HUMAN OLIGODENDROCYTES GROWN IN 3D AS A MODEL TO STUDY OLIGODENDROCYTE INJURY INDUCED BY THE LYME DISEASE SPIROCHETE BORRELIA BURGDORFERI.

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We are studying the pathogenesis of Lyme neuroborreliosis, a disease of both the central and peripheral nervous systems that is caused by the spirochete Borrelia burgdorferi (Bb). As a possible mechanism for Lyme neuroborreliosis we are evaluating the central hypothesis that Bb and/or the inflammatory mediators induced in the central nervous system (CNS) by Bb contribute to neuronal and/or glial apoptosis. Recently we reported that the interaction of the Lyme disease spirochete Borrelia burgdorferi with brain parenchyma elicits inflammatory mediators from glial cells and glial (oligodendrocyte) and neuronal apoptosis. Numerous transcripts of genes that regulate inflammation as well as both oligodendrocyte and neuronal apoptosis were significantly perturbed, as assessed by DNA microarray analysis. Concomitantly, significant proportions of both oligodendrocytes and neurons undergoing apoptosis were present in spirochete-stimulated brain tissues ex vivo, and in the frontal cortex of stereotactically inoculated rhesus monkeys in vivo. Oligodendrocytes, which are the myelin-producing cells in the CNS, are the major targets of injury in multiple sclerosis (MS), a CNS disease that often shows clinical similarities with Lyme neuroborreliosis. In order to study the molecular mechanisms involved in oligodendrocyte death as induced by Bb in more detail, we are evaluating the suitability of a human cell line of primary oligodendrocytes MO3.13. The aim of this study is to compare MO3.13 grown in both the conventional two-dimensional (2D) in vitro tissue cultures, and three dimensional (3D) rotatory wall vessel (RWV) cultures (Synthecon Inc.). 3D culture has been reported to enhance the phenotype of cells to one that is closer to that seen in vivo. The expression of characteristic oligodendrocyte markers (myelin basic protein, MBP), myelin proteolipid protein (mPLP), oligodendrocyte marker (O4), galactoceramide (GALC) and 2’,3’-cyclic nucleotide 3’-phosphodiesterase (CNPase) were evaluated by immunofluorescence staining and confocal microscopy. MBP expression was also studied using flow cytometric evaluation. Enhanced expression of MBP and mPLP was observed in differentiated oligodendrocytes (grown in medium containing phorbol myristate acetate and devoid of serum) both in 2D and 3D as compared to those in undifferentiated growth medium, while the expression of O4, GALC and CNPase was unaffected. Glial fibrillary acidic protein (GFAP) that is known to be expressed in undifferentiated MO3.13 cells and decrease in differentiated cells in 2D cultures showed a similar pattern in 3D cultures. Importantly, the morphology of cells grown in 3D following differentiation showed more pronounced processes and MBP expression (indicators of enhanced differentiation) as compared to that seen in 2D differentiated cells. Preliminary flow cytometric evaluation showed enhanced MBP expression in the 3D cultures as compared to that seen in 2D cultures even prior to differentiation. In addition, we have observed that live Bb induce 2.5 fold higher levels of oligodendrocyte apoptosis as compared to background levels in 2D differentiated MO3.13 cells in 48 hours, as measured by the flow cytometric evaluation of MBP-positive cells showing active caspase-3 activity. This observation supports our hypothesis that the Lyme disease spirochete can induce apoptosis of oligodendrocytes. We are presently evaluating this
phenomenon in the 3D system. In conclusion, the human oligodendrocyte cell line MO3.13 grown in 3D is both a novel and more appropriate in vitro model to study oligodendrocyte injury, a phenomenon possibly contributing to the pathogenesis of Lyme neuroborreliosis.

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