Abstract

Multiple Sclerosis (MS) is characterized by lesions of the Central Nervous System (CNS). These lesions are characterized by an inflammatory response, marked by an increase in macrophages and microglia to help clear the accumulating lipids in myelin debris. Apolipoprotein E (APOE) aids in mediating lipoprotein metabolism. There are three isoforms of APOE present in the human population (APOE2, APOE3, and APOE4) and, due to structural variations, these isoforms have differences in binding to lipids and cell receptors. Therefore, we speculate that these isoforms contribute to the pathogenesis of MS and regulate remyelination differently. Our LFB-PAS staining shows significantly impaired remyelination in APOE2 and APOE4 mice. Further, APOE3 macrophages ingested significantly more cells in comparison to their WT counterparts. These findings suggest that the presence of APOE3 plays an inhibitory role in remyelination. Finally, from secretome analysis, we found upregulated candidates in APOE3 post-phagocytosis macrophages that promote neural conduction and clearance of myelin debris, suggesting a compensatory mechanism for remyelination in the presence of APOE3.

Background

The APOE protein is suggested to mediate the uptake and redistribution of cholesterol in the Central Nervous System.1 Lipid bound by APOE can be delivered to cells through endocytosis via members of the LDL receptor family. In Multiple Sclerosis (MS), myelin debris accumulates as the axons of neurons become demyelinated. Defects in APOE have been shown to cause the accumulation of lipoproteins which may contribute to an attenuation of remyelination.2, 3 There are three different isoforms of APOE in the human population: APOE2, APOE3, and APOE4. The differences are in two critical amino acid positions (112 and 158).3 These structural variations among the isoforms affect their ability to bind to lipids and cell receptors.4 APOE allele has the highest frequency in the population at approximately 78% and is found in the majority of a subpopulation of MS patients.4,5 The goal of this project was to understand how different APOE isoforms affect the remyelination process with an emphasis on APOE3.

Figure 1. Structures of human APOE isoforms (adapted from Mahley 2009). A) A critical salt bridge in APOE2 rearranges several amino acids to vary its structure from APOE3. B) The location of amino acid Arg-62 differs in APOE3 from APOE4. In APOE4, Arg-62 is more exposed in helix 2, allowing for easier interaction with Glu-255.

Methods

0-week mice 5-week mice 5+2 week mice

Cuprimine (demyelination) Diet Recovery (remyelination)

Figure 2. Cuprimine Administration for histology. Mice were fed 0.2% cuprine diet for 5 weeks to induce full demyelination in the medial corpus callosum. Mice were then given normal diet for 2 weeks for recovery prior to tissue collection. Sections were deparaffinized at 5 um and stained with LFB-PAS to assess for myelination level.

Figure 3. APOE3 and APOE4 showed impaired remyelination. Brain samples were obtained 2 weeks post-demyelination to measure levels of remyelination across different isoforms of APOE and APOE2.6, *p<0.05, ***p<0.001. Statistical significance were obtained by one-way ANOVA, followed by Tukey’s multiple comparison test.

Figure 4. APOE3 macrophages show deficiency in their ability to phagocytize apoptotic cells. Wild-type (WT) and APOE3 peritoneal exudate macrophages were plated and stimulated by apoptotic thymocytes for 30 minutes prior to quantification using confocal microscopy. Completed by another lab member. N=3, *p<0.05, **p<0.01. Statistical significance were obtained by one-way ANOVA, followed by Tukey’s multiple comparison test.

Results

Figure 5. Heat-map of the secretome profile of post-phagocytosis macrophages of WT and APOE3. Supernatants of wild-type (WT) and APOE3 macrophages were collected 24 hours post-phagocytosis of apoptotic cells. Secretomes were analyzed at the UNC Proteomics Core. Red indicates a higher expression in APOE3 secretome, and blue represents a higher expression in the WT. Proteins are clustered using the log2-fold change values.

Table 1. Top candidates in the secretion of post-efferocytosis WT and APOE3 macrophages. Candidates with an absolute value in log2-fold change (FC) higher than 2 were listed for their relevant function.

Conclusion

- In vivo staining showed that APOE3 and APOE4 inhibit remyelination [Figure 3].
- APOE3 macrophages showed a diminished ability to phagocytize apoptotic cells when compared to WT macrophages [Figure 4].
- Secretome factors upregulated in post-phagocytic APOE3 macrophages were annotated from online databases, which suggested their functions are associated with promoting the restoration of normal energy levels to produce neural conduction and clearing myelin debris with impaired lipid metabolism [Table 1, Figure 5].

Future Directions

- LFB-PAS staining of S1+1 wk and S3+3 wk brains will help to establish the progression of recovery in mice with different human isoforms.
- Immunohistostaining will be used to localize the secretome factors in the murine brain during the cuprizone treatment and recovery period to corroborate their presence.
- Factors upregulated in the APOE3 and WT secretomes will be tested for their effects on oligodendrocyte precursor cell differentiation.
- Phagocytosis assays are used to assess how APOE2, APOE3, APOE4, and APOE2 macrophages phagocytize apoptotic cells differently.

References