Methods: Reversible, blue light (BL) stimulated, optogenetic A1 protein expression plasmids, containing wild-type A1, tagged with both the optogene Cryptochrome 2 and mCherry, were transfected into HEK293T cells and a differentiated neuronal cell line and used to examine the effects of RNA oligo treatment on protein dynamics and downstream cellular pathway functions in real-time. Using this in vitro optogenetic paradigm of A1 dysfunction, we gathered evidence on how RNA oligo treatment affects A1 self-association clustering and downstream neuronal morphology and viability.
Results: Imitating an acute environmental cell stress using a BL stimulus followed by a steady period of recovery, our data show that RNA oligo treatment significantly decreased the kinetics of cytoplasmic A1 cluster formation [half-maximal formation time (minutes): Oligo=100; no treatment (NT)=62] and significantly decreased the number of cells with A1 clustering (percent cells w/ A1 clusters: Oligo=33%; NT=50%). We found that RNA oligo treatment stabilized the structure of A1 upon binding (avg. protein denaturation temp.: Oligo=62°C; NT=61°C), and significantly increased the quantity of A1 clusters (avg. clusters/cell: Oligo=19 ; NT=5) and decreased their size (avg. cluster size (μm2): Oligo=0.31; NT=0.95). Finally, we show that neuron morphology and viability were perturbed with A1 cluster formation.
Conclusions: Using an in vitro optogenetic approach, this study presents evidence that A1 clustering negatively affects neuronal morphology, and that RNA oligo treatment attenuates MS-associated A1 dysfunction, indicating that A1 protein dysfunction perturbation may affect NDG in MS.

P198 The Selective Sphingosine-1-Phosphate Receptor 1 (S1P1) Modulator Ponesimod Enhances Murine Oligodendrocyte Precursor Cell (OPC) Differentiation and Retains OPC Migration

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Background: Sphingosine-1-phosphate receptor (S1PR) modulators are clinically applied to target relapse remitting multiple sclerosis (MS) and the early phase of progressive MS when inflammation still prevails. S1P1 receptor modulation (functional antagonism) is known to prevent lymphocytes egression from lymph nodes, hence hampering neuroinflammation in MS. Recent findings suggest a potential additional role for S1P1 receptor modulation in neuroprotection and remyelination.
Objectives: As the Giα-coupled S1P1 is the most prominently expressed S1P receptor in oligodendrocyte precursor cells (OPCs), we hypothesized that functional antagonism by the S1P1-monoselective modulator ponesimod induces OPC differentiation.
Methods: Primary mouse OPCs were harvested via the shake-off method and treated in vitro with the S1P1-selective modulator ponesimod (3nM - 3000nM), the S1P5-selective modulator A971432 (3nM - 3000nM) or a phosphorylated form of the non-selective modulator fingolimod (3000nM). Migration was evaluated in the agarose drop migration assay (ADMA), while differentiation was quantified using a fluorescent immunohistochemical staining for the oligodendrocyte marker O4 and the differentiation marker myelin basic protein (MBP).
Results: Ponesimod and A971432 did not affect migration independently, the combination however inhibited OPC migration as did the 3000nM fingolimod-phosphate. Treatment with ponesimod (300nm) or A971432 (1000uM) displayed a significant increase in MPB protein expression.
Conclusions: We report that the S1P1 monoselective modulator ponesimod and the S1P5 monoselective modulator A971432 separately did not affect primary mouse OPC migration whereas the combination of the S1P1 and S1P5 modulators demonstrates impaired OPC migration. OPC differentiation was increased by the higher concentrations of ponesimod. S1P1 monoselective modulation holds promise for further research into the remyelinating capacity of the modulators such as ponesimod.

P199 Deficiency in B Cell Maturation Antigen reveals sex differences in Experimental Autoimmune Encephalomyelitis

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Background: Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS) leading to neuronal damage. The disease is heterogeneous with respect to onset and clinical course, being more prevalent in women than in men with a ratio of 3:1. Further sex differences have been observed with males having a more severe and debilitating disease course compared to females. The complex interplay of immune cells, sex hormones, or genetic differences could be contributing to these clinical phenomena, which remains to be elucidated. In experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, several reports have demonstrated no sex differences in disease onset or severity in C57BL/6 mice. Herein we report that deficiency in B Cell Maturation Antigen (BCMA) in C57BL/6 mice reveals a sex difference in EAE. BCMA plays a prominent role in B cell function, however, the role BCMA plays in MS and EAE is unknown.
Objectives: The goal of this project was to determine the effect BCMA has on EAE in male and female mice.
Methods: EAE was induced in male and female BCMA+/+ and BCMA-/- mice with an immunization of MOG35-55 in complete Freund's adjuvant. Immune cell analysis was performed using flow cytometry and cytokines were quantified using ELISA. Bone marrow transplant was used to determine if sex chromosomes play a role in the sex differences observed in the BCMA-/- mice. Castration and ovariectomy surgeries were performed to study the effect of sex hormones in determining the sex differences in the BCMA-/- mice.
Results: We found that the severity of EAE was not different in males and females in BCMA+/+ mice. Strikingly in the BCMA-/- mice, EAE severity was significantly increased in males compared to females. The increased EAE in the BCMA-/- males