16.7 Preliminary work done so far.

We have established Mueller cell cultures and work with MIOM1 cell line bearing Mueller cell characteristics on a regular basis (Fig.1). The laboratory has also established neurosphere assay for subsequent passaging, differentiation and characterization from ciliary body and iris epithelial cells with precursor properties (Fig.2). We find, both CB1 and CB2 receptors are expressed by Mueller glia and modulated during injury by endocannabinoids (Fig.3). Our preliminary data shows low level of basal expression of Notch pathway components (preliminary data, Fig 4). 24 hour incubation with LPS induces a rise in mRNA of Notch 1, Hes5, Hey2, Wnt5a and Delta1. Both AEA and 2-AG suppress this rise on co-incubation. Thus AEA and 2-AG reverse injury-induced changes. Furthermore, we show that endocannabinoids can suppress at the protein levels of Notch1 and 2, as well as Hes1 under these conditions. Intriguingly, protein levels of the aforementioned proteins are induced higher in comparison to LPS alone at 8 hours. This suggests that AEA and 2-AG affect in a time-dependent manner and may induce Mueller cells into cell cycle progression, early on their effect causing de-differentiation. Activation of the Notch pathway is known to result in changes in cell fate, including self-renewal of stem cells or differentiation along a particular lineage. At the later phase the fall in Notch components may enhance differentiation. Concomitantly, the Jak/STAT pathway shows similar time-dependent changes in expression (preliminary data, Fig 5).

Figure 1. Primary human Mueller cell culture stained for CRALBP (Mueller glia) and GFAP (astrocytes). Immunofluorescence in MIO-M1 cells of the same antigens.
Figure 2. Neurospheres generated from precursor cells in ciliary body (A) and Iris (B) tissue. Cells were grown for 6 days to generate the spheres.

Figure 3. CB1 and CB2 receptors are expressed in retina, explants and human Mueller cells at mRNA (A) and protein (B) level. The levels of the CB1 and CB2 receptors get modulated during inflammation by endocannabinoids (C and D, respectively). (unpublished data, manuscript submitted)

Figure 4. Notch pathway components get modulated by endocannabinoids. mRNA levels of Notch pathway components get regulated on 24 hour incubation with endocannabinoids, in LPS-inflamed Mueller glia. Note that AEA and 2-AG both have similar effects in suppressing Notch components.
Validation at the protein level of some of these components confirm downregulation at 24 hours. Intriguingly, at the early stages (8 hours) of incubation the protein levels of these same components are unregulated in comparison to only LPS treatment and control. This suggests endocannabinoids affect Notch components in a time dependent manner.

Figure 5. Expression of Jak/Stat mirror changes seen in Notch 1 and 2 at both 8 hours and 24 hours. At 8 hours phosphorylated Jak1 and Stat3 are high, while they are suppressed by endocannabinoids at 24 hours.
References


