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Editorial

Microglia: Newly discovered complexity could lead to targeted therapy for neonatal white matter injury and dysmaturation

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1. Introduction

This Commentary is stimulated by a recent report by Hammond et al. [1] which utilized break-through molecular techniques to characterize microglia in multiple regions of developing brain. The work delineated a heretofore unrecognized complexity in microglial phenotypes that has implications for normal brain development, disease, dysmaturation and potential therapies. In particular the work has special relevance to the fundamental basis of the neurological disability in very premature infants, i.e., a combination of initial cerebral white matter injury (WMI), and especially, the subsequent dysmaturation involving both white matter and neuro-axonal structures [2, 3]. In the following I will discuss first the development of microglia in brain, their critical role in the genesis of both the white matter injury (WMI) and of the subsequent dysmaturation in premature brain, the pioneering new work of Hammond et al. re: microglial phenotypes, and the implications of the new data for targeted therapy in the premature infant.

2. Microglia in developing brain

Microglia are derived principally from bone marrow-derived monocytes and enter the CNS in the first trimester. These cells become prominent in the human forebrain at 16 – 22 weeks' gestation and migrate progressively through the white matter from 20–35 weeks and then to cerebral cortex [4–7]. Thus, the cerebral white matter is heavily populated with microglia during a period when various important maturational events are occurring and when a variety of pro-inflammatory states (e.g., hypoxia, ischemia, systemic inflammation) can lead to activation to destructive microglial phenotypes and WMI; such activated microglia have been termed M1.

Microglia not in an active pro-inflammatory state (M2) play important roles in such aspects of brain maturation as axonal development, oligodendroglial development – myelination, vascularization, synaptic development and pruning, and neural circuit

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formation [8]. Because of these crucial developmental roles of so-called M2 microglia, diversion of these "normal" cells to a microglial phenotype with primarily pro-inflammatory (M1) functions could contribute importantly to the disturbed maturational events observed subsequently in premature brain (see later) [3].

3. Microglia in cerebral white matter injury and subsequent dysmaturation

The spectrum of cerebral WMI in the modern era is quite different than that in previous years. Specifically, the relative frequency and severity of the two essential components of WMI, i.e., focal periventricular necroses and diffuse white matter abnormality, have changed in the modern era. Thus, overt focal periventricular necroses are now uncommon, and WMI consists predominately of few or no focal necroses and principally a diffuse lesion in cerebral white matter [9]. The latter includes, initially, death of pre-myelinating oligodendrocytes (pre-OLs) followed by replenishment of the pre-OL pool, but a subsequent impairment of pre-OL maturation (and ultimately, hypomyelination) [3, 10]. The pre-OL dysmaturation is considered to lead to (1) axonal dysmaturation, via impaired trophic interactions with pre-OLs, and (2) impaired development of cerebral cortex and thalamus, via anterograde and retrograde trans-synaptic effects caused by the axonal disturbances [3, 10]. The *key mediators of the critical pre-OL dysmaturation is the marked gliosis that is the hallmark of the diffuse lesion.* The *gliosis consists of* "*activated*" *microglia and reactive astrocytes*. The mechanisms by which the gliosis leads to the dysmaturation of pre-OLs and neuronal-axonal structures have been discussed elsewhere [3, 10, 11]. The pro-inflammatory microglia are critically involved by release of reactive oxygen/nitrogen species and cytokines that then act on pre-OLs. Additionally, these pro-inflammatory microglia have been shown recently to induce formation of neurotoxic reactive astrocytes [12, 13]. These astrocytes secrete cytokines and other molecular products important in the pre-OL maturational failure. Finally, the shift in microglial phenotype from an anti-inflammatory to a pro-inflammatory, activated phenotype diverts the critical roles of the former, "normal" microglia in OL development. These roles involve release of critical growth factors and related proteins, cytokines, and iron [8].

4. Newly discovered complexity of microglia in developing brain

The discovery that the long-standing bimodal characterization of microglia as pro-inflammatory (activated) (M1) or anti-inflammatory (M2) is too simplistic was pioneered by the recent work of Hammond et al. [1, 8]. In the landmark study, using recently developed high throughput techniques to delineate RNA sequences in single cells, Hammond et al. analyzed over 76,000 microglia in developing mouse brain [1]. This approach of defining single cell transcriptomics led to the discovery of many distinct microglial subpopulations with unique molecular signatures. These distinct subpopulations changed over the course of development and exhibited regional specificity [1]. Microglia were found to be most diverse during early development. At least nine distinct microglial subpopulations could be defined. Notably a distinct microglial phenotype was localized to developing white matter tracts. The genetic signatures allowed delineation of key metabolic pathways important to each distinct microglial subpopulation. The subpopulation localized to developing white matter tracts, termed axon-tract associated microglia (ATM), have molecular signatures consistent with involvement in axonal development just prior to myelination, i.e., the time period occurring in premature brain. The data raise the possibility that specific interventions could target such specific microglia, when activated, and potentially convert a harmful phenotype to a "normal", developmentally important phenotype.

How could microglial phenotype be manipulated? Interventions designed to convert brain microglia from a pro-inflammatory to an anti-inflammatory phenotype have been studied in a variety of animal models. Immunomodulating agents that cross the blood-brain barrier (minocycline, melatonin) have been identified [14], but their safety and efficacy in the premature infant for *long-term use* are not established [15]. The latter point is critical because the dysmaturational events in premature brain occur over months and perhaps longer (see later). Moreover, the use of such broad-spectrum immunomodulatory agents affect *all* microglia and could have unwanted effects. Apart from OL and axonal development, microglia during the premature and early infancy periods are involved in such critical developmental events as synaptic formation, sculpting and connectivity [8]. The *specific microglia involved in the dysmaturation of premature brain* should be targeted.

As just noted, the duration of intervention likely required to alter microglial phenotype may be relatively long. Thus, available MRI evidence suggests that dysmaturation of premature brain continues for many months and likely longer [16]. Although neuropathological data in human infants are scanty, available information suggests that diffuse white matter gliosis is present for at least many months after the premature period and likely longer [17–20]. Indeed, there is precedent in human neuropathology for microglia to be *chronically activated*, e.g., after traumatic brain injury. In the latter setting, these cells are considered important in subsequent degeneration of axons and neurons years later and to play a role in the enhanced incidence of such degenerative disorders, such as Alzheimer's and Parkinson's disease.

The specificity of microglial targeting could become a reality in view of the work of Hammond et al. [1]. For example, although not yet proven, the specific microglia likely involved in the white matter disturbance that leads to white matter (pre-OL) and neuro-axonal dysmaturation in the premature infant (see earlier) is the sub-type identified in developing white matter and related axonal tracts. Hammond et al. [1] have shown that these cells have a unique transcriptional signature and associated distinguishing metabolic signatures, as noted earlier. These *characteristics provide specific targets to be modulated*. One approach would be to identify distinctive microRNAs (miRNA) in these cells, (MicroRNAs are short noncoding RNA molecules involved in the regulation of gene expression.) Recent research has identified a variety of *pharmacological agents that can mimic or decrease expression of endogenous miRNAs* [21]. In one animal model *intravenous* delivery of a specific miRNA (miR-124) via miR-124-enriched *exosomes* promoted change of microglia from an inflammatory to anti-inflammatory phenotype and provoked neurogenesis [22]. (Exosomes are a type of extracellular vesicle that can carry proteins, lipids, and various types of RNA.) Another approach would be to construct *dendrimers* that specifically target the microglia of interest. Dendrimers are unique nanoparticles synthesized for a variety of functions, including targeted delivery of therapeutic agents to brain [23, 24]. Their small size and tailorable surface functional groups perhaps could be designed to target specific microglia. Drugs to target the specific distinguishing metabolic characteristics of "activated" microglia can be incorporated into the dendrimer. Several recent models of ischemia- or inflammation-induced fetal or neonatal brain injury indicate potential value of this approach [25–29]. Thus, the particular future challenge is to target only specific "activated" microglia, such as those in developing white matter tracts identified by Hammond et al. [1], which appear to be excellent candidates as the principal culprits in pre-OL and axonal dysmaturation in WMI of the premature infant. Future work will be of great interest, but the foundation appears to be laid by the recent work [1].

5. Conclusions

This Commentary relates to the recent discovery by new advanced molecular techniques that, in contrast to the previous binary notion of "inflammatory" and "anti-inflammatory" phenotypes, many (at least nine) distinct microglial subpopulations with unique molecular signatures can be identified in developing brain. These subpopulations exhibit developmental and regional specificity. One distinct microglial phenotype was localized to developing white matter tracts. In view of the importance of microglia in the genesis of cerebral WMI and subsequent dysmaturation in premature brain, the findings suggest that targeting such a glial population could have a major beneficial role in preventing or mitigating the unfavorable outcomes in such infants. Potential interventions include intravenous delivery of specific pharmacological agents targeting miRNAs, as well as delivery of other therapeutic agents (drugs, cytokines, particular nucleic acids, proteins, lipids, etc.) by such unique carriers as exosomes and dendrimers.

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