Role of macrophage phenotype and macrophage/DRG site-specific interactions on neurite outgrowth in adult DRG neurons in vitro

Echevarria et al.

Franklin D. Echevarria and Richard E. Zigmond
Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH

Following a peripheral nerve injury, macrophages infiltrate and accumulate at two distinct areas of the peripheral nerve projection; the peripheral ganglia and the distal axotomized nerve segment. It is well characterized that macrophage accumulation at the distal nerve segment following injury is to promote Wallerian degeneration, a necessary component of PNS regeneration. While the reason for accumulation at the ganglia is not well characterized, studies from our lab and others suggest it promotes regeneration. Indeed, macrophages are implicated in destroy/repair functions elsewhere in the body, and it is thought that their phenotype directs these opposing actions. Specifically, pro-inflammatory/destructive macrophages are designated M1, while anti-inflammatory/reparative macrophages are designated M2. Despite advancements, it is still not well understood: 1) Whether macrophage interacting exclusively with the cell body of the ganglia or its axon influences regeneration and 2) Whether macrophage phenotype is also involved in this process. To answer these questions, we utilized a dissociated mouse dorsal root ganglion (DRG) culture system to model peripheral nerve regeneration, and macrophages derived from bone-marrow precursor cells (bone marrow derive macrophages; BMDM). We examined the role of BMDM phenotype by obtaining M0 (unstimulated), M1 (stimulated with LPS/IFNγ), and M2 (stimulated with IL-4) BMDMs and applied them directly or indirectly (via well-insert) to DRG neurons for 48 hours. Interestingly, direct application of M1 BMDMs led to decreased neurite outgrowth compared to media, M0, and M2 treatment, while indirect application led to increased neurite outgrowth compared to media and M0 treatment. This suggests that M1 BMDMs may release factors that facilitate neurite outgrowth but that direct contact between M1 BMDMs and DRGs negates this influence. To test whether site-specific BMDM/DRG interactions influences DRG outgrowth, we used XONA microfluidic devices to allow us to manipulate the DRG cell body and its axon individually. 24 hours after plating DRGs in the soma chamber of the MFD, M1 or M2 BMDMs were plated exclusively in the axon chamber, where the macrophages could interact with DRG axons, but not the DRG cell body. 48 hours after BMDM addition, neurite outgrowth was assessed. Interestingly, we saw no difference in neurite outgrowth suggesting macrophage phenotype has no effect on regeneration at the level

of the axon. Experiments looking at adding BMDMs to DRG cell bodies are currently underway.