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Early Host Responses to Prion Infection: Development of In Vivo and In Vitro Assays

George A. Carlson, Ph.D., Principal Investigator

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INTRODUCTION

A misfolded form of prion protein (PrP^{Sc}) is the functional component of infectious prions and is derived posttranslationally from a benign cellular isoform of unknown function (PrP^C). A long incubation time is a hallmark of prion disease and during this preclinical phase of infection prion replication occurs without any obvious harm to the organism or to the infected cells. Advances in genomics and proteomics provide the opportunity to search for specific patterns of change in gene and protein expression that occur after prion infection. We hypothesize that a specific constellation of changes in mRNA and protein expression will prove to be a more sensitive indicator of prion exposure than current assays exclusively focussed on PrP. We are measuring changes in mRNA in blood cells and in serum glycoprotein concentrations that are induced by prion infection in mice. A prion-specific signature would form the basis for a blood-based screen for prion infected individuals. We also are exploring mouse CNS stem cell-containing neurospheres cultures as a genetically tractable model for prion infection. Neurosphere cultures or their derivatives may be capable of supporting prion replication and serve as a test system for infectious prion particles.

BODY

Progress towards completing each specific sub-task in the Statement of Work are indicated in **bold type** below:

Task 1. Determine whether there are specific changes in mRNA and protein expression profiles in the blood of prion-infected mice. (Months 1-30)

- a. Expand our colonies of mice to provide sufficient numbers of C57BL/6J, B6.I-1, FVB-*Prnp*^{tm1}, (FVB x FVB. *Prnp*^{tm1})F1, FVB/NCr, and FVB-Tg(MoPrP-A)B4053 to provide sufficient numbers for the experiment. There will be 14 groups of mice with 20 mice per group—a total of 280 mice. (Months 1-6) **Successfully completed.**
- b. Establish reproducibility of mRNA isolation from blood cells and glycoproteins from serum using 10 inbred C57BL/6J mice. (Months 1-6) **Successfully completed.**
- c. Inoculate these genetically defined mice with one of two different prion strains and bleed (200 μ l) at regular intervals (7, 14 or 28 days) throughout the pre-clinical incubation period and after clinical signs appear. (Months 6-24) **In Progress.**
- d. Perform DNA microarray and serum glycoprotein analyses, analyze the data and determine whether there are expression profiles unique to each host-agent combination and/or whether a genotype-independent and agent-independent profile specific to prion exposure can be detected. Each timepoint will consist of 4 replicates per group. (Months 6-36) **In Progress.**
- e. Changes in mRNA expression suggestive of specificity for prion infection will be re-evaluated using larger volumes of blood pooled from relevant groups of mice. Up to 200 mice will be available for this purpose. (Months 12-36) **In Progress.**

Task 2. Using existing mouse neurosphere lines, determine whether CNS stem cells can provide an in vitro model for prion infection.

- 1) Import and establish cultures of mouse CNS neurosphere lines provided by our collaborators at StemCells, Inc. and the Salk Institute. (Months 1-2) **Successfully completed.**
- 2) Assess levels of PrP^C expression by immunoblotting and immunofluorescence in the neurosphere lines in comparison to N2A cells that can be infected with prions. (Months 2-8) **Successfully completed.**
- 3) Infect PrP^C-expressing neurosphere lines in culture with RML scrapie isolate. Assess viability, proliferation and differentiation at each passage (approximately every 2 weeks). Harvest cultures, prepare protein isolates and determine whether proteinase K-resistant PrP^{Sc} increases over the amount present immediately after infection. (Months 6-24) **In progress.**
- 4) Determine whether prions are propagated in infected neurosphere cultures by incubation time analysis in mice. Up to 250 mice will be available for these studies. (Months 12-36). **In progress.**
- 5) Using mRNA expression profiling and quantitation of secreted or released glycoproteins identifies a subset of prion-specific changes identified in blood. (Months 18-36). **Not yet initiated.**

Task 1. Over 140 expression arrays have been run at the Institute for Systems Biology on RNA from peripheral blood of mice infected with either the RML or 301V strains of scrapie at McLaughlin Research Institute. Preliminary studies optimized methods of RNA isolation to ensure reproducibility of standardized reference RNAs. Results to date have not revealed a consistent signature indicative of prion infection at early time points after inoculation. However, as clinical illness approaches, approximately 80 differentially expressed genes were detected, indicating the feasibility of identifying changes in blood cells. To enhance sensitivity and our ability to detect subtle changes in gene expression in presymptomatic animals we have switched from arrays constructed at ISB to Affymetrix chips. Preliminary results are encouraging. We have also decided to examine changes in blood white cell populations with time after infection by flow cytometry. We also are piloting preparing RNA from enriched populations of peripheral blood cells to detect changes that would be masked in the mixed population.

Task 2. We have demonstrated by several techniques that mouse CNS stem cell or neurosphere cultures express levels of PrP^C comparable to those of the infectible N2A cell line. We have extended these studies beyond using the existing cell lines proposed in the application to creating CNS stem cell cultures from different strains of mice and from transgenic mice expressing high levels of PrP^C. Cell lines from Tg4053 mice, which express high levels of mouse PrP, can be infected and replicate scrapie prions. This result is very exciting and indicates the feasibility of developing sensitive assays for infectious prions from other animals including human, cow, deer, and elk using cell lines from transgenic mice expressing PrP transgenes from these species. Preliminary results using cell lines from non-transgenic mice also are encouraging and suggest that prions can replicate in CNS stem cells expressing normal levels of PrP. This would provide the opportunity to use cell lines derived from various species to directly test prion susceptibility.

KEY RESEARCH ACCOMPLISHMENTS

- Optimized RNA isolation to ensure reproducibility on microarrays
- Ran and analyzed over 140 peripheral blood RNA samples for differential gene expression
- Demonstrated differential gene expression in peripheral blood of clinically ill mice
- Optimized procedure for analysis of differential expression of plasma glycoproteins and began time course studies
- Demonstrated differences in glycoproteins in plasma of clinically ill mice
- Established CNS stem cell cultures from fetal brain of six different lines of mice
- Demonstrated that CNS stem cell cultures express PrP^C
- Demonstrated production of PrP^{Sc} in a CNS stem cell line from a transgenic mouse expressing high levels of PrP, indicating the potential for a tissue culture assay for infectious prions.

REPORTABLE OUTCOMES

In addition to the cell lines outlined above, two abstracts have resulted from research supported by this award. The abstracts are included with this report.

CONCLUSIONS

Based on our results to date, we are confident that when the study is complete we will be able to conclude whether there are signature differences in gene expression or plasma glycoproteins in presymptomatic, prion-infected animals that can be used to identify prion infected individuals.

Our most exciting result is that mouse CNS stem cell cultures appear to replicate scrapie prions and have potential as an assay for infectious particles.

APPENDICES

Two abstracts from the 9th International Conference on Alzheimer's Disease and Related Disorders are attached.



The 9th International Conference on
Alzheimer's Disease and Related Disorders
Presented by the Alzheimer's Association
July 17-22, 2004

Abstract Submission Site

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Use of Expression Arrays to Prioritize and RNAi to Test Prion Incubation Time Modifier Gene Candidates

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Background: Although the most dramatic control of prion incubation time in the mouse is exerted by alternative alleles of the prion protein (PrP) gene, other loci strongly influence the length of the interval between prion inoculation and onset of disease. Crosses between CAST/Ei and SJL/J mice, which share PrP gene coding sequences but differ in incubation time, and quantitative trait analysis were used to identify to regions on Chromosomes 9 and 11 that harbor prion incubation time modifier genes. **Objective:** The goal is to identify the genes within these chromosomal intervals that underlie differential prion susceptibility. **Method:** Differences in activity of allelic gene products are responsible for the majority of quantitative trait loci and some activity differences may reflect level of mRNA expression. SJL strains congenic for either the Chromosome 9 or Chromosome 11 interval from long incubation time CAST mice were produced by backcrossing and marker-assisted selection. Microarray analysis is used to compare gene expression in brains and spleens of scrapie prion-infected and non-infected congenic pairs. Differentially expressed genes mapping within the congenic intervals or active in pathways involving genes in these intervals are modifier gene candidates. The scrapie-infected neuroblastoma cell line ScN2a provides a substrate to test whether lowering expression of a candidate gene reduces production of the disease-specific PrP^{Sc} isoform. **Results:** The effectiveness of RNA interference (RNAi) was demonstrated by the ability of anti-PrP constructs expressing fluorescent protein as an indicator of transformation and a hairpin substrate for production of small inhibitory RNAs to suppress expression of PrP as detected by immunofluorescence. Such RNAi

Sc

directed against PrP also suppressed production of proteinase K-resistant PrP by ScN2a cells. **Conclusions:** RNAi provides sensitive means to evaluate the effects of reduced gene expression on prion replication. (Supported by NINDS program project grant NS41997. R.K is the recipient of Department of Defense National Prion Research Career Transition Award.)
Author Disclosure Block: R. Kumar, None.

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Presentation Preference : Poster

Topic (Complete): Related Neurodegenerative Conditions - Other Prion Disease

Keyword (Complete): prion proteins ; gene expression ; gene mapping

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We encourage you to submit your abstract to both the Imaging Consortium and the International Conference.

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The 9th International Conference on
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Presented by the Alzheimer's Association
July 17-22, 2004

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Expression of Prion Protein in Mouse CNS Stem Cells and their Progeny

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Background: Prion replication involves posttranslational conversion of the normal cellular isoform of prion protein, PrP^C, to the disease-specific PrP^{Sc} isoform. Much of our knowledge of the cellular mechanisms of prion replication comes from only a few cell lines, such as ScN2a, that are capable of supporting scrapie prion infection. Although such in vitro culture systems are valuable, they are poorly suited for studies on the mechanisms of genetic susceptibility to prion disease or prion pathogenesis. CNS stem cell lines can be isolated easily from fetal brain and maintained as neurospheres. If CNS stem cell lines or their differentiated progeny prove capable of supporting prion replication, they would provide an in vitro system for dissecting the mode of action of prion disease modifier genes. **Objective:** Replication of scrapie prion infectivity requires expression of PrP^C. The goal of this study was to analyze expression of PrP^C in neurospheres isolated from a variety of inbred mouse strains and transgenic mice. **Methods:** Fetal brain cells (E15) were isolated and cultured in N2-media supplemented with EGF, FGF2, LIF and N-acetylcysteine to generate neurospheres. Fluorescent antibodies against nestin, vimentin and CD-24 were used as markers for putative CNS stem cells, while antibodies against MAP2 and GFAP identified differentiated neurons and astrocytes. Coexpression of these antigens and PrP was detected with polyclonal (R073) and monoclonal (D13, D18) antibodies against PrP. **Results:** Almost all cells that were strongly positive for CNS stem cell markers also expressed PrP. PrP positivity was maintained in cells with reduced expression of stem cell markers and low expression of MAP2 or GFAP. Cells strongly positive for the neuron marker MAP2 expressed high levels of PrP, while GFAP-positive astrocytes were only weakly stained with anti-PrP antibodies. **Conclusion:** PrP is abundantly expressed in neurosphere cultures. Studies to

determine whether these cultures can support prion replication are underway. If CNS-stem cells prove susceptible to prion infection, they could provide a means to test prion susceptibility in other species, including humans and bovines. (Supported by NINDS program project grant NS41997 and by a grant from the National Prion Research Program of the Department of Defense.)

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