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Cholesterol fuels microglia in chronic stroke

Stefano Pluchino & Cory M. Willis

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Cholesterol accumulation in microglia drives persistent inflammation after stroke. In this issue of *Nature Metabolism*, Zhao et al. suggest that enhancing microglial cholesterol catabolism may offer a promising strategy to reduce brain damage and improve recovery.

Stroke remains a major global health challenge, leaving many survivors with long-term disabilities despite advances in acute treatments such as thrombectomy and thrombolysis[1]. A key player in the brain's response to stroke is the microglia[2], the resident immune cell of the central nervous system. Microglia are known to have a complex and often contradictory role following stroke and can be both protective and harmful[3]. In the initial hours after a stroke, microglia migrate to the damaged area, where they can paradoxically worsen injury by releasing inflammatory substances. However, microglia are also essential for clearing debris and promoting tissue repair. Unfortunately, this initial beneficial phase can transition into a long-lasting state of chronic inflammation, hindering the brain's ability to heal and recover[3]. The mechanisms underlying this shift towards chronic inflammation have been challenging to elucidate. Scientists are increasingly recognizing the importance of disrupted myelin[4], cholesterol accumulation[5] and impaired waste clearance[6] following a stroke, but how these factors specifically contribute to sustained inflammation and microglial dysfunction remains largely unclear.

In this issue of *Nature Metabolism*, Zhao and colleagues[7] shed light on this critical question, uncovering a novel metabolic pathway involving cholesterol accumulation within microglia that drives chronic neuroinflammation after ischaemic stroke. Specifically, they used a mouse model of ischaemic stroke known as the middle cerebral artery occlusion (MCAO) model, which mimics large vessel occlusion in patients, allowing investigation of reperfusion treatments. The authors performed a comprehensive analysis of brain tissue from both sexes at various time points after stroke, spanning from the acute phase (days 3 and 7) to the chronic phase (days 14, 30, 90 and 180). Magnetic resonance imaging revealed widespread brain damage, including myelin breakdown, in the striatum across all time points. Further investigation revealed a substantial accumulation of microglia filled with lipid droplets, giving them a 'foamy' appearance (Fig. 1) that is reminiscent of the foamy macrophages that drive the progression of atherosclerosis[8], where cells engulf cholesterol and transform into foam-like structures.

To understand the specific types of lipid accumulating in these microglia, the authors combined targeted lipidomic mass spectrometry analysis and bulk RNA sequencing on CD11b$^+$ flow cytometry-sorted microglia at the acute (3 days) and chronic (30 and 90 days) stages post-stroke. Their findings revealed a marked increase in cholesterol esters, a major component of lipid droplets, in the lesioned areas of the brain at all time points. Consistent with the altered cholesterol ester storage, bulk RNA sequencing showed substantial changes in

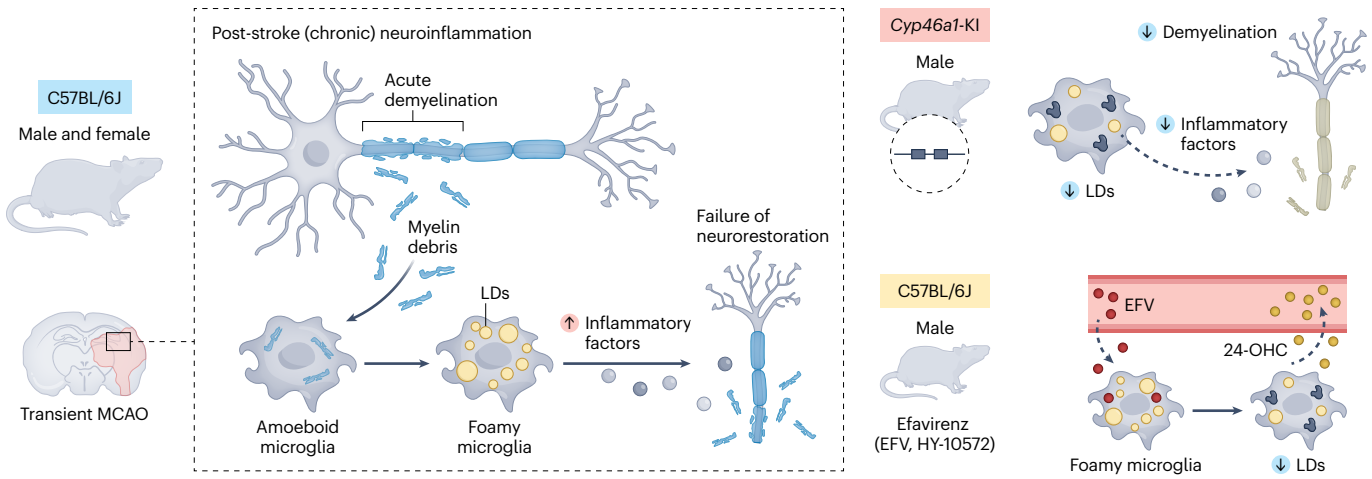

Fig. 1 | Cholesterol accumulation drives accumulation of foamy microglia in chronic stroke. The transient MCAO model of ischaemic stroke in mice results in myelin destruction and extracellular cholesterol build-up. Reactive amoeboid microglia take up and accumulate cholesterol-rich lipid droplets (LDs), forming foamy microglia that secrete pro-inflammatory factors, thereby impeding inflammation resolution (left). Genetic overexpression of *Cyp46a1* in microglia in the newly generated *Cx3cr1*$^{+/creER}$*;R26-LSL-Cyp46a1*$^{wt/mut}$ (*Cyp46a1* knock-in (KI)) mice converts cholesterol into 24-OHC, facilitating its efflux into the bloodstream, reducing inflammation and promoting recovery (top right). Pharmacological activation with efavirenz (EFV; 0.09 mg per kg per day) via oral gavage from day 7 to day 74 after MCAO in C57BL/6J mice replicates this effect, leading to increased CYP46A1 activity and 24-OHC efflux, which also mitigates inflammation and supports restoration (bottom right).

cholesterol metabolism-associated genes, such as increased expression of *Soat1* and *Nceh1*, which encode enzymes involved in cholesterol ester synthesis and hydrolysis, respectively – suggesting active regulation of cholesterol ester metabolism by microglia after stroke. In addition to cholesterol esters, free cholesterol also accumulated in microglia within the lesioned area only during the chronic stage, eventually forming cholesterol crystals[9], which are a hallmark of dysregulated cholesterol metabolism.

To gain a deeper understanding of the microglial responses, the researchers performed single-cell RNA sequencing. They identified two distinct clusters of foamy microglia characterized by elevated expression of genes involved in cholesterol metabolism (*Abca1* and *Lpl*) and phagocytosis (*Trem2* and *Apoe*). Pseudotime trajectory analysis revealed that expression of cholesterol metabolism genes preceded that of inflammatory genes (*Ccl3* and *Cxcl14*). This suggests a temporal relationship in which cholesterol uptake and accumulation may initiate the sustained pro-inflammatory phenotype of microglia. Therefore, early cholesterol-induced metabolic reprogramming seems to drive and maintain this pro-inflammatory state during the chronic stage.

Next, the authors investigated whether this cholesterol build-up was indeed sufficient to trigger a chronic pro-inflammatory phenotype. They injected cholesterol crystals or free cholesterol directly into mouse brains to simulate the extracellular cholesterol deposition observed during ischaemic stroke. The injection of cholesterol crystals or free cholesterol induced prolonged activation of microglia in demyelinated lesions at the injection sites. Bulk RNA sequencing of microglia following injection of cholesterol crystals revealed increased expression of inflammatory markers (*Il1b* and *Il1a*) and cholesterol metabolism-related genes (*Soat1* and *Nceh1*). The chemical depletion of microglia using PLX5622 resulted in a reduction of the lesion area. These findings support the hypothesis that excess cholesterol (cholesterol crystals or free cholesterol) alone in the brain parenchyma is sufficient to trigger microglial activation and lesions like those seen after stroke.

To identify a potential therapeutic target, the authors focused on cytochrome P450 family 46 subfamily A member 1 (CYP46A1), an enzyme that converts cholesterol into 24S-hydroxycholesterol (24S-OHC), thus facilitating cholesterol removal from the brain[10]. They hypothesized that enhancing CYP46A1 activity would aid in clearing excess cholesterol from microglia and reduce inflammation. Using both genetic and pharmacological approaches, they first genetically engineered mice to overexpress *Cyp46a1* specifically in *Cx3cr1*$^{+}$ microglia. Second, they treated mice with efavirenz, an FDA-approved drug known to activate CYP46A1. Both strategies led to substantial reductions in lesion volume, improvements in myelin repair, and notable recovery in motor and cognitive functions, as well as a decrease in lipid droplet accumulation within microglia. These findings present a compelling case for targeting microglial cholesterol metabolism as a therapeutic strategy – not only for post-stroke neuroinflammation (Fig. 1) but also potentially in multiple sclerosis and other neuroinflammatory disorders involving demyelination[11].

Although the evidence is promising, it is important to acknowledge limitations that warrant further study for clinical translation. First, markers such as CD11b and IBA1 that are used to identify microglia are not entirely specific, as they are also expressed by other myeloid cells such as macrophages. Future research using more refined techniques such as dual reporter mice[12] could more precisely distinguish microglia from infiltrating macrophages. Second, the microglia-depleting agent PLX5622 also affects macrophages and does not eliminate all microglia[13]. The observed compensatory lipid uptake by astrocytes highlights the complex cellular interactions in the brain and raises questions about whether the increase in microglia itself or their dysregulated cholesterol metabolism is the critical factor in pathology. Third, this study does not definitively determine whether the interventions preserve myelin or promote remyelination. Future studies tracking oligodendrocyte progenitor cells and assessing myelin regeneration[14] would be valuable. Finally, given cholesterol's vital and ubiquitous role in cellular function, developing targeted, cell-specific therapies will be important to avoid unintended effects. Further research on long-term safety and efficacy is also essential.

In conclusion, Zhao and colleagues present a compelling story of how cholesterol accumulation in microglia drives sustained inflammation after stroke. Further research into how cellular metabolism and mitochondrial function influence microglial transitional states and pathogenicity[15] is necessary, but their findings offer promising avenues for developing targeted therapies to promote brain repair and improve outcomes for stroke survivors.

Stefano Pluchino ✉ **& Cory M. Willis**
Department of Clinical Neurosciences and NIHR Biomedical Research Centre, University of Cambridge, Cambridge, UK.
✉e-mail: spp24@cam.ac.uk

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References

1. Feigin, V. L. et al. *Int. J. Stroke* **20**, 132–144 (2025).
2. Zhao, S. C. et al. *Acta Pharmacol. Sin.* **38**, 445–458 (2017).
3. Planas, A. M. *Glia* **72**, 1016–1053 (2024).
4. Cheng, Y. J. et al. *Brain* **147**, 1294–1311 (2024).
5. Wei, W. et al. *Adv. Sci.* **11**, e2306863 (2024).
6. Arbaizar-Rovirosa, M. et al. *EMBO Mol. Med.* **15**, e17175 (2023).
7. Zhao, Q. et al. *Nat. Metab.* https://doi.org/10.1038/s42255-025-01379-7 (2025).
8. Stroope, C. et al. *Nat. Metab.* **6**, 617–638 (2024).
9. Maxfield, F. R. & Tabas, I. *Nature* **438**, 612–621 (2005).
10. Russell, D. W., Halford, R. W., Ramirez, D. M. O., Shah, R. & Kotti, T. *Annu. Rev. Biochem.* **78**, 1017–1040 (2009).
11. Ionescu, R. B. et al. *Cell Stem Cell* **31**, 1574–1590.e11 (2024).
12. Jordão, M. J. C. et al. *Science* **363**, eaat7554 (2019).
13. Bosch, A. J. T. et al. *Diabetologia* **66**, 2292–2306 (2023).
14. Baaklini, C. S. et al. *Cell Rep.* **42**, 113574 (2023).
15. Peruzzotti-Jametti, L. et al. *Nature* **628**, 195–203 (2024).

Competing interests

S.P. is founder, CSO and shareholder (>5%) of CITC Ltd. C.M.W has no competing interests to declare.