HUMAN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES: A CRITICAL SCIENTIFIC INVESTMENT

Final Report
of the
National Prion Research Program

US Army Medical Research and Materiel Command
Congressionally Directed Medical Research Programs

Fort Detrick, Maryland

2007

Reviewers

The *Final Report of the NPRP* has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this review is to provide candid and critical comments that will assist this organization in making its report as sound as possible and to ensure that the report meets the standards for objectivity, evidence, and responsiveness. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

Dr. Roger Dodd
Dr. Bruno Oesch
Dr. Suzette Priola
Dr. Robert Will

Although the reviewers listed above have provided many constructive comments, they were not asked to endorse the recommendations. Responsibility for the final content of this *Final Report of the NPRP* rests with the Congressionally Directed Medical Research Programs.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MD 21702-5012

REPLY TO
ATTENTION OF

JAN 2008

Office of the Commanding General

Dear Colleagues:

It is my pleasure to acknowledge the work of the Congressionally Directed Medical Research Programs (CDMRP) in leading the way for the Department of Defense (DOD) in prion science. Their planning and execution of the National Prion Research Program (NPRP) was critical in responding to the need to expand the scientific foundation of Transmissible Spongiform Encephalopathies (TSEs). This *Final Report of the NPRP* will serve as a synopsis of the U.S. Army Medical Research and Materiel Command's five-year effort to provide a framework for advancing prion science and identify key projects in this scientific portfolio that would reap the greatest investment in the detection, prevention and treatment of prion diseases.

First, I commend the Institute of Medicine's (IOM) Committee on TSEs: Assessment of Relevant Science for delineating the critical issues that impact basic biomedical research on TSEs. Informed by the IOM's guidance, the CDMRP created a comprehensive scientific plan, prioritized the DOD's research needs, and set a path for progress with the vision to eliminate the occurrence of human TSEs. The CDMRP's vision and deliberate execution of their strategic plan has resulted in the success of the NPRP. Second, I extend my deepest appreciation to the scientists and investigators whose dedication and selfless commitment to their research endeavors have significantly contributed to unlocking the mysteries in this perplexing disease. Their incredible work in this field has extended the evidence for preventing, mitigating and treating prion diseases. Finally, I congratulate the 2007 NPRP Summit participants for assessing the current state of the science in prion disease and making insightful recommendations for future scientific investments. The Summit was the culmination of the NPRP and demonstrated that scientific-technical partnerships are essential to producing cutting-edge basic and applied research.

The *Final Report* summarizes the accomplishments of the funded investigators, delineates ongoing knowledge gaps, and informs decision-makers of future research needs. Continued support of these scientific efforts will allow the maintenance and leveraging of the momentum gained by the initial investment in critical scientific research on the nature of prions. This support will ensure fulfillment of the program's goal to reduce the threat of human TSE infection.

Sincerely,

George W. Weightman
Major General, Medical Corps
Commanding General

US Army Medical Research and Materiel Command
Congressionally Directed Medical Research Programs

National Prion Research Program

Vision: eliminate the occurrence of human transmissible spongiform encephalopathies.

Mission: develop a diagnostic test to detect the presence of prion disease.

Introduction

The Congressionally Directed Medical Research Programs (CDMRP) emerged from heightened public awareness, and increased interest in the community concerning various health issues. These efforts convinced Congress to direct the Department of Defense to manage programs that focus on specific diseases. A unique partnership, forged among the public, Congress, and the military, led to the establishment of CDMRP within the US Army Medical Research and Materiel Command (USAMRMC) in fiscal year 1993 (FY93) to manage these programs. CDMRP has grown to encompass multiple targeted programs and has received $4.36 billion in appropriations through FY07.

During FY02, concerns over potential contamination of the food and blood supply prompted the U.S. Congress (Joint Appropriations Conference Committee Report No. 107-350) to provide the largest single appropriation in history for research on prion disease. The National Prion Research Program (NPRP) was established with $42.5 million from Congress. The FY02 Senate Appropriations Committee Report 107-109, pages 148-149, also stated that the "priority goal… is to rapidly develop a diagnostic test to detect the presence of prion disease." The Congressional language recognized that there was "currently no test to confirm the presence of the disease" and that this disease "represents a significant threat to the U.S. population." The direction from Congress was to create a coordinated effort with other Federal agencies in an attempt to develop complementary programs of research and avoid duplicative activities. This influx of funding provided the funds to support established investigators as well as those new to the field to collectively focus on understanding prion disease and developing the components of diagnostic tools. With this increased funding, there was hope of leveraging knowledge to answer the many questions associated with this disease.

This *Final Report of the NPRP* examines the accomplishments of the NPRP with respect to the guidance set by Congress and in response to the recommendations made by the Institute of Medicine's Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science. This report discusses the results of the October 2007 NPRP Summit that was convened by CDMRP to address ongoing critical knowledge gaps in the development of ante-mortem diagnostic tools for prion disease and to explore future courses of action for achieving the goal of having a diagnostic test for the detection of prion disease. The *Final Report* includes conclusions made by the Summit participants regarding the state of the science and recommendations to inform decision makers regarding future policy.

Overview of Prion Diseases

Transmissible spongiform encephalopathies (TSEs) are a group of related diseases including Creutzfeldt-Jacob disease (CJD) and its new variant (vCJD), kuru (all three affect humans), scrapie in sheep and goats, bovine spongiform encephalopathy (BSE; "mad cow disease"), and chronic wasting disease (CWD) in deer and elk. These diseases have long incubation periods of years or decades, cause progressive neurological degeneration, evoke no obvious inflammation or immune response, and are invariably fatal. The current disease theory attributes TSEs to prions, normal cell membrane proteins that can form atypical three-dimensional configurations. Upon infection, the incoming misfolded prion proteins (PrP^{Sc}) trigger a cascade converting

normal prion protein (PrP^C) or a partially unfolded PrP^C intermediate arising from normal conformational fluctuations to a misfolded form, PrP^{Sc}. The misfolded prion proteins aggregate in the central nervous system and lead to the symptoms of prion diseases. The initiation of the first prion protein misfolding is unknown, but one model proposes that the conformational change of PrP^C to PrP^{Sc} is thermodynamically controlled with PrP^C conformation favored at equilibrium. PrP^{Sc} is favored when it adds onto a seed or fibril-like aggregate of PrP^{Sc} proteins. This model predicts that fibril-like prion aggregates can replicate by fragmentation. Thus, transferring misfolded prion proteins to a new host by blood transfusion, tissue transplantation, contact with contaminated surgical instruments, or tissue ingestion can transmit the disease. In sporadic prion diseases, the origin of the prion protein misfolding is unknown. In familial prion diseases, several heritable mutations in the prion protein gene lead to accumulation of misfolded prion protein and subsequent disease manifestations.

Scrapie, a prion disease of sheep, has been recognized for centuries, and it was historically controlled by culling infected animals. When prion disease in cattle (BSE) was identified in the 1980s, it was also controlled by culling infected animals. Prion diseases in humans are relatively rare. Incidence of sporadic CJD (sCJD) in humans is about 1 in 1 million; about 10% of all human TSE cases are familial (i.e. about 1 in 10 million). In 2003, it was estimated that iatrogenic transmission through dura mater or corneal transplants, pituitary hormones, and instruments used in neurosurgery contaminated with prion-infected material had resulted in 250 cases of CJD worldwide. Modification of surgical procedures has eliminated or significantly limited this mode of transmission. Transmission of the human prion disease kuru by ingestion of brain tissue was recognized in the 1950s, and cessation of ritual cannibalism eliminated this mode of transmission. In all of the above examples, transmission is from human to human, or intraspecies transmission.

The potential impact of prion diseases on human health was greatly magnified by the recognition that interspecies transfer of BSE to humans by beef ingestion resulted in vCJD. While changes in animal feed constituents and slaughter practices appear to have curtailed vCJD, there is concern that CWD of free-ranging deer and elk in the U.S. might also cross the species barrier. Thus, consuming venison could be a source of human prion disease. Whether BSE and CWD represent interspecies scrapie transfer or are newly arisen prion diseases is unknown. Therefore, the possibility of transmission of prion disease through other food animals cannot be ruled out. There is evidence that vCJD can be transmitted through blood transfusion. There is likely a pool of unknown size of asymptomatic individuals infected with vCJD, and there may be asymptomatic individuals infected with the CWD equivalent. These circumstances represent a potential threat to blood, blood products, and plasma supplies.

These threats to the food and blood supplies are serious because the incubation time for prion diseases is so long and there is no reliable ante-mortem diagnostic test, *i.e.,* a test that can be performed to detect the disease while the person or animal is alive. Definitive diagnosis can be only made by examining central nervous system tissue at autopsy. Prions do not elicit an obvious immune response, so detection by measuring an immune response in the host has not been possible. Developing a diagnostic test to detect prions in peripheral tissues is dependent on surmounting two major impediments: (1) PrP^{Sc} has exactly the same amino acid sequence as PrP^C and (2) PrP^{Sc} represents only 0.001% of the total prion protein in an affected host.

National Prion Research Program

Background

Health threats posed by prion disease appear to involve the food and blood supplies and put military beneficiaries in affected areas overseas at risk. The 1985 outbreak of BSE in cattle in the United Kingdom placed overseas servicemen and women and their families who consumed beef from domestic cattle at a higher risk than those who were stationed stateside, where BSE had not occurred previously. Since then, the areas of concern have expanded to include any location in which cattle have been diagnosed with prion disease and anywhere beef may have been shipped from these areas for human consumption. People who have ingested contaminated beef may unknowingly transmit prions via blood donations. Concerns over potential contamination of the food and blood supply prompted the US Congress to provide the largest single appropriation in history for prion-related research, $42.5 million to CDMRP in FY02. Under the direction of the CDMRP, the DOD NPRP was established by Congress to address the significant threat posed by prion disease for humans and animals. The priority of the NPRP, as delineated by the Senate Appropriations, was to rapidly develop an ante-mortem diagnostic test, *i.e.*, a test that can be performed to detect the disease while the person or animal is alive, to detect the presence of prion disease. See Appendix A for a list of abbreviations used in this report.

Program Execution

The NPRP is managed through the USAMRMC, Office of the CDMRP. The program was designed according to the CDMRP's successful research model. A USAMRMC Steering Committee was convened to address transmissible spongiform encephalopathy-related issues specific to military missions and to support the NPRP based on the USAMRMC's experience in infectious disease detection and diagnosis. A stakeholders' meeting was held in which military, scientific, regulatory, industry, and public health participants provided input on the major issues in prion research. Based upon the stakeholders' recommendations, an Integration Panel (IP) (composed of prion experts from the military, scientific, regulatory, industry, and public health communities) was formed to determine the vision and investment strategy for the NPRP.

To assist the DOD in their execution of the funding and administration of the NPRP, the IOM formed the Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science to provide guidance to the CDMRP for the NPRP. In response to a request from USAMRMC, the IOM released an interim report on *Advancing Prion Science: Guidance for the National Prion Research Program* in January 2003, and the final report in November 2003, as delineated in Appendix B. The report delineated 26 recommendations for prion research in seven areas: (1) basic research, (2) diagnostics, (3) blood testing, (4) surveillance, (5) prevention and treatment, (6) research infrastructure, and (7) military health.

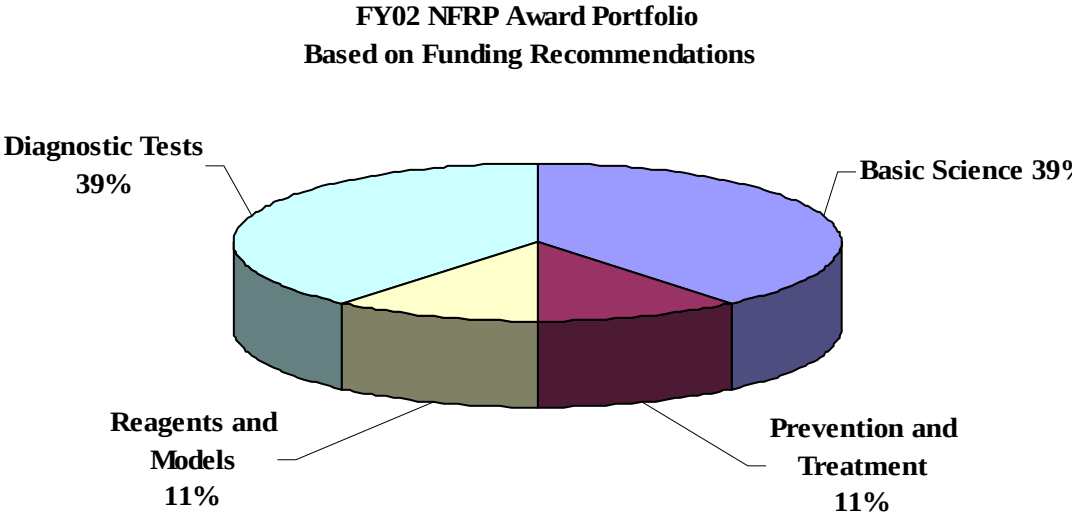
The IOM noted that it considered all recommendations important, but recognized that limited resources for prion research necessitated funding priorities. Appendix B provides the entire list of recommendations with the highest priorities highlighted. The recommendations by functional

area were assigned to one of three priorities, with 1 being for the most critical TSE research needs, based on the following criteria:

- Impact on public health
- Impact on protecting animal (especially cattle) health
- Impact on protecting the U.S. economy (BSE/CWD)
- Potential for scientific breakthroughs
- Support for stepwise progression of prion science
- Return on investment
- Feasibility of success

The NPRP Portfolio

Review of solicited proposals was conducted according to the two-tier review model recommended in 1993 to the USAMRMC by the National Academy of Sciences Institute of Medicine (IOM) (IOM. 1993. Strategies for Managing the Breast Cancer Research Program: A Report to the U.S. Army Medical Research and Development Command, IOM Committee to Advise the Department of Defense on Its Fiscal Year 1993 Breast Cancer Program. Washington, DC: National Academy Press.) Research areas of emphasis were supported by an interim IOM report requested by USAMRMC. In response to a Program Announcement (a request from the DOD for research proposals to study prions) released in August 2002, 136 submissions were received. NPRP funded 38 of those submissions supporting research focused on improving diagnostic testing; performing basic science research; developing prion research infrastructure in the area of reagents and models; and studying prevention and treatment approaches to limiting risks to the military (see chart below).

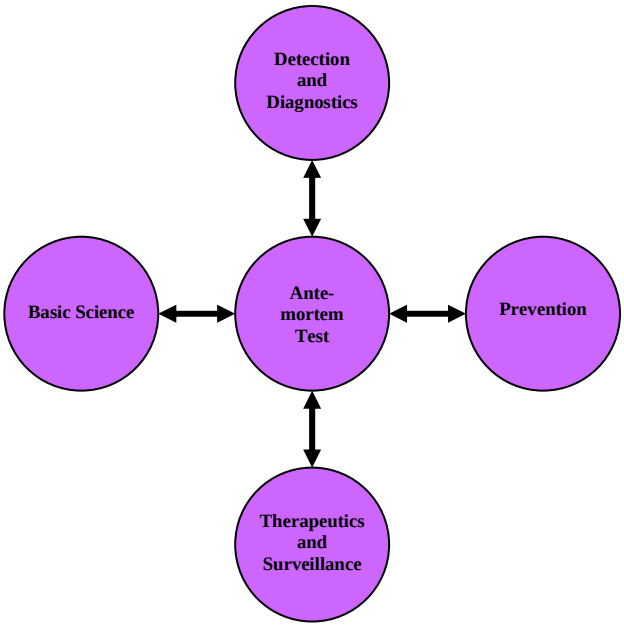


The 38 funded proposals recommended for funding by members of the NPRP Integration Panel represented an exceptionally broad range of projects bringing new methodologies and additional expertise to the basic biology (understanding of the nature and symptoms of the disease that will facilitate the science of diagnostic testing.), detection, prevention, and treatment of prion diseases. Fifteen awards were directed toward the development of sensitive and reproducible ante-mortem diagnostic tests and/or the development of new biomarkers for the disease. A wide range of detection platforms and methodologies were used including new immunological, physical and chemical detection methods. Four awards were directed toward developing experimental models to facilitate more sensitive and rapid detection of the disease agent in living systems. The development of tests that could detect prion in blood or tissues directly supported the goal of assessing risk to the military. Fifteen awards were made for research in basic science focusing on biology, epidemiology, and etiology of prion diseases. Four studies were funded that focused on methods to prevent infection or progression of prion disease. The studies included developing prion-resistant instruments, vaccines, inhibitory RNA molecules, monoclonal antibodies and traditional drug screens

All of these awards provided important insight into methods for the detection, treatment and prevention of prion diseases. Awardees demonstrated innovation in their approaches and collaboration through academic and industry partnerships. The work within and between basic science, detection and diagnostics, prevention, and therapeutics and surveillance for prion diseases will further the progress toward achieving a sensitive and reproducible ante-mortem prion test. Of the 38 awards, 4 were career transition awards, 17 were idea awards, 16 were investigator–initiated awards, and 1 was a prion techniques fellowship award. The 38 award recipients began their research in mid-2003, with performance periods ranging from 3 to 5 years. See Appendix C for a list of the award recipients.

2005 NPRP Investigators Meeting

The CDMRP hosted the NPRP Investigators Meeting on 8 and 9 December 2005 to present results of DOD-funded studies and assess progress of the program. The meeting was successful in bringing together all funded investigators to share their results and exchange knowledge and ideas that could lead to novel approaches to difficult research problems. Investigators were studying a variety of prion diseases to include: CJD, BSE, scrapie, and CWD. Presentations at this meeting as well as recent publications by NPRP-funded investigators demonstrated that the investment in the NPRP has had a significant impact in expanding the prion research community, addressing the primary goal of developing an ante-mortem diagnostic test and adding to the scientific knowledge of prion disease.



Real progress has been made toward the goal of developing an ante-mortem diagnostic test. Major impediments to prion detection in the peripheral tissue have been overcome. Although PrP^{Sc} exists in extremely low concentrations in some peripheral tissue, several investigators reported successful development of methods to concentrate and purify PrP^{Sc} from these tissues. Investigators reported on the development of detection methods that were both highly sensitive and selective for structural features specific to PrP^{Sc} or to PrP^{Sc} aggregates. Some detection methods are based on binding by monoclonal antibodies or aptamers, single-stranded nucleic acid ligands selected using an *in vitro* procedure termed systemic evolution of ligands by exponential (SELEX) enrichment. Others are based on spectrometric methods such as matrix assisted laser desorption/ionization (MALDI), liquid chromatography/electrospray, ionization Fourier transform mass spectrometry (FTMS), or tryptophan fluorescence.

Gene expression microarray analysis was used to identify a “genetic signature” that could be used as surrogate markers of prion disease. By using mouse model systems in which the disease course is well characterized, several investigators identified expression signatures as a well as

candidate biomarkers that were detectable before clinical signs or pathological changes developed. The direct identification of differentially-expressed proteins through mass spectrometry methods was also reported.

Several NPRP-funded investigators are examining prion protein structure through the use of mass spectrometry, fluorescence spectrometry, nuclear magnetic resonance, and monoclonal antibodies to specific conformational regions. These investigators have begun to identify regions of the prion protein required for aggregate formation, an important step in the disease process. One investigator has developed a theoretical model for aggregate formation based on prion trimers and incorporating domain swapping. This model is consistent with the remarkable temperature stability of the infectious prion structure and helps to explain the absence of PrP^{Sc} monomers.

NPRP-funded investigators studied interspecies transmission of CWD by comparative sequence analysis. This research has identified genotypic differences in prion protein that may affect susceptibility to CWD, as has been shown for scrapie. Identifying the effects of prion protein structural differences on infectivity for CWD may have applications to many prion diseases.

With the use of cell cultures, NPRP-funded investigators are studying prion disease. Murine hypothalamus cell lines are being used to study disease progression as well as to develop rapid *in vitro* infection assays. Cell lines can also be used to test prion disease prophylaxis and treatment. A mouse neurosphere cell line infected with mouse scrapie and a deer cell line persistently infected with CWD were developed in NPRP-funded projects. The latter cells are being used in *in vitro* testing for promising anti-TSE agents including pentosan polysulfate and porphyrins. At least one such porphyrin is shown in follow-up *in vivo* experiments to substantially increase survival times in mice intracerebrally inoculated with scrapie.

Funding also increased research resources to support scientists working in the prion field. One NPRP award supports a flock of sheep that are highly susceptible to scrapie, which can serve as a uniform source of infected material, allowing for standardization of detection assays among different laboratories. Prion research capacity building is supported by the funding of 15 new investigators in this field as well as 40 pre- and post-doctoral trainees, increasing the intellectual foundation of the prion research field.

The research supported by the NPRP has made a substantial impact on the primary goal of developing an ante-mortem diagnostic test and laid the groundwork for answering many associated questions with these diseases. See Appendix D for the abstracts presented at the 2005 December Investigators Meeting.

2007 NPRP Summit

Although an enormous amount of scientific progress in the understanding of prion diseases has been achieved through the NPRP, there remain many unanswered critical public health questions. The NPRP was structured to fund basic biomedical research with studies focusing on diagnostics, prevention, and therapeutics/surveillance research. (See Appendix E: NPRP Funded Investigators' Accomplishments by Year and Research Area for a list of studies.) As a capstone to the NPRP, the CDMRP hosted the 2007 NPRP Summit inviting participants from the international prion community. (See Appendix F: 2007 NPRP Summit Participants.) The goal of the NPRP Summit was to identify the gaps remaining since the original charge from Congress. The participants were to summarize the current state of prion science; identify the critical areas future prion research needs to address; identify what needs to be done for the prion research to move forward; and develop a plan for moving forward in developing ante-mortem diagnostic tests.

The NPRP Summit created a forum for exchange to address research efforts across the field. The Summit participants examined the current state of the science and the progress that has been made to date. The participants discussed the many challenges and roadblocks to discoveries that confront this science and its community of researchers. The Summit participants engaged in workgroup sessions to identify ways to build a stronger alliance among the community of prion researchers and presented recommendations for the way ahead in research. There were 4 workgroup sessions that were held concurrently. The workgroups were organized to review the current science in Basic Biology (Workgroup 1), Detection & Diagnostics (Workgroup 2), Prevention (Workgroup 3), and Therapeutics & Surveillance (Workgroup 4). The reports from each of the workgroup sessions were summarized by the moderators. (See Appendix G: 2007 Summit Agenda.) Recommendations were generated from the workgroups as well as from questionnaires.

Basic Biology (Workgroup 1)

Dr. Byron Caughey, NIH, NIAID, Rocky Mountain Laboratories, Hamilton, MT, was the moderator for the Basic Biology workgroup.

Although progress has been made, the function of normal prion protein (PrP^{C}) remains elusive. More work is needed to understand which of the many diverse ligands that bind PrP^{C} contribute to its function. PrP^{C} may have different functions in different cells (e.g. neuronal versus non-neuronal cells) and researchers still need to formulate a unifying principle of prion protein function if possible. Understanding the normal function of PrP^{C} is essential for understanding how it may contribute to the neurotoxicity and neurodegeneration that are hallmarks of TSE disease. Techniques to discriminate between normal, infectious and/or neurotoxic forms of prion protein are needed. Also needed are more targeted, sophisticated assays to determine neurotoxicity.

The precise nature of the infectious TSE agent needs to be better defined given that the relationship between abnormal prion protein (PrP^{Sc}) and infectivity remains poorly understood. Major issues include deciphering the diversity of PrP^{Sc} structures and determining which prion protein forms are most significant in terms of TSE infectivity and neurotoxicity. Are different

forms of PrP^{Sc} the same functionally in different tissues, such as the blood or the brain? This issue is particularly important for the development of TSE specific, sensitive diagnostics. Higher resolution structural information is still needed to understand the pathogenic (disease-causing) prion protein forms. There is a need for further practical structural analysis of the disease-associated prion protein (e.g. NMR, X-ray crystallography etc.) as well as theoretical computational model building which is a new, unexplored tool.

The workgroup agreed that there is a need to further study the evidence that synthetic recombinant prions have been generated in vitro. The role of other molecules in TSE infectivity remains unresolved. There are cofactors believed to be involved in prion disease, including polyanionic molecules such as nucleic acid. The polyanion requirement associated with prions is an area that needs particular focus. A minority opinion was expressed that a nucleic acid as a viral component may have a role in prion disease; however, the consensus opinion was that the most recent data do not support a replicating virus in TSE infection.

Tremendous progress has been made in understanding how prions replicate and spread in cells in vitro. There is also progress in developing cell culture assays for studying different species of TSE infectivity. However, further work is needed to understand the underlying mechanisms of the disease process including where prions reside in a cell, and how different strains and species of prions interact with cells. The basis of prion strains, diversity of conformations, and stability against fragmentation and degradation (different half lives) is important and requires more research..

How different strains of prions spread naturally within a host and between species is only partly understood and remains a critical issue for controlling the spread of prions to humans. How prions may be spread through the environment needs further study; the mechanisms of transmission may be different for different animal species.

The role of the immune system in prion disease is not well understood. It is not known whether innate immune mechanisms are involved, or if prions can be cleared naturally from infected tissue.

Recommendations from Workgroup 1

- **Develop a clearer understanding of the role played by the normal prion protein and its associated ligands.** If researchers had a better understanding of how normal prion protein functioned, they would gain valuable insight into how neurotoxicity and neurodegeneration are induced during TSE disease. This in turn would lead to new therapeutic approaches for prion diseases where, currently, disease symptoms present in the nervous system when it is too late to provide effective treatment. Such information would also lead to new targets for TSE diagnostics and the development of new diagnostic tests. It is likely that the knowledge gained would also benefit other neurodegenerative diseases of protein misfolding such as Alzheimer's disease.

- **Develop a clearer understanding of the precise nature of the infectious prion particle and the role played by abnormal prion protein**. Understanding the

structure of the infectious particle is essential for developing diagnostic tests that are specific for the forms of abnormal prion protein present during TSE infection. Due to potential contamination issues with common use equipment, structural studies in particular require expensive and highly sophisticated equipment that can be dedicated to prion research only. Identification of abnormal prion protein structures or co-factors essential for prion infectivity would provide another set of targets for TSE diagnostics and therapeutics.

- **Continue basic research to develop a more rapid, sensitive diagnostic tool.** Continued basic science research into the mechanisms underlying the development and spread of prion diseases is absolutely essential for the development of effective ante-mortem TSE tests and new anti-TSE therapeutics. Additionally, although prions can be detected in blood using some techniques, the level of sensitivity needs to be improved and/or the reproducibility needs to be increased, especially for diagnostic purposes. There continues to be a need for a validated, rapid method for the detection of prions that is sensitive enough to detect the low level of prions likely present in blood and other peripheral tissues and fluids.

- **Establish and implement procedures to prevent and contain the spread of prions.** Defining the medically and agriculturally relevant routes of prion spread either within the host or between animal species is essential for controlling prion diseases as a human health problem. If the research community is to more widely use techniques to amplify prions, such as the protein misfolding cyclic amplification (PMCA) reaction, that enhance studies of prion protein structure, replication, and pathology, it needs to implement procedures to detect, limit, and inactivate accidental contamination from the newly amplified material.

Detection and Diagnostics (Workgroup 2)

Dr. Alex Raeber, Prionics, AG, Switzerland was the moderator for the Detection and Diagnostics workgroup.

The workgroup noted that no fewer than 20 test concepts had been funded by the NPRP. However, many are far from being applied, *e.g.*, used prior to blood transfusion to test for potential infectivity. Researchers need to agree as to what constitutes proof-of-concept for these tests. The introduction of a test is a multi-stage process: (1) sufficient proof-of-concept to justify development of the prototype, (2) test development, and (3) prototype testing. If the results justify manufacture there is progress to the production, testing and validation stages and finally, if the test proves robust, to commercial production. There are different stakeholders at different stages and Congress and the public need to understand the complexity of test development and validation.

The workgroup discussed the most significant knowledge gaps. Most diagnostic tests developed over the last 5 years are not yet at the proof-of-concept stage. Those that are should move forward if continued support is available. The availability of reference samples for markers and for infectivity is limited. For the best candidate tests need to move ahead; this will require technology transfer, which involves manpower and resources and sharing of intellectual

property. The workgroup prioritized research goals to include detection of prions in hospital settings and on surgical instruments. Group members suggested the need to combine methods such as PMCA with other detection methods. Combined tests are likely to be required for an ideal diagnostic method.

The other area of research emphasis is in cell culture models. Cell culture models are especially needed in the study of human material from vCJD and CWD. A broader set of non-prion markers are needed as the current surrogate markers develop late in the disease period. The following comments were made about blood sampling and blood donor safety with regard to development of a specific and sensitive prion blood test.

Recommendations from Workgroup 2

- **Conduct FDA Center for Biologics Evaluation and Research (CBER) meeting on donor blood safety.** There is no test yet available to verify that donated blood is free of prions. The research community would benefit from an FDA CBER meeting to define targets, which will vary according to the application such as for clinical diagnostic tests or for blood screening. Close communication between the FDA and the prion research community will ensure that experiments are valid and appropriate. For blood donors, the concept is to have a two-stage process. If a donor is positive in the first, highly sensitive test, the blood would be rejected for transfusion. A second confirmatory test would be performed, and if also positive the donor would be counseled. Clinical diagnostic testing should be separated from blood screening.

- **Encourage patients and families to provide samples.** There is a need for more samples from both living and deceased patients affected by TSE, as well as those persons at high risk for harboring TSE. The research community will have to make sure patients understand how the tests will be used, and address privacy (from insurance agencies and employers) concerns. Researchers should partner with CJD organizations to enhance sample collection.

- **Create Reference Centers.** There is a critical need for reference centers for tissues, blood, and transgenic mouse strains capable of propagating prions from different species. Creation of a national TSE tissue bank could promote research efforts to test and validate TSE agents and evaluate promising diagnostics methods.

Prevention (Workgroup 3)

Dr. Bruno Oesch, Prionics, AG, Switzerland was the moderator for the Prevention Workgroup.

Prevention is defined as any activity which reduces the burden of mortality or morbidity from disease. Defining the areas where we can try to prevent the spread of the disease in animals is the best focus. It is important to maintain and improve surveillance in the various species affected by prions: humans, cattle, sheep, deer, elk, and possibly mice and voles. Surveillance is needed on the entry of infectious material into animal feed and subsequently the food chain that may affect humans. Animal feed may consist of many different elements that may contain the

prion protein. Meat and bone meal (MBM) from some animal species is used for animal feed to other species. Also used for animal feed are "specified risk materials" (SRM), which includes the skull, brain, eyes, tonsils, spinal cord and the nerves of animals. SRM is considered high risk for its potential to contain prion proteins. Banning the use of SRM for both human and animal food was critical in halting the epidemic of BSE in the UK and Europe. The success of the ban illustrates the central importance of SRM spread of BSE and underscores the need to monitor and/or prevent the entry of SRM into the food chain.

There is a concern that human infection could occur through prion contaminated blood transfusions, medical instruments, and tissue transplants. The research community needs to develop new methods for inactivation of prion proteins that are compatible with maintaining the viability of biological material for human use. The goal of prevention is to reduce the amount of prion contaminated material to which human may be exposed.

The workgroup members suggested that the current surveillance methods need to be maintained and improved. Some group members expressed concern for the level of resources needed being too low to continue current levels of surveillance.

Animal identification is the first step in surveillance. Traceback is a method by which an affected animal can be tracked back to its origin and the potential source of infection. Traceback is done through the National Animal Identification System (NAIS). The USDA representative suggested that traceback was important to reduce the spread of prion disease in livestock. Issues of the business cost for traceback are also a consideration.

Recommendations from Workgroup 3

- **Develop policy on CWD surveillance.** CWD occurs naturally in the wild among deer, elk, and moose. It poses a threat to hunters and others who consume meat from these animals. Deer meat (venison) is sometimes sent by hunters to commercial processors to be preserved as sausage and jerky (dried meat). If the venison is from a deer with CWD, it could contaminate processing machinery and spread the disease to previously uncontaminated meat products and then to unsuspecting consumers. Another threat is that deer are frequently raised on deer farms for hunting purposes and not monitored for CWD. Deer from farms can be sold and transported across state lines to other commercial farms, posing a significant risk of spreading CWD even further. A federal surveillance and monitoring program for CWD in wild and commercial herds is needed.

- **Conduct studies on surgical equipment contamination.** The risk of asymptomatic prion contamination occurring in hospitals is not known. The risk of blood and tissue from patients with vCJD contaminating surgical rooms and equipment, as well as methods to inactivate the agent on surgical equipment need to be developed. The data need to be improved and based on today's hospital environment, e.g., current heat, cleaning, inactivation steps should be reviewed. Prion researchers should initiate laboratory studies to assess the efficiency of hospital cleaning methods using human strains.

- **Conduct studies on inactivation.** A more systematic approach to studying inactivation of prion material is needed. Autoclaving of whole intact animals is not sufficient. It is unclear what combination of high temperature and pressure is needed to inactivate prions as residual infectivity can still be detectable. Further study needs to be done on the properties of prions as there appear to be a range of different forms, which may have distinct biological characteristics.

- **Review the use of MBM and SRM.** MBM and SRM are valuable sources of protein in animal feed; they are also potential sources of infection. Surveillance of animals receiving MBM and SRM that are likely to become part of the human supply of food may reduce the risk of transmission.

Therapeutics and Surveillance (Workgroup 4)

Dr. Phil Minor, National Institute for Biological Standards and Control, Potters Bar, UK was the moderator for the Therapeutics and Surveillance workgroup session.

With the lack of an effective vCJD treatment, surveillance is essential to control transmission. Surveillance studies are a high priority and remain critical to tracking the disease. The workgroup had an active discussion on BSE surveillance. It was suggested that they should treat the US and Canada in the same way by using a high level of surveillance in both countries. BSE is currently classified as a select agent. The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules for the possession, use, and transfer of select agents and toxins (42 C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121) in the Federal Register on March 18, 2005. BSE was listed by the USDA as one of several select agents and toxins that could be used during acts of terrorism. Other examples include avian influenza and foot-and-mouth disease viruses. The issues surrounding select agents were discussed and lead to suggestions for a containment policy.

Tracking of human TSE through autopsies has increased due to support and education of physicians and family members of the deceased. Hopefully, the autopsied tissues will lead to a better understanding of pathological pathways and contribute to the development of diagnostics. Surveillance for prion disease through autopsies is a problem due to difficulty obtaining consent, and not being able to control the risk of infection from autopsied materials. Government agencies could address those issues by increasing support and allocating resources to educate family members, physicians and medical examiners on the need for more autopsies, as well as continuing support for research on decontaminating autopsy laboratories.

Better diagnostics are key to successful treatments in the future. One issue that will need clarification is who should receive prophylactic measures. Should it be assumed any blood donor could be potentially infectious with prion disease? Do all blood product recipients have the same risk of incurring infection? Without better diagnostics to determine the nature and extent of prion disease, it is difficult to focus on prophylactic therapeutics.

In terms of clinical trials, the researchers need to establish a consensus on a standard clinical trial design for general use and there is a need to coordinate the clinical trials. If clinical trial

designs are coordinated in advance among the researchers, results that support surveillance and treatment guidelines will be reached sooner.

In terms of vaccines, the workgroup said vaccines were an important consideration. Studying the immune response during infection is of interest. If the Government had a vaccine or other alternate intervention to stop CWD transmission, this would be of great importance.

In relation to CWD and hunters, Government regulators such as State Fish and Wildlife or Game officers need to better promote the requirement to turn in deer heads to determine the real prevalence of the disease. If ante mortem tests were available, State Fish and Wildlife offices could offer them to hunters before they slaughter deer for consumption. However, the repercussions for testing include the socio-economic effects of finding increased prevalence of CWD on the hunting industry.

Recommendations from Workgroup 4

- **Reconsider the research safety restrictions related to BSE.** The administrative requirements to track every sample, criminal penalties, and inspections associated with the select agent status of BSE make research on it very difficult. The select agent status and Biological Safety Lab (BSL) level required should be based on the type of material being studied and the stage of the experiment, rather than indiscriminately applied to all materials and experiment components. Cattle with BSE should be managed under BSL-2 requirements, not the more restrictive BSL-3 requirements. If management standards are reduced to reflect the nature of the disease, BSE research will be easier and less expensive to perform, while continuing to be safe.

- **Improve containment measure standard.** Regulators need to revisit containment measures for BSE and scrapie following the most up to date scientific principles. The regulators need to establish a high level committee to devise a standard that is for unique TSE diseases.

Other recommendations

- Establish a unified community of US researchers, similar to *Neuro Prion* in Europe, in order to improve idea and materials exchange.

- Increase the number of clinical researchers to push further new technologies and methods as agents for clinical applications.

When asked, the Summit participants provided these additional perspectives on the following issues: (1) obstacles facing prion investigators, (2) areas of research that, if supported, could make a significant impact on prion diseases, and (3) areas of research which will contribute the most to creating an ante-mortem diagnostic tool for prion disease.

What obstacles (scientific, knowledge gaps, clinical, funding) are researchers facing?

- Incomplete understanding of the actual infectious materials (analyte) that best correlates with disease development (pathogenic mechanisms) such as minimal infectious dose
- Lack of reliable assays for prions in blood, urine, cerebral spinal fluid (CSF), and other body fluids
- Incomplete understanding of the nature and activity of "accessory" factors (polyanions, nucleic acids, planar organic compounds) on formation of infectious material
- Unclear understanding of prion strains in CWD and detection (pre-symptomatic)
- Lack of a practical rapid ante-mortem confirmatory diagnostic test, including a test that distinguishes between sporadic CJD and iatrogenic CJD
- Lack of high resolution structural information such as knowledge of strains, conversion, ($PrP^{C} \rightarrow PrP^{Sc}$), cell biology, structure of PrP^{Sc}, function of PrP^{C}
- Limited access to standardized reagents, including infectious material, and limited availability of transgenic animals
- Lack of well characterized reference samples (standard) for test validation
- Lack of data on prion disinfection efficacy in current hospital disinfection procedures for surgical instruments
- Overly strict biosafety level measures and restrictive use of laboratory and large animal containment

What prion disease research areas, if funded, could make a significant impact on the prevention, detection, diagnosis, and treatment of these diseases?

- Development of promising diagnostics (PMCA) involving combinatorial diagnostics (i.e. more than one approach) to improve specificity and sensitivity
- Identification of which infective species in blood is critical in order to target diagnostics/screening to appropriate molecules
- Detailed typing of prion strains in human CJD, BSE, and other animal prion diseases
- Research in molecular and cellular progression of disease
- Epidemiology in wild species and pathogenesis across species (comparative)
- Environmental surveillance and prevention of human transmission of CWD and BSE
- Inactivation/removal of infectivity that is compatible with biomedical instruments to ensure safety for use of surgical instruments, transplantation of organs and cells, blood transfusion
- Rapid infectivity assays (not just prion protein assays) either in culture or by molecular assays of agent associated components
- Evaluation of all (not just prion protein) molecules of the most infectious fractions
- Treatment regimens including vaccine production
- Specified Risk Material (SRM) disposal
- Research into atypical scrapie/BSE and CWD transmission to humans
- Reagents and assays for prion detection and diagnostics
- Identification of ligands that would be useful for brain imaging / diagnostics (retinal imaging also a possibility)
- Structural probes and modeling

What areas of research will contribute the most to the effort of creating an ante-mortem diagnostic tool for prion diseases?

- Basic biology of the structure of PrP^{Sc} (pathogenesis); more knowledge in prion strains in natural hosts
- Perfecting PMCA – simplifying, speeding up the test and increasing its specificity – possibly in combination with another test
- Amplification technologies to improve sensitivity (other ligands, in addition to PMCA), for example coupling to PMCA antibody immobilization and capture followed by PCR of attached nucleic acids
- Better and more reagents and assays for prion detection to increase both the specificity and sensitivity
- Understanding the properties of the agent in blood and its presence in CNS and other tissues and fluids
- Identifying tissues involved early in the disease process
- Verify "spontaneous" generation of infectivity
- Infection of primates (monkeys) with BSE/vCJD to obtain reference materials
- Development of imaging technologies, similar to those used in the diagnosis of Alzheimer's Disease

Clearly, noticeable progress has been made in advancing the science of prion disease. However, there was a concern voiced at the 2007 NPRP Summit that the lack of continued commitment will stall and potentially halt the momentum that has been gained in the basic understanding of prion disease and have a significant impact on the prevention, detection, diagnosis and treatment of prion diseases. The prolonged incubation times of TSE diseases which, even in the fastest experimental models of disease can be months or even years, inevitably slow the pace of TSE research and reagent development, especially when compared to other infectious diseases of known viral or bacterial origin. Thus, many promising projects that were initiated through the NPRP have just started to produce useful results. In general, the Summit participants believe that support of the ongoing basic and applied research is critical and holds the great potential for progress in finding the answers to crucial questions about prion diseases especially in the development of diagnostics. Many diagnostic reagents, strategies, and detection technologies were employed for ante-mortem diagnostics test development but most of these were only taken through the initial stage of development. Without additional resources, many of these efforts will languish because their development is too premature to be picked up by a commercialization enterprise. Support is particularly important in the advancement of young researchers and scientists who do not have access to the materials or lack required infrastructure to support their scientific work.

Conclusion

Overall, CDMRP has effectively executed and managed the scientific investment made by the U.S. Congress. This investment has successfully increased the research capacity and globally expanded the scientific community in prion diseases. Significant progress has been made toward the goal of developing an ante-mortem diagnostic test for prion disease. The research conducted by scientists within the U.S. and around the world has strengthened relations with other countries and achieved synergy among the international community, U.S. federal agencies, academia, and industry. Major impediments to the detection of misfolded prion proteins in peripheral tissue have been overcome. However, several obstacles still face scientists. Existing technologies must be optimized and new technologies must be used from fields outside of the usual expertise of prion investigators. Access to human and animal blood and tissue samples and establishment of reference samples for validation or positive controls is critical to research efforts. Avenues to share such resources must be encouraged. It is clear that nothing will improve the prospect of obtaining an ante-mortem diagnostic test more than an increased understanding of the prion agent, the pathogenesis and transmission of the disease at a basic level. Research in these areas is ongoing and many new investigators have entered this field due to initial support from NPRP. This investment must be sustained.

The scientific community would benefit from continued support in order to leverage the momentum gained by its initial investment in the critical scientific research on the nature of prions. This will ensure fulfillment of its goal to reduce the threat of human TSE infection in the United States and within the military community. Science requires long term commitment to allow proof-of-concept projects to move to a stage of development where those that are successful can be picked up by industry. Through the NPRP, the DOD has contributed to the evolving global knowledge of prion diseases and advancing prion science. Congress, through the DOD, now has the unique opportunity to continue its leadership in prion research and establish proven diagnostic tests so as to prevent the future spread of TSEs to humans.

APPENDIXES

A. Abbreviations
B. IOM Recommendations for the NPRP
C. NPRP Funded Awards
D. Abstracts from the 2005 NPRP Investigators Meeting
E. NPRP Funded Investigator Accomplishments by Year and Research Area
F. 2007 NPRP Summit Participants
G. 2007 NPRP Summit Agenda

A

Abbreviations

BSE	bovine spongiform encephalopathy
CDI	conformation-dependent immunodiagnostic assay
CDMRP	Congressionally Directed Medical Research Programs
CHO	Chinese Hamster Ovary
CJD	Creutzfeldt-Jakob disease
CNS	Central Nervous System
CSF	cerebrospinal fluid
CWD	chronic wasting disease
DGEs	differential expressed genes
DNA	deoxyribonucleic acid
DOD	U.S. Department of Defense
ELISA	enzyme-linked immunosorbent assay
FDC	follicular dendritic cell
FTMS	Fourier transform mass spectroscopy
HDAC	histone deacetylase
IHC	immunohistochemistry
IOM	Institute of Medicine
IU	Infectious Unit
MALDI	matrix-assisted laser desorption/ionization
NIH	National Institutes of Health
NPRP	National Prion Research Program
PMCA	protein misfolding cyclic amplification
PrP	prion protein
PrP^{C}	protease-sensitive cellular prion protein
PrP^{res}	protease-resistant protein associated with prion disease
PrP^{Sc}	protein associated with prion disease, limited resistance to proteinase K
recMoPrP	recombinant mouse prion protein
RNA	ribonucleic acid
SAFs	scrapie associated fibrils
SBIR	Small Business Innovation Research
sCJD	sporadic Creutzfeldt-Jakob disease
SELEX	systemic evolution of ligands by exponential
siRNA	small interfering RNA
vCJD	variant Creutzfeldt-Jakob disease
SRM	specified risk material
STTR	Small Business Technology Transfer Research
Tg	transgenic
TPC	Technical Planning Committee
TSE	transmissible spongiform encephalopathy
USAMRMC	United States Army Medical Research and Materiel Command
vCJD	variant Creutzfeldt-Jakob disease

B

Institute of Medicine Recommendations for the NPRP

Advancing Prion Science:
Guidance for the National Prion Research Program[1]

Recommendation	**Priority (1 = highest)**
Basic Research	
Fund basic research to elucidate the: **(1) Structural features of prions,** **(2) Molecular mechanisms of prion replication,** **(3) Mechanisms of pathogenesis of transmissible spongiform encephalopathies,** **(4) Physiological function of PrP^{C}.**	**1**
Improving Diagnostics	
Fund research to:	
Develop new assays most likely to achieve quantum leaps in the quality of prion detection tools, rather than incremental improvements to existing tests. Any efforts to improve existing tests should aim to increase their sensitivities by several orders of magnitude (at least 10^3). The optimal test should detect less than 1 infectious unit (IU) of PrP^{Sc} per unit of ultimate product used (e.g., 1 liter of blood or 100 grams of beef).	**1**
Improve in vitro techniques that amplify small amounts of PrP^{Sc} to enhance the sensitivities of diagnostic tests.	**2**
Develop novel methods and reagents that detect or bind to prions, including new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules. This research could lead not only to better diagnostics, but also to better therapeutic and prophylactic strategies.	**1**
Identify surrogate markers or signatures for the detection of prions or prion diseases.	**3**
Improve techniques for propagating prions in cultured cells and develop new in vitro cell systems as a means to assay and study prions.	**2**
Develop functional imaging for the presence of PrP^{Sc} in brain tissue, leading to an early	**3**

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diagnostic test similar to the imaging diagnostic being developed for Alzheimer's disease.

Testing Blood for Evidence of TSEs

Fund research (1) to determine the amount of sporadic Creutzfeldt-Jakob disease (sCJD) prions and variant Creutzfeldt-Jakob disease (vCJD) prions in human blood and (2) to estimate the amount of PrP^{Sc} corresponding to one infectious unit of sCJD and vCJD prions in human blood. **1**

U.S. Surveillance for TSEs

Provide funds to:

Promote an increase in the proportion of cases of human neurodegenerative disease, especially suspected cases of transmissible spongiform encephalopathy (TSE) that are recognized and autopsied. **2**

Increase the number and diversity of epidemiological studies on human TSEs in the United States. In particular, support research to identify potential cases of vCJD and new human TSEs possibly caused by the agent of chronic wasting disease. **2**

Support the development of a nationwide surveillance system for chronic wasting disease in the United States. **2**

Expand research on the natural history, prevalence, distribution, exposure and transmissible characteristics, host susceptibility, and host range of transmissible spongiform encephalopathies, especially chronic wasting disease. **1**

Assessment of Strategies to Prevent and Treat TSEs

Fund research to improve rapid, accurate, and affordable screening assays for central nervous system (CNS) tissue such that the assays can specifically identify CNS material from cattle in processed meat products. **2**

Fund risk assessments that characterize the exposure of hunters, cervid processing establishments, and consumers to the infectious agent of chronic wasting disease. **3**

Fund research to:

Develop novel methods for removing prions from or inactivating prions in blood products and tissues in vitro, using physical, chemical, or immune mechanisms alone or in combination. **2**

Develop standard assays for the detection of PrP^{Sc} or TSE infectivity on the surfaces of reusable medical instruments and materials, as well as research to develop better methods to disinfect such instruments and materials. **2**

Develop standard test methods for detecting prion contamination in environmental samples. **3**

Identify safe, cost-effective disposal mechanisms for animals and rendered waste infected with agents of TSEs. This research would best be conducted with a multidisciplinary approach involving experts in such fields as prion biology, biochemistry, environmental engineering, and commercial disposal technology. **2**

Develop new therapeutic agents, including antibodies that either block the conversion of PrP^{C} to PrP^{Sc} or disrupt the molecular mechanisms of pathogenesis of transmissible spongiform encephalopathies after this conversion has taken place. The most promising approach appears to be rational drug design, which begins with knowledge of the tertiary structure of the protein or molecule that the therapeutic agent will target. **1**

Prion Research Infrastructure

Provide funds to:

Attract and train more investigators in prion disease research. In addition, for investigators conducting prion bioassay research, provide grants for 5 to 7 year periods. **1**

Boost the capacity of the U.S. infrastructure for research on TSEs by expanding or upgrading existing laboratories, animal facilities, and containment laboratories (biological safety levels 2 and 3), and by building new ones. **1**

Develop scientifically based biological safety level standards for laboratories conducting research that involves infectious agents known to cause transmissible spongiform encephalopathies. **2**

Support new or established TSE repositories that contain a collection of reference materials and genetically engineered animals (including transgenic mice), as well as reagents useful for developing TSE diagnostics and for other TSE research. All registered investigators involved in prion research should have access to these collections. **1**

Support the U.S. Food and Drug Administration's development of characteristics of panels of reference reagents needed to evaluate the performance characteristics of tests designed to detect prion protein and TSE infectivity. These panels would be used to confirm the performance characteristics of test kits before they are approved for public use, as well as to perform quality control on test kit lots before their release to the market. **3**

Enable U.S.-based investigators of TSEs to collaborate or train with TSE investigators internationally and to use TSE research facilities abroad. Exploiting such opportunities will expand the range of TSE research that U.S. scientists can conduct.	**3**

Risks to the U.S. Military

Use existing passive surveillance systems to monitor the incidence of Creutzfeldt-Jakob disease and vCJD among individuals receiving medical care from health systems of the U.S. Department of Defense and the Department of Veterans Affairs.	**3**

C

NPRP Funding Awards

Title	Institution	Budget
Alternative Molecular and Cellular Approaches to the Diagnosis, Transmission and Prevention of Creutzfeldt-Jakob Disease	Yale University	$1,361,445
Development of Rapid, Simple and Sensitive Capture-ELISA for the Diagnosis of TSE	Case Western Reserve University	$2,494,370
Ante-mortem Prion Diagnostics	The Regents of the University of California	$2,399,439
Prion Transport to Secondary Lymphoreticular System Tissues	Creighton University	$443,176
Early Host Responses to Prion Infection: Development of in Vivo and in Vitro Assays	McLaughlin Research Institute	$2,241,625
Experimental and Theoretical Approaches to PrP^{Sc} Detection in Biological Fluids and Ante-mortem Diagnosis of TSE Based on Laser-Induced Fluorescence Immunoassays	State University of New York, Downstate Medical Center	$1,549,998
Integument: Prion Fate and Diagnostic Potential	King's College London	$35,743
Propagation of Mammalian Prions in Yeast	Washington University	$575,550
Development of Aptamer Beacons for Ante-mortem Diagnosis of Chronic Wasting Disease	Oklahoma State University	$569,250
The Role of a Novel Topological Form of the Prion Protein in Prion Disease	Washington University	$445,800
Development of Assay for Prion-Specific Detection and Ante-mortem Diagnosis	Case Western Reserve University	$1,006,885
Efficient and Rapid Development of Transgenic Hamster Models of TSEs Using a Radical New Technology	Baltimore Research and Education Foundation, Inc.	$402,565
Physical Characterization of a Highly Infectious Monodisperse Preparation of TSE Infectivity as a Substrate for Diagnostic Development	Baltimore Research and Education Foundation, Inc.	$401,939
Investigation of Immunization Strategies Against Chronic Wasting Disease in Deer and Elk	University Hospital of Zürich	$555,000
Migratory Leukocytes in the Pathogenesis and Diagnosis of Prion Disease	South Dakota State University	$264,031

Title	Institution	Budget
Environmental Impact of Chronic Wasting Disease	University of Wisconsin	$2,377,595
Combination Therapy for Prion Diseases Using Synthetic Analogs and Natural Products of Acridine and Polyamines	Pharmadyn, Inc.	$447,141
The Ante-mortem Detection and Conformational Switches of Prion Proteins	The Salk Institute for Biological Studies	$999,588
Diagnosing Prion Diseases: A Genomic and Proteomic Approach to Biomarker Discovery	University of Wisconsin	$541,815
Genetic Susceptibility and Biological Characterization of Chronic Wasting Disease	University of Wisconsin	$2,281,436
Elucidation of Prion Protein Conformational Changes Associated with Infectivity by Fluorescence Spectroscopy	University of Montana	$501,840
Deconstructing Prion Biogenesis, Elimination and Neurotoxicity	Whitehead Institute for Biomedical Research	$2,408,508
Pre-Clinical Detection of PrP-Scrapie in Blood	Lawrence Livermore National Laboratory	$1,155,000
High-Throughput Screening of Compounds for Anti-Transmissible Spongiform Encephalopathy Activity Using Cell-Culture and Cell-Free Models and Infected Animals	National Institutes of Health	$993,700
Development of Methods for the Real-Time and Rapid Identification and Detection of TSE in Living Animals Using Fluorescence Spectroscopy of the Eye	Iowa State University	$549,021
New Structural Approaches to Understand the Disease Related Forms of the Prion Protein	University of California Berkeley	$563,688
Development of Anti-Prion Surgical Instruments and Highly Sensitive Prion Diagnostic Probes by Ion Implantation Technique	University of Dundee	$247,807
PrP^{Sc}-Specific Reagents for the Diagnosis and Therapy of Prion Infection	The Scripps Research Institute	$1,500,000
Ultra-Sensitive Detection of Prion Protein in Blood Using Isothermal Amplification Technology	University of Maryland, Baltimore	$415,600
Diagnostic, Prognostic, and Therapeutically Relevant Prion Co-Factors: An Approach Based on Functional Genomics	University Hospital of Zurich	$1,508,719
Development of High Affinity Ligands and Methods to Detect Prions	University of Minnesota, Twin Cities	$377,598
Theoretical Modeling of Molecular Mechanisms, Time Scales, and Strains in	The Regents of the University of California	

Title	Institution	Budget
Prion Diseases		$516,831
A Combinatorial Approach of Gene Silencing and Expression Profiling in Deciphering the Roles of Prion and Auxiliary Molecules in Aberrant Prion Replication	McLaughlin Research Institute for Biomedical Sciences	$121,800
Epidemiology of Chronic Wasting Disease: PrP^{res} Detection, Shedding, and Environmental Contamination	University of Wyoming	$2,000,852
Establishing C. elegans as a Model Organism for Prion Research	Northwestern University	$399,817
Development of a Rapid and Sensitive Test for the Detection of Prions in Cultured Cells	Hebrew University of Jerusalem	$454,320
Development of an Assay for the Detection of PrP^{res} in Blood and Urine Based on PMCA Assay and ELISA Methods	Baltimore Research and Education Foundation, Inc.	$1,492,444
Structural Inheritance in Yeast	University of Washington	$560,324

D

Abstracts from the 2005 NPRP Investigators Meeting
(In Order of Investigator Last Name)

Judd Aiken
University of Wisconsin, Madison, WI
Award: DAMD17-03-1-0369

Absorption of the Infectious Prion Protein to Soil Minerals

Sheep scrapie and cervid chronic wasting disease (CWD) are unique among transmissible spongiform encephalopathies (TSEs) because they are horizontally transmitted. The misfolded isoform of the prion protein (PrPSc), the likely etiological agent of TSEs, is resistant to inactivation and TSE infectivity persists when buried for at least three years. PrPSc is thought to be shed into the environment from scrapie- and CWD-infected animals. The agent also likely enters the soil when carcasses of infected animals decompose. Healthy sheep or deer have become TSE infected after occupying land or paddocks previously containing diseased animals or carcasses, suggesting environmental transmission of disease. We examined the potential for soil to serve as a TSE reservoir by studying the association of PrPSc with common soil minerals. We demonstrate substantial PrPSc adsorption to two clay minerals and lesser sorption to quartz surfaces. The interaction between PrPSc and montmorillonite (Mte) clay was strong, making desorption of PrPSc from Mte surfaces difficult. Several extractants, including strong chaotropic agents like guanidine or urea (8 M each), failed to remove PrPSc from Mte surfaces. Of the tested compounds only sodium dodecyl sulphate effectively extracted the PrPSc. PrPSc desorbed from Mte was cleaved at an N-terminal site. Despite the remarkably avid adsorption to Mte, sorbed PrPSc remained infectious. Our results suggest that soil, and particularly clay minerals, could contribute to the environmental transmission of scrapie and CWD.

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University of Wisconsin, Madison WI
Award: DAMD17-03-1-0369

Interspecies Transmission of CWD Disease Agent

Given the resistance of the prion disease agent to inactivation and a rapidly expanding CWD endemic region, it is reasonable to assume that significant amounts of CWD are accumulating in the environment. While direct transmission of CWD to humans and agricultural animals are of great concern, they are not the only routes for CWD to cause health and economic concerns. Any species susceptible to CWD agent has the potential to serve as a vector for transmission. One of our goals is to assess the interspecies transmission risk from CWD contamination in the environment. We are approaching this goal using two methods, direct inoculation of species with disease agent and in vitro conversion assays. A number of other species have been experimentally infected with CWD (mice, hamsters, skunks, ground squirrels); none have demonstrated any symptoms of clinical disease. Direct inoculations are not, however, feasible for the majority of the wild mammals native to CWD endemic areas. We are sequencing, cloning and expressing the Prnp gene from a wide variety of mammals that are likely to come in contact with CWD disease agent and are using these gene products in in-vitro conversion assays.

Judd Aiken
University of Wisconsin, Madison, WI
Award: DAMD17-03-1-0294

PrP Genotype May Affect Susceptibility to CWD

The linkage of the prion protein (PrP) gene, Prnp, to transmissible spongiform encephalopathy (TSE) susceptibility has been well-documented in mice, sheep, and humans. Variability in Prnp also likely affects the susceptibility of cervids to chronic wasting disease (CWD). In elk, variability at Prnp codon 132 has been shown to significantly influence susceptibility to CWD (O'Rourke et al., 1999). Molecular analyses also suggest that the CWD susceptibility of white-tailed deer is influenced by polymorphisms at codon 96 and codon 226, although the relatively small sample sizes in early studies limited conclusions (Johnson et al. 2003; O'Rourke et al. 2004). By expanding the number of animals analyzed (292 CWD-positive and 153 CWD-negative deer from the CWD endemic region of Wisconsin), we have demonstrated a statistically significant bias in the Prnp genotypes between the CWD-positive and -negative populations. The predominant Prnp allele in the CWD-positive and -negative populations encode for Q, G, Q at codons 95, 96, and 226, respectively. The G96S allele was the second most prevalent allele in the population. Of the possible combinations of Prnp alleles, deer homozygous or heterozygous for the G96S allele and deer heterozygous for the Q95H allele were significantly under-represented in the CWD-positive population compared to CWD-negative deer. To determine whether the presence of the G96S allele affected progression of the disease, the intensity and distribution of PrPCWD staining in the obex of the medulla oblongata was assessed and then correlated with identified Prnp genotypes. The significant reduction in the mean levels of PrPCWD staining in the obex of CWD-positive deer with the G96S allele suggests disease progression is retarded in these deer. Although the majority (>95%) of the deer in Wisconsin are genetically susceptible to CWD, there are two Prnp alleles (present at low abundance) that appear to reduce susceptibility to CWD.

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Award: DAMD17-03-1-0291

Transmissible spongiform encephalopathy (TSE) diseases caused by prion infection are a group of fatal neurodegenerative disorders such as BSE, CWD, Scrapie and CJD. These disorders are believed to occur through the accumulation of an abnormal pathogenic isoform (PrP^{Sc}) of cellular protein (PrP^{c}). However, the molecular mechanism of disease pathology is not fully understood. Also, all validated diagnostics of the diseases rely on the immunochemical detection of PrP^{Sc}, precluding diagnosis of presymptomatic prion infections. Although the differential expression of a number of transcripts in CNS tissue at late stage of the infection has been reported, a useful biomarker has not yet been identified. Therefore, gene expression was comprehensively analyzed in different stages of prion-infected mice. Eight-week-old mice (C57B1/6) were infected intraperitoneally with the RML strain of mouse prion. At 108, 158 and 198 days post-inoculation, mice were sacrificed and gene expression profiles in the brain, PBMC and spleen were obtained using Affymetrix gene arrays. Of the 45,000 genes whose expression was measured, numbers of genes were up or down-regulated at different time points. The patterns were also different depending on the organs. Presence of pathogenic isoform of prion (PrP^{Sc}) in brains and spleens was detected by Western blot analysis. Also, expression of some important genes including glial fibrilliary acidic protein was confirmed by iqRT-PCR. Changes in transcript levels correlated with pathological changes induced by prion infection including astrocytosis and neurodegeneration.

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Award: DAMD17-03-1-0319

Prion Transport to Secondary Lymphoreticular System Tissues

Following peripheral routes of prion infection, prions are first detected in follicular dendritic cells in secondary lymphoreticular system tissues (e.g.spleen and lymph node) prior to neuroinvasion. However, the mechanism by which prions are transported to secondary LRS tissues is unclear. We have identified a prion strain that is unable to be transported to and/or replicate in spleen and lymph nodes. Following intraperitoneal inoculation of the drowsy (DY) strain of hamster-adapted transmissible mink encephalopathy (TME), we are unable to detect DY PrPSc in spleen, medial iliac lymph node or mesenteric lymph node by Western blot or immunohistochemistry. In addition, intracerebral inoculation of these tissues into recipient hamsters resulted in the animals remaining clinically normal for 400 days postinfection. This is the first identification of a LRS replication deficient prion strain and our ongoing studies are aimed at understanding why DY TME is not transported to and/or replicates in spleen and lymph nodes. Our working hypothesis is that macrophages are responsible for clearance of DY TME from the host and that components of the innate immune system may opsonize PrPSc and contributes to phagocytosis by macrophages. To investigate this hypothesis, we have incubated PrPSc from DY infected hamsters with macrophages and compared the ability of DY PrPSc to be phagocytosed and cleared compared to the hyper (HY) strain of TME, which can replicate in spleen and lymph nodes. Preliminary studies indicate strain-specific differences between HY and DY TME and suggest that DY PrPSc may be cleared more efficiently than HY PrPSc. In addition, we have been able to successfully immunoprecipitate HY and DY PrPSc using the 15B3 antibody (a generous gift from Alex Rabier and Bruno Oesch, Prionics AG, Switzerland) and currently investigating if innate immune components interact with PrPSc.

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Structural Studies of Misfolded Prp Conformers

TSE are fatal neurodegenerative diseases affecting humans and animals, characterized by the accumulation within the central nervous system of an abnormally folded conformer (PrPSc) of the cellular prion protein (PrPC). Unlike the structure of PrPC, which has been determined by NMR studies of recombinant PrP species; only predictive models of PrPSc conformation are available.

Novel strategies to decipher PrPSc structure are needed. Toward this goal, two motif-grafted recombinant antibodies, termed 89-112 and 136-158, have been derived that specifically recognize disease-associated forms of PrP from multiple species. We aim to employ these molecules to derive co-crystals of antibody complexed to abnormal PrP conformers produced in vivo and in vitro.
During initial studies, we have grown crystals of a Fab fragment of the 89-112 reagent that diffract to 2.5Å. Structural information forthcoming from this data set will further increase our understanding of the interaction between motif-grafted antibodies and PrPSc, thereby advancing the rational design and development of reagents for the diagnosis of prion infections.

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Development of Aptamer Beacons for Antemortem Diagnosis of Chronic Wasting Disease

A need exists for rapid and specific ante-mortem probes for the chronic wasting disease-associated isoform PrPCWD of the normal prion protein PrPC of elk and deer. Short single-strand oligonucleotide species termed aptamers may be selectable that are able to differentially bind PrPCWD. Current methods of TSE diagnosis rely upon antibody recognition of the mis-folded PrPD isoform of PrPC, and antibodies thus far generated are unable to differentiate the PrPC from the misfolded isoform PrPD. Others have selected RNA aptamers that recognize PrPC and inhibit its conversion to PrPD and one that binds to PrPD 10-fold higher than to PrPC, however, an aptamer that readily differentiates PrPD from PrPC has not thus far been identified. Aptamers are selected from oligonucleotide pools consisting of low copy numbers of highly random sequence species by affinity partitioning and PCR amplification termed SELEX. Two barriers to SELEX selection of aptamers that differentially recognize PrPCWD are 1) target presentation of the folding motifs of PrPCWD not present in PrPC such that oligonucleotide species that recognize these motifs are enrichmed over those that recognize motifs common to PrPC and PrPCWD, and 2) efficient partitioning and PCR amplification of PrPCWD-specific aptamer species over non-specifically bound oligonucleotide species. To address the first of these barriers, a novel crossover SELEX was employed whereby the pool of oligonucleotides was directed toward recognition of PrPCWD and removal of species that recognize PrPC. To improve partitioning of PrPCWD-specific aptamer species, a novel electrophoretic dialysis partitioning step coupled with use of a non-extendable reverse primer tag were employed to improve SELEX efficiency. The reduced aptamer pools from these methods have been cloned and will be sequenced for evaluation of specificity for PrPCWD. The next phase of this project will involve coupling selected aptamers with detection modalities such as beacon or surface plasmon resonance technologies.

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Molecular Signatures of Prion Infection

Currently, prion infection in humans and livestock is diagnosed by clinical signs, the presence of mis-folded, proteinase K-resistant PrPSc in the brain, and histopathology. Development of improved methods for detection of the disease-specific PrPSc isoform is one approach towards development of an ante-mortem blood test to identify prion-infected individuals. Using the mouse as a model, we have applied global genomic and proteomic analyses to develop a network-based framework integrating molecular signatures of mRNA, proteins, and protein-protein interactions in brain, spleen, and blood. Identification of differentially expressed genes (DEGs) used the Affymetrix Mouse GeneChip 430A 2.0; differentially expressed proteins (DEPs) in plasma were profiled using liquid chromatography and mass spectroscopy (LC/MS) and LC/MS/MS. To focus on networks specifically perturbed by prion infection, two distinct prions strains and seven mouse strains were analyzed. Analysis to date (07/25/05) has revealed 57 DEGs in brain common to four of the mouse strain-prion strain combinations. Of these, 25 are expressed before clinical signs or pathological changes develop, making them good candidates for early diagnostic markers. Twelve of these genes encode secreted proteins that may be detectable in plasma. We have recently infected neurosphere cultures with prions with little apparent cytopathic effect; this new model will permit discrimination of genes altered due to prion replication from genes related to the pathological response of the host. Neurosphere lines have been isolated from the same prion strain-mouse strain combinations used in our global analyses to identify prion-specific signatures. A network model to understand relationships among differentially expressed genes and proteins and their biological significance is under development.

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Mouse Neurosphere Cultures for Bioassay of Prion Infectivity and for the Study of Prion Replication and Disease Susceptibility

Few cell lines can be infected with prions, precluding in vitro analysis of the mechanisms underlying genetic differences in susceptibility to infection. Similarly, some of these cell lines, mouse N2a cells for example, are resistant to prion strains that can readily transmit disease to mice. With one exception, sensitive bioassay of prions requires inoculation of mice with incubation times ranging from months to over a year. Neurosphere lines grow as aggregates and contain CNS stem cell activity; we now report that these cultures can be infected with prions. Using a defined, serum-free medium, cell lines were isolated from brains dissected from fetuses at embryonic day 12 to 15. In addition to expressing the stem cell-associated marker nestin, most cells from PrP transgenic or from wild-type mice express the normal isoform of PrP (PrPC), which is essential for prion replication. RML scrapie brain homogenate was added to neurosphere cultures from FVB, FVB transgenic mice that overexpress mouse PrP (Tg4053), and FVB mice with a targeted null mutation in the PrP gene (Prnp). Presence of the proteinase K-resistant, misfolded PrPSc isoform was measured at each passage by Western, dot, or cell blots. A dramatic rise in PrPSc with time was observed in the Tg4053 cells while the level PrPSc decayed to undetectable levels in the cultures of cells lacking PrP; levels of PrPSc in FVB cultures persisted and then increased over several passages. Prions produced in culture were transmissible to mice and produced typical scrapie pathology. Intracellular aggregates of PrP were seen in infected cultures. To date, infection of Tg4053 neurospheres by prion isolate diluted 1 to 50,000 has been demonstrated. Neurosphere lines from transgenic mice overexpressing PrP may provide a sensitive in vitro bioassay not only for mouse prions but also for those from other species, including humans.

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Inhibition of Protease-Resistant Prion Protein in a Transformed Deer Cell Line Infected with Chronic Wasting Disease

Chronic wasting disease (CWD) is an emerging transmissible spongiform encephalopathy (prion disease) of North American cervids, i.e., mule deer, white-tailed deer and elk (wapiti). To facilitate in vitro studies of CWD, we have developed a transformed deer cell line that is persistently infected with CWD. Primary cultures derived from uninfected mule deer brain tissue were transformed by transfection with a plasmid containing the SV40 virus genome. A transformed cell line (MDB) was exposed to microsomes prepared from the brainstem of a CWD-affected mule deer. CWD-associated, protease-resistant prion protein (PrPCWD) was used as an indicator of CWD infection. Although no PrPCWD was detected in any of these cultures after two passes, dilution cloning of cells yielded one PrPCWD-positive clone out of 51. This clone, designated MDB-CWD, has maintained stable PrPCWD production through 32 serial passes thus far. A second round of dilution cloning yielded 20 PrPCWD-positive subclones out of 30 and a third round yielded 8 positives out of 11. The MDB-CWD cell line was positive for fibronectin and negative for microtubule-associated protein 2 (a neuronal marker) and glial fibrillary acidic protein (an activated astrocyte marker), consistent with derivation from brain fibroblasts (e.g., meningeal fibroblasts). Two inhibitors of rodent scrapie PrP-res accumulation, pentosan polysulfate and a porphyrin compound, indium (III) meso-tetra(4-sulfonatophenyl)porphine chloride, potently blocked PrPCWD accumulation in MDB-CWD cells. This demonstrates the utility of this cell line in a rapid in vitro screening assay for PrPCWD inhibitors and suggests that these compounds have potential to be active against CWD in vivo.

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Development of Prion-Specific Reagents

Prion diseases, a group of fatal neurodegenerative disorders, are characterized by the presence of the abnormal scrapie isoform of prion protein (PrPSc) in affected brains. A conformational change is believed to convert the normal cellular prion protein (PrPC) into PrPSc. Detection of PrPSc for diagnosis and prophylaxis is impaired because available antibodies recognizing epitopes on PrP fail to distinguish between PrPSc and PrPC. Accurate and rapid diagnosis of prion disease has become especially important with the occurrence of bovine spongiform encephalopathy in many countries, including the United States, and the emergence of variant Creutzfeldt-Jakob disease (CJD). Here we report the development of several molecules, designated prion-specific reagents (PSRs) that are specific to the disease-associated PrP. These include the anti-DNA antibody OCD4, the gene 5 protein well-established as a DNA binding protein (g5p), and two short peptides. The peptides are 12 and 15 amino acid residues long, and are derived from the sequence of the Kringle domains in plasminogen, the full length version of which was recently reported to bind disease-associated, but not normal, PrP. Using affinity capture methods, we show that PSRs are able to capture PrP from the brain homogenate of humans and animals affected by prion diseases including sporadic and variant CJD, scrapie, chronic wasting disease, and bovine spongiform encephalopathy, but not unaffected controls. Further, these reactions appear to be conformation-dependent. PSRs capture significantly more disease-associated PrP than similar experiments performed with widely used anti-PrP antibodies. Our finding that these prion-specific reagents specifically target disease-associated PrP in a wide variety of species and disease phenotypes opens new avenues in the study and diagnosis of prion diseases.

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The Application of Amplification Methods to Detect Ultra-Low Levels of Pathologic Prion Protein

Pathologic prion protein (PrPsc), implicated in transmissible spongiform encephalopathies in humans and animals, is detected by antibody-based tests or bioassays to confirm the diagnosis of prion diseases. Presently, the Western Blot or ELISA is used to test nervous tissue where concentrations of PrPsc are relatively high, but cannot detect prion in blood where levels are considered to be much lower. Therefore, highly sensitive methods are needed to detect infectious prion in the pre-clinical stage of infection to improve the safety of blood donations and to protect the food supply from contaminated beef. The immuno-polymerase chain reaction (IPCR) is a technique whereby the exponential amplification ability of PCR is coupled to the serologic detection of proteins by an ELISA. We have developed a real-time IPCR method capable of detecting recombinant hamster PrP down to 100 attogram/mL concentrations, and in PK-digested scrapie infected hamster brain homogenates diluted to 10-8 (approximately 10-100 infectious units) with a semi-quantitative dose response. This level of detection is up to 1 million-fold more sensitive than standard Western Blot and ELISA methods and poises IPCR as a method capable of detecting PrPsc in samples from infected animals and humans in the pre-clinical phase of infection. Also, these studies show that unless PK-digestion of samples is optimized, a highly sensitive assay such as IPCR may incorrectly define a sample as falsely positive. The IPCR method can be performed in laboratories that have the capability to perform routine molecular testing (e.g., HIV viral load), can be performed at less than $10 per test, and can be configured for high throughput. Efforts have also been extended to translate the IPCR method to an RNA-polymerase isothermal amplification strategy. This study was supported in part by the Department of Defense, HSRRB Log Number A-12174, NP020120.

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Theoretical Modeling of Prion Structures and Kinetics

Overview of the research of my group on: (i) novel molecular level models for the minimal prion infectious unit and consequences for strains and inherited forms of the prion disease; (ii) studies of metal binding to the prion protein and an exploration of the possible role of the nonoctarepeat copper in protecting against prion disease; (iii) kinetics of prion aggregation for both mammalian and yeast prions; (iv) interaction of amyloid peptides with model membranes.

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Developing nano-LC Mass Spectrometry and Single Molecule Optical Assays for Pre-clinical Detection of PrP-Scrapie in Blood

While the presence of PrPSc - the misfolded protein marker for scrapie - has never been directly detected in blood, its demonstrated presence in lymphoid tissues and the observation that blood from infected sheep can induce scrapie in otherwise healthy animals, indicate that a transmissible agent is present in the blood and may be associated with the PrPSc protein. Thus, in principle, the presence of abnormal PrPSc should be detectable through a blood test, provided the detection method has sufficient sensitivity and specificity. In this project, we are developing ultra sensitive detection techniques, including single molecule optical techniques and nano-liquid chromatography mass spectrometry-based proteomic analysis to detect PrPSc at pico- to femto-molar levels in blood fractions.

Samples were obtained from naturally infected sheep, raised for this project and diagnosed through detection of PrPSc in the lymphoid tissue and/or in placental tissue collected at parturition. Mass spectrometric studies were conducted on blood fractions from both infected and uninfected sheep. The samples were digested with proteinase K and were analyzed using a multidimensional nano electrospray liquid chromatography system coupled to a 9.4 Tesla Fourier Transform mass spectrometer. Spiked samples were used to establish a lower limit of detection for ~29kDa whole proteins. These yielded an upper bound on the naturally occurring concentration of PrPSc in each blood fraction. Results will be presented demonstrating the method of detecting protein aggregates using fluorescence correlation spectroscopy (FCS) and time correlated single photon counting techniques (TCSPC). Initial efforts are focused on the detection of beta-Amyloid fragments during the analytical method development. Future application for the detection using antibodies or aptamers tagged with fluorophores will be discussed.

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Saccharomyces Cerevisiae as a Model System to Investigate Prion Biology

The baker's yeast Saccharomyces cerevisiae offers a number of potential advantages for the study of prion biology. We have tested the feasibility of using S. cerevisiae expressing PrP as an assay system for propagating and quantitating mammalian prions. In addition, we have used yeast to investigate two different hypotheses regarding the normal, physiological function of PrPC. The starting point for these studies was our development of DPAPB-PrP, a form of PrP carrying a modified signal peptide that is efficiently targeted to the secretory pathway in yeast.

Using two different methods, we have tried infecting spheroplasts from yeast expressing DPAPB-PrP with rodent prions, and then tested for production of PrPSc by Western blotting in bulk as well as clonal cultures. Thus far, we have not been able to detect amplification of PrPSc in these cultures.

We have also used the yeast system to investigate a role for PrPC in copper metabolism (1). We tested whether PrP expression altered the growth deficiency phenotypes of yeast strains harboring deletions in genes that encode key components of copper utilization pathways, including transporters, chaperones, pumps, reductases, and cuproenzymes. We found that PrP did not rescue any of these mutant phenotypes, arguing against a direct role for the protein in copper utilization.

Finally, we utilized yeast to test the hypothesis that PrPC functions to protect cells from apoptotic stress (2). The mammalian pro-apoptotic protein, Bax, confers a lethal phenotype when expressed in yeast under control of a galactose-inducible promoter. We found that DPAPB-PrP potently suppressed Bax-induced cell death in yeast. In contrast, cytosolic PrP (23-231) failed to rescue growth of Bax-expressing yeast, indicating that protective activity requires targeting of PrP to the secretory pathway. Deletion of the octapeptide repeat region did not affect the rescuing activity of PrP, but deletion of a charged region encompassing residues 23-31 partially eliminated activity. We also tested several PrP mutants associated with human familial prion diseases, and found that only a mutant containing 9 extra octapeptide repeats failed to suppress Bax-induced cell death. These findings establish a simple and genetically tractable system for assaying a putative biological activity of PrPC. With the use of appropriately designed genetic screens and selections, it should now be possible to identify proteins from yeast and mammals that alter the cytoprotective activity of PrP and that may also interact physically with PrP.

1. Li, A., Dong, J., and Harris, D.A. (2004) J. Biol. Chem. 279:29469-29477.
2. Li, A., and Harris, D.A. (2005) J. Biol. Chem. 280:17430-17434.

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Ante-mortem Biomarkers of Prion Disease

Definitive prion diseases diagnosis is currently limited to post-mortem assays for protease-resistance prion protein. By utilizing mass spectrometry based protein profiling and bioinformatics we present a method by which a pre-mortem test can be developed using cerebrospinal fluid (CSF) and/or serum of animals infected with prion diseases. Analysis of CSF from infected/uninfected hamsters is carried out on a matrix assisted laser desorption/ionization – Fourier transform mass spectrometer utilizing a novel pulse-sequence for the analysis of multiple masses simultaneously. Following mass spectrographic analysis, data is deisotoped, calibrated, and classified by machine-learning algorithms identifying differentially expressed proteins in infected or control samples. Finally, data is displayed using software developed in house to illustrate differentially expressed features. Using this strategy, we have been able to classify protein profiles from prion-infected animals better than chance. These learning algorithms have identified several peaks, which may be indicative of prion infection.

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Effect of Tafenoquine and Mefloquine on Animal and Yeast Prions

In view of the effectiveness of anti-malarial drugs on inhibiting PrPSc formation in infected cells, we have tested over two hundred aminoquinoline and acridine analogs, and found that tafenoquine (TF) and mefloquine (MF) (Lariam, Roche) are the most potent compounds against two different strains of mouse prions 22L and RML. Both TF and MF were able to inhibit PrPSc formation in the 22L- and RML-infected ScN2a cells after five days of growth in the presence of the drugs. The activity was greater against RML than 22L infected cells for both TF and MF. The IC50 value for TF was 332 ng/mL (0.72 microM) in the RML-infected cells, compared to > 1000 ng/mL (> 2.2 microM) for the 22L-infected cells. Whereas MF showed an IC50 of 224 ng/mL (0.54 micoM) in the RML-infected cells, the IC50 was 482 ng/mL (1.2 microM) in the 22L-infected cells. Quinacrine, an anti-malarial drug currently in human trials against CJD, has an IC50 value of ~0.4 microM against PrPSc from RML-infected cells, similar to the activity of MF and TF.

Among other potent anti-malarial compounds screened, it was found that artemisinin has only slight activity against RML-PrPSc in infected cells at 10 microM. Quinidine has an IC50 value against RML PrPSc below 10 microM, but without 22L inhibition at the same concentration. Primaquine showed complete inhibition of RML-PrPSc at 10 microM but only slight activity against 22L at 10 microM. Quinine had an IC50 value against RML-PrPSc of about 10 microM but showed only slight inhibition against 22L-PrPSc at that concentration. It seems that differences in the prion strains would affect the ability of the compounds to inhibit them in infected cells. Taken together MF and TF have better activity than these other tested antimalarial compounds. MF is now being tested in mice infected with prions. We have also developed and adapted yeast prions to test anti-prion compounds in high-through-put capacity, and both TF and MF were checked for their effect on yeast prions and to establish their mechanism of actions. A test against yeast prions is another way to test compounds in vitro for potential in vivo activity without having to do expensive and time-consuming cell and/or animal studies. Surprisingly, both TF and MF were able to inhibit the amyloid formation of the yeast prion protein (Sup-NM) in a dose-dependent manner, using thiofavin T measurements, and also able to inhibit almost completely the amyloid polymerization. This effect on the yeast prion was further supported by their inhibitory effect on the induction of [PSI+], the yeast prion protein Sup35, in yeasts.

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Award: DAMD17-03-1-0291

Ante-mortem Biomarkers of Prion Disease

Definitive prion diseases diagnosis is currently limited to post-mortem assays for protease-resistance prion protein. By utilizing mass spectrometry based protein profiling and bioinformatics we present a method by which a pre-mortem test can be developed using cerebrospinal fluid (CSF) and/or serum of animals infected with prion diseases. Analysis of CSF from infected/uninfected hamsters is carried out on a matrix assisted laser desorption/ionization – Fourier transform mass spectrometer utilizing a novel pulse-sequence for the analysis of multiple masses simultaneously. Following mass spectrographic analysis, data is deisotoped, calibrated, and classified by machine-learning algorithms identifying differentially expressed proteins in infected or control samples. Finally, data is displayed using software developed in house to illustrate differentially expressed features. Using this strategy, we have been able to classify protein profiles from prion-infected animals better than chance. These learning algorithms have identified several peaks, which may be indicative of prion infection.

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Potent Anti-Scrapie Activities of Degenerate Phosphorothioate Oligonucleotides

Although transmissible spongiform encephalopathies (TSEs) are incurable, a key therapeutic approach is to prevent normal prion protein (PrP-sen) conversion to the disease-specific form (PrP-res). Degenerate phosphorothioate oligonucleotides (PS-ONs) have been found to be low nM PrP-res inhibitors with strong anti-scrapie activity in vivo. Comparisons of the cell culture PrP-res inhibitory activity of various PS-ONs and analogs thereof indicated that hydrophobicity and size were important while base composition was only minimally influential. Labeled PS-ONs bound avidly to recombinant PrP-sen as measured by fluorescence polarization, but could be displaced by sulfated glycan PrP-res inhibitors indicating overlapping binding sites. Labeled PS-ONs also bound PrP-sen on live mouse neuroblastoma and SN56 cells and were internalized. This binding likely accounts for the anti-scrapie activity. Prophylactic PS-ON treatments more than tripled scrapie survival periods in mice. Survival times also increased when PS-ONs were mixed with scrapie brain inoculum. With these anti-scrapie activities and much lower anticoagulant activity than pentosan polysulfate, degenerate PS-ONs are attractive new compounds for treating TSEs.

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ATP-Binding Cassette (ABCA1) Protein Is Differentially Expressed In Prion Disease and affects PrP Expression

Background: Although alternative alleles of the mouse prion protein (PrP) gene have the greatest influence on prion susceptibility, other genes strongly influence prion incubation time. Objective: Our goal is to identify and validate genes whose differential expression is shared among various mouse strains during progression of scrapie. Method: Microarray analysis was used to identify differentially expressed genes (DEGs) in brain, spleen and blood during disease progression in three different mouse strains (CAST/Ei, SJL/J and C57BL/6J). RNAi-mediated knock down was used to determine whether a DEG affected PrP expression. Results: ABCA1 emerged as a common DEG in all three mice. ABCA1 mRNA expression is 2-2.5 fold higher in terminally ill mice than age matched controls; overexpression was confirmed at protein level in cerebellum and cerebrum. ABCA1 is a transmembrane protein and involved in cholesterol transport. Since PrPc is localized in cholesterol rich caveolae-like domains and cholesterol depletion by chemical means leads to decreased formation of PrPsc from PrPc, ABCA1 could play an important role in prion disease. Overexpression and RNAi-mediated knockdown of ABCA1 led to an increase and decrease of prion protein expression in cultured N2a cells. However, PrP null, wild type and transgenic mice overexpressing PrP did not differ in ABCA1 expression, possibly due to developmental compensation by related ABC superfamily members; alternatively, PrP could be downstream of ABCA1. Overexpression of caveolin, a gene postulated to act downstream of ABCA1, caused a several fold increase in PrP expression. Conclusion: Our study suggests that the DEG ABCA1 may play important roles in regulating the level of PrP expression and in prion disease.

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Assay Development for Chronic Wasting Disease Prion

The characterization of the transmission mechanisms of Chronic Wasting Disease (CWD) requires the development of an ultra-sensitive detection assay. Collaboration was established with Dr. Chad Mirkin group from Northwestern University to utilize their ultra-sensitive protein detection technology named Bio-Bar Code (BBCA) in order to develop ultra-sensitive assays for the PrP protein in all types of tissues, fluids, and protein conformations. The bio-bar code assay is a sandwich immunoassay, which uses magnetic beads with attached monoclonal antibodies for protein separation and nano-particles with attached DNA and polyclonal antibodies for signal amplification. Presently, antibodies to PrP for the bio-bar code assay have been acquired or are being created, and preliminary assays have been run showing potential protein detection limits approaching three attomolar with purified PrPres . These initial tests indicate that the antibody combinations are critical to the development of a successful assay. Preliminary results are promising; however several issues need to be overcome including negative control signal minimization, signal development robustness, and assay variability. All of these problems hinge on antibody combination characteristics. While several standard antibodies including R35, 6H4, 12B2, and F99 have yielded promising results, new antibodies are being developed in conjunction with Dr. Kathryn O'Rourke of the USDA and other collaborators hold even greater promise for the development of an ultra-sensitive assay. Once the assay is developed, important pathological questions, especially transmission mechanisms, will be discovered.

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C. Elegans as a Model Organism for Prion Research

We have initiated a study to establish C. elegans as a model organism for prion research. By ectopic expression, we have examined the ability of yeast prion proteins to aggregate and to form toxic species in C. elegans. We found that transgenic animals that express different mutants of SUP35, the chromosomal determinant of the yeast prion [PSI+], exhibit dramatic differences in aggregation patterns. Mutations that promote [PSI+] formation in yeast are shown to promote Sup35-GFP aggregation in C. elegans whereas mutations that abolish [PSI+] formation in yeast also abolish Sup35-GFP aggregation. Moreover, a severe cellular toxicity is shown to associate with Sup35-GFP aggregation. Our findings suggest that the protein folding machineries are similar in yeast and C. elegans. By expressing mammalian prion protein in C. elegans, we have also investigated the possibility of establishing C. elegans as a model organism for studying the etiology of mammalian prion diseases. We have observed strong protein aggregation patterns in animals that express the cytosol form of mouse prion protein, mPrPcyto, which lacks the signal sequence and the GPI anchor of mPrP. Interestingly, the toxic phenotypes caused by mPrPcyto expression are strikingly different from what reported for polyQ aggregation or for Sup35-GFP expression in C. elegans. The mPrPcyto effects are pleiotropic, ranging from non-detectable, small in size, defective in mobility, sterile, to dumpy-like. Further characterization of this unique pleiotropic effect caused by mPrPcyto and examination of possible infectious particle formation of yeast prion proteins in C. elegans will be discussed.

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Surrogate Markers and Beyond

Sheep scrapie and human CJD infectious agents behave as persistent latent viruses that escape adaptive immune (B and T cell) defenses. However, myeloid microglial responses to a foreign agent can be detected. This is particularly apparent with the more virulent rodent adapted FU-CJD strain, as well as in BSE-linked human vCJD cases (1). In animals with FU-CJD, microglia activation can also precede pathological changes in host prion protein (PrP). To find if microglia elaborated defensive "innate immune" factors, we used array technology to identify known markers of inflammation and infection. Microglia were isolated from FU-CJD infected and control brain and showed 1) microglia are as infectious as brain even though they display no abnormal PrP, 2) infected microglia produce high levels of innate inflammatory transcripts, and 3) PrP amyloid does not elicit these complex transcriptional responses (2,3). Furthermore, a specific group of brain transcripts were elevated early, and preceded host PrP changes and clinical signs (4,5). These markers may also help differentiate distinct agent strains, such as sCJD and vCJD agents.

We also developed a fast and reproducible in vitro system for infection by various TSE agents of low and high virulence. Previous culture models have been plagued by problems of instability, and none have produced high infectious titers of human derived CJD agents. Titers of the FU-CJD agent in vitro are as high as in degenerating brain (6). A co-culture strategy also made it possible to demonstrate effective interference between different CJD and scrapie strains. Interference was not dependent on the presence of immune system cells, and was also unrelated to the presence or absence of pathologic PrP. Rapid assays of infectivity are now possible, and co-culture strategies should be useful for detection of infected white blood cells without PrP pathology. These cultures will also be advantageous for identifying intrinsic, strain-defining molecules.

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Structural Changes in Prion Protein as Monitored by Tryptophan Fluorescence

Numerous single Trp-containing mutants of the truncated form (residues 90-231) of hamster prion protein (PrP) have been over-expressed in E. coli and purified. The local protein environment of the tryptophan of each mutant has been investigated using fluorescence emission spectroscopy, with excitation of Trp at 295 nm. For each mutant, the fluorescence spectrum of the á-form (PrPsen) was compared with that of the sample after it been chemically converted to a soluble â-rich oligomeric form (representing PrPres).

Upon conversion, several mutants show large shifts (~7 nm) in the fluorescence wavelength maximum and/or the signal intensity, while others did not. This has helped us to identify sequence positions that are sensitive to the structural changes in PrP that occur upon conversion. Trp at these positions might directly undergo a change in secondary structure after conversion. Alternatively, the Trp may lie along the subunit interface in the oligomeric form, which affects the Trp micro-environment. In either case, combining tryptophan scanning with fluorescence spectroscopy will be helpful in elucidating structural details of PrPres.

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Development of Methods for the Real-Time and Rapid Identification and Detection of TSE in Living Animals Using Fluorescence Spectroscopy of the Eye

Transmissible spongiform encephalopathies (TSEs) are thought to be caused by the accumulation of abnormal protease-resistant proteins called prions, which are found in aging central nervous system tissue and in the eyes. Other protease-resistant compounds, collectively called lipofuscins, also accumulate in CNS. Lipofuscins accumulate in the eye, especially in the diseased eye. An increase in lipofuscin accumulation is known to occur in human Creutzfeldt-Jakob disease victims and in other cases of experimental TSEs. Lipofuscins are fluorescent compounds with characteristic optical spectra. Some individual lipofuscin compounds (especially from the eye) have been studied in detail with regard to optical and chemical properties. The spinal cord and brain also have been observed to be fluorescent under certain wavelengths of light. This is due in part to lipofuscin accumulation in this tissue. The literature indicates that abnormal TSE prions also display characteristic optical spectra. Preliminary data indicate that the fluorescent spectra of scrapie-infected sheep brain differ substantially from that of the non-infected sheep brain. The purpose of this study is to test the hypothesis that this spectral difference is the result of altered lipofuscin and/or prion spectral properties. Lipofuscins and prions may serve as useful fluorescent markers, which are correlated with the occurrence of TSEs and can be detected by spectroscopy. Significant results and conclusions obtained to date are:

- Spectra from the various parts of sheep eyes are very rich in detail as a function of excitation wavelength.
- For purposes of comparison, we present spectra from three parts of the eye at excitation wavelengths λex = 410, 470, and 520 nm: retina; optic nerve; outer tissue (sclera); and lens.

It appears that while the optic nerve presents the richest spectra with the most detail, the retina and the sclera show the largest differences in spectral features when comparisons are made between the healthy and the infected populations.

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Antemortem Prion Diagnostics

Prions cause bovine spongiform encephalopathy (BSE) of cattle, Creutzfeldt-Jakob disease (CJD) of humans, scrapie of sheep, and chronic wasting disease (CWD) of deer and elk. Four new concepts have emerged from studies of prions. First, prions are infectious proteins that are devoid of nucleic acid. Second, prion diseases may be manifest as infectious, genetic, and sporadic disorders. Third, prion diseases result from the accumulation of PrPSc, the conformation of which differs substantially from that of its precursor PrPC. Fourth, PrPSc can exist in a variety of different conformations, each of which seems to specify a particular disease phenotype. That the mammalian prion contains only PrPSc, thus supporting the hypothesis that prions are infectious proteins, has recently been demonstrated by using recombinant (rec) PrP produced in *E. coli* (Legname, Nguyen et al. 2005). Late in the course of disease, both humans and animals develop signs and symptoms of CNS dysfunction. At this advanced stage, PrPSc is generally detectable in the CNS when sensitive tests, such as the conformation-dependent immunoassay (CDI), are used (Safar, Geschwind et al. 2005). Towards developing a sensitive and specific presymptomatic diagnostic test for prion disease, we have made advances in understanding the biology of disease progression, elucidating chemical conditions for prion protein (PrP) precipitation, and establishing diagnostic protocols. Using SELDI-TOF MS and a ProteinChip platform in mouse models of prion disease, protein profiles of fractionated brain homogenates were produced from symptomatic CD-1 mice infected with RML prions and compared to controls; 24 protein biomarkers were identified. Additionally, we established a FACS protocol for fractionating human white blood cells (WBCs) from CJD patients. PrPSc in human WBCs will be measured using the CDI. In parallel work aimed to increase the sensitivity of the CDI by precipitation of prions, Keggin-type polyoxometalate (POM) complexes demonstrated superior ability to precipitate selectively disease-causing PrPSc. We propose that prion aggregation may involve multivalent electrostatic interactions between the POM anions and positively charged cleft sites of PrPSc. Using POMs, we are continuing to adapt the CDI to human plasma using samples from healthy controls and patients dying of sporadic CJD. From these studies, we conclude that a protease-sensitive (s) form of PrPSc is present at low concentrations in the plasma of patients with sporadic CJD. Whether or not there is sufficient sPrPSc present in the plasma of CJD patients so that it can be used as a diagnostic marker remains to be established.

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Development of a Naturally Infected Sheep Scrapie Resource for TSE Assay Development

Transfusion transmitted TSE infections have now been documented in hamsters, mice, sheep and humans and from asymptomatic as well as symptomatic donors. We have shown that TSE-infected rodent blood contains only 10 ID/ml during clinical disease and less at earlier times. The corresponding concentration of infection-specific prion protein would be £ 0.1 pg/ml. Detection will likely require concentration from an entire single unit donation of 500 mls.

The only practical source of TSE infected blood in 500 ml quantities is from scrapie infected sheep. In a collaboration with Marie Bulgin, DVM at the Caine Veterinary Center, Idaho, Rick Kascsak, Ph.D. and Richard Rubenstein, Ph.D. at the Institute for Basic Research, NY and Linda Detwiler, DVM we have developed a powerful new resource for blood assay development. A flock of 100 animals has been bred for high susceptibility to scrapie in which most animals become symptomatic by 2 to 3 years of life. Additional animals are inoculated by the oral route to define preclinical incubation times. Control tissue are obtained from a scrapie-free certified flock being bred for the same genetics as the infected flock. Two highly reactive monoclonal antibodies that recognize the sheep prion protein have been developed for immunoassay development and a transgenic mouse overexpressing the sheep prion protein may enable titration of sheep infectivity in mice. The incubation time of the first transmissions has been < 200 days suggesting a high efficiency of transmission.

Scrapie infected sheep show far more tissue, and physiological variability than laboratory rodents. To reduce the confounding influences of individual samples we have prepared large pools of sheep brain homogenate (now being titered in the transgenic) and sheep plasma both of which are serving as standard reference materials. The sheep brain homogenate has proven extremely potent, causing infections in less than one year in some genotypes even when administered orally.

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Note in explanation:
This abstract reflects the revised statement of work for a proposal that originally was directed at the construction of transgenic hamsters. While we are still interested in doing this, and there are some promising new developments in this area, the original method has not worked and the new developments are not ready for an attempt with this application. In consultation with LtCol Carpenter we have revised the statement of work to support the development of this core resource. The NPRP meeting would give us an excellent opportunity to make the rest of the TSE community aware of this resource. Its continued support will depend upon broadening the user base for the resource. RGR

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Development of a Diagnostic for the Detection of PrPres in Blood and Urine

We have developed a sensitive assay based on the ORIGEN electrochemiluminescence technology that is capable of detecting 5 pg/ml prion protein at twice background levels in the presence of plasma. Comparison with other published assay formats indicates that all of the most sensitive assays have similar twice background sensitivities in the presence of plasma. We therefore propose twice background sensitivity as a standard point of comparison between assays. From our infectivity measurements in blood, we deduce that we will need at least 50 fold greater sensitivity to detect endogenous infection-specific prion protein in blood. This can be achieved either by increasing the sensitivity of the assay or concentrating the prion protein before assay.

Our assay development efforts have utilized our laboratory's quantitative model for blood-borne TSE infectivity in hamsters as well as naturally infected blood from scrapie infected sheep. Infected sheep blood can be obtained in 500 ml quantities equal to those in a human blood donation. This has enabled us to explore several methods of concentrating the prion protein from human scale blood collections prior to assay including precipitants, immuno-affinity, and synthetic ligand-affinity capture methods.

A blood-based diagnostic must also be capable of discriminating infection-specific prion protein from a >10,000 fold excess of native prion protein. We have been exploiting the ORIGEN assay to characterize and optimized protease digestion conditions, differential separation methods, and a novel immunological method.

Urine would be an ideal substrate for TSE diagnosis but initial reports of infection-specific prion protein in urine have proved to be an artifact. However, it is now claimed that urine contains TSE infectivity. We are attempting to verify this claim by applying the same highly sensitive and precise methods that we have developed for assaying TSE infectivity in blood to urine.

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Fluorescent Immunoassay Development for PrP^{Sc} Detection and Antemortem Diagnosis of TSEs

The overall goal of our project is to develop methods of high-sensitivity and high-specificity for ante mortem diagnosis of TSEs by detection of PrPSc in biological fluids. We have focused on development of immunological methods to isolate blood PrP from normal and scrapie-infected animals and the assembly of fiber-based fluorescence detection system. By employing magnetic bead-based immunocapture protocols, plasma PrP from normal and scrapie-infected animals was successfully isolated and can be detected directly by Western blot. In terms of resistance of PrP to proteinase K (PK) digestion, there was no marked difference in plasma derived PrP between normal and infected animals, although more extensive conditions should be tested. In parallel with the assay development we have designed and constructed an optical fiber-based detection instrument for use with the fluorescence based assay systems under development. This instrument takes advantage of the large effective numerical aperture of optical fibers to detect fluorescence from the sample through 4p steradians, for maximum collection efficiency. For ease of use, we have designed the system around standard, disposable micro-capillaries as a sample container, with the detection head re-configurable for acceptance of various volume size capillaries. The sample is excited along the axis of the capillary, and in the current configuration can be excited with multiple laser wavelengths. We are using phase sensitive detection (i.e. lock-in amplification) to achieve high sensitivity, which can be done for four separate detection channels if desired.

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Quantification of PrPSc Using Mass Spectrometry and Standard Peptides

In order to apply mass spectrometry to analyze PrPSc amounts in a given sample, some points had to be considered. Cellular PrP and PrPSc have the exactly same primary amino acid sequence. Since PrPSc is known to be PK resistant, PK digestion can be used to remove PrPC and, at the same time, reduce the complexity of the sample which is a prerequisite of doing mass spectrometry. To quantify PrPSc we focus on the smaller of the two cystein containing tryptic peptides denominated PrPep (VVEQMCVTQYQK).

Isotopic labeling procedure (ICAT) and the LC-MS/MS compatibility of PrPep were made with PrPep containing different modification on the cysteins. We found the heavy and light ICAT labeled PrPep to co-elute and the alkylated PrPep to be a potential internal standard. Oxidation on the methionine results in a reduction of PrPep precursor ion intensity but can be reversed by reduction with N-â-Mercaptoacetamid.

We performed first experiments using uninfected brain homogenate spiked with specific amounts of ICAT labeled PrPep. We could identify and quantify the labeled PrPep by analyzing the data with the suitable software (SEQUEST) indicating the applicability of the workflow. These tests were followed by spike experiments using infectious brain homogenate. Currently we are working on the sample preparation protocols with focus on the tryptic digest to increase the signal intensity of the PrPSc peptide. To improve the sensitivity on the MS side we started to establish SRM (Selected Reaction Monitoring) to quantify the peptide on MSMS level. This method allows to just focus on specific ions and to trace them in a complex mixture.

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The Antemortem Detection of Prion Proteins

The aim of our research is to develop an antemortem test for prion diseases based on a non-immunological, cell-based biological assay for the detection of PrP amyloid species. In addition, we focus on elucidating the infectious entity of prions and the molecular mechanism of generating prion infectivity. Blood from animals with prion disease contain low levels of prion infectivity, which primarily resides in the "buffy coat" fraction that contains lymphocytes and mononuclear cells. We believe that detecting individual infected white blood cells (WBCs) may increase the detection sensitivity by over 100-fold when compared with a method using pooled cells. We initially showed that a tetrazolium dye called MTT is able to detect biologically active amyloidogenic proteins, including prion proteins in single cells. This assay worked fine on cultured nerve and glial cells, but failed to be useful for the detection of prion infected lymphoid cells because of the high background in these cells. Since this assay ultimately turned out not to work, we have developed an alternative method that combines WBC isolation and cell blotting of PrP^{sc} to detect individual cells that contain PrP^{sc}. A sensitivity study shows that as low as 10 to 50 prion-infected cells can be detected, suggesting that it may have the potential to be used for the antemortem detection of prion infection in blood. In the second part of the project we initiated structural studies on HET-s, the prion protein of the fungus, P. anserina. The structural basis of prion infectivity and the origin and mechanism of infectivity has been elusive because acquisition of atomic resolution structural properties of amyloid fibrils represents an unsolved technical challenge. The C-terminal prion domain of HET-s comprises residues 218-289. Amyloid fibrils of HET-s(218-289) are necessary and sufficient for the induction and propagation of prion infectivity. We used fluorescence measurements, quenched hydrogen exchange NMR and solid state NMR to determine the sequence specific positions of secondary structure elements of the amyloid fibrils of HET-s(218-289). This information revealed four beta-strands constituted by two pseudo repeat sequences, each forming a beta-strand-turn-beta-strand motif. We showed that this conformation is the functional and infectious entity of the HET-s prion by using a structure-based mutagenesis approach. These results correlate for the first time distinct structural elements with prion infectivity.

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Spongiform Encephalopathy and Prion Plaque Generation by Mouse Transgenesis

Chronic wasting disease (CWD) is a naturally-occurring, geographically widespread prion disease in captive and free-ranging North American deer and elk, with unknown potential as a human health threat. The elk PrPC structure contains a well-defined loop connecting the second strand of the beta sheet with alpha helix 2 (amino acids 165-175) which is rigidified by two local hydrogen bond networks. These hydrogen bond networks are absent from mouse PrPC [1]. We have expressed a PrPC mutant in transgenic mice that mimics the elk "rigid loop" (RL). These transgenic mice develop a spontaneous neurologic disease with 100% penetrance characterized by vacuolar change, gliosis, microglial activation, and PrP plaques in the brain, similar to deer with CWD or patients with variant CJD or Gerstmann-Straussler-Scheinker syndrome (GSS), and typical of a transmissible spongiform encephalopathy.

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Molecular Characterization of Motif-Grafted PrPSc-Specific Antibodies

Antibody scaffolds containing grafted polypeptides comprised either of PrP amino acid residues 89-112 or of PrP residues 136-158, bind specifically and with high affinity, to PrPSc and PrP27-30 from prion-infected humans and rodents, but not to PrPC. Characterizing the nature of the specific binding interaction between the motif-grafted antibody reagents and disease-associated PrP conformers may yield insights into the formation of the PrPC-PrPSc interface, a key step in the assembly of the prion replicative complex, and enable the design and development of reagents for the diagnosis of prion infection.

Binding experiments indicate that the PrPSc epitope recognized by IgG 136-158 is proteinaceous, whereas recognition of PrPSc and PrP27-30 in brain homogenates by IgG 89-112 was diminished by pre-treatment of the homogenate with DNase, but not RNase. Moreover, binding of IgG 89-112 to the abnormal PrP conformers was readily inhibited in the presence of excess DNA. These observations suggest that binding to PrPSc and PrP27-30 by IgG 89-112 is at least partially dependent upon the presence of DNA, implying the existence of a complex containing PrPSc and DNA in homogenates of prion-infected brains. To identify key residues within the 89-112 PrP sequence graft contributing to the PrPSc binding interaction, we have generated an additional series of antibodies containing truncated and mutated PrP grafts. Binding studies using these reagents have identified a core PrPSc binding motif composed of PrP amino acids 95-105, within which lysine residues at positions 101 and 104 appear to perform a critical role.

An extensive panel of novel motif-grafted antibodies containing other regions of PrP peptide sequence has been generated to identify possible additional sites of interaction between PrPC and PrPSc. The reactivity of these novel reagents with different PrP conformers will be presented.

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Gene Expression Analysis of Prion-Infected Mice

Selection and Evaluation of DNA Aptamers against PrPC and PrPSc as Conformation-Specific Tools in Scrapie Diagnostics

DNA aptamers were selected by systematic evolution of ligands by exponential enrichment (SELEX) using a 40-mer aptamer library flanked by 28-mer primer-binding sites that theoretically represented approximately 1024 distinct nucleic acid species. Eight aptamers selected against recombinant human prion protein (rhuPrPC 23-231) bound to mammalian PrPC and not to PrPSc. These aptamers bound to PrPC immunoprecipitated from healthy sheep, calf, piglet and deer, and to PrPC expressed in mouse neuroblastoma cells, but did not bind to proteinase K digested mouse neuroblastoma cells or PrP-null cells in chemiluminescent gel-shift and dot blot analyses. PrPC-specific aptamers recognized a conformational motif in the 23-89 region of the molecule and the binding was aptamer sequence and structure dependent. Double-ligand sandwich protocols applying aptamer-aptamer and aptamer-monoclonal antibody (with binding specificities in the 90-231 molecule) combinations could detect down to 1-ng/ml rhuPrPC 23-231; these aptamers also bound to plasma and milk PrPC isoforms. Six aptamers selected against hamster drowsy PrPSc bound to both PrPSc and 23-231 and 90-231 PrPC molecules in gel shift assays. In conclusion, the selected DNA aptamers that bind specifically to rhuPrPC, mammalian PrPC, and PrPSc have been characterized and can be applied for prion test of biological samples as diagnostic tools.

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Molecular chaperones and prion disease

Prion diseases are devastating diseases of the central nervous system. Although there are many unsolved issues in prion diseases, it is widely accepted that the conformational conversion of the prion protein (PrP) triggers neuronal dysfunction and cell death. Molecular chaperones are protein re-modeling factors that are particularly important during environmental stresses, such as heat or other conditions that lead to protein misfolding. Surprisingly little attention has been given to the role of molecular chaperones in mammalian prion diseases, despite the well documented effects of molecular chaperones on the conformational conversion of yeast prions. We have initiated a project exploring the role of molecular chaperones in mammalian prion diseases. As a starting point, we have inoculated Heat Shock Factor 1 (Hsf1) KO mice, which are incapable of mounting the classic stress response (Xiao et al., 1999). Hsf1 is a transcription factor that mediates the stress response by up-regulating a variety of molecular chaperones. We have inoculated both Hsf1 KO and wild-type mice with a range of doses of infectious prions (RML strain). Hsf1 KO mice succumb to prion disease significantly faster than controls, indicating an important role for the stress response, and possibly molecular chaperones, in prion pathogenesis.

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Neurodegenerative Illness In Transgenic Mice Expressing A Transmembrane Form Of The Prion Protein

Although PrPSc is widely believed to be the infectious form of the prion protein, it may not be the proximate cause of neuronal cell death in prion diseases. CtmPrP is a single-span transmembrane version of the prion protein produced during translocation of PrP into the ER, and this PrP isoform has been proposed as a candidate for a neurotoxic intermediate underlying prion-induced pathogenesis. To investigate this hypothesis, we have constructed transgenic mice that express L9R-3AV PrP, a mutant prion protein that is synthesized exclusively in the CtmPrP form in transfected cells. These mice develop a fatal neurological illness characterized by ataxia and marked neuronal loss in the cerebellum and hippocampus. Tg(L9R-3AV) PrP does not have any of the biochemical properties of PrPSc. We have demonstrated that Tg(L9R-3AV) neurons produce CtmPrP by several assays, including use of an antibody specific for CtmPrP previously characterized in our laboratory. CtmPrP in cultured primary neurons is localized to the Golgi apparatus, rather than to the endoplasmic reticulum as in transfected cell lines; this observation suggests a possible locus of CtmPrP-mediated toxicity. Most surprisingly, the severity of the neurodegenerative phenotype induced by Tg(L9R-3AV) PrP expression is strongly dependent on co-expression of endogenous, wild-type PrP. Our results provide new insights into the cell biology of CtmPrP, the mechanism by which it induces neurodegeneration, and possible cellular activities of PrPC.

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An Aggregation Specific Elisa: Detection Of Conformational Difference Between Recombinant PrP Protein Dimers And PrPSc Aggregates

The conversion of the normal cellular prion protein, PrPC into the protease resistant, scrapie PrPSc aggregate is the cause of prion diseases. We developed a novel ELISA that is specific for PrP aggregate, by screening 30 anti-PrP monoclonal antibodies (Mabs) for their ability to react with recombinant mouse, ovine, bovine or human PrP dimers. One Mab that reacts with all four recombinant PrP dimers also reacts with PrPSc aggregates in ME7, 139A or 22L-infected mouse brains. The PrPSc aggregate is proteinase K resistant, has a mass of 2000-kDa or more, and is present at a time when no protease resistant PrP is detectable. This simple and sensitive assay provides the basis for the development of a diagnostic test for prion diseases in other species. Finally, the principle of the aggregate specific ELISA we have developed may be applicable to other diseases caused by abnormal protein aggregation, such as Alzheimer's disease or Parkinson's disease.

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Rapid and Sensitive Detection of Prions in GT1-1 Cells

Our goal is to devise a rapid, cell culture-based prion bioassay by stepwise improving the infection of cells, the propagation of prions therein, and the detection of infected cells. We have concentrated on the mouse hypothalamic line GT1-1, which is very susceptible to mouse RML scrapie. Better ways to infect these cells, including the absorption of prions to steel particles, are being evaluated.

Expressing a 3F4 epitope-labeled mouse PrP in these cells solved the inoculum background problem. We are now able to detect de novo PrPSc (and hence infected cells) hours after their exposure to the medium of infected ScGT1 cells. We have devised several ways to increase PrPSc in these cells, including (i) treatment with brain-derived neurotrophic factor (BDNF, J Neurosci. 2005 25:8451-6) and (ii) decreasing lysosomal degradation of PrPSc with cathepsin inhibitors (J Virol. 2004 78:4776-82). A three-fold PrPSc increase was achieved by treating the cells with the DNA active drug trichostatin A (TSA) for 16 h prior to detection. TSA increased the expression of PrP under the control of a CMV promoter. We are evaluating several methods to detect PrPSc, including western and dot blots, and colony blotting. A broad spectrum, lipofuscin-like fluorescent background in GT1-1 cells hampers the immunofluorescent detection of PrPSc. We are exploring whether "quantum dots" fluorophores, with their very narrow-peaked spectra, may help solve this problem. Improvements needed to further improve the sensitivity and the generality of this system, and possible methods to achieve them, will be discussed

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Towards Purification and Characterization of the TSE Infectious Agent

The Transmissible spongiform encephalopathy (TSE) diseases have proved particularly difficult to study because of the heterogeneous form of the infectivity. For rational development of diagnostics and therapies, a better understanding of the structure of the elemental unit of infectivity is needed. To that end our laboratory used brain homogenate from infected hamsters to create a novel preparation of the TSE agent in a highly dispersed form capable of passing through 15 nm nanofilters. Now we are purifying the dispersed infectivity away from other molecular contaminants. It is important that at every step of our purification, we verify that the infectivity is not being inactivated and is being maintained in a dispersed state. We therefore established an assay for dispersion and tested infectivity using the hamster bioassay. Nuclease and protease enzyme treatments reduced the levels of some contaminants in the preparation without inactivating infectivity or causing aggregation. We applied density gradient equilibrium ultracentrifugation to separate particles in our treated, dispersed preparation based on the buoyant density of the infectivity. CsCl, a common density media, proved unsuitable for our purposes because it caused aggregation of PrPres. Despite this loss of dispersion, two sharp peaks of PrPres and TSE infectivity were formed in the CsCl gradient. Subsequent studies with low ionic strength media have succeeded in concentrating PrPres and purifying it away from greater than 99% of total protein while maintaining its small average particle size. Nucleic acids in the preparation have been reduced by at least 97%. The purified dispersed material is being titered by bioassay and the process is being scaled up.

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Award: DAMD17-03-1-0476

New Approaches to Understand the Structure of Disease Related Forms of the PrP

To gain insights into the organization of the prion protein, PrP, in its disease associated 'scrapie' form (that is aggregated, at least when isolated), we are applying solid state NMR and hydrogen exchange methods to PrP constructs. Our focus has been on a peptide model, residues 89-143 with a P101L mutation, designated P55, that has been shown to induce prion disease in animals expressing PrP with the P101L mutation, but only when the peptide is inoculated in an aggregated, fibrillar form. This peptide is the smallest construct of PrP that has shown clear activity in an in vivo assay. Although smaller than PrP itself, the relatively broad resonances observed in solid state NMR limit our ability to interpret spectra from uniformly labeled samples. Selective labeling by chemical synthesis has been applied, but cost is limiting for many labeled amino acids. We have explored selective incorporation using in vitro translation and also in vivo expression. The in vitro approach has better selectivity, but while P55 could be produced, the amount was small. With expression in E. coli we have produced and purified semi-selectively labeled P55 in larger quantities and are carrying out solid state NMR studies of to probe local conformation, and organization in fibrils. The kinetics of the fibrillization process of the P55 peptide have been studied to optimize conditions for fibril formation, and to enable structural comparison of initial small fibrils with larger mature ones. Results and their relation to our current understanding of the structure of P55 in fibrils will be discussed. To complement the solid state NMR, and to provide a method for analyzing full length PrP in aggregated states we are determining rates of hydrogen exchange of backbone amides. Analysis is being done both with mass spectroscopy (requiring very small amounts of sample but with limited resolution in which amides are exchanging), and also NMR spectroscopy of dissolved fibrils (high sequence resolution but requiring large amounts of purified and isotopically labeled protein). Fully 13C/15N labeled peptide was produced and the backbone resonances were assigned in mixed organic/water solutions. The protocols for doing deuterium exchange into the fibrils (through which the NH correlation peaks disappear) have been established. The methods and measurements will be described. Mass spec detection of hydrogen exchange has also been pursued. Initial experiments indicate limited fragmentation by pepsin (the protease of choice to initially break up the protein into fragments). We are pursuing addition of other proteases to increase the coverage of PrP that can be achieved. Our results will be presented. The combination of solid state NMR and hydrogen exchange are being used to define conformations and interactions of the peptide backbone that will lead to a model for P55 in the fibril state. The utility of this information for analyzing the behavior of PrP in vivo will be described.

Alan J. Young
South Dakota State University, Brookings, SD
Award: DAMD17-03-1-0399

Migratory Leukocytes in the Pathogenesis and Progression of Scrapie

Transmissible spongiform encephalopathies (TSEs) are infectious neurological diseases that are increasingly of human health concern . Although the oldest of these diseases - scrapie - has been recognized for nearly 300 years, many details of prion pathogenesis remain unclear. Methods to detect and eliminate infectious prions (PrPSc) from biological samples are limited due to incomplete knowledge of disease pathogenesis. Although the primary cell type associated with TSEs is the neural cell, immune cells also form an important reservoir of disease. Specifically, follicular dendritic cells (FDCs) and B cells in germinal centers are the earliest identifiable source of PrPSc propagation. Given that B cells are migratory cells, continually recirculating between the blood and the tissues via the lymph, they could contribute to blood-associated infectivity. We have previously described two equally represented but phenotypically distinct subsets of B cells in the peripheral blood of ruminants. Using animals experimentally affected with scrapie, we have found disease-associated changes in the distribution and phenotype of these two populations. In acute studies, differentiation of these two subsets appears to be affected by prion infection such that significantly reduced numbers of B cells are released from prion-stimulated as opposed to control lymph nodes. In addition, preliminary studies indicate a specific role for ruminant γδ-T cells in the response to prion infection. More importantly, we identified PrPSc associated with leukocytes migrating through both the afferent and efferent lymphatics which drained sites of prion protein inoculation. In vitro FDC-B cell coculture from scrapie-infected animals indicates that at least some of these cells are recirculating, blood-borne B cells. These data clearly support a role for migratory cells in prion pathogenesis and may potentially be exploited as novel diagnostic techniques.

Dr Qi Zhao
University of Dundee, Dundee, Scotland
Award: DAMD17-03-1-0423

Development of Anti-Prion Surgical Instrument by Ion Implantation Technique

Currently there is no accepted procedure for the removal of prion infectivity from surgical instruments as prion proteins are readily and tightly bound to the instrument surfaces, which significantly increases the risks of iatrogenic transmission of CJD. The purpose of this research is to minimize prion adhesion forces by altering the surface energy of surgical steels using ion implantation techniques, so that prions can be removed both easily and completely from the surfaces.

In this study, the total interaction energy between prion particles and stainless steel surfaces were calculated using the extended DLVO theory and the required surface energy of modified steel surfaces for minimizing prion protein adhesion was derived. The stainless steel 316L plates and crystal sensors were implanted with F, Si, and H under various ion implanted energy and dose. The surface energy values of the implanted surfaces and proteins were measured using Dataphysics OCA-20 contact angle analyzer. The adhesion of prion equivalent proteins on ion implanted surfaces in a real time was measured using a Quartz crystal microbalance (QCM-D300) with high sensitivity 5 ng/cm2. The experimental results showed that the F implanted surface with surface energy 21.1 mN/m inhibits prion protein adhesion significantly, compared with stainless steel 316L and H and Si ion implanted surfaces, which is consistent with our modeling results. The proteins can be removed/cleaned easily from F implanted surface, and no protein residual was observed with SEM after cleaning. While some protein residual was observed on stainless steel surfaces, H implanted surface and Si implanted surface after cleaning under identical conditions. The infectivity of ion implanted surfaces was also assayed using cell culture.

E

Investigators' Accomplishments By Year and Research Area

The following table provides information on the research accomplishments resulting from funding through the NPRP and for Small Business Innovation Research (SBIR) grants managed by the CDMRP

Research Area: Basic Biology

Year	Accomplishment/Findings	Investigator (Grant)	Institution
2002	Microglia from Creutzfeldt-Jakob disease-infected brains are infectious and show specific mRNA activation profiles. CJD infected microglia displayed morphological changes indicative of cellular activation.	Manuelidis (NP020028)	Yale University
2002	Case Western Reserve University Unique inflammatory RNA profiles of microglia in CJD/Microglia from CJD-infected brains are infectious and show specific mRNA activation profile.	Manuelidis (NP020028)	Yale University
2002	Demonstrated a shortening of incubation time for human prions in mice expressing a chimeric mouse-human prion protein transgene.	Pruisner (NP020038)	UCSF
2004	Development of high affinity aptamers, pieces of DNA and RNA, which bind to 23-89 residues of the prion protein.	Sreevatsan (NP020123)	University of Minnesota
2004	Recombinant mouse prion protein (recMoPrP) produced in Escherichia coli was polymerized into amyloid fibrils that represent a subset of beta sheet-rich structures. Fibrils consisting of recMoPrP(89-230) were inoculated intracerebrally into transgenic (Tg) mice expressing MoPrP(89-231). The mice developed neurologic dysfunction between 380 and 660 days after inoculation.	Pruisner (NP020038)	UCSF
2004	Demonstrated upregulation of 10 interferon-sensitive genes at very early stages of infection before abnormal prion protein and clinical signs of CJD were detectable. The induction of interferon-sensitive genes without appreciable interferon synthesis is similar to viral	Manuelidis (NP020028)	Yale University

Year	Accomplishment/Findings	Investigator (Grant)	Institution
	infection.		
2004	Demonstrated that the synthetic peptide-induced disease can be serially passaged in Tg196 mice and that the PrP conformers accompanying disease progression are conformationally indistinguishable from MoPrPSc(P101L) found in Tg2866 mice developing spontaneous prion disease.	Pruisner (NP020038)	UCSF
2004	Development of an animal model showing in vivo movement of labeled scrapie associated fibrils (SAFs) across the intestinal mucosa. SAFs were shown to penetrate beyond the luminal surface.	Felts (NP020059)	King's College London
2004	A virus behind the mask of prions? Biological features of prion protein similar to conventional viral particles.	Manuelidis (NP020028)	Yale University
2005	Knockdown of ATP-binding cassette protein A1 (ABCA1) expression by siRNA approach reduces prion replication by reducing normal prion protein expression.	Kumar (NP020147)	McLaughlin Institute
2005	Identified differential expressed genes (DGEs) based on temporal patterns of expression that are shared in five mouse strain-prion strain combination.	Carlson (NP020044)	McLaughlin Institute
2005	Demonstrated that a transmembrane form of the prion protein (Ctm PrP) is localized in the golgi apparatus of neurons.	Stewart (NP020064)	Washington University
2005	Elucidated the structure of copper-prion complex and mechanism of copper inhibition of infectious prion conversion.	Cox (NP020132)	UC Davis
2005	Demonstrated that neurospheres may offer new approaches to studying the replication of prions/tool for the study of genes modulating prion susceptibility.	Carlson (NP020044)	McLaughlin Institute
2005	Beta-sheet structure shown as the infectious conformation of HET-s prion of the yeast Podospora anserina.	Schubert (NP020089)	Salk Institute for Biological Studies
2005	Elucidated the structure of copper-prion complex and mechanism of copper inhibition of infectious prion	Cox (NP020132)	UC Davis

Year	Accomplishment/Findings	Investigator (Grant)	Institution
	conversion.		
2005	Showed that infection of mice with an attenuated CJD agent (SY-CJD) interferes with superinfection by a more virulent human-derived CJD agent (FU-CJD).	Manuelidis (NP020028)	Yale University
2005	Demonstrated the effect of disease-related mutations on the anti-apoptotic activity of PrP using a yeast model. Mammalian prion protein suppresses Bax-induced cell death in yeast.	Harris (NP020061)	Washington University
2005	Demonstrated the coexistence of multiple PrP^{Sc} types in individuals with Creutzfeldt-Jakob disease.	Aguzzi (NP020121)	University of Zurich
2005	Generated Tg mice expressing full-length bovine prion protein.	Pruisner (NP020038)	UCSF
2005	Demonstrated neurodegenerative illness in Tg mice expressing a transmembrane form of the prion protein.	Stewart (NP020064)	Washington University
2005	Identified differentially expressed genes in prion-infected neurospheres.	Carlson (NP020044)	McLaughlin Institute
2006	DNA aptamers selected against recombinant prion proteins bind mammalian normal prion protein and not abnormal misfolded prion protein.	Sreevatsan (NP020123)	University of Minnesota
2006	Generated the Tg mice expressing prion protein from Rocky Mountain elk, white tailed deer and mule deer.	Pruisner (NP020038)	UCSF
2006	Showed chronic lymphocytic inflammation enables prion accumulation in otherwise prion-free organs.	Aguzzi (NP020121)	University of Zurich
2006	Demonstrated substantial differences in proteinase K-digested PrP^{Sc} fragments from chronic wasting disease of elk and deer and Creutzfeldt-Jakob disease point to non-transmissibility of CWD from cervids to humans.	Chen (NP020069)	Case Western Reserve University
2006	Demonstrated that naturally occurring prion protein has the potential to bind metals and that copper bound to normal prion protein may protect it from conversion to misfolded prion protein.	Taraboulos (NP020163)	Hebrew University of Jerusalem
2006	First demonstration of prions in skeletal muscles of deer with chronic wasting disease putting humans who	Sigurdson (NP020081)	University Hospital

Year	Accomplishment/Findings	Investigator (Grant)	Institution
	consume or handle such meat at risk.		Zurich
2006	Showed that prions adhered to both soil and soil minerals (quartz, montmorillonite, and kaolinite). Showed that prions adsorbed to montmorillonite remained infectious after intracerebral inoculation into Syrian hamsters.	Aiken (NP020087)	University of Wisconsin
2006	Found heat shock factor regulating yeast prion de novo appearance and strain inheritance.	Li (NP0201600)	Northwestern University
2006	First demonstration of PrP^{Sc} in mammary glands of sheep affected by scrapie and mastitis suggesting the possibility of horizontal prion spread within flocks.	Sigurdson (NP020081)	University Hospital Zurich
2006	Demonstrated that two polymorphisms G96S and Q95H in white-tailed deer were linked to a reduced susceptibility to CWD.	McKenzie (NP020094)	University of Wisconsin
2006	Demonstrated that conformational polymorphism of prion protein within a single amyloid fibril can be detected using a novel dual color immuno-conformational assay using an array of antibodies recognizing different PrP epitopes.	Robert Williamson (NP020119)	The Scripps Research Institute
2007	Demonstrated virus like arrays of ~ 25nm particles are the cause of TSE.	Manuelidis (NP020028)	Yale University

Research Area: Detection and Diagnosis

Year	Accomplishment	Investigator (Grant)	Institution
2004	Development of high affinity aptamers, pieces of DNA and RNA, which bind to 23-89 residues of the prion protein. (MOVE TO DETECTION/DIAGNOSTICS)	Sreevatsan (NP020123)	University of Minnesota
2004	Antibodies grafted with replicative interface sequences of cellular PrP specifically bind to PrP^{Sc} and not normal PrP, preventing PrP:PrP^{Sc} interaction required for replication.	Williamson (NP020119)	The Scripps Research Institute
2004	Detected Immunoglobulins in urine of hamsters with Scrapie using the anti-mouse IgG secondary antibody.	Pruisner (NP020038)	UCSF

Year	Accomplishment	Investigator (Grant)	Institution
2004	Developed a follicular dendritic cell (FDC)-based in vitro cell culture assay derived from susceptible deer and elk CWD to amplify prion in blood, brain, and lymph nodes.	SBIR	Rural Technologies
2004	Developed a cell culture-based assay involving engineering of a cow B-lymphocyte cell line to surface express high levels of elk PrP^{C} fused to yellow fluorescent protein.	SBIR	Nomadics, Inc
2005	Identified new markers for defining different stages of progressive disease in CJD infected mouse brain such as (a) elevation of serum amyloid A3 and L-selectin as early as 20 days after intracellular inoculation; (b) upregulation of MIP-1alpha, MIP-1beta, MCP1, IL1alpha and TNFalpha well before prion protein abnormalities that begin only after 80 days; and (c) symptomatic neurodegenerative disease after 100-110 days.	Manuelidis (NP020028)	Yale University
2005	Developed and patented a prototype optical fiber-based fluorescent system for detection of extremely low concentration PrP^{Sc} in biological fluids from infected animals. Also demonstrated for the first time PrP^{Sc} isolation from blood plasma.	Rubenstein (NP020048)	New York State University
2005	Developed Misfolded Protein Diagnostic (MPD) technology for the detection of the infectious form of prion protein by using conformationally active peptides that are sequence-matched to the target prion protein. These peptides undergo conformational changes resulting in fluorescent signals when exposed to infectious prion proteins.	SBIR	Adlyfe, Inc
2005	Optimized Surface-Enhanced Laser Desorption/Ionization time-of-flight (SELDI-TOF) mass spectrometry-based proteomics for the identification of potential surrogate markers for sheep scrapie.	SBIR	Rural Technologies
2005	Developed a novel aggregation-specific ELISA (AS-ELISA) that is specific for disease associated PrP^{Sc} aggregates present in the brains of mice infected with any one of the three strains of mouse PrP^{Sc}: ME7,	Sy (NP020030)	Case Western Reserve University

Year	Accomplishment	Investigator (Grant)	Institution
	139A, or 22L.		
2005	Designed unique PCR primers capable of amplifying and detecting disease-specific genetic material i.e. circulating nucleic acid sequences in serum and plasma samples of human prion disease.	SBIR	Chronix Biomedical
2005	Developed a simple, rapid Western blotting method for the reliable detection of 1-2 ng/ml of prion protein in human urine. The average prion concentration in healthy individuals is 7 ng/ml.	Chen (NP020069)	Case Western Reserve University
2005	Compared conformation-dependent immunodiagnostic assay (CDI) with histological and immunohistochemistry (IHC) methods for the diagnosis of CJD in 18 brain regions from 8 patients and demonstrated that CDI is the most sensitive method for diagnosis of CJD.	Pruisner (NP020038)	UCSF
2005	Developed a molecular imprinting technique, conducting polymer-based sensors for the detection of CWD infectious protein in cervid tissues and fluids. Demonstrated detection sensitivity at about 4-5 ppm.	SBIR	TDA Research Inc
2005	Developed a Mab and lectin ELISA assay that distinguishes sCJD PrP^{Sc} from vCJD PrP^{Sc}.	Sy (NP020030)	Case Western Reserve University
2005	First demonstration of PrP^{Sc} isolation from blood plasma using magnetic bead-based immunoassay coupled with laser-induced fluorescence spectrofluorometry	Rubenstein (NP020048)	New York State University
2005	Developed a new, extremely sensitive analytical technique capable of detecting and quantifying proteins by coupling immunoassays with Multi-Photon Detection (MPD) for identifying prion biomarkers.	SBIR	Bio Traces, Inc
2006	Developed immortalized cell lines for prion propagation using a primary cervid cell culture transformed with a vector carrying the gene encoding the human telomerase reverse transcriptase.	SBIR	DNA Solution
2006	Developed a bio-bar code, bead-based ELISA assay for detecting purified prion protein that successfully determines the concentration of protease sensitive prion protein in urine.	Lewis (NP020152)	University of Wyoming
2006	Developed a real-time immuno-PCR to detect ultra-low levels of pathologic prion protein in scrapie infected hamster brain homogenates.	Constantine (NP020120)	University of Maryland Baltimore

Year	Accomplishment	Investigator (Grant)	Institution
2006	Developed a high-throughput dot blot assay for detection of PrPSc in sheep.	Caughey (NP020114)	NIH Rocky Mountain Laboratories
2006	Developed CNS stem cell cultures grown as neurospheres that can be infected with prions for developing a sensitive bioassay for mouse prions	Carlson (NP020044)	McLaughlin Institute
2006	Defined the details of the ELISA plasma-based prion detection assay in hamster infected with scrapie. Optimized and refined the proteinase K (PK) conditions for digestion of endogenous plasma prion protein.	Rohwer (NP020178)	Baltimore Research and Education Foundation
2006	Developed NanoLC Proteomics System for LC separation of complex protein mixtures that could detect 100's of fmols of proteins in the 20-30kDa range.	De Yoreo (NP020111)	Lawrence Livermore National Laboratory
2006	Developed a fluorescence spectroscopy method to detect accumulated prion and lipofuschin in the retina, lens and sclera of the eye of scrapie infected sheep.	Petrich (NP020116)	Iowa State University
2006	Developed high-throughput assays for human CJD using new, rapid tissue culture assays that can be used for agent strain identification and titration.	Manuelidis (NP020028)	Yale University
2006	Demonstrated a novel PrP conformational antibody that specifically recognizes pathologic prion protein.	Williamson (NP020119)	The Scripps Research Institute
2006	Developed a MALDI-FTMS technique and bioinformatics software "specmap" for protein profiling of cerebrospinal fluid and serum from hamsters and mice. Identified a 2730 kDa protein that predicted disease in 11 out of 13 animals.	Aiken (NP020090)	University of Wisconsin
2006	Developed a method for identifying individual prion-infected white blood cells (WBC's) of mice using WBC isolation and cell blotting of PrP^{Sc}. Demonstrated detection limits as low as 10 prion-infected cells in 5 X 105 WBC's.	Schubert (NP020089)	Salk Institute for Biological Studies
2006	Developed a protocol aptamo-PCR method using a magnetic bead capture of PrP^{Sc} and amplification of aptamers bound to PrP^{Sc}. Optimized conditions for dual-color fluorescence to enable prion detection at a	Sreevatsan (NP020123)	University of Minnesota

Year	Accomplishment	Investigator (Grant)	Institution
	single molecule level.		
2006	Developed an Anti-DNA antibody OCD4 that detects PrP^{Sc} but not endogenous PrP (PrP^{C}) in humans and animals.	Su Chen (NP020069)	Case Western Reserve University
2006	Identified 10 putative genes from the blood of mice in which the up-regulation is correlated with disease presence.	Aiken (NP020090)	University of Wisconsin
2006	Developed fluorescence correlation spectroscopy method to detect PrP in the picomolar range.	De Yoreo (NP020111)	Lawrence Livermore National Laboratory

Research Area: Prevention and Treatment

Year	Accomplishment	Investigator (Grant)	Institution
2004	Demonstrated that repeated CpG oligodeoxynucleotide administration destroys lymphoid compartments that replicate prions.	Aguzzi (NP020121)	University of Zurich
2005	Demonstrated that HDAC Inhibitors of the MEK1/2 signaling pathway affected prion-infected cells	Taraboulos (NP020163)	Hebrew University of Jerusalem
2005	Demonstrated that tannic acid, pentosan polysulfate and Fe(III) deuteroporphyrin 2,4-bisethyleneglycol are potent inhibitors of mouse and sheep PrPSc.	Caughey (NP020114)	NIH Rocky Mountain Laboratories
2006	Demonstrated that intracerebral injection of Fe(III) meso-tetra (4-sulfonatophenyl) porphyrin increased survival time of mice after intracerebral prion infection.	Caughey (NP020114)	NIH Rocky Mountain Laboratories
2006	Showed that cholinesterase inhibitors huprine X and Y purged PrPSc in scrapie-infected Rov9 cells.	Chiang (NP020088)	Pharmadyn, Inc
2006	Demonstrated that degenerate phosphorothioate oligonucleotides exhibited anti-scrapie activities in vivo.	Caughey (NP020114)	NIH Rocky Mountain Laboratories
2006	Demonstrated that mefloquine, an anti-malaria drug with anti-prion activity in vitro, lacked activity in vivo.	Caughey (NP020114)	NIH Rocky Mountain

Year	Accomplishment	Investigator (Grant)	Institution
			Laboratories
2006	Showed that antimalarial drugs tafenoquine and mefloquine were the most potent compounds against two different strains of mouse prions 22L and RMl in infected N2a cells.	Chiang (NP020088)	Pharmadyn, Inc

Research Area: Prevention and Treatment

Year	Accomplishment	Investigator (Grant)	Institution
2002	Demonstrated that murine CNS stem cell cultures grown as neurospheres can be infected with prions.	Carlson (NP020044)	IBRP McLaughlin Institute
2004	Developed software programs for the yeast gene deletions.	Kennedy (NP020184)	University Washington
2004	Developed follicular dendritic cell (FDC) culture system and several FDC cell lines from normal and infected sheep.	Young (NP020085)	South Dakota State University
2005	Produced and purified recombinant sheep-PrP fragments 23-231 and 90-231.	Sreevatsan (NP020123)	University of Minnesota
2006	Developed embryonic stem cell lines carrying different PrP mutations. Created Tg mouse lines expressing cyPrP.	Lindquist (NP020105)	Whitehead Institute for Biomedical Research
2005	Developed FDC cell lines from ileal Peyer's patch and retropharyngeal lymph node.	Young (NP020085)	South Dakota State University
2005	Developed mouse neuroblastoma N2a cells infected with the RML and 22L strains of mouse prions.	Chiang (NP020088)	Pharmadyn, Inc
2005	Generated CHO cell lines expressing wild-type prion protein (PrP) and the accumulated undegraded cytoplasmic PrP (cyPrP).	Lindquist (NP020105)	Whitehead Institute for Biomedical Research
2006	Established a rapid and sensitive cell culture system for the detection of prions based on the GT1-MHM2 cell system, allowing detection of nascent abnormal prion protein in infected cells within hours of the infection.	Taraboulos (NP020163)	Hebrew University
2006	Constructed a set of C. elegans expression plasmids for	Li	Northwestern

Year	Accomplishment	Investigator (Grant)	Institution
	prion proteins.	(NP020160)	University
2006	Developed a deer cell line infected with chronic wasting disease PrP to facilitate in vitro testing of drugs.	Caughey (NP020114)	NIH Rocky Mountain Laboratories
2006	Developed murine-adapted CWD, i.e., CWD in transgenic mice overexpressing murine PrP (as opposed to deer or elk PrP) for pathogenesis, diagnostic, and therapeutic studies in mice.	Sigurdson (NP020081)	University Hospital Zurich
2006	Established flock of genotyped sheep. Established repository of prion infected blood and plasma samples stocked for use by the TSE research community.	Rohwer (NP020078)	Baltimore Research and Education Foundation
2006	Developed bioinformatics tool "Specmap" for a comprehensive proteomic analysis package for protein profiling of cerebrospinal fluid and serum.	Aiken (NP020090)	University of Wisconsin

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2007 NPRP Summit Participants

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CDMRP Staff

Dr. Carole Christian
Dr. Katherine Moore
Ms. Patricia Roth

G

2007 National Prion Research Program Summit

AGENDA

Tuesday, October 2, 2007
Welcome *Dr. Salvatore Cirone* *Program Director, Health Science Policy* *Office of the Assistant Secretary of Defense for Health Affairs*
Introductory Remarks and Charge to the Summit Participants *Colonel Janet Harris, U.S. Army, Nurse Corps* *Commander, Congressionally Directed Medical Research Programs (CDMRP)*
Opening Session - Keynote Address ***"Perspectives on Prion Research"*** *Dr. Robert Rohwer* *Director of the Molecular Neurovirology Laboratory at the Veterans Affairs Medical Center in Baltimore* *Associate Professor of Neurology at the University of Maryland, School of Medicine, MD*
Wednesday, October 3, 2007
Introduction to Workgroup *Dr. David Asher* *Chief and Supervisory Medical Officer in the Laboratory of Bacterial, Parasitic and Unconventional Agents within FDA's Division of Emerging and Transfusion-Transmitted Diseases, Office of Blood Research and Review, Center for Biologics Evaluation and Research*
Progress in Basic Biology *Dr. Byron Caughey* *Senior Investigator in the Laboratory of Persistent Viral Diseases* *Rocky Mountain Laboratories, National Institutes of Health (NIH)*
Progress in Detection and Diagnostics *Dr. Alex Raeber* *Head of Research & Development* *Prionics, AG*
Progress in Prevention *Dr. Bruno Oesch* *Chairman of the Board of Directors* *Prionics AG*

Progress in Therapeutics and Surveillance
Dr. Phil Minor
Head of Division of Virology
National Institute for Biological Standards and Control

Workgroup Sessions
Session 1:Basic Biology, Dr. Caughey, Moderator
Session 2:Detection and Diagnostics, Dr. Raeber, Moderator
Session 3: Prevention, Dr. Oesch, Moderator
Session 4:Therapeutics and Surveillance, Dr. Minor, Moderator

CDMRP National Prion Research Program Storyboard
Dr. Nrusingha Mishra
Director of Grants, CDMRP

Workgroup Sessions
Session1: Basic Biology, Dr. Caughey, Moderator
Session 2: Detection and Diagnostics, Dr. Raeber, Moderator
Session 3: Prevention, Dr. Oesch, Moderator
Sessions 4:Therapeutics and Surveillance, Dr. Minor, Moderator

Thursday, October 4, 2007

Wrap-Up
Dr. Suzette Priola
Rocky Mountain Laboratories, NIH

Workgroup Reports and Recommendations
Moderators

Closing Plenary
Dr. Robert Will
Professor of Neurology, University of Edinburgh
Director, National Cruetzfeldt-Jakob Disease Surveillance Unit
Consultant Neurologist & Senior Lecturer, Department of Neurosciences, Western General Hospital, Edinburgh

Closing Remarks
Captain Melissa Kaime, U.S. Navy, Medical Corps
Deputy Commander, CDMRP

Adjourn