Origin and Differentiation of Nerve-Associated Macrophages

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*J Immunol* 2020; 204:271-279; doi: 10.4049/jimmunol.1901077
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The mature peripheral nervous system is a steady network structure yet shows remarkable regenerative properties. The interaction of peripheral nerves with myeloid cells has largely been investigated in the context of damage, following trauma or infection. Recently, specific macrophages dedicated to homeostatic peripheral nerves have come into focus. These macrophages are defined by tissue and nerve type, are seeded in part prenatally, and self-maintain via proliferation. Thus, they are markedly distinct from monocyte-derived macrophages invading after local disturbance of nerve integrity. The phenotypic and transcriptional adaptation of macrophages to the discrete nervous niche may exert axon guidance and nerve regeneration and thus contribute to the stability of the peripheral nervous network. Deciphering these conserved macrophage–nerve interactions offers new translational perspectives for chronic diseases of the peripheral nervous system, such as diabetic neuropathy and pain.

**Origin and Differentiation of Nerve-Associated Macrophages**

Julia Kolter,* Katrin Kierdorf,†‡§ and Philipp Henneke*,†

The Journal of Immunology, 2020, 204: 271–279.

In the last decade, several breakthrough discoveries have transformed our understanding of tissue macrophages. In contrast to the historical view that macrophages are generally seeded by circulating myeloid progenitors or hematopoietic stem cells (HSCs), novel experimental approaches have revealed heterogeneity in origin and renewal and indicated specialization of macrophage subsets within one tissue (1–4). This diversity offers solutions for the diverse tasks associated with the complex three-dimensional tissue organization. As an example, the skin extends from the microbially colonized epidermis to the subcutis and-dimensional tissue organization. As an example, the skin extends from the microbially colonized epidermis to the subcutis and-self-maintain via proliferation. Thus, they are markedly distinct from monocyte-derived macrophages invading after local disturbance of nerve integrity. The phenotypic and transcriptional adaptation of macrophages to the discrete nervous niche may exert axon guidance and nerve regeneration and thus contribute to the stability of the peripheral nervous network. Deciphering these conserved macrophage–nerve interactions offers new translational perspectives for chronic diseases of the peripheral nervous system, such as diabetic neuropathy and pain. *The Journal of Immunology, 2020, 204: 271–279.*

### Description of NAMs in different tissues

**Sciatic nerve.** In 1977, Arvidson (7) detected resident epi- and endoneurial cells with macrophage features in the murine sciatic nerve after injection of HRP. Subsequent studies described them to be elongated and orientated along the longitudinal nerve axis and to constitute 2–9% of all endoneurial cells (5, 6, 9, 10). They are typically located near blood vessels or interstitially in the endoneurial space (6, 9, 11, 12). Substantial progress in the characterization of these cells was achieved by the group of Reinhard Kiefer in the early 2000s, for example, by addressing their exchange in mouse and rat bone marrow chimera (12–17). In Wallerian degeneration after nerve injury, resident macrophages showed morphological changes, phagocytosis of myelin, and proliferation, yet the differences to recruited (i.e., monocyte-derived) macrophages were found to be rather subtle (12–14).

**Intestine.** The gastrointestinal tract is innervated by the intrinsic enteric nervous system (ENS) as well as extrinsic sympathetic and parasympathetic ganglia (18). Macrophages are abundant throughout the resting lamina propria and between the circular and longitudinal muscle layers of the muscularis externa.

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Received for publication September 3, 2019. Accepted for publication October 27, 2019.

This work was supported by grants from the German Research Foundation (HE3127/9-1, HE3127/12-1, and SFB/TRR167) and the German Ministry of Education (Grants 01EO0803, 01GL1746A, and 01EK1602A).

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Steady-state muscularis macrophages (MMs) exhibit a tissue-protective phenotype, based on the expression of markers such as Retnla, Cd163, Mrc1, and Il-10 (22), and they reside in close proximity to myenteric plexus ganglia (23, 24). In 2014, an anatomical interaction of MMs with enteric neurons was uncovered. Macrophage-derived BMP2 acts on BMPR+ enteric neurons, which then change the smooth muscle contraction pattern and thereby regulate intestinal motility (25). Enteric neurons in turn produce CSF1, indicating a reciprocal interaction between the nerves and their associated CSF1R-dependent macrophages. In addition, MMs express β2-adrenergic receptors, which can interact with tyrosine hydroxylase (TH)+ neurons of sympathetic ganglia innervating the intestine (22). Norepinephrine (NE) signaling was suggested to decrease the proinflammatory LPS response and enhance the tissue-protective phenotype of MMs upon luminal bacterial infection (22, 26). MMs might also prevent neuronal damage in Salmonella spp. infection via a protective program that involves β2-adrenergic signaling and arginase 1 (F. Matheis, P. A. Muller, C. L. Graves, I. Gabanyi, Z. J. Kerner, D. Costa-Borges, and D. Mucida, manuscript posted on bioRxiv).

It was furthermore established that MMs comprise four subpopulations, which differ in their anatomical localization. Serosal MMs are not in contact with neurons, stellate myenteric plexus MMs are mostly associated with neuronal cell bodies, and the bipolar MMs of the circular muscles and of the deep muscular plexus run in parallel with nerve fibers (22). However, the heterogeneity of MMs cannot yet be resolved by immunophenotypic markers, which currently precludes specific experimental analysis. Additionally, macrophages were recently described to reside in proximity to submucosal neurons of the lamina propria (27). The subset-specific transcriptomes and functions remain to be resolved.

Adipose tissue. NAMs have been described in adipose tissues of mice and humans. In the mouse, macrophages expressing the CX3C chemokine receptor 1 (CX3CR1 or fractalkine receptor) have been identified in close proximity to sympathetic nerve fibers in brown adipose tissue (BAT) (28). However, assigning distinct properties to NAMs in BAT is hampered by the fact that interstitial macrophages are also partly CX3CR1+. In the white adipose tissue, sympathetic NAMs have been described as well (29, 30). Earlier studies suggested that macrophages are a source of NE, the main neurotransmitter of the sympathetic nervous system, in response to cold stimulation (31). However, this hypothesis has been recently challenged because adipose tissue macrophages do not express TH, a key enzyme of the catecholamine synthesis (29, 32, 33). Instead, sympathetic NAMs express SLC6A2, a NE transporter, and monoamine deoxidase A, a degrading enzyme, and were shown to import and metabolize NE and thereby contribute to its clearance (29, 30).

Skin. The majority of nerve fibers in the skin are myelinated and nonmyelinated sensory nerves, which transmit signals from environmental stimuli as well as pain and itch to the dorsal root ganglia and the CNS. These fibers mostly signal via neuropeptides, including substance P and calcitonin gene-related peptide (34–36). Autonomic TH+ sympathetic nerves, which innervate blood and lymphatic vessels as well as glands, hair follicles, and muscles, constitute a minority of the...
We recently identified cutaneous nerve fibers (34, 35). In contrast to other tissues, they were separable from their stromal counterparts by strong expression of the fractalkine receptor because other dermal macrophages are CX3CR1low-int in adult mice. These sensory NAMs (sNAMs) display a strikingly elongated morphology with a length of up to 200 µm (Fig. 2). Their protrusions are in direct contact with nerve axons. sNAMs are abundant in thick nerves, where they intertwine with axon bundles, and on nerve junctions, but also occupy single fibers. Because they constitute only 2–3% of the total dermal macrophage population (37), they may evade identification, for instance by unbiased single cell sequencing without prior enrichment by flow cytometry.

Others. In addition to nerve axons, macrophages are also found in close approximation with neurons in the sensory ganglia, predominantly outside of the basal lamina of satellite cells (6) as well as the paravertebral ganglia of the sympathetic nervous system (29) and scattered throughout the dorsal root ganglia (39–42). In the cornea, which is highly innervated by sensory nerves, a distinct association of CX3CR1+ macrophages with peripheral nerve trunks was identified (43). CX3CR1+ macrophages have furthermore been found around bronchiole-associated nerve bundles of the lung and sympathetic nerves of the epicardial surface of the heart (38), indicating that NAMs exist across ontogenetically distinct organs.

Ontogeny of PNS macrophages

During development, embryonic progenitors from the yolk sac and the fetal liver give rise to tissue-resident macrophages. They are postnatally maintained by self-renewal in some tissues (e.g., the brain and the liver) (44–48), yet are replaced in other tissues by circulating myeloid progenitors to a varying degree (20, 49, 50). Early studies employing the transplantation of bone marrow from fluorescent reporter mice in full body irradiated mice suggested that up to 75% of macrophages associated with sciatic nerves and dorsal root ganglia are replaced by bone marrow progenitors within a few months (11, 12, 14, 15). However, because tissues were not shielded during lethal irradiation, macrophage recruitment and proliferation may have been influenced by radiation-induced tissue and immune cell damage in these studies, similar to previous experience in CNS-associated macrophages (CAMs) and microglia (51, 52). In the skin, sNAMs are prenatally seeded and do not depend on adult hematopoiesis for their maintenance (37). They exhibited very low exchange rates in bone marrow chimera with tissue shielding during irradiation and an average lifespan of more than 6 mo. Even after depletion of the population by a CSF1R inhibitor, most sNAMs were reconstituted by endogenous proliferation rather than by monocyte influx (37). Nevertheless, bone marrow–derived myeloid progenitors are generally capable of colonizing nerves in low numbers and give rise to NAMs when axons regrow after experimental nerve injury (27, 37).

In the intestine, macrophages are generally thought to be prenatally seeded and diluted out by a strong influx of monocytes around the time of weaning (20, 53, 54). However, MMts and lamina propria macrophages were not discriminated in these studies. During murine development, MMts colonize the fetal bowel at E12.5 before enteric neurons differentiate in the colon (55), and a recent study indicates that long-lived embryonic macrophages persist in the submucosal...
region of the lamina propria and adjacent to myenteric neurons of the muscularis externa (27). In the submucosa, embryonic macrophages colocalize to certain microanatomical structures, such as enteric neurons and nerve fibers of the submucosal plexus, blood vessels, Paneth cells, and Peyers patches (27). Notably, macrophages adjacent to submucosal and myenteric neurons are not replaced by bone marrow--derived monocytes under homeostatic conditions (27). Accordingly, embryonic descendence with low replacement by circulating myeloid progenitors and long-term maintenance under homeostasis might be common features of NAMs across different tissues and nerves.

Inheritance and local adaptation of NAMs

Two models on the commitment of macrophages to the PNS are conceivable. First, NAMs may represent macrophages of the respective tissue that have functionally and spatially adapted to the nervous niche. Second, NAMs may be part of a distinct lineage of PNS macrophages, similar to those in the CNS. In other words, one may ask whether NAMs in the skin are specialized dermal macrophages or rather “tissue-independent” PNS macrophages.

Immunofluorescence images of NAMs in different tissues reveal a remarkable similarity in terms of their bipolar elongated morphology with few ramifications, which run in parallel to the nerve axons (Fig. 2). Similar to CNS macrophages, all populations described so far highly express the fractalkine receptor CX3CR1 (25, 27–29, 37, 51) (Table I). Fractalkine is implicated in neuronal cross-talk (56, 57) and transmits survival cues to macrophages and patrolling monocytes (58, 59).

Interestingly, MMs in the mouse intestine, which colocalize with CSF1-producing enteric neurons (see above), do not require the ENS to colonize the muscularis externa. They develop normally with respect to numbers and phenotype in inherited ENS absence (i.e., in Hirschsprung disease) (55). This suggests that resident MMs occupy nerves in the tissue, once this niche is opening. Similarly, we observed that, after mechanical skin injury, resprouting nerve axons were populated by macrophages with a sNAM-like phenotype (37). Fate-mapping analysis revealed that these macrophages derived neither from the original nerve-resident population nor from circulating monocytes. Instead, interstitial macrophages appear to be capable of adapting to the unoccupied nerve axons by upregulation of CX3CR1 and other sNAM markers and adopting the morphology and scanning behavior. Thus, the specification of NAMs likely depends on cues derived from the PNS.

Tissue nerves in turn vary in terms of surface molecule expression, the usage of neurotransmitters, the degree of myelination by glial Schwann cells, and their general anatomical buildup (e.g., single fibers versus large nerve bundles). It thus seems likely that the nerve type, which is colonized, impacts the phenotype of NAMs. For instance, the NE myelination machinery of sympathetic NAMs and the adrenergic receptors of enteric macrophages are not expressed by macrophages on sensory nerves in the skin (Table I). Therefore, the protein expression profile of neurons likely provides a specific receptor–ligand interface for macrophages, which shapes their phenotype. This reciprocal interaction in combination with the spatially restricted environment may facilitate the low turnover and long-term maintenance of PNS macrophages, as observed in most investigated tissues (27, 28, 37). Finally, the complexity of large peripheral nerves with peri- and epineurial sheaths enclosing axon bundles and supplying blood vessels will likely yield heterogeneous macrophage populations related to these distinct compartments. This is analogous to the CNS, in which parenchymal microglia coexist with perivascular, choroid plexus, and meningeal macrophages (51).

Functions in homeostasis

Macrophages have been characterized as a local surveillance system for nerves, which, among other functions, clear extracellular particles from the endoneurial space of peripheral nerves (6). However, the lack of specific depletion strategies for these macrophages has been an obstacle for more specific functional insights.

Intravital two-photon microscopy showed that sNAMs scan sensory nerve axons in the skin by extension and retraction of protrusions (37), resembling the movements of microglia (60). Strikingly, sNAMs “slid” along axons and interacted with neighboring macrophages. The functional purpose of macrophage movements along sensory nerves has not been fully resolved, yet they likely contribute to surveillance and thereby the integrity of the nerve fibers. In the intestine, depletion of local macrophages in the submucosal and myenteric plexus over 7 d resulted in a reduced density of enteric neurons (27), suggesting that macrophages indeed provide essential cues for nerve homeostasis. Accordingly, neuron-induced ion secretion and gastrointestinal motility were affected in the absence of macrophages (25, 27). In BAT, genetic deficiency of the nuclear transcription regulator Mecp2 in macrophages impaired thermogenesis, which was attributed to reduced sympathetic innervation (28). Mecp2-deficient macrophages produced elevated amounts of PlexinA4, a receptor that interacts with semaphorins to direct axon guidance (61–63). This finding is analogous to sNAMs in the dermis, which highly express PlexinA4 as well (37). Hence, macrophages might contribute to axon growth and guidance of peripheral nerves by the provision of soluble factors or other molecular cues. As indicated above, sympathetic NAMs also play a role in NE catabolism in adipose tissue (29, 30). This clearance mechanism may prevent spillover of NE from sympathetic neurons to neighboring tissues and thereby limit catecholamine--induced lipolysis of adipocytes (29, 30, 64), hence contributing to obesity. Notably, macrophages show increased NE degradation in age-related chronic inflammation, which is associated with reduced lipolysis in ageing mice (30). The effect depends on inflammasome activation via NLRP3. Thus, resident macrophages relay inflammatory signals and act as mediators between the immune and the respective nervous system. This intercellular signaling network most likely impacts the inflammatory and metabolic state of the surrounding tissue. The concept integrates well into the growing body of knowledge on neuroimmune crosstalk. Underlying mechanisms and molecular pathways were recently reviewed in detail elsewhere (65–68).

Nerve damage and repair

In contrast to the CNS, neurons of the PNS are capable of functional regeneration, even in adulthood (69, 70). Damage of peripheral nerves results in infiltration of macrophages in
virtually every type of disease that affects axon or myelin integrity of the PNS (6, 9, 71). Because a priori resident and newly recruited macrophages cannot yet be discriminated by immunological markers, most studies on nerve repair have evaluated the global role of macrophages (both resident and infiltrating) in this process. In general, it is accepted that macrophages participate in the repair response after nerve injury, especially in the process of Wallerian degeneration of the distal nerve segment after axotomy, and in nerve regeneration.

The underlying processes have been comprehensively summarized elsewhere (72–75). However, only a few studies have analyzed the specific contribution of macrophages that were already resident on nerves before damage. It was described that endoneurial macrophages proliferate and phagocytose myelin in a sciatic nerve crush model at early stages, before other macrophages are recruited into the lesioned nerve ∼3–4 d after injury (12, 15). In skin injury, CX3CR1-based lineage tracing showed that resident sNAMs accumulate and proliferate in nerve endings at the wound margin (37). These macrophages contain myelin-filled vacuoles and do not receive input by recruited bone marrow–derived progenitors. The myelin sheath at the respective nerve endings was trimmed at the site of injury, enabling axon sprouting of lesioned nerves after injury (37). Finally, the lesioning of skin sensory nerves by localized laser injury led to immediate directed movement of nearby NAMs (37). Macrophages shielded the lesion site with multiple protrusions in response to the injury. A similar behavior has been observed in microglia, in which it is considered to serve neuroprotective purposes (60, 76).

Interestingly, a comparable “cloaking” mechanism by perineurial macrophages was suggested to prevent inflammatory factors, guidance of neurotrophic factors, regulation of lipolysis and thermogenesis (43), and maintenance of enteric neurons; regulation of intestinal peristalsis via BMP2 secretion (37) (37) (78–80, 82).

Comparison to CNS macrophages

Macrophages of the CNS have been described almost a century ago, when Pio del Rio-Hortega identified microglia in the CNS parenchyma. Whereas microglia are the only resident macrophages in the parenchyma, various CAM populations reside in the CNS interfaces, including the meninges, the perivascular space along the vessels, and the choroid plexus in the ventricles. The function and ontogeny of microglia and CAMs have been extensively studied and are reviewed elsewhere (78–80). Interestingly, NAMs share some aspects with their CNS-resident relatives, even though they represent highly adapted macrophage

Table I. Comparison of NAMs in different tissues and microglia

<table>
<thead>
<tr>
<th>Localization</th>
<th>Sciatic Nerve</th>
<th>Adipose Tissue</th>
<th>Intestine</th>
<th>Skin</th>
<th>Microglia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve type</td>
<td>Perivascular and endoneurial space</td>
<td>Brown and white adipose tissue</td>
<td>Muscularis externa</td>
<td>Sensory nerves</td>
<td>In contact with CNS neurons and glia in all anatomical brain regions</td>
</tr>
<tr>
<td>Morphology</td>
<td>Slim, elongated; frequently ramified</td>
<td>Profuse, extending pseudopodia</td>
<td>Bipolar cell bodies in parallel to nerve fibers; stellate in ganglia</td>
<td>Bipolar, elongated (up to 200 µm)</td>
<td>Small cell soma; highly ramified</td>
</tr>
<tr>
<td>CSF1R dependency</td>
<td>?</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>?</td>
<td>?</td>
<td>&gt;80% (adult)</td>
<td>100% (lineage-defining marker)</td>
<td>~20–30% (in steady-state)</td>
</tr>
<tr>
<td>MHC class II</td>
<td>~60%</td>
<td></td>
<td></td>
<td></td>
<td>~10–30%</td>
</tr>
<tr>
<td>CD206 (mannose receptor)</td>
<td>?</td>
<td>?</td>
<td></td>
<td></td>
<td>Iba1, CD11b, MerTK, CD45, CD64, F4/80, CD14</td>
</tr>
<tr>
<td>Markers for identification</td>
<td>CD68, Iba-1</td>
<td>CD11b, MerTK, CD45, CD64, F4/80, CD11c</td>
<td>CD11b, MerTK, CD45, CD64, F4/80, CD11c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene signature</td>
<td>Slc6a2a, Mann</td>
<td>Adhr2, Bmp2Fcr5, Ape1, Cx3, Clqa, Hscb</td>
<td>Plexn4, Tgfbr1, Axl, B2m4, Cd49</td>
<td>Tmem119, Hesch, P2ry12, Fcrl, Tgfbr1, Olfn13, Siglech, Sparc, Gpr34, Sall1</td>
<td></td>
</tr>
<tr>
<td>Ontogeny</td>
<td>Unknown, large input by HSCs</td>
<td>Embryonic; independent of HSCs</td>
<td>Embryonic; fetal liver; independent of HSCs</td>
<td>Long lived; self-maintaining</td>
<td>Embryonic; yolk sac; independent of HSCs</td>
</tr>
<tr>
<td>Lifespan</td>
<td>75% exchange within 36 wk</td>
<td>BAT; extended half-life (&gt;8 m)</td>
<td>Long lived (&gt;35 wk); self-maintaining</td>
<td>Nerve surveillance; axon resprouting after nerve injury</td>
<td>Long lifespan; self-maintaining with clonal expansion</td>
</tr>
<tr>
<td>Function</td>
<td>Involved in Wallerian degeneration after nerve injury</td>
<td>Clearance of NE; regulation of lipolysis and thermogenesis</td>
<td>Maintenance of enteric neurons; regulation of intestinal peristalsis via BMP2 secretion</td>
<td>Nerve surveillance; axon resprouting after nerve injury</td>
<td>Phagocytosis, supply of neurotrophic factors, guidance of developing vasculature, synaptic pruning</td>
</tr>
</tbody>
</table>

*Evaluated in lethally irradiated bone marrow chimera.
populations with many different specifications (Table I). In contrast to the CNS, which is considered to be an immune-privileged organ and largely shielded by the blood–brain barrier from the periphery, the PNS is in extensive contact and exchange with surrounding tissues. Similar to CAMs, the morphology of NAMs is adapted to their anatomic niche. The elongated morphology, which extends along axons and nerve fibers, resembles that of perivascular macrophages along CNS vessels and NAMs in the dura mater (51, 81), but is in contrast to the ramified morphology of microglia or the star-shaped macrophages in the stroma of the choroid plexus (51) (Fig. 2). sNAMs show an extensive scavenging activity with continuous extension and retraction of their processes along the axons (37). This behavior has also been described for resting microglia in the CNS (60) and for CAMs in the meninges and perivascular space (82, 83). Furthermore, NAMs share typical immunophenotypic markers with microglia, CAMs, and other tissue macrophages, such as CD11b, F4/80, CD64, or MerTK (Table I). CX3CR1 is highly expressed on microglia and CAMs in the adult CNS but downregulated in other adult tissue macrophages (e.g., Kupffer cells) (48). Surprisingly, sNAMs in the dermis show high levels of CX3CR1 throughout adulthood, whereas other dermal macrophages only express low or intermediate levels (37). In contrast to CAMs, which express the mannose receptor CD206 or lymphatic vessel endothelial hyaluronic acid receptor 1 (82), these markers are not found on sNAMs in the dorsi nor on interstitial NAMs in the lung (37, 38).

CNS macrophages (except in the stromal choroid plexus and dura mater) have been demonstrated to be solely derived from an early erythromyeloid progenitor (EMP) from the yolk sac (2, 51, 84), whereas other tissue macrophages seem to arise from a later wave of EMPs, which first colonize the fetal liver (2, 85). Recently, sNAMs in the dorsi have been identified to be seeded prenatally and to be maintained with minor or no input of HSC-derived progenitors (37). This indicates that although PNS-resident macrophages are ontogenetically similar to most CNS macrophages, they could be derived from different waves of EMP-derived progenitors. Most likely, this difference can be explained by the differential progression of CNS and PNS development during organogenesis. However, the exact developmental stage at which peripheral nerves are colonized by tissue macrophages remains to be resolved.

Similar to microglia, NAMs are generally long lived and maintained via endogenous proliferation (27, 28, 37). However, their exact turnover time is not established yet and may differ between tissues. For their maintenance, most tissue macrophages depend on growth factor signaling via CSF1R. Microglia and CAMs express low levels of CSF1R on their surface but highly depend on CSF1R signaling (86, 87), in particular the ligands IL-34 and partially CSF1 (88, 89) in the adult CNS. CSF1R inhibition also results in the loss of dermal sNAMs and MM in the intestine (25, 37). However, it has not been fully resolved which growth factors maintain NAMs in general and whether they differ between tissues, similar to what was found for microglia in different brain regions (90, 91).

In further analogy to NAMs, microglia express a variety of neurotransmitter receptors (e.g., glutamatergic receptors) (92). Additionally, similar to NAMs, which contribute to peripheral nerve outgrowth and maintenance, microglia are often attributed to be “the gardeners” of the CNS. They continuously monitor neuronal networks and help to wire and mature neuronal connections during development (57, 92). The phagocytosis of cell debris and myelin is another function shared between CNS and PNS macrophages. Microglia remove myelin during injury or inflammation and therefore facilitate tissue repair and remyelination (93). Yet, whereas microglia are also essential for oligodendrocyte progenitor maintenance in the adult CNS and myelogenesis during development (94), the role of NAMs during the development and myelination of peripheral nerves is not yet understood.

**NAMs in human diseases**

Macrophages were identified in large nerves and in sensory and autonomic ganglia of healthy humans several decades ago (6, 95, 96). Recently, macrophages, which closely resembled their murine counterparts, were identified in human sympathetic ganglia and dural nerves (29, 37).

Besides their beneficial contributions to neuronal maintenance and repair as discussed above, macrophages also contribute to the initiation of immune-mediated demyelination in diseases such as Guillain-Barré syndrome and chronic inflammatory demyelinating neuropathy, at least in animal models (6, 72, 74, 97). In Guillain-Barré syndrome patients, macrophage-associated demyelination can be detected in nerve biopsies (9, 98). Moreover, the depletion of macrophages improves disease outcome in experimental allergic neuritis, the animal model for Guillain-Barré syndrome (99–101), and attenuates neuropathic pain following nerve injury (102–104). In ageing mice, degenerative changes of peripheral nerves could be prevented by long-term depletion of macrophages (105). Additionally, macrophages contribute to immune-mediated diseases affecting the CNS, such as chronic itch (106), inflammatory bowel disease (53, 107), and rheumatoid arthritis (108–110).

In all of these studies, the specific contributions of resident and recruited macrophages in nerves remain unknown. Given that manipulation of neuroimmune interactions shows great translational potential (66, 111–113), further studies on NAMs in humans and their ontogeny and function are urgently required.

**Conclusions**

Macrophages have emerged as morphologically and functionally distinct subsets in large peripheral nerves and diverse tissues such as skin, intestine, and adipose tissue. In line with their striking morphological adaptation to the PNS niche and with unique transcriptomes that distinguish them from neighboring tissue macrophages, specific functional duties can be expected. Although organ- and nerve-specific cues are likely to influence distinct NAM identities, the respective knowledge is still in its infancy.

It appears that homeostatic NAMs receive a substantial prenatal input, are relatively long lived, and maintained via endogenous proliferation rather than turnover by circulating progenitors. Currently, the degree of similarity between NAMs from different tissues and nerves remains an important puzzle because site-by-site comparisons are missing. Uncovering overlapping essential genes seems worthwhile to allow for the
design of specific genetic targeting techniques and pharmacological manipulation. As most NAMs were analyzed as part of a larger tissue macrophage population, identification of selective markers would enable a more specific functional analysis (e.g., in PNS development and maintenance). In tissues in which specific markers are absent, the application of laser capture microdissection may help to resolve distinct subsets.

Following nerve injury, highly differentiated and tissue-adapted macrophages can acquire properties of NAMs and fill novel niches, at least in the skin. In contrast, the direct contribution of monocyte-derived macrophages may be limited. It remains to be established whether the replacement or expansion of homeostatic PNS macrophages imprints a lasting mark on the nerve interface (e.g., after regeneration).

Together, the evolutionary-conserved positioning of macrophages in peripheral nerves of rodents and humans across different tissues implies critical functions in nerve protection and homeostasis. However, because of their localization, they might also contribute to immune-related diseases of the PNS, such as neuropathy, neuralgia, and itch. Future investigations should help to shed light on these functions and their potential benefit for PNS regeneration.

Disclosures

The authors have no financial conflicts of interest.

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