NEUROSCIENCE

Pain-resolving microglia

An emergent subgroup of spinal cord microglia mediates recovery from persistent pain

By **George Sideris-Lampretsas** *and* **Marzia Malcangio**

Neuropathic pain after peripheral nerve damage is a lasting condition that generally persists even when the cause of damage disappears (*1*). The immune system is integral to the development of neuropathic pain: In the spinal cord, microglia—the central nervous system–resident macrophages—respond to neuronal activity and set up a positive feedback loop with neurons that promotes pain onset. Thus, disruption of microglia-neuron communication is being considered as a strategy to produce analgesia. On page 86 of this issue, Kohno *et al.* (*2*) describe a spinal cord–resident pool of microglia that emerges during pain maintenance and contributes to the resolution of neuropathic pain in mice. Microglial heterogeneity is a well-accepted concept that is underexplored in the context of chronic pain (*3*). The finding that spinal cord microglia acquire spatial and temporal transcriptional heterogeneity that affects pain could identify new therapeutic strategies to relieve pain.

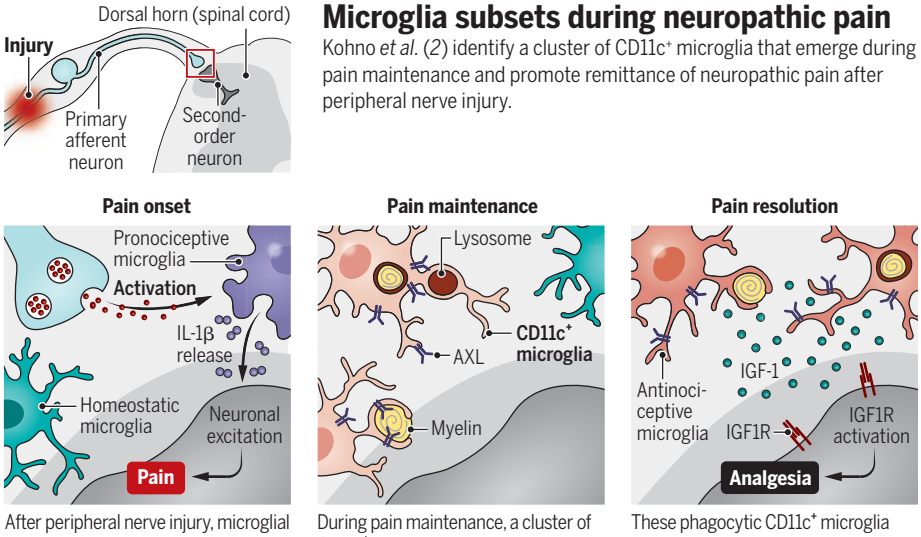
In response to peripheral nerve trauma, the nociceptive system is activated. Nociception is the response of the nervous system, including the spinal cord, to noxious stimulation and tissue damage. Painful stimuli are detected by peripheral nerve fibers and transmitted to their central terminal in the dorsal horn of the spinal cord. There, microglia promptly proliferate and up-regulate the expression of various genes that are implicated in pronociceptive pathways (*4*). Microglia play a pronociceptive role at the onset of pain, in a sex-dependent manner: The involvement of microglial signaling is reliable in male mice, whereas some pathways appear not to be effective in female mice (*5*). However, these cells are considered less relevant during the maintenance of neuropathic pain (*4*).

By contrast, the study of Kohno *et al.* shows that in the maintenance phase, an emergent CD11c$^+$ microglial pool engages in clearance of myelin. The prevention of CD11c$^+$ microglia emergence is sufficient for pain to be maintained in a mouse model of neuropathic pain, indicating that they are essential for pain remission. These microglia develop an antinociceptive function through the release of anti-inflammatory mediators, such as insulin-like growth factor 1 (IGF-1). Thus, under neuropathic conditions, microglia can serve diverse roles in a temporal manner alongside pain development and remittance. Some microglia initially respond to neuronal activation and contribute to pain onset; then, with time, this distinct pool of microglia appears, expands, and eventually establishes a new microglia-to-neuron communication that is associated with pain resolution (see the figure).

Wolfson Centre for Age-Related Diseases, King's College London, London, UK. Email: george.sideris@kcl.ac.uk; marzia.malcangio@kcl.ac.uk

Microglia subsets during neuropathic pain

Kohno *et al.* (*2*) identify a cluster of CD11c$^+$ microglia that emerge during pain maintenance and promote remittance of neuropathic pain after peripheral nerve injury.



After peripheral nerve injury, microglial activation in the dorsal horn engages pronociceptive communication with neurons by releasing cytokines [such as interleukin-1β (IL-1β)] that facilitate neuronal activity.

During pain maintenance, a cluster of CD11c$^+$ microglia emerge, express the receptor AXL, and engulf myelin debris from degenerating processes that affect injured neurons.

These phagocytic CD11c$^+$ microglia release insulin-like growth factor 1 (IGF-1), which exerts antinociceptive action through activation of neuronal IGF-1 receptor (IGF1R).

What is the local cue that triggers antinociceptive microglia emergence during neuropathic pain? In brain development and disease, CD11c$^+$ microglia appear in response to disturbance of homeostasis, and they check neuronal health by increasing phagocytic potentials (*6*). Kohno *et al.* found that at 2 weeks after nerve injury in mice, CD11c$^+$ microglia express the receptor tyrosine kinase AXL, which alongside the receptor tyrosine kinase MER detects and engulfs amyloid-β aggregates in the brains of people with Alzheimer's disease (*7*). Kohno *et al.* observe the CD11c$^+$ microglial population engulfing myelin basic protein, which suggests that they are devoted to taking up debris resulting from loss of myelin integrity in some fiber terminals. At this time, CD11c$^+$ microglia are not yet antinociceptive. Loss of myelin integrity may be the result of degenerative processes affecting injured neurons that terminate in the spinal cord. Alternatively, loss of myelin integrity may be mediated by increased neuronal excitation in response to injury; hence, microglial modulation of myelin integrity may indirectly control neural circuit functionality (*8*).

By 5 weeks after nerve injury, when pain has fully resolved in the mouse model, CD11c$^+$ microglia express IGF-1, which confers a sex-independent antinociceptive effect to microglia in these neuropathic conditions. Thus, throughout the pain-maintenance phase, CD11c$^+$ phagocytic microglia gradually become antinociceptive and eventually contribute to the resolution of nociceptive hypersensitivity through release of IGF-1. Because the receptor for IGF-1 is expressed by neurons in the dorsal horn but also by oligodendrocytes, astrocytes, endothelial cells, and microglia, there remains scope for further studies on cellular targets of CD11c$^+$ microglia-derived IGF-1.

Microglia can change function in response to regional cues through fast and effective regulation of gene expression. For example, transcriptomics changes in neurodegenera-

GRAPHIC: KELLIE HOLOSKI/*SCIENCE*

tive conditions show that subsets of microglia shift from a dynamic homeostatic profile and acquire a specific disease-associated microglia (DAM) transcriptional signature (*9*). In pain remission, CD11c$^+$ microglia show some similarities to DAMs because they differentially express genes such as apolipoprotein E (*Apoe*) and *Axl*. Nevertheless, CD11c$^+$ microglia in the neuropathic spinal cord of mice have a distinct transcriptional profile from the DAM subset. This transcriptional profile is associated with antinociceptive microglial functional output. A cluster of microglia reminiscent of the antinociceptive CD11c$^+$ microglia that express *Apoe*, *Axl*, and *Igf1* are also detected in the spinal cord of mice 5 months after peripheral nerve injury (*10*). These results support the suggestion that a subset of microglia expressing a well-preserved network of transcripts appears weeks after pain onset, with Kohno *et al.* finding that these microglia have antinociceptive properties.

One of the most valuable implications of this work is how this new mechanism that stimulates the emergence of pain-killing microglia can be translated into effective treatments. Better understanding of the role of IGF1 and APOE in antinociceptive microglia would facilitate the design of therapeutic paradigms that promote analgesia. Furthermore, definition of the context required for the emergence of antinociceptive microglia would be invaluable to determine how to induce protective microglia in chronic pain conditions. Therefore, studies on pain remission states that are not characterized by loss of myelin integrity, and hence are not associated with enhanced microglial phagocytic activity, would illustrate whether microglia can then acquire an antinociceptive phenotype. It remains to be shown whether the constant uptake of myelin might eventually cause phagocytic exhaustion and microglia dystrophy, which will affect neuronal health. Overall, the study of Kohno *et al.* highlights that it is time to reassess the role of microglia in pain as a therapeutic target for the effective treatment of chronic pain. ■

REFERENCES AND NOTES

1. M. Costigan *et al.*, *Annu. Rev. Neurosci.* **32**, 1 (2009).
2. K. Kohno *et al.*, *Science* **376**, 86 (2022).
3. G. Sideris-Lampretsas, M. Malcangio, *Brain Behav. Immun.* **96**, 279 (2021).
4. G. Chen *et al.*, *Neuron* **100**, 1292 (2018).
5. R. E. Sorge *et al.*, *Nat. Neurosci.* **18**, 1081 (2015).
6. A. Benmamar-Badel *et al.*, *Front. Immunol.* **11**, 430 (2020).
7. Y. Huang *et al.*, *Nat. Immunol.* **22**, 586 (2021).
8. E. N. Santos, R. D. Fields, *Sci. Adv.* **7**, eabk1131 (2021).
9. H. Keren-Shaul *et al.*, *Cell* **169**, 1276 (2017).
10. S. Tansley *et al.*, *Nat. Commun.* **13**, 843 (2022).

ACKNOWLEDGMENTS

We acknowledge funds from the European Union Horizon 2020 research and innovation programme "TOBeATPAIN" under Marie Skłodowska-Curie grant agreement 764860.

10.1126/science.abo5592

GENOMICS

A next-generation human genome sequence

A near-complete sequence outlines a path for a more inclusive reference

By **Deanna M. Church**

Twenty-one years ago, two initial versions of a human genome sequence were published by Celera Genomics (*1*) and the Human Genome Project (HGP) (*2*). These assemblies were incomplete and replete with errors, but despite these flaws, the value of having a human genomic reference assembly was clear. The assembly produced by the HGP went on to be "finished" (*3*) and has been continually updated over the past decade (*4*). Despite the scientific and economic value of the reference (*5*), it has many shortcomings, including that it is not actually finished. On page 44 of this issue, Nurk *et al.* (*6*) provide the most complete reference assembly for any mammal. This new human reference is poised to have its own substantial impact on genome analysis and represents an important step to assembly models that represent all humans, which will better support personalized medicine, population genome analysis, and genome editing.

The current version of the human genome reference assembly, GRCh38.p14 (GRCh38), has millions of bases represented by the letter "N," which means that the actual base residing at that location is unknown. There are also 169 sequences that cannot confidently be ordered or oriented within the assembly, typically owing to their repetitive nature, and that get carried along as a bag of sequences that are hard to analyze. Biologically important regions—such as the short arms of acrocentric chromosomes, centromeres, and several duplicated euchromatic regions—are not represented, are represented by model sequences, or are incorrectly represented. These sequences account for ~8% of the human genome. Until recently, limitations of sequencing technology, primarily that the sequencers could read no more than about 1000 bases at a time, it was impossible to correctly assemble the sequence reads of these regions. But they have important biological functions and, in some cases, these regions are associated with human disease.

Inscripta, Inc., Boulder, CO, USA.
Email: deanna.church@inscripta.com

When the idea of sequencing the human genome was initiated, it was not a universally popular idea, nor was there a clear technological blueprint of how the project would be completed (*7*). The initial focus was on mapping-based approaches to better understand the human genome and to further develop sequencing technology. There were public debates on the best way to proceed, with some pushing for an approach that required no mapping, just chopping up the whole genome and sequencing it in a "whole-genome shotgun" sequencing approach (*8*); others argued against this (*9*). The HGP opted for a more structured approach. This involved cloning genomic DNA into pieces that could be grown in bacteria (clones) and indexed in 96-well plates. Clones from these libraries were first mapped to chromosome regions, then individually sequenced. The finished clones were assembled to create the chromosome sequences found in the assembly. Celera Genomics opted for the shotgun approach, which was also used by the Telomere-to-Telomere (T2T) Consortium and is the dominant method today. The comparison of the two assemblies (*10*) led to a better understanding of both approaches and of the human genome sequence.

An important attribute of the human reference assembly is that the source DNA was derived from multiple individuals. One of the first lessons in genetics is that humans inherit one set of chromosomes from each parent. This leads to a duplication of each chromosome, except for the sex chromosomes in individuals harboring an XY configuration. Each pair of chromosomes are not exact duplicates; there can be hundreds of thousands of differences between them, including the presence or absence of large stretches of sequence on one chromosome. The sequence on an individual chromosome is called a haplotype. One reason the bacterial cloning approach was preferred by the HGP was "because each clone represents a single haplotype, problems caused by the presence of polymorphisms are eliminated" [(*9*), p. 411]. That DNA from many donors was pooled in both approaches suggests that the extent of individual variation was not fully appreciated at the time. For example, when two clones