activation marker for microglia in multiple settings. This proliferation leads to the restoration of homeostasis. Throughout these changes, the other cell populations present in the CNS (bottom) remain apparently unaffected, even in the near absence of microglia. Credit: Marina Corral Spence/Springer Nature

Huang et al.[1] used several methods, including single-cell RNA sequencing, to show that nestin expression is indeed activated in the abundant proliferating microglia arising during repopulation (Fig. 1).

In one of the most exhaustive searches for the origin of a cell type to date, Huang et al.[1] also performed lineage tracing with markers for oligodendrocyte progenitors, astrocytes, and three different types of neurons. In fact, as they used markers that are not fully specific to these cells, they may have also ended up tracing stromal cells (which, like astrocytes, express GLAST[5]) and most likely pericytes, which, like oligodendrocyte precursor cells, are known to express Cspg4 (NG2). In none of these experiments were the reconstituted microglia labeled, and while it is impossible to be absolutely comprehensive (for example, endothelial and ependymal cells were not tested), one can at least conclude that it is highly unlikely that the CNS harbors the neuroectodermal microglial progenitor that was favored in the literature 20 years ago[6].

Huang et al.[1] delivered the final, definitive blow to the idea of a non-microglial progenitor by performing the converse experiment, using a strain of mouse in which inducible Cre is under the control of the promoter for a myelomonocytic marker, Cx3Cr1CreER^{T2} mice. This strategy transiently labels all myelomonocytic cells, but as most of them are short lived, the only cells still labeled 2 months after activating the transgene are long-lived tissue-resident cells such as microglia[7]. At this point, the ablation and repopulation protocol was carried out, resulting in all new microglia carrying the label and thus formally proving that Cx3cr1-positive cells are the source of repopulation.

In addition, Huang et al.[1] analyzed the changes that occurred in whole-brain gene expression following microglial ablation. Surprisingly, except for the absence of microglia-specific transcripts in CSFR1-inhibitor-treated brains, the whole-brain transcriptome was largely undisturbed. After 60 days of recovery, only three transcripts were differentially expressed between treated and untreated brains. Notably, regenerated microglia also responded to brain injury and neuroinflammatory stimuli in a similar fashion to resident microglia. Thus, while the prolonged absence of microglia may have a deleterious effect on brain health, acute microglial ablation and regeneration has minimal immediate or prolonged impact on brain homeostasis.

As is often the case with effective science, we are left with more questions than we started with. Are the rare surviving microglia simply those forming the 'tail' of a Gaussian distribution curve representing the extent of dependency on CSFR1 signaling, or are they a special, preordained subset of hardy cells that serves as a reserve population? What are the signals that induce the fast cycling behavior of microglia upon withdrawal of the CSFR1 inhibitor? And, most importantly, how do these cells know to stop once they restore their numbers to the levels observed at steady state? Clearly a 'counting mechanism' exists for microglia, just as it does for a number of other tissue-resident cell types, and understanding how it works will be a necessary breakthrough if we are to understand biological processes ranging from regenerative systems to cancer.

Another set of questions relates to the low bar that was required to claim a non-hematopoietic progenitor for microglia. The hypothesis that microglia were mesodermal in origin, first expressed by del Río Hortega at the beginning of the century[8], was tested not long after the demonstration of these cells' self-renewal ability. Classical chicken–quail chimera studies pointed to the yolk sac as the location where they arise[9]. This origin of microglia from embryonic or fetal hematopoiesis, now amply confirmed by several groups using modern lineage tracing[10], seems a strong counter to the existence of a non-hematopoietic progenitor in the adult CNS. And yet such a progenitor was claimed on the basis of the questionable idea that regenerating microglia could not possibly cycle fast enough to repopulate the CNS on their own, even though the required 5–6 h per cycle that they calculate (a calculation disputed by Huang et al.[1]) has precedent in cell cycles as fast as 2 h that have been reported for T cells responding to an infection in vivo[11].

The expression of a purported progenitor marker, nestin, was accepted as corroborating evidence that microglial progenitors were not hematopoietic in nature. In the CNS during homeostasis, the expression of nestin, an intermediate filament, is restricted to populations of neural stem or progenitor cells. In fact, the term 'nestin' is an acronym for 'neuroectodermal stem cell marker'. However, nestin is not specific to a lineage, having been detected in proliferating cells in a multitude of developing, adult, and cancer tissues including, for example, myogenic[12] and endothelial cells, as well as subsets of pericytes[13]. Indeed, reports on nestin expression in activated microglia, as well as in bone-marrow-derived macrophages, were quickly published[14,15] after the study by Elmore et al.[2], highlighting the ease with which this protein is detected—if only one looks.

In summary, this work leaves little doubt that, following depletion of microglia, this population recovers at breakneck speeds starting from the few cells that escaped death. This will, we hope, free the field from having to revisit this issue and allow it to focus on more relevant questions pertinent to microglia functions. These are currently shrouded in as much myth as the identity of their adult progenitors. For example, rivers of ink have been spent, with little data support, describing microglial polarization states and whether these cells are protective or detrimental in specific pathologies. The combination of modern lineage tracing techniques, allowing the unambiguous distinction of microglia from other myelomonocytic cells, and deep transcriptomics should yield clear answers in the near future. ❐

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Competing interests

The authors declare no competing interests.