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TITLE: **The Effect of Age on the susceptibility and Severity of Demyelination**

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14. ABSTRACT <u>Purpose:</u> The purpose of this investigation is to determine if aged mice are more susceptible to the induction of EAE an animal model for MS. <u>Scope:</u> EAE was induced in a cohort of mice (8 weeks old) which is the usual age for induction of chronic experimental allergic encephalomyelitis (EAE), an animal model for multiple sclerosis. In addition cohorts of 2 aged C57Bl/6 mice (16 months,18 months and 20 months) were induced for the disease. A control mouse for each age was injected with all regents except for the myelin protein required to induce disease, myelin oligodendrocyte protein. The animals were then evaluated for the onset and severity of disease. <u>Major Findings:</u> None of the controls got sick up to 14 days after the initiation of EAE as expected. The onset of disease in the control cohort was at the expected time (9 days) after the initiation of EAE. However, on average the onset of EAE in all the aged animals occurred 2 to 6 days after the onset of EAE in the control group. The peak clinical disease score was 2.5 for the control group while in every aged cohort the clinical scores were 0.5 to 1.5 more severe. This preliminary data indicates that with increasing age EAE disease onset is later and more severe.					
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Introduction

Subject: The subject of this investigation is the effect of age on the onset and severity of EAE an animal model. Based on recent observations (Shepherd et al, 2010) of decreased paranodal integrity with age we believe that increased age and subsequent loss of the myelin-axon integrity may account for the increased severity and susceptibility to progressive MS with increasing age. We propose that decreased paranodal stability as evidenced by fewer transverse bands which anchor myelin to the axon makes the aged CNS more susceptible to the loss of myelin leading to a progressive clinical course, demyelination and subsequent axonal loss. This same scenario should be replicated in the animal model of MS which is the subject of this investigation.

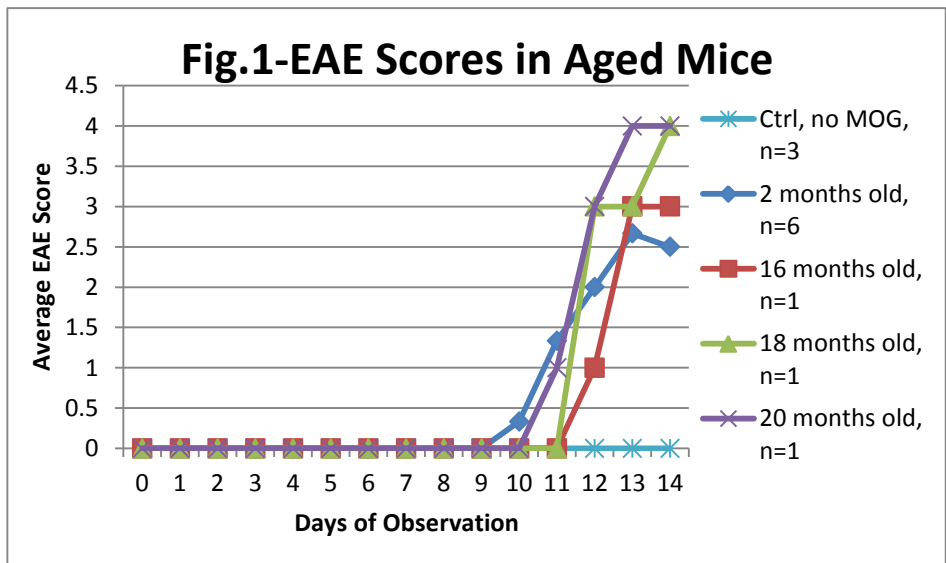
Purpose: The purpose of this investigation is to evaluate the effect of age on the onset and severity of EAE an animal model for MS.

Scope: We induced EAE in cohorts of the normal aged mice used for these studies (2 months) as well as cohorts of animals aged 16 months 18 months and 20 months followed by a determination of the onset and severity of disease in the “old” and normal aged cohorts.

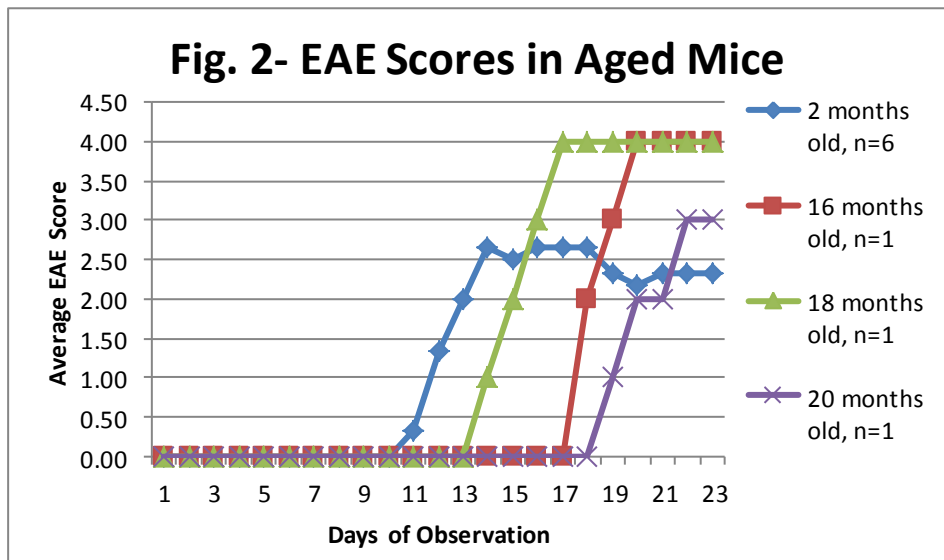
Body:

I have applied for and obtained a one year extension of this grant; the studies we have done to date are preliminary and not publishable. This is because the aged mice are often difficult to obtain and difficult to maintain but recently the flow of aged mice has improved and

we will have publishable results soon. Therefore this should not be viewed as the absolute final report but a report of the progress to date. As previously noted, we induced EAE in 2 mice of each aged cohort except for the controls which we used 6 mice to be sure we had an accurate baseline. The clinical scores are shown in the graphs below.



Note that the 2 month old animals began to get sick on day 10 and reached a maximum score of 2.5. In contrast the 16 month old did not get sick until day 12 while the 18 and 20 month old animals got rapidly sick on day 12. What is particularly impressive is how rapidly the older animals (18 and 20 months) became sick quickly reaching stage 4 in a day or two while the youngest of the “old” animals reached a score of 3 over a period of 2 days. This is in stark contrast to the 2 month old animals which took 4 days to reach their peak stage of disease.



The same trends evident in Figure 1 are also seen in Fig.2. In this case the onset of disease was even more delayed relative to a 10 day onset in the 2 month old mice. Note that there was a 4 day delay in the case of the 18 month old mouse, an 8 day delay in the case of the 16 month old mouse and a 9 day delay in the case of the 20 month old mouse. Once again the final clinical state relative to the 2.5 for the control 2 month old mice was 4 for both the 18 month old and the 16 month old mouse and 3 for the 20 month old mouse. Obviously to be confident of these trends the experiment must be repeated with more animals. We are

currently evaluating the morphology of the EAE-affected aged mice by EM. Preliminary observations suggest a more pronounced demyelination and immune infiltration in the older mice relative to the younger control mice (2 month old).

Key Research Accomplishments:

- Older animals have a delayed onset of disease
- Older animals have a more rapid course of disease
- Older animals rapidly progress to a more severe clinical score
- Older animals have a more pronounced demyelination

Reportable outcomes:

There are no reportable outcomes to date as further experiments to verify initial observations are currently in progress.

Conclusions:

Our initial hypothesis that older animals would get “sicker quicker” has not been experimentally verified. Instead the older animals got sick at a later time point relative to younger animals. However the final severity of the disease and the rate at which the animals progressed to this more severe clinical status was much more rapid than with the younger animals that did not get as sick. It also took the younger animals a greater period of time to reach their final clinical state.

References:

Shepherd MN, Pomicter AD, Velazco CS, Henderson SC, Dupree JL (2010) Paranodal reorganization results in the depletion of transverse bands in the aged central nervous system. *Neurobiol Aging*. 2010 Jan;33(1):203.e13-24. doi: 0.1016/j.neurobiolaging.2010.08.001. Epub 2010 Oct 2

Appendices: None