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USING CRYOPRESERVED ADULT MYENTERIC NEURONAL CELLS FOR CELL CULTURE BASED NEUROTOXICITY STUDIES.

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The ability to cryopreserve adult neuronal cells for use in cell culture experiments would represent a significant advance in research. We have developed a method for neuronal cell cryopreservation, where the frozen adult myenteric cells, when thawed and grown in primary cell culture display normal morphology and neurochemistry. On this basis, we employed cryopreserved myenteric neuronal cells isolated from male Sprague Dawley rat ileum to examine the role of macrophages in neurotoxicity associated with gut inflammation. Many components have been implicated in inflammatory bowel disease (IBD), among which macrophages play a more prominent role. Myenteric neurons cultured for 7d and then co-cultured (8 chamber slides) 24hr with non-activated RAW 264.7 monocyte/macrophage cell line under control conditions, showed normal viability and survival. In co-cultures exposed to the bacterial toxin (LPS; 24hr), we observed significant changes in the RAW cells, including vast morphological changes, increase in adhesion, and increased nitric oxide (NO) production typical of activated macrophage cells. With LPS treatment, co-cultures displayed more than 50% loss in neurons. LPS treatment of just myenteric neuronal cell cultures was without effect. L-NAME reduced the NO production and neurodegeneration in co-cultures. These data show the viability of cryopreserved myenteric neuronal cells to study cell-cell interaction and neurotoxicity in primary culture. The ability of activated macrophages to induce neuronal cell death is striking. Our results also suggest that induction of NO production in activated macrophages may be important in inflammation-dependent neurodegeneration.