Role of Complement Factor H Polymorphism and Diet in Neuroinflammation

Samuel Parnell BS, Ade Kasumu PhD, Bogale Aredo PhD, Xiao Chen MD, Rafael Ufret-Vincenty MD

Department of Ophthalmology, UT Southwestern Medical Center

ABSTRACT

Background: Multiple lines of evidence point toward an important role for complement factor H (Cfh) in neuroinflammation. Neuropathological evidence of activated microglia and activated astrocytes has been found in the brains of both Parkinson's disease and Alzheimer's disease patients. Cfh has also been found to be present in amyloid-beta plaques in Alzheimer's disease. Finally, although still somewhat controversial, some studies have found that the Y402H polymorphism in Cfh seems to influence the risk of Alzheimer's disease. Our laboratory has developed a mouse model of early AMD based on expressing variant Cfh molecules in mice. The Cfh transgenic mice develop deposits under the retinal pigment epithelium (under the retina), which resemble early changes seen on AMD. We believe these findings indicate that the variant Cfh molecules are less able to control chronic low grade inflammation at the tissue level. We propose to use these mice to determine if Cfh variants lead to: 1. increased chronic low grade inflammation also in the brain, 2. increased oxidative damage in the brain, and/or 3. increased levels of beta amyloid deposits in the aging brain.

Methods: Young (6 month old) and aging (18 month old) Cfh transgenic and control B6 mice were divided into groups and fed either a control diet or a high-fat diet for 5 months. Brains were then collected after perfusion with 4% paraformaldehyde, weighed, and post-fixed overnight in the same fixative. Brain sections (30 µm thickness) were obtained utilizing a Leitz sledge microtome with a freezing stage. Immunohistochemistry was performed on the sections with antibodies specific for inflammatory, oxidative stress, and microglial markers. Stained images were visualized using a Carl Zeiss fluorescence microscope with an objective lens of 10x. To ensure a similar anatomic location for all samples, the dentate gyrus region was photographed and analyzed. The number of microglia over the photographed field were counted and averaged in order to obtain a cells/field value for each mouse subgroup. Statistical analysis was then performed on the data in order to determine the correlation between the number and activation of microglia in the dentate gyrus and the age, genotype, and diet of the mice.

Results: Many of the tested antibodies, particularly those associated with oxidative stress, did not stain the sections, perhaps due to the fixation method. However, the anti-TREM2 and anti-Iba 1 antibodies stained well. There was no difference in the number of Iba1+ microglia in the dentate gyrus of CfhTg vs. B6 mice (p=0.607). Younger mice seemed to have higher numbers of these cells compared to older mice (73.3 vs. 52.6 microglia/field; p=0.0352). In addition, the mice fed a high fat diet appeared to have less microglia per field compared to the mice fed a normal control diet (53.1 vs. 72.8 microglia/field; p=0.0469). TREM2 is considered to be a marker for microglial activation. Neither age (p=0.65), nor a high fat diet (p=0.435) appeared to affect the level of TREM2 expression. However, there was a trend towards higher numbers of TREM2+ cells in CfhTg mice compared to B6 mice (p=0.17), particularly in the old group of mice (p=0.12). The CfhTg brains weighed less than the corresponding B6 brains (0.447 vs. 0.482g; p=0.00024). Mice fed the high fat diet had higher brain mass compared to those fed the normal diet, regardless of the genotype. The mean brain weight for high fat diet mice was 0.468g compared to 0.441g for normal diet mice (p=0.229).

Conclusions: There may be an increased number of activated microglial cells in CfhTg vs. B6 mice. Furthermore, the Cfh transgenic mice brains weigh less than the corresponding B6 mice brains. More brains will be analyzed to determine if these findings can be corroborated. Increased microglial activation and a decrease in brain mass can be due to increased levels of oxidative stress or tissue injury. Alternative methods of tissue collection will be explored in order to maximize the antigen recognition by antibodies. Also, additional commercially available antibodies against amyloid beta, malondialdehyde and nitrotyrosine will be tested.

INTRODUCTION

The complement system is an important part of the innate immune system that functions to initiate an inflammatory response to protect against foreign cells and pathogens. This inflammatory response occurs via opsonization by phagocytes, chemotaxis of leukocytes and monocytes, osmotic cell lysis, and the release of CD5a anaphylatoxin. However, complement also has the potential incite inflammation that can damage host tissue. Thus complement regulatory proteins, such as complement factor H, are present in blood and on the surface of host cells to prevent activation of the complement system. Recent evidence points to the dysregulation of the complement system playing a significant role in multiple diseases. Specifically, mutations in complement factor H (Cfh) have been associated with atypical hemolytic uremic syndrome, and Y402H polymorphisms of factor H have been associated with age related macular degeneration.

In a broader sense, evidence points to Cfh having an important role in general neuroinflammation. Neuropathological evidence of activated microglia and activated astrocytes has been found in the brains of both Parkinson's disease and Alzheimer's disease patients. Cfh has also been found to be present in amyloid-beta plaques in Alzheimer's disease. Finally, although still somewhat controversial, some studies have found that the Y402H polymorphism in Cfh seems to influence the risk of Alzheimer's disease. The objective of our study is to determine if mice with Cfh polymorphisms have increased markers of inflammation, oxidative stress, and beta amyloid deposits. High fat diets were also fed to half of the control and Cfh transgenic mice to determine what role diet has on complement activation in the brain.

RESULTS: Evaluation Mouse Brain Weight and Presence of Microglia in Mouse Brain Samples

Analysis of Mouse Brain Weight

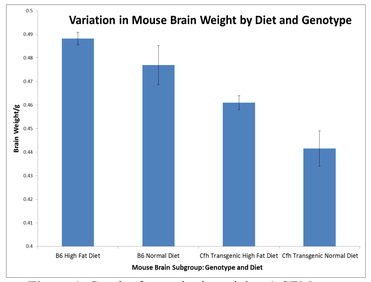

Figure 1: Graph of mean brain weight +/- SEM for 18 month old mice based on diet and genotype

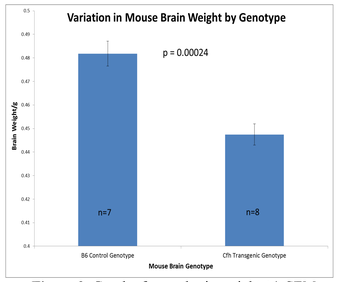

Figure 2: Graph of mean brain weight +/- SEM for 18 month old mice based on genotype

From Figures 1 and 2 it is evident that CfhTg brains had a small, but statistically significant decrease in brain weight compared to B6 controls (0.447g vs. 0.482; p=0.00024). In addition, the mice fed the high fat diet had a trend towards increased brain mass compared to those fed the normal diet, regardless of the genotype. The mean brain weight for high fat diet mice was 0.468g compared to 0.441g for normal diet mice (p=0.229). The decrease in brain weight in the Cfh transgenic mice moved us to postulate the hypothesis that the Cfh variant may lead to CNS oxidative stress and tissue injury, and that it could be associated with increased inflammation and microglial activation. To address this hypothesis we began by investigating the presence and activation of microglia in brain samples, and correlating these parameters with genotype and diet.

The presence of microglia in the dentate gyrus region of the brain was highlighted in the brain sections by staining with anti-Iba1 antibody, shown in fluorescent green in Figure 3. Additionally, the numerous branching processes on the microglia aid in determining the identity of the cells on the brain sections. Sixteen mouse brains were stained with anti-Iba1 antibody and analyzed to determine how the number of microglia per field varied with age, diet, and the complement factor H polymorphism. Figure 2 depicts the average number of microglia per field in the dentate gyrus for the different mouse subgroups. Upon statistical analysis of the brain sections stained with anti-Iba1 antibody, we determined that there was no significant difference in the number of Iba1+ microglia in the dentate gyrus of Cfh transgenic mice vs. B6 control mice (p=0.607). Younger mice seemed to have higher numbers of these cells compared to older mice (73.3 vs. 52.6 microglia/field; p=0.0352). In addition, the mice fed a high fat diet appeared to have less microglia per field compared to the mice fed a normal control diet (53.1 vs. 72.8 microglia/field; p=0.0469).

Analysis of Anti-Iba1 Antibody Stained Mouse Dentate Gyrus

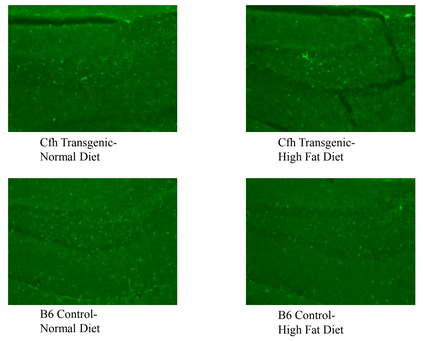

Figure 3: Images of 30µm sections of dentate gyrus from 18 month old mice specimens stained with anti-Iba1 antibody

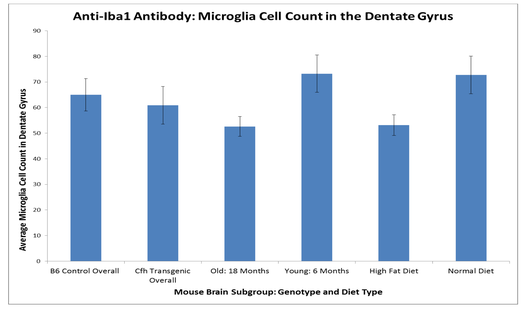

Figure 4: Graph of average microglia/field in the dentate gyrus for mouse population subgroups +/- SEM

RESULTS: Evaluation of the Activation of Microglia in Mouse Brain Samples

Analysis of Anti-TREM2 Antibody Stained Mouse Dentate Gyrus

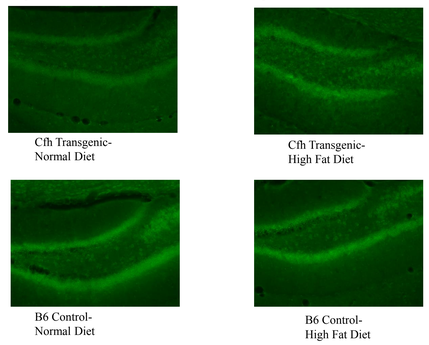

Figure 5: Images of 30µm sections of dentate gyrus from 18 month old mice specimens stained with anti-TREM2 antibody

The images above are mouse dentate gyrus sections stained with anti-TREM2 antibody. Triggering receptor expressed on myeloid cells 2 (TREM2) is a membrane-bound receptor expressed by microglia that enhances phagocytosis. Active microglia express TREM2 and are involved in debris clearance and phagocytosis in the central nervous system. The cells stained above contain TREM2 receptors on their surface and thus are active microglia. Twenty two mouse brains were stained with anti-TREM2 antibody and analyzed to determine how the number of activated microglia per field varied with age, diet, and the complement factor H polymorphism. The graphs to the right show how the average number of activated microglia per field in the dentate gyrus varied for the different mouse subgroups examined.

Neither age (p=0.65), nor a high fat diet (p=0.435) appeared to significantly affect the level of TREM2 expression. However, there was a trend towards higher numbers of TREM2+ cells in CfhTg mice compared to B6 mice (68.7 vs. 61.3 activated microglia/field; p=0.17), particularly in the 18 month old group of mice (69.8 vs. 57.8 activated microglia/field; p=0.12).

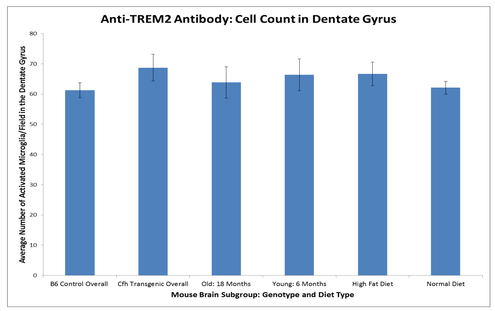

Figure 6: Graph of average number of activated microglia/field in the dentate gyrus for mouse population subgroups +/- SEM

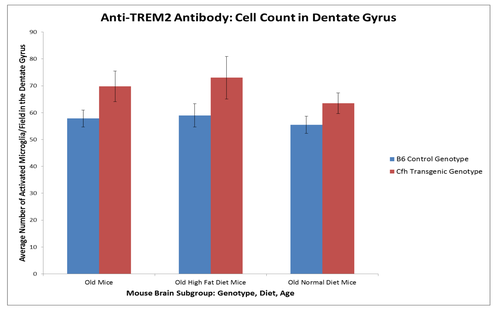

Figure 7: Graph of average number of activated microglia/field in the dentate gyrus for 18 month old mice +/- SEM

MATERIALS AND METHODS

- Transgenic mice expressing chimeric Cfh molecules consisting of mouse short consensus repeats (SCRs) 1-5, followed by human SCRs 6-8, followed by mouse SCRs 9-20, were generated. These mice expressed the Y402H variant of Cfh that has been found to be a risk factor for AMD and develop deposits under the retinal pigment epithelium, which resemble early changes seen on AMD.
- Young (6 month old) and aging (18m old) Cfh transgenic and control B6 mice were divided into groups and fed either a control diet or a high-fat diet for 5 months.
- Brains of the mice were collected after perfusion with 4% paraformaldehyde and post-fixed overnight in the same fixative. After the brains were collected, they were weighed and post fixed overnight in the same
- Brain sections (30 µm thickness) were obtained utilizing a Leitz sledge microtome with a freezing stage.
- Immunohistochemistry was then performed on the sections with antibodies specific for inflammatory, oxidative stress, and microglial markers. The specific antibodies that successfully stained and that were used for statistical analysis are listed below.
  - Anti-Iba1, Rabbit 1:100, Wako Chemicals USA, Marker of Microglia
  - Anti Biotinylated TREM2, Rabbit 1:50, Bioss USA, Marker of Microglial Activation
- Stained images were visualized using a Carl Zeiss fluorescence microscope with an objective lens of 10x. To ensure a similar anatomic location for all samples, the dentate gyrus region was photographed and analyzed.
- Using samples stained with the anti-Iba1 and biotinylated anti-TREM2 antibodies, the number of microglia over the photographed field were counted and averaged in order to obtain a cells/field value for each mouse subgroup.
- Finally, statistical analysis was performed on the data in order to determine the correlation between the number and activation of microglia in the dentate gyrus and the age, genotype, and diet of the mice.

CONCLUSION

CfhTg mice have reduced brain weight compared to age-matched B6 mice. There was no difference in the number of Iba-1+ microglia in Tg vs. B6 brains. However, the number of cells staining positive for TREM2, a marker of microglial activation, showed a trend towards being increased in Cfh transgenic vs. B6 mouse brains. A decrease in brain mass, combined with an increase in microglial activation may suggest that Cfh variants may be associated with increased oxidative stress and tissue injury in the brain. More brains will be analyzed to determine if these findings can be corroborated. Alternative methods of tissue collection will be explored in order to maximize the antigen recognition by antibodies. Also, additional commercially available antibodies against amyloid beta, malondialdehyde and nitrotyrosine will be tested.

REFERENCES

1. Hoffman G, Le W, Sita LV. The Importance of Titrating Antibodies for Immunocytochemical Methods. Current Protocols in Neuroscience. 2008;Unit 2.12

2. Kelly J. Complement System. 2008. Immune System. National Institutes of HealthWeb. 5 Jan 2014.

3. McGeer EG, Klegeris A, McGeer PL. Inflammation, the complement system and the diseases of aging. Neurobiol Aging. 2005;26 Suppl 1:94-7

4. Mooijaart SP, Koeijvoets KM, Sijbrands EJ, Daha MR, Westendorp RG. Complement Factor H polymorphism Y402H associates with inflammation, visual acuity, and cardiovascular mortality in the elderly population at large. Experimental Gerontology. 2007;42 (11): 1116–22.

5. Ufret-Vincenty RL, Aredo B, Liu X, McMahon A, Chen PW, Sun H, Niederkorn JY, Kedzierski W. Transgenic mice expressing variants of complement factor H develop AMD-like retinal findings. Invest Ophthalmol Vis Sci. 2010 Nov;51(11):5878-87.

6. Zetterberg M, Landgren S, Andersson ME, Palmér MS, Gustafson DR, Skoog I, Minthon L, Thelle DS, Wallin A, Bogdanovic N, Andreasen N, Blennow K, Zetterberg H. Association of complement factor H Y402H gene polymorphism with Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet. 2008;147B(6):720-6

7. Zipfel PF, Misselwitz J, Licht C, Skerka C. The role of defective complement control in hemolytic uremic syndrome. Semin. Thromb. Hemost. 2006;32 (2): 146–54.