**Role of cIAP2 in activation of microglia**

**Introduction:**

Multiple Sclerosis(MS) is an autoimmune disease of the Central Nervous System(CNS) portrayed by demyelination, axonal degeneration, and cell death of oligodendrocytes. Although current research believe that MS is caused by an unidentified environmental factor in a person who is predisposed to respond, there is no proven, identifiable cause of MS. It can be diagnosed in patients at any age, but most are between the ages of 20-50. MS is more common in women than men in fact women are two to three times more likely to be diagnosed with MS. Some of the symptoms a patient with MS shows are numbness, tingling, pain, etc. In the CNS, inflammation is caused by the immune system resulting in damage to myelin. Myelin is a sheath protecting and insulating nerve fibers. A patient would begin showing symptoms of both numbness and tingling as the neuronal signals in the CNS are changed or stopped entirely, which comes as a result of damaged or destroyed myelin. This destruction of myelin in the CNS is coupled with activated microglia. Microglia are glial cells that function as immune cells of the CNS. These glial cells play a role in inflammatory and immune responses. Microglia secrete many pro-inflammatory and anti-inflammatory cytokines such as tumor-necrosis factor-α(TNF-α) and interlukin-1 (IL-1). These are both signaling proteins involved in inflammation and regulation of immune cells. TNF-α signaling can lead to multiple outcomes including cell death as well as the NF-kB pathway. TNF-α binds to TNFR1 or R2 which are both ligand binding receptors which causes a conformational change in the receptor resulting to the dissociation of a TNFR inhibitory protein, the silencer of death domain protein. This dissociation allows for the recruitment of the intracellular “death signaling induced signaling complex(DISC) proteins, TNFR-associated death domain protein(TRADD), Fas associated protein with death domain(FADD), and the TNFR-associated factor(TRAF). These proteins create a concrete frame work which recruit pro-caspase-8, which when cleaved releases an activated form of caspase-8 resulting to cell death. However, there is another regulator of cell death which is done by the inhibitors of apoptosis proteins(IAPs) which act directly with TRAF. In the absence of cIAP2, there was an increased amount of cell death that was observed. However, research done by the Kordula showed post-EAE in the absence of cIAP2, oligodendrocytes died and resulting in an increase in activated microglia. This brought light to the underlying question as to why do all cells except for microglia die.

**Experiment:**

An experiment that would be useful in getting a step closer into making progress into answering the question would be a Lactate dehydrogenase(LDH) Cytotoxicity Assay. This would be done with microglial cells obtained from both wildtype cIAP2 knockout mice and plate them. Each well should have approximately 200,000 cells per well. Each well will get its own proteins; control, IL-1, TNF-α, IL-1 and S1P, and TNF-α and S1P. These proteins will have a different effect on the microglial cells. LDH is an enzyme found in all living cells which catalyzes the conversion of lactate to pyruvate as well as NAD+ to NADH. It also does the reverse reactions as well. A larger number of LDH activity in a cell is directly related to the number of damaged cells. The steps involved in this assay start with the cell culture. This particular assay requires a 96 well culture plate in order to test each compound. Each well requires 200 ul of culture media as well as the test proteins being used, which are mentioned above. It is recommended to make 3 replicates of each test sample as well as making three negative control and three positive control groups. Prepare working reaction buffer which would be made with sodium lactate, INT salt, and substrate mix to PBS. After adding this into the 96 well plate, take the plate and read it through an absorbance reader at 490 and take the reading. The media should look orange, meaning that the more orange detected the more damaged and lysed. Napierska, et. al, dud the same LDH assay in order to measure cell toxicity in different concentrations of silicon in human endothelial cells. The results showed that the higher the concentrations of silicon showed higher cytotoxicity.

**Discussion:**

The expected results for the experiment should show an increased amount of cell survival when the protein S1P is added. S1P is believed to be involved in the recruitment of microglia Therefore, it is believed that S1P is the protein saving the microglia from cell death resulting in the microglia being able to do its role as a cell, which is to act as an immune defense mechanism in the CNS. Overall, this should show an increase cell survival in oligodendrocytes as well, the previous cells which were dying post-EAE. Some of the limitations that could be observed would be the failure of explanation of what is going on signaling wise. The cell death assay would only show death versus survival and not give much insight into how the S1P protein is working and where it is working.

Some other experiments that could answer that question would be to identify whether the S1P protein is attaching to the DISC proteins by inhibiting some of the DISC proteins, and in order to amplify this a Western Blot could be run in order to see if the S1P is attaching to the DISC. The biggest aspect the results could be used for from the LDH-cytotoxicity would be useful in drug therapy. However, in a research perspective it would be more important to understand the signaling mechanism as well as what the S1P is doing in the TNF signaling. Obviously, as time goes on there will be more experiments to come as the research continues.

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