The Function of APOE During Remyelination

Leyla Ozelkan[1,2], Liwen Zhou[1], Laura Herring[3] and Glenn K. Matsushima[1,4,5]
[1]UNC Neuroscience Center, [2]Department of Biology, [3]UNC Hooker Proteomics Core Facility, [4]Department of Microbiology and Immunology, [5]Department of Integrative Biology and Genome Sciences

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 APOE3 and APOE4 mice. Further, APOE3 macrophages ingested significantly less apoptotic 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. [2] 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, 6] 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] APOE3 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.

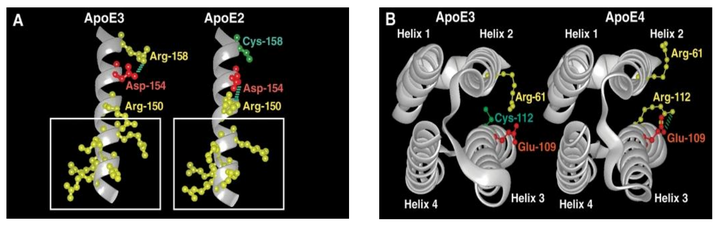

Figure 1. Structures of human APOE isoforms (adapted from Mahley 2009). A) A critical salt bridge in APOE2 rearranges several amino acids to vary its structure from APOE3. B) The location of amino acid Arg-61 differs in APOE3 from APOE4. In APOE4, Arg-61 is more exposed in helix 2, allowing for easier interaction with Glu-255.

Methods

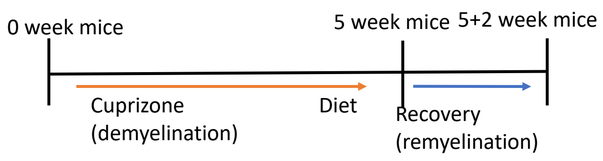

Figure 2. Cuprizone Administration for histology. Mice were fed 0.2% cuprizone diet for 5 weeks to induce full demyelination in the medial corpus callosum. Mice were then given normal diet for 2 weeks for recovery prior to tissue collection. Brain sections were obtained at 5 um and stained with LFB-PAS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

Results

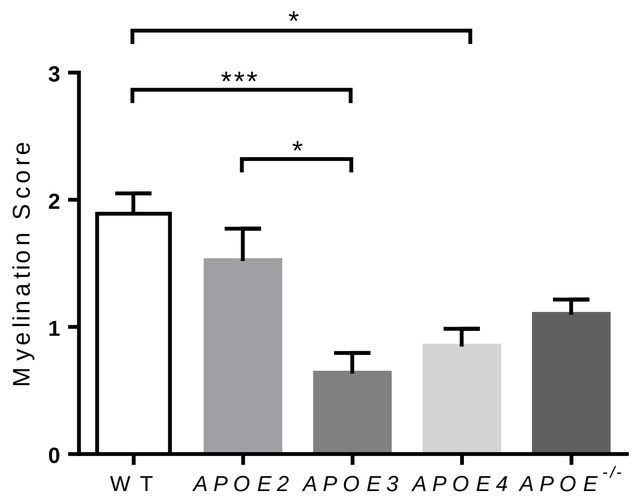

Figure 3. APOE3 and APOE4 showed impaired remyelination. Brain samples were obtained 2 weeks post-demyelination to measure levels of remyelination across different isoforms of APOE and *APOE*$^{-/-}$. n=4-5. *p<0.05, ***p<0.001. Statistical significance were obtained by one-way ANOVA, followed by Tukey's multiple comparison *t*-test.

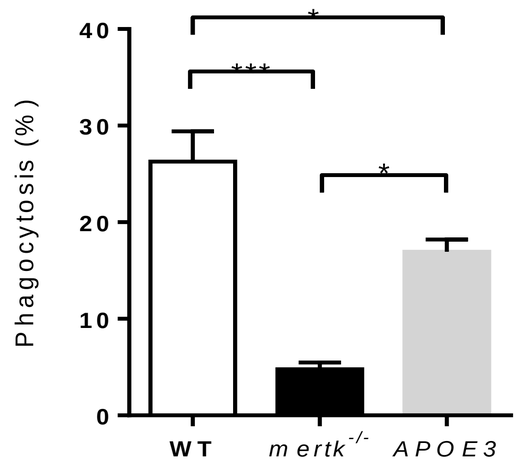

Figure 4: APOE3 macrophages show deficiency in their ability to phagocytize apoptotic cells. Wild-type (WT) and APOE3 peritoneal exudate macrophages were plated and stimulated by apoptotic thymocytes for 30 minutes prior to quantification using confocal microscopy. Completed by another lab member. N=3. *p<0.05, *p<0.001. Statistical significance were obtained by one-way ANOVA, followed by Tukey's multiple comparison *t*-test.

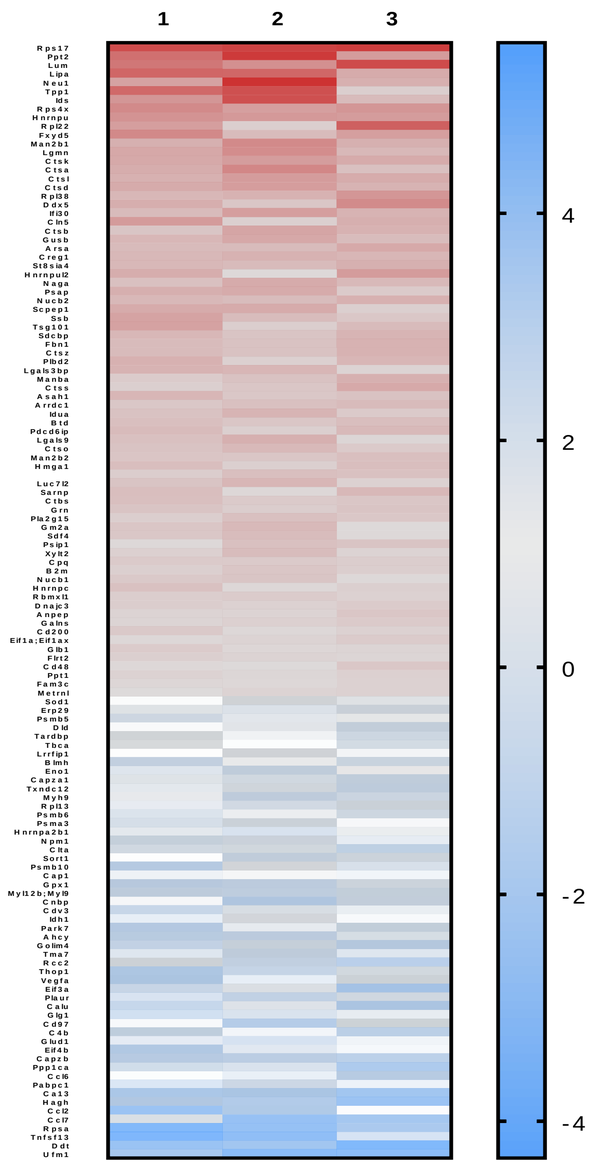

Figure 5. Heat-map of the secrotome profile of post-phagocytosis macrophages of WT and APOE3. Supernatants of wild-type (WT) and APOE3 macrophages were collected 24 hours post-phagocytosis of apoptotic cells. Secrotomes were analyzed at the UNC Proteomics Core. Red indicates a higher expression in APOE3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

Protein Name	Gere Names	LOG FC (*APOE3:* WT)	Relevant Reported Function
40S ribosomal protein S17	Rps17	4.861	Structural constituent of a ribosome
Lysosomal thioesterase PPT2	Ppt2	3.638	Lipid metabolism in the lysosome.
Lumican	Lum	3.522	Involved in the formation of connective tissue (collagen, fibrils, anchor connective tissue, etc.).
Lysosomal acid lipase/cholesteryl ester hydrolase	Lipa	3.110	Regulation of lipid metabolism.
Sialidase-1	Neu1	3.025	Aids in lysosomal compartmentalization, stability, and catalytic activation. Also regulates plasma lipoproteins.
Tripeptidyl-peptidase 1	Tpp1	2.941	Lysosomal enzyme responsible for clearance of intraneuronal aggregates.
Iduronate 2-sulfatase	Ids	2.715	Needed for lysosomal degradation of heparin sulfate and dermatan sulfate.
40S ribosomal protein S4, X isoform	Rps4x	2.412	X-linked protein important for translation.
Heterogeneous nuclear ribonucleoprotein U	Hnrnpu	2.316	Involved in cell and transcription/translation stability.
60S ribosomal protein L22	Rpl22	2.244	Translation and protein binding.
FXYD domain-containing ion transport regulator 5	Fxyd5	2.019	Na+-K+-ATPase regulator.
Carbonic anhydrase 13	Ca13	-2.066	Catalyze the reversible hydration of carbon dioxide to regulate pH.
Hydroxyacylglutathione hydrolase, mitochondrial	Hagh	-2.071	Enzyme involved in the catabolic processes of glycolysis.
C-C motif chemokine 2	Ccl2	-2.121	inflammatory factor that shows chemotactic activity for monocytes and memory T cells.
C-C motif chemokine 7	Ccl7	-2.134	Attracts monocytes and eosinophils.
40S ribosomal protein SA	Rpsa	-3.284	Role in translation + potential role in crossing the blood brain barrier.
Tumor necrosis factor ligand superfamily member 13	Tnfsf13	-3.285	Regulates cell growth and ability of macrophage to enter injury site.
D-dopachrome decarboxylase	Ddt	-3.367	Binds cytokines and proteases.
Ubiquitin-fold modifier 1	Ufm1	-3.394	Post-translational modifier.

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 5+1 wk and 5+3 wk brains will help to establish the progression of recovery in mice with different human APOE 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 will be used to assess how *APOE2*, *APOE3*, *APOE4*, and *APOE*$^{-/-}$ macrophages phagocytize apoptotic cells differently.

References

1. Laws, S. M., Hone, E., Gandy, S., & Martins, R. N. (2003). Expanding the association between the APOE gene and the risk of Alzheimers disease: possible roles for APOE promoter polymorphisms and alterations in APOE transcription. *Journal of Neurochemistry*, *84*(6), 1215–1236. doi: 10.1046/j.1471-4159.2003.01615.x
2. Baaklini, C. S., Rawji, K. S., Duncan, G. J., Ho, M. F. S., & Plemel, J. R. (2019). Central Nervous System Remyelination: Roles of Glia and Innate Immune Cells. *Frontiers in Molecular Neuroscience*, *12*. doi: 10.3389/fnmol.2019.00225
3. Sigurdsson, A. F. (2018, October 31). Apolipoprotein E – ApoE Explained. Retrieved January 20, 2019, from https://www.docsopinion.com/2016/08/22/apolipoprotein-e/
4. Phillips, M. C. (2014). Apolipoprotein E isoforms and lipoprotein metabolism. *IUBMB Life*, *66*(9), 616–623. doi: 10.1002/iub.1314
5. Sadeghi, H. M., Sabzghabaee, A. M., Mousavian, Z., Saadatnia, M., Shirani, S., & Moazen, F. (2011). Polymorphism of Apo lipoprotein E gene and the risk of multiple sclerosis. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences*, *16*(12), 1519–1524.
6. Rebeck, G. W. (2017). The role of APOE on lipid homeostasis and inflammation in normal brains. *Journal of Lipid Research*, *58*(8), 1493–1499. doi: 10.1194/jlr.r075408