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INSIGHTS

Engineering mighty microglia

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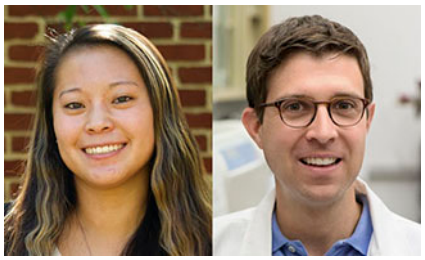
Defective microglial responses underlie many neurological disorders. Recent efforts to swap out dysfunctional microglia with optimized replacements have been derailed by safety issues and transplantation inefficiencies. In this issue, Chadarevian et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20220857) designed a novel strategy that enables improved engraftment of human microglia.

Chadarevian et al. (2023) report that a point mutation in human colony-stimulating factor 1 receptor (CSF1R) enables widespread engraftment of human microglia in adult mice with no gross differences in microglial gene expression, morphology, or immune response. Given that the current strategies to replace microglia are inefficient and hazardous, this newly developed microglia replacement strategy could offer a much-needed therapeutic approach to treat a multitude of neuropathological diseases.

Microglia are brain-resident macrophages that play critical roles in neurodevelopment, protection against infections, and the containment and disposal of neurotoxic material (Ennerfelt and Lukens, 2020; Frost and Schafer, 2016). Mounting evidence indicates that dysregulated microglial responses are centrally involved in a spectrum of neurological disorders ranging from autism to Alzheimer's disease (Colonna and Butovsky, 2017; Keren-Shaul et al., 2017; Lukens and Eyo, 2022). Furthering our understanding of how microglia function in the brain, in addition to engineering new ways to restore or replace faulty microglia, are crucial next steps to advancing therapies and treatments for nervous system disorders.

When microglia become problematic in the brain, the most obvious course of action would simply be to take them out of the picture altogether and replace them with microglia better equipped to restore brain health. Depleting existing microglia to create a suitable engraftment niche is generally thought to be a requirement of effective microglia replacement strategies. Current microglia depletion strategies often require the use of irradiation and chemotherapeutic agents to allow the transplanted cells to retain a foothold in the brain (de Vasconcelos and Lacerda, 2022). Such approaches have serious side effects that include global immunosuppression, susceptibility to infection, and increased cancer risk, and thus, improved strategies are needed.

The current approach to replace microglia in humans involves hematopoietic stem cell transplantation (HSCT), which attempts to restore defective microglia function by replacing them with peripheral macrophages (see panel A of figure). However, the harvesting of donor cells and the aggressive immunosuppressive conditioning regime required prior to infusion limits the efficacy and practicality of HSCT, in addition to having high mortality overall and slow replacement rates (de Vasconcelos and Lacerda, 2022). Peripheral macrophages transplanted in the brain have also been shown to remain transcriptionally and functionally distinct from the microglia that they aim to replace (Cronk et al., 2018; Shibuya et al., 2022), and the long-term effects of peripheral macrophage engraftment in the brain remain to be determined. Therefore, improved microglial replacement strategies are needed to realize the full potential of this approach in the treatment of neurological disease.



Insights from Adeline E. Walsh and John R. Lukens.

Continuous CSF1R signaling is required for microglial proliferation and survival. CSF1R-inhibiting drugs such as Plexxikon (PLX) 3397 have also proven to be extremely effective in rapidly depleting microglia from the brain (Elmore et al., 2014; Green et al., 2020). Unfortunately, CSF1R inhibitor pretreatment to eliminate the vast majority of endogenous microglia has not been found to significantly improve long-term donor cell engraftment in the adult brain (Abud et al., 2017). Chadarevian and colleagues hypothesized that they could overcome this hurdle by genetically engineering a CSF1R variant that would be resistant to inhibitor treatment. In this scenario, coupling the transplantation of these engineered microglia with CSF1R inhibitor treatment would enable the donor microglia to outcompete their

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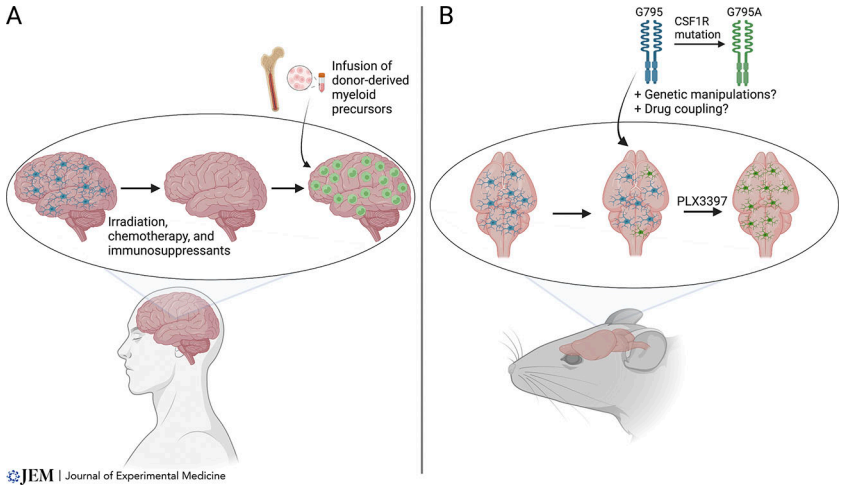

Microglia engineered to harbor an inhibitor-resistant mutation in human CSF1R offer an improved strategy to replace faulty microglia in neurological diseases. (A) The current methods to deplete and replace microglia in humans involves HSCT. This method requires intense immunosuppressive pretreatments to promote engraftment, and the resulting peripheral macrophages and myeloid cells do not fully recapitulate endogenous microglia. (B) Microglia engineered to possess a G795A mutation in CSF1R successfully engraft the adult mouse brain and are resistant to subsequent CSF1R inhibitor (PLX3397) treatment, thus allowing for efficient replacement of microglia. The beneficial properties of these engineered microglia could potentially be bolstered by targeting other neurological disease–related genes such as *TREM2*, *CD33*, or *PLCG2* in tandem.

endogenous counterparts by conferring them resistance to depletion.

The authors first performed an in vitro screen whereby they individually introduced 12 amino acid substitutions into the kinase domain of human CSF1R at residues predicted to hinder PLX3397 binding. Here, they identified that a G to A substitution at position 795 of human CSF1R protein confers potent resistance to PLX3397 treatment without altering cell growth or survival rates in conditionally immortalized macrophages (CIMs). These G795A CSF1R-expressing CIMs efficiently engrafted into the brains of PLX-treated, human CSF1-expressing neonatal and adult mice (see panel B of figure). Remarkably, the engrafted CIMs persisted even after the cessation of PLX treatment and limited the re-emergence of the native microglia population. These results are noteworthy as previous studies demonstrated that endogenous microglia usually repopulate once PLX treatment ends (Chadarevian et al., 2023; Elmore et al., 2014). This new finding may allow for long-term microglia replacement without having to continually inhibit CSF1R signaling, which may cause dangerous immunosuppression.

The group then looked to apply their findings to human-induced pluripotent stem cell–derived microglia (hiMG) to increase their work's translatability (Abud et al., 2017). Using CRISPR technology, the group introduced the G795A mutation into hiMGs and then transplanted them into mouse brains. The group confirmed that the xenografted G795A hiMGs (xMGs) had similar gene expression profiles compared to wild-type microglia, responded to an immune challenge in a similar manner to wild-type controls, and expanded in the presence of PLX to replace endogenous microglia and fully occupy the brains of neonatal and adult mice. Overall, these findings indicate that xMGs maintain microglial identity and function, which suggests that this microglia transplantation technology could potentially be harnessed as a neurologic disease therapeutic in the future.

Notably, Chadarevian and colleagues demonstrated that the G795A xMGs were able to engraft the non-depleted adult mouse brain without PLX pretreatment, albeit at low levels. Remarkably, the microglia that did successfully engraft persisted and migrated from the injection sites during continuous PLX treatment and expressed the canonical microglial marker P2RY12 30 d after PLX cessation. This suggests that microglia depletion before engraftment could be bypassed in future therapeutic endeavors.

This safer and more efficient method to swap out microglia could broaden the use of microglial replacement therapies, widen treatment windows, and ultimately ameliorate the harmful roles of microglia in many disease contexts. Moreover, other neurological disease–related molecules could also be targeted in tandem with CSF1R variants to further optimize the neuroprotective properties of the transplanted microglia using this strategy. For example, neuroprotective mutations in *TREM2*, *CD33*, and *PLCG2* could also be coupled with the CSF1R G795A variant to generate omnipresent microglia that are capable of limiting or reversing Alzheimer's disease progression.

Although these findings present exciting therapeutic potential, there are several key limitations of the study that would need to be further investigated should this work advance to a more clinically applicable stage. At the basic science level, more work should be done to elucidate how the G795A CSF1R mutation affects in vivo microglial responses. Although it was shown that these G795A CSF1R microglia express homeostatic markers upon brain colonization and can respond normally to an immune stimulus, microglia functions extend beyond these parameters. For instance, it remains to be seen if these microglia can phagocytose debris, maintain key cell–cell interactions, and retain the same metabolic profile as their endogenous counterparts. Additionally, although xMGs persisted within engrafted regions 30 d after PLX treatment ended, there were a few regions of the brain that had low success of xMG engraftment. These regions were eventually repopulated by endogenous microglia. Future work is needed to ascertain why certain regions were less successful in xMG engraftment than others and whether the endogenous microglia that do return are in small enough numbers to see a positive treatment effect from the transplanted cells.

There is also the question of when in the course of disease that microglia should be depleted and then replaced. For instance, it is possible that there is a point during disease progression where brain damage becomes too extensive and swapping in new microglia may not be beneficial. More studies in mouse models of specific diseases are also needed. These future studies should involve scaling the timetable of the experiments to a human-relevant layout and performing longer-term studies to determine

the enduring impact of xMG engraftment on brain function and overall health.

In summary, Chadarevian et al. (2023) engrafted PLX-resistant human microglia with a G795A mutation in CSF1R, allowing for robust and selective replacement of endogenous microglia without the need for bone marrow harvests or prior immunosuppressive treatments. Their data show that replacing endogenous microglia with human-derived microglia-like cells is possible. Although its future in the clinic remains a few years down the road, their findings provide a major step forward in the development of microglia transplantation strategies to treat neurological diseases.

Disclosures: The authors declare no competing interests exist.

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