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

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, erythro-myeloid 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 erythro-myeloid 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, Kuffer 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 perineurium 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-Barre 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 in 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 phagocytic 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 the 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? Or is the 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 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 another 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 naive to self-duplicate? No studies to date explored the functional dichotomy of macrophages focusing specifically on self-renewing vs. non self-renewing populations. Therefore, 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 their homeostatic function. The general function of self-renewing macrophages was shown to reduce the number of enteric neurons, highlighting their roles in nerve homeostasis.
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.

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Mueller M, Leonhard C, Wacker K, Ringstetsh EB, Okabe M, Hickey WF, Kiefer R (2001) Rapid response of identified resident endoneurial macrophages to nerve injury: 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, whereas 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 subpopulations (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.

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