Self-renewal of peripheral nerve resident macrophage: does it represent a unique activation status?

Maricris Bautista, Anand Krishnan*

Macrophages are immune cells of myeloid origin and are present in almost all tissues. They perform a wide variety of functions contributing to tissue development, homeostasis, pathogenesis, and repair (Wynn et al., 2013). Strikingly, macrophages residing at different tissues, and at different compartments of an individual tissue, demonstrate enormously diverse molecular characteristics (Gordon and Taylor, 2005). Querying this exceptional molecular heterogeneity challenged the long-standing theory that adult tissue macrophages derive solely from circulating monocytes. Indeed, lineage tracing and fate mapping studies using chimeric and cre-floxed animal models, followed by RNA-sequencing, convincingly demonstrated that tissue resident macrophages also consist of a pool that originates from yolk-sac progenitor cells (YPC) (Ginhoux and Guilliams, 2016; De Schepper et al., 2018). For example, initial macrophages arise from primitive progenitor cells in the yolk-sac at embryonic day (E) 8.5. These macrophages migrate into developing tissues to generate long-term resident tissue macrophages. At E10.5, erythromyeloid progenitor cells from the yolk-sac enter the fetal liver giving rise to hematopoietic stem cells (HSCs), which will eventually generate fetal liver monocytes. The fetal liver monocytes then migrate into developing peripheral tissues to establish another pool of long-term resident tissue macrophages. In addition, the erythromyeloid progenitor cells seed bone marrow, and the resulting bone marrow HSCs then maintain the uninterrupted supply of circulating monocyte-derived macrophages in adult tissues (Ginhoux and Guilliams, 2016; Goldmann et al., 2016). Overall, adult tissues encompass a mix of YPC-derived and circulating monocyte-derived macrophages and they are molecularly distinct. Some examples of YPC derived macrophages include microglia, alveolar macrophages, Langerhans cells, Kupffer cells, peritoneal macrophages, cardiac macrophages, and a subset of macrophages in the peripheral nervous system (PNS) (Wynn et al., 2013; Wang et al., 2020; Ydens et al., 2020).

Macrophages are an integral part of the PNS. The PNS resident macrophages distribute themselves to the epineurium and endoneurium of the nerve (Ydens et al., 2020). Their functions include supporting nerve homeostasis, phagocytosis of axonal and myelin debris during Wallerian degeneration, and generation of vascular endothelial growth factor for nerve repair-related angiogenesis. They also secrete several cytokines, including CCL-2, to attract more macrophages to the nerve repair site. Furthermore, they contribute to neuropathic pain and autoimmune demyelinating diseases such as Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy (Kiefer et al., 2001). Overall, macrophages elicit a mix of beneficial and harmful functions. Do these mixed functions originate from a single population of macrophages? Or are there separate identities to the heroes and villains?

PNS macrophages can be functionally classified into the pro-inflammatory M1 and anti-inflammatory M2 types. However, considering their ontogeny, they can be classified into YPC-derived and circulating monocyte-derived macrophages (Wang et al., 2020; Ydens et al., 2020). Assigning a unique heroic or villainous identity to these different classes of macrophages, especially for therapeutic purposes, is hindered by several reasons. For example, M1 and M2 are transient states of macrophages. Therapeutic wiring of macrophages based on their transient states may be challenging if the targeted population's major functions are distinct from the one associated with their transient state. Next, although recent studies sequenced the genetic profile of YPC-derived long-term resident and circulating monocyte-derived short-term resident macrophages (Wang et al., 2020; Ydens et al., 2020), no reliable markers are established to easily distinguish these two populations, necessitating the requirement of sophisticated models for functional studies. Interestingly, lineage tracing and sequencing studies revealed additional hierarchical and molecular complexities even within the long-term resident PNS macrophages. For instance, independent studies demonstrated that long-term resident PNS macrophages originate from either early wave (primitive progenitors) or late wave (fetal liver HSC) YPCs, indicating their multiple ontogenies (Wang et al., 2020; Ydens et al., 2020). Likewise, Ydens et al. (2020) demonstrated that macrophages residing at the epineurium and endoneurium of the sciatic nerve (SN) are different and have distinct molecular features. Wang et al. (2020) showed that resting SN and dorsal root ganglia (DRG) resident macrophages have diverse molecular profiles. The major conclusion emerging from these studies is that, in addition to their ontogeny, tissue location may also dictate the molecular architecture of PNS macrophages.

The next question is about the functional orientation of the long-term resident PNS macrophages. Well, no studies to date explored functional diversity of the long-term resident PNS macrophages at the steady state. It is noteworthy that 90% of the macrophages in injured nerves are comprised of recruited macrophages and they play a major role in tissue clearing and vascular endothelial growth factor production during Wallerian degeneration and nerve regeneration, respectively (Ydens et al., 2020). Nonetheless, long-term resident SN macrophages were also shown to perform phagocytotic activities (Mueller et al., 2001). Similarly, Wang et al. (2020) demonstrated that YPC-derived long-term resident SN macrophages at the steady-state carry a molecular signature that supports angiogenesis, suggesting that they may also hold a fair share in contributing to nerve regeneration if required. Interestingly, endoneurial macrophages in the SN demonstrate a differential repair response compared to epineurial macrophages, indicating site-specific functional orientation. For example, endoneurial macrophages participate in nerve regeneration by actively releasing chemoattractive cytokines and recruiting circulating monocyte-macrophages (Ydens et al., 2020). Therefore, it may be hard to predict functional orientation of macrophages from their ontogeny. While macrophage functions could be predicted from their molecular signature, such molecular correlations may be challenging for routine predictions because of their exceptional tissue site- and context-dependent diversity. It is likely that both long-term resident and infiltrating macrophages perform closely related functions, if not homeostatic tasks. In such a case, what could be a marker to distinguish the good and the bad cells within this mixed population? Do their ability to self-renew serve as a marker of functional orientation? Do their self-renewal indicate a unique activation status?

Although initial studies considered macrophages as mature and terminally differentiated cells, recent studies demonstrated that they self-renew (De Schepper et al., 2018). For example, in the PNS tissues, SN resident macrophages self-renew in response to nerve injury (Mueller et al., 2001). Similarly, we found that resident macrophages in the normal and injured DRGs self-renew (Krishnan et al., 2018). Considering self-renewal as a mechanism for long-term maintenance, it has been widely believed that self-renewal capacity is limited solely to the long-term resident tissue macrophages. For example, the fetal liver HSC-derived macrophages that constitute a pool of long-term resident SN macrophages are capable to self-renew. Ontogenically, the fetal liver HSC-macrophage generation involves monocyte intermediates, and if they could self-renew, the circulating monocyte-macrophage may also have self-renewal capacity. This raises a question of whether infiltrating monocyte-macrophages comprise of two sub-populations: one with self-renewal capacity and the other without it? It is also possible that infiltrating macrophages at certain context acquire self-renewal capacity in response to specific factors present in the PNS milieu. We found that macrophages rely on colony stimulating factor receptor 1 signaling for local proliferation in the DRGs (Krishnan et al., 2018). However, our study did not address if the local proliferation emanates from long-term resident or infiltrating monocyte-macrophages. Additional studies are required to understand whether circulating monocyte-macrophages are also as potent as YPC-derived macrophages in their proliferative potential.

Are the self-renewing macrophages functionally different from their counterparts that are naïve to self-duplicate? No studies to date explored the functional dichotomy of macrophages focusing specifically on self-renewing vs non-self-renewing populations. A general consensus has been arrived on assigning tissue homeostatic functions to the long-term resident tissue macrophages (Ginhoux and Guilliams, 2016). Hence, one functional attribute to the self-renewing population could be tissue homeostasis. In the gut, depletion of self-renewing macrophages was shown to reduce the number of enteric neurons, highlighting their roles in nerve homeostasis

(De Schepper et al., 2018). Mueller et al. (2001) demonstrated that self-renewing resident macrophages rapidly respond to SN injury by altering their morphology and clearing myelin debris. Temporally, this response occurred as early as 2 days post injury, even before circulating monocyte-macrophages arrive at the site, indicating that self-renewing macrophages can engage in phagocytic activities. Mueller et al. (2001, 2003) also demonstrated expression of MHC class II antigens in self-renewing macrophages, supporting the idea that this population also performs immunoregulatory functions. It is, however, noted that the circulating monocyte-macrophages are more efficient in performing phagocytotic functions compared to their long-term resident counterparts (Mueller et al., 2001). Ultimately, the burning question is, do the circulating monocyte-macrophages also acquire self-renewal capacity? More studies are required to find an answer to this question.

If both long-term resident and a sub-population of infiltrating macrophages show self-renewal, then the currently known functions of PNS macrophages may require a revisit with specific focus on self-renewing *vs*. non-self-renewing populations. However, a major challenge will be to identify unique markers to segregate them into two different bins. Timed expression of induced fluorescence proteins (eGFP or eYFP) in Cx3cr1^{+} macrophages is an option, wherein several months after the timed expression, the fluorescent protein labeled Cx3cr1^{+} non-self-renewing macrophages will be depleted from the system due to their natural turnover, while the Cx3cr1^{+} self-renewing population, due to their ability to retain the fluorescent protein during self-renewal, will be distinguishable. Such models have been used to delineate macrophage ontogeny (Goldmann et al., 2016; De Schepper et al., 2018). While the fluorescence model mentioned above may be time-consuming, several other approaches may also be considered to distinguish the two populations. We demonstrated that PNS resident macrophage self-renewal is dependent on CSF1R (Krishnan et al., 2018), and hence the difference in CSF1R expression (high/low) in the self-renewing *vs* non-self-renewing macrophages may help to segregate them. Alternatively, microglia, the central nervous system correlates of PNS macrophages, use interleukin 34 signals for self-renewal, and hence, the dependency of PNS macrophages to interleukin 34 may be worth examining to specifically pull out the self-renewing population. Furthermore, the expression of the cell cycle marker Ki67 and incorporation of exogenously administered nucleoside analog BrdU may mark the self-renewing population. However, Ki67 is a transient marker and may not label a temporary quiescent state of mitotically competent macrophages. On the other hand, systemic administration protocols may insert BrdU into dividing HSCs in the bone marrow too, and therefore, the non-self-renewing macrophage generated from the BrdU-inserted HSCs-monocyte may also retain BrdU and may interfere with the identification of self-renewing population. Markers that differentially expressed in mitotically competent cells along with macrophage-specific markers may help.

Long-term PNS resident macrophages self-renew, albeit at a slow turnover rate. After nerve injury, they enter a state of more frequent proliferation, perhaps for constituting a local army for nerve repair (**Figure 1**).

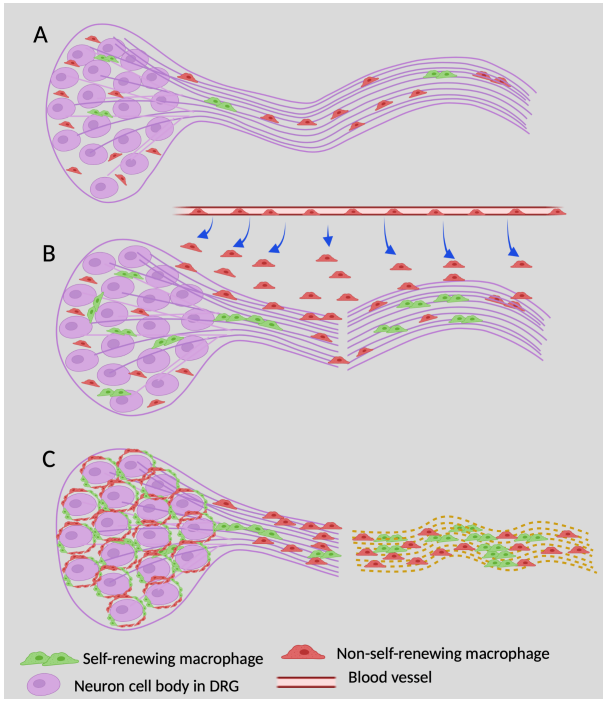

Figure 1 | Macrophage dynamics in the peripheral nerve.
(A) Macrophages, both competent (green) and incompetent (red) for self-renewal, are present in peripheral nerve and DRG. (B) Nerve injury induces influx of circulating monocyte-macrophages into nerve and DRG. It also triggers more frequent self-renewal of macrophages. However, whether both long-term resident and infiltrating population self-renew is not known. (C) Three to four days after nerve injury, both self-renewing and non-self-renewing macrophages increase in abundance in the nerve and DRG. In the DRG, this mixed population of macrophages orient around neuronal cell bodies as a protective sheath. DRG: Dorsal root ganglia.

The occurrence of these proliferative events within the mixed beds of long-term resident and infiltrating macrophages makes it difficult to identify if self-renewal capacity is limited to one population. Considering self-renewing macrophages as a separate group and revisiting their functional roles in peripheral nerves may clarify the complex biology of PNS macrophages.

This work was supported by the Natural Sciences and Engineering Research Council (NSERC), Canada (Discovery Grant No. RGPIN-2020-05862, to AK).

Maricris Bautista, Anand Krishnan*
Department of Anatomy, Physiology, and Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada (Bautista M, Krishnan A)
Cameco MS Neuroscience Research Centre (CMSNRC), University of Saskatchewan, Saskatoon, SK, Canada (Bautista M, Krishnan A)
***Correspondence to:** Anand Krishnan, PhD, anand.krishnan@usask.ca.
https://orcid.org/0000-0002-0064-942X (Anand Krishnan)

Date of submission: May 13, 2021
Date of decision: July 2, 2021
Date of acceptance: July 27, 2021
Date of web publication: September 17, 2021

https://doi.org/10.4103/1673-5374.324845
How to cite this article: *Bautista M, Krishnan A (2022) Self-renewal of peripheral nerve resident macrophage: does it represent a unique activation status? Neural Regen Res 17(5):999-1000.*
Copyright license agreement: *The Copyright License Agreement has been signed by both authors before publication.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

References

De Schepper S, Verheijden S, Aguilera-Lizarraga J, Viola MF, Boesmans W, Stakenborg N, Voytyuk I, Schmidt I, Boeckx B, Dierckx de Casterlé I, Baekelandt V, Gonzalez Dominguez E, Mack M, Depoortere I, De Strooper B, Sprangers B, Himmelreich U, Soenen S, Guilliams M, Vanden Berghe P, et al. (2018) Self-maintaining gut macrophages are essential for intestinal homeostasis. Cell 175:400-415.e13.
Ginhoux F, Guilliams M (2016) Tissue-resident macrophage ontogeny and homeostasis. Immunity 44:439-449.
Goldmann T, Wieghofer P, Jordão MJ, Prutek F, Hagemeyer N, Frenzel K, Amann L, Staszewski O, Kierdorf K, Krueger M, Locatelli G, Hochgerner H, Zeiser R, Epelman S, Geissmann F, Priller J, Rossi FM, Bechmann I, Kerschensteiner M, Linnarsson S, et al. (2016) Origin, fate and dynamics of macrophages at central nervous system interfaces. Nat Immunol 17:797-805.
Gordon S, Taylor PR (2005) Monocyte and macrophage heterogeneity. Nat Rev Immunol 5:953-964.
Kiefer R, Kieseier BC, Stoll G, Hartung HP (2001) The role of macrophages in immune-mediated damage to the peripheral nervous system. Prog Neurobiol 64:109-127.
Krishnan A, Bhavanam S, Zochodne D (2018) An intimate role for adult dorsal root ganglia resident cycling cells in the generation of local macrophages and satellite glial cells. J Neuropathol Exp Neurol 77:929-941.
Mueller M, Wacker K, Ringelstein EB, Hickey WF, Imai Y, Kiefer R (2001) Rapid response of identified resident endoneurial macrophages to nerve injury. Am J Pathol 159:2187-2197.
Mueller M, Leonhard C, Wacker K, Ringelstein EB, Okabe M, Hickey WF, Kiefer R (2003) Macrophage response to peripheral nerve injury: the quantitative contribution of resident and hematogenous macrophages. Lab Invest 83:175-185.
Wang PL, Yim AKY, Kim KW, Avey D, Czepielewski RS, Colonna M, Milbrandt J, Randolph GJ (2020) Peripheral nerve resident macrophages share tissue-specific programming and features of activated microglia. Nat Commun 11:2552.
Wynn TA, Chawla A, Pollard JW (2013) Macrophage biology in development, homeostasis and disease. Nature 496:445-455.
Ydens E, Amann L, Asselbergh B, Scott CL, Martens L, Sichien D, Mossad O, Blank T, De Prijck S, Low D, Masuda T, Saeys Y, Timmerman V, Stumm R, Ginhoux F, Prinz M, Janssens S, Guilliams M (2020) Profiling peripheral nerve macrophages reveals two macrophage subsets with distinct localization, transcriptome and response to injury. Nat Neurosci 23:676-689.

C-Editors: Zhao M, Zhao LJ, Wang L; T-Editor: Jia Y