Do phagocytotic mechanisms regulate soluble factor secretion in microglia?

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Microglia are responsible for phagocytosis in the brain: Phagocytosis, one of the major mechanisms of innate immune defense, is the process by which several types of cells in the immune system recognize, engulf, and digest large particles, such as pathogens and cell debris. In the brain, microglia play phagocytotic roles to regulate the micro-environment of brains under both physiological and pathological conditions. For example, during development, microglia help develop functional synaptic connections by pruning excessively produced synapses. Also, during the recovery phase after brain injury, microglia participate in repairing processes by phagocytosis. The removal of dead/damaged cells by microglia is an important step for brain recovery because compensatory neurogenesis and angiogenesis cannot be fully achieved when the obstacles (i.e., dead cells) remain in the injured brain region. Furthermore, microglia may also participate in pathophysiological mechanisms in brain by secreting soluble factors upon activation. Microglia can release both beneficial and detrimental factors after brain injury depending on the context, but it is still mostly unknown whether and how phagocytotic activity regulates the mechanisms by which microglia produce and secrete these factors. A recent study by our group (Hamanaka et al., 2020) highlights the possibility that the phagocytotic process changes the pattern of the secretome in microglia. At least in *in vitro* cell culture conditions, microglial response in soluble factor secretion after phagocytosis differs depending on the types of particles/substances that microglia encounter. In this perspective, we briefly introduce the roles of microglia in the brain, focusing on how they contribute to the maintenance of the brain micro-environment, and then we discuss how phagocytosis can regulate soluble factor secretion in microglia. Please note that the reader is encouraged to seek detailed reviews (Underhill and Goodridge, 2012; Fu et al., 2014; Hu et al., 2015; Galloway et al., 2019) that describe and summarize microglial function; due to the space limitation, this perspective does not cite the primary literatures for microglial roles in physiological and pathological conditions.

Microglial phagocytosis in central nervous system (CNS): Microglia constitute 10–20% of the glial cells in the brain and spinal cord, and they are a major cell type of phagocytic cells in the CNS. Under normal physiological conditions, microglia stay in an inactivated/resting state characterized by small cell bodies and highly ramified branching processes. During development, microglia prune synaptic connections by phagocytosing excessively produced synapses to build up correct and functional synaptic connections. In addition, microglia phagocytose apoptotic cells accumulated in the developing brain to maintain brain homeostasis. Even after the developing phase, abundant synapse loss and myelin degeneration are constantly generated as a part of the physiological processes, and again, microglia play a role in eliminating the impaired myelin debris, synapses, and other apoptotic cells by phagocytosis. Under these physiological conditions, microglial phagocytosis contributes to maintain brain homeostasis and does not activate pro-inflammatory cascades. On the other hand, in response to injury or invasion of pathogen, microglia are sometimes transformed into the pro-inflammatory state (please see the next paragraph for more detail regarding the activation state of microglia), which is involved in multiple inflammatory responses. They migrate to and accumulate at the site of damaged brain region by chemotaxis, and there, depending on the types of foreign materials present at the site, they use specific receptors to initiate phagocytosis. In general, the process of phagocytosis in microglia consists of multiple steps through cytoskeletal reorganization; (i) sensing and moving toward foreign materials, (ii) adhering to the materials, (iii) engulfing them, and (iv) digesting them in the phagosome. After brain injury, removal of cell debris by microglial phagocytosis is considered beneficial, because dead/damaged cells would physically disturb the compensatory neurogenesis and angiogenesis. In addition, aside from pathogens and cell debris, amyloid beta is another substance that can be subject to microglial phagocytosis. Amyloid beta is confirmed to accumulate in Alzheimer's disease (AD) brains, and therefore, microglial phagocytosis is now proposed as a therapeutic target for AD (Wisniewski and Goni, 2015). It should be noted here that human and mouse microglia contribute differently to pathological conditions of neurodegenerative diseases, including AD pathology (Friedman et al., 2018). Therefore, we need to keep in mind that the mechanisms of phagocytosis may differ between species. Regardless, although microglial phagocytosis plays a role in maintaining brain homeostasis, microglia have been traditionally considered deleterious cells in CNS diseases, mostly because they secrete several noxious factors after brain damage and thus exacerbate inflammatory cascades.

Microglia as a source of soluble factors: Microglia are a major cell type of phagocytic cells in the brain, and microglial phagocytosis generally works beneficially. However, the roles of microglia under diseased or injured conditions may not be so straightforward. Once resting microglia respond to external stimuli, they are known to exhibit two different types of activated forms, depending on the stimuli and their surrounding conditions; one is the M1 state that secretes harmful pro-inflammatory factors (e.g., reactive oxygen species, heat shock protein 60, interleukin 6 (IL-6), etc.), and the other is the M2 state that secretes beneficial anti-inflammatory factors (e.g. CXCL13, insulin growth factor-1, IL-10, etc.). Therefore, although microglia play a central role in phagocytosis at/around the lesion area in removing detrimental substances, depending on the soluble factors they secrete, they could have either negative or positive effects on neighboring cells and the surrounding micro-environment. Microglial phenotype changes, including their activation state, are closely related to the patterns of soluble factor repertoire from microglia, and these factors are strong modulators for brain functions. Notably, compared to the roles of microglia-derived factors in brain damage and repair, little is known about the types of secretome from microglia after phagocytosis. However, after phagocytosing apoptotic cells through a phosphatidylserine-mediated recognition mechanism, microglia were shown to secrete an anti-inflammatory factor prostaglandin E2 (Zhang et al., 2006). Therefore, it is possible that the process of phagocytosis could regulate soluble factor secretion from microglia.

Relationship between phagocytosis and soluble factor secretion in microglia: Thus far, the mechanisms and steps of phagocytosis and the roles of microglia-derived factors in brain function have been extensively studied. However, how and whether phagocytosis regulates soluble factor secretion from microglia is still mostly understudied and unknown. In the context of the relationship between phagocytosis and soluble factor secretion, our group recently proposed the possibility that the secretion pattern from microglia may depend on the substances of phagocytosis (Hamanaka et al., 2020). In the study, we prepared primary rat microglial cultures and subjected them to three kinds of substrates - *E. coli* bioparticles, cell debris, and amyloid beta - as bait for microglial phagocytosis. Cultured microglia phagocytosed all these substrates, but interestingly, only microglia that were treated with *E. coli* bioparticles secreted neurovascular mediator matrix metalloproteinase-9 (MMP-9) after the event of phagocytosis. To further identify which step of phagocytosis is required for MMP-9 secretion after *E. coli* bioparticle treatment, we subjected cultured microglia to lipopolysaccharide, the major component of the outer membrane of *E. coli* that is not phagocytosed by microglia. Lipopolysaccharide induced MMP-9 secretion from microglia through the same intracellular signaling cascade (e.g., Toll-like receptor 4-mitogen activated protein kinase pathway), as observed in the case of *E. coli* bioparticle treatment. These findings support the idea that the pattern of the microglial secretome may depend on the substances subject to phagocytosis. The study demonstrated that the "engulfing" step of phagocytosis may not be a critical trigger for MMP-9 secretion; rather, adherence to *E. coli* before initiating the process of engulfing the substance plays an important role in MMP-9 secretion from microglia, though it is still possible that the engulfing step can also be a modulator for the pattern of secreting factors from microglia. In major phagocytotic cells, live Gram-negative bacteria triggered inflammatory responses, including interferon β and IL-1β production; however, after phagocytosis, heat-killed bacteria did not induce these responses (Sander et al., 2011). Importantly, these

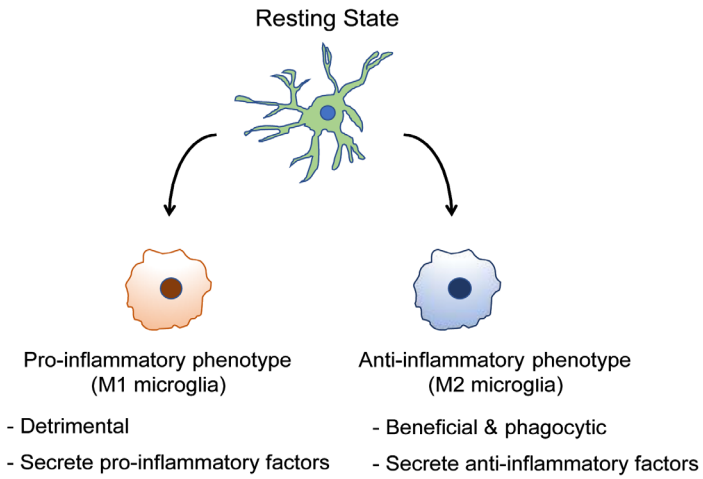

Figure 1 | **Phagocytosis and soluble factor secretion are two major functions in microglia.**
Microglia in the resting state serve as "sentinels" by surveying their neighboring conditions in the brain. Once microglia are activated, they become so-called pro-inflammatory M1 microglia or anti-inflammatory M2 microglia, depending on the types of stimuli. Between the two phenotypes of microglia, M2 microglia are thought to be responsible for phagocytosis. However, emerging literature now suggest that beyond the concept of M1/M2 microglia, heterogeneous populations of activated microglia appear after brain injury. As discussed in this perspective, it is still mostly unknown how the process of phagocytosis regulates the patterns of soluble factor secretion from microglia. Future studies are warranted to investigate this mechanism for the purpose of understanding the nuanced mechanisms of microglial activation under physiological and pathological conditions.

different responses were due to intracellular mechanisms, i.e. only live bacteria induced inflammasome activation, which is required for processing of IL-1β. It is not yet confirmed that the same mechanism is enclosed in microglia, but these studies indicate that phagocytotic mechanisms are closely related to the selection of the microglial secretome pattern.

Conclusion and future remarks: Phagocytosis and the secretion of soluble factors are two major functions in microglia (**Figure 1**). Despite this fact, surprisingly, the relationship between phagocytosis and soluble factor secretion has been relatively understudied in our field. As discussed in this perspective, the phagocytotic process may regulate the selection of the microglial secretome that contributes to the modulation of the neighboring micro-environment. Therefore, future studies are indeed warranted to investigate the precise mechanisms by which phagocytosis changes the pattern of soluble factor secretion from microglia. However, how will these studies help us develop our understanding of mechanisms regarding neuroregeneration, which is the main research focus of this journal? A hint may lie in the fact that beyond the traditional M1/M2 microglia polarization, it is now widely well-accepted that microglia exhibit more heterogeneous phenotypes (Lee et al., 2019). For example, microglia show different characteristics depending on the region, such as cerebral white matter vs gray matter. Cerebral white matter contains significantly higher numbers of microglia, and white matter microglia express more phagocytosis-related proteins such as CD68 and CD86 compared to gray matter (Zrzavy et al., 2018). This difference may partly come from the surrounding environment of microglia, because compared to gray matter debris, white matter debris is more preferably engulfed by phagocytotic cells (Huizinga et al., 2012). Cerebral white matter is comprised of myelinated axons that connect neurons in various regions of the brain, and white matter dysfunction is one of the major characteristics of vascular-related CNS diseases or aging, often leading to cognitive decline. Under diseased conditions, the activities of microglia become dysregulated, leading to abnormalities such as reduced phagocytic capacity (Deczkowska et al., 2018). Considering the relationship between phagocytosis and soluble factor secretion in microglia, we may hypothesize that once microglia lose their ability to phagocytose in white matter, they no longer regulate the neighboring homeostasis, as they cannot produce sufficient soluble factors due to the lack of signaling from phagocytosis-dependent mechanisms. During the recovery or chronic phase of CNS diseases, the brain tends to activate compensatory mechanisms, such as neuroregeneration, e.g., axonal remodeling/repairing along with re-myelination in white matter. Microglia could participate in these positive responses by secreting a broad spectrum of trophic factors, but this function of white matter microglia may be easily disrupted because of reduced phagocytic capacity. The papers introduced in this perspective emphasize the importance of further investigation into how phagocytotic mechanisms regulate the selection of soluble factors from microglia.

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