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ABSTRACT

In the original proposal, we proposed a use of new transposon technology developed by TOSK Inc. to introduce the human, sheep, white tail deer, bovine, and mouse prion genes into golden Syrian hamsters. TOSK Inc. failed to produce a working technology to achieve these goals and therefore we lost a year of research and proposed a new statement of work. This annual report will summarize why we have proposed a revised statement of work and re-state the approved statement of work. With a re-direction of funds and an approved one year no-cost extension, we have proposed to accomplish the following goals: 1) Maintain a scrapie infected sheep flock in Idaho, 2) Use transgenic mice (with the sheep PrP gene) to titer the infectivity of plasma from the sheep flock, 3) Pool, and bring to homogeneity the sheep plasma and separately the frozen whole blood that we have collected over the last five years and redistribute each into aliquots that will serve as a common TSE infected blood reference material, 4) Develop a protocol for distribution of sheep resource materials to academic requesters and a price list for distribution of the same materials to commercial interests, 5)Continue to develop the short incubation time model of sheep scrapie by breeding for VVQQ genotype and infecting with inocula prepared from the first passage transmissions

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INTRODUCTION

In the original proposal titled "Efficient and Rapid Development of Transgenic Hamster Models of TSEs Using a Radical New Technology," we proposed a use of radical new transposon technology developed by TOSK Inc. to introduce the human, sheep, white tail deer, bovine, and mouse prion genes into golden Syrian hamsters. The original specific aims were:

1) To make a proof of principal of the new TOSK transposon technology by obtaining the first ever transgenics of hamsters.
2) To obtain transgenic hamsters with a high level of susceptibility to human, sheep, deer, and cattle TSEs.
3) To test the level and spectrum of susceptibility of the transgenic animals obtained in aim 2 to infection by TSE agents obtained from humans, sheep, deer, and cattle.
4) To test the ability of the transgenic hamsters carrying the human and sheep PrP genes to detect TSE infectivity in blood from individuals infected with CJD, vCJD, and sheep scrapie.

This annual report will summarize why we have proposed a revised statement of work, re-state the approved new statement of work, and formally request a one year no-cost extension to accomplish the goals set forth in the new statement of work. The year long no-cost extension is required due to the time lost from the lack of progress from

TOSK on the original statement of work. Our first yearly progress report details the achievements and difficulties for each of the above specific aims.

Briefly, since the initial introduction of the TOSK transposon technology, no further details of the company's experiments have been published. In spite of our prior agreements with TOSK established before the submission of the NPRP proposal, multiple attempts to contact TOSK about their transgenic services once the award had been made were unsuccessful. TOSK has, in 2004, suspended transposon-based transgenics services without explanation. To date no hamster transgenic has been produced either by conventional or transposon elements based techniques. This possibility was considered in the NPRP proposal with the fall back being production of conventional mouse transgenics with the sufficient susceptibility to the host species to detect blood borne infectivity.

BODY

As part of our NIH contract through our subcontractor and collaborator Richard Rubenstein, we have had a head start in that effort as mouse transgenics carrying sheep PrP, human PrP, and elk PrP have already been developed at the IBR. The IBR transgenics had to be re-derived by Harlan to rid them of mouse hepatitis and other pathogens circulating in the IBR facility before they could be transferred to our laboratory. This has now been completed for the sheep and human PrP transgenics and we are currently receiving approximately 25 animals per month of each strain. Using funds from our NIH contract, we have inoculated approximately 200 sheep PrP transgenic mice. We have recently observed the first transmission of the disease to this mouse at an incubation period of 6 months with scrapie infected brain material. This is a relatively short incubation time for a mouse model, indicating a relatively efficient cross-species transmission. This has encouraged us to proceed with a limiting dilution titration of naturally infected whole blood and plasma from scrapie infected sheep which we are accumulating animals for now. If we get transmissions from blood, we will have achieved one of the ultimate goals of the original proposal and by demonstrating the presence of TSE infectivity in blood in a cross-species transmission from a natural infection we will have established a critical missing element for the investigation of natural infections and the development of blood-based diagnostics for TSE infections.

This mouse, if it proves sufficiently sensitive to detect TSE infectivity in sheep blood, would validate our efforts over the last five years to develop a practical model in sheep for studying blood-borne TSE infectivity on a scale comparable to human use of blood. While we were the first to make quantitative measurements of the titer of TSE infectivity in blood, plasma and other blood fractions and components, the method that we developed that makes this possible, "limiting dilution titration", requires large numbers of animals, which is doable in rodents but would be prohibitively expensive and impracticably protracted for any but the highest priority experiments in large animals. At the same time the very low titer of TSE infectivity in blood, and the small volumes of blood obtainable from individual rodents, conspire against rodent models for the development of blood-based diagnostics. To take advantage of the best features of both systems, what is needed are transgenic rodents with the same sensitivity to infection by

scrapie from sheep as same-species transmissions between sheep. With such a transgenic, it would be possible to use sheep as a source of large, unit scale, quantities of blood while quantifying the infectivity levels in mice. While it would also be highly desirable to have a transgenic infectible by the low levels of infectivity in human blood, such an animal would not provide the same level of access to large quantities of infected blood for assay development and validation. This is because, CJD infections are rare and blood from CJD infected persons is even rarer.

We now have in place all of the other critical elements for using sheep blood as substrate for assay development and we propose here to apply the remaining funds in this award to consolidating this model into a core resource for the TSE assay development by the larger TSE community. The elements currently in place include:

1. Dr. Marie Bulgin's flock of naturally infected sheep at the Caine Veterinary Center of the University of Idaho, has been supported by a subcontract from our NIH contract for assay development for the last five years. We are currently supporting a flock of 50 animals that we have been breeding for the highly susceptible AA QQ genotype. This flock produces 5 to 10 cases of naturally acquired sheep scrapie per year. We also inoculate approximately 10 animals per year so that we can obtain blood at predictable intervals during the infection.

2. Dr. Linda Detwiler's flock of uninfected scrapie-free certified sheep that are being bred for the AAQQ genotype. While the AAQQ genotype was highly prevalent just five years ago, especially in the common black face sheep, it is rapidly disappearing due to selective breeding to increase resistance to scrapie. This is especially true in scrapie-free certified flocks which are now almost entirely AARR. This was creating a crisis in obtaining credible control tissue which has now been relieved by Dr. Detwiler's purchase of a scrapie-free certified flock and her commitment to breeding AAQQ animals for use as controls in scrapie research projects. It should be noted that this is a high risk endeavor on her part, as it will be much more challenging to maintain a scrapie free flock with the AAQQ genotype. We will purchase blood and/or animals from her flock on an as needed basis, but will genotype her animals in conjunction with the Idaho flock.

3. One and one half liters of a highly potent 20% brain homogenate capable of transmitting the infection to sheep by oral inoculations of less than 100 μl/animal. We use the oral route as it is the most likely route by which the natural cases are acquired.

4. Two new monoclonal antibodies that are strongly reactive with sheep PrP that have been developed by Rick Kascsak as a collaborator and subcontractor to our NIH contract.

5. Western blot, Elisa, and Delphia assays for sheep PrP have been developed using these reagents.

6. Over the last five years we have collected and stored over 40 liters of plasma from sheep symptomatic for scrapie with the intention of preparing a reference material from the pooled material for use in assay development and validation and for comparison of blood-based diagnostics for TSEs. At first we were working blind, without proof that sheep blood actually contained infectivity. Fiona Houston's transfusion experiments have now shown that sheep blood does contain infectivity and is capable of transmitting the disease when transfused in unit quantities. It is our hope that the sheep PrP transgenic will now enable us to quantitate the infectivity in this pool.

7. We have also serendipitously uncovered a remarkably short incubation time strain of sheep scrapie. When the pooled inoculum described in (3.) above is administered orally to sheep with the VVQQ genotype they develop clinical scrapie in less than 6 months, whereas a typical case takes at least 18 months even after intracerebral inoculation. This result has now been confirmed by Jurgen Richt and his colleagues at the USDA Agricultural Research Service in Ames, IA. The ARS will strain type the first passage animals and inoculum in inbred mice. This strain brings rodent-like incubation times to sheep which could greatly facilitate sheep to sheep infectivity experiments as well as expedite the production of infected tissues.

The NIH contract that has enabled us to establish this flock of sheep with naturally acquired scrapie is scheduled to end on August 31st 2005. The flock has continuously increased in value as we have developed and added the reagents that were needed to facilitate the use of sheep tissues for TSE research. The flock is currently serving as a resource center for TSE infected blood and brain for the TSE research community and we predict that demand will continue to grow as we continue to characterize the sheep infection using the new reagents that we have developed. As it gains more users it may be possible to support the resource through charges for reagents. Far more desirable would be a government contract promising continuity of funding for what should be considered a core resource for TSE investigations.

In summary, the following is the approved revised statement of work:

1) Support the scrapie infected flock at the Caine Veterinary Center at a sustainable, if reduced, number of animals consistent with the funding. We would recommend halving the total number of animals to fifty. Support the continued genetic monitoring of Dr. Detwiler's control flock.

2) Pool, and bring to homogeneity the sheep plasma and separately the frozen whole blood that we have collected over the last five years and redistribute each into aliquots that will serve as a common TSE infected blood reference material.

3) Develop a protocol for distribution of sheep resource materials to academic requesters and a price list for distribution of the same materials to commercial interests.

4) Use the transgenic mice carrying the sheep PrP gene that we have developed, to measure the infectivity in the sheep plasma pool and the whole blood pool described in (2.) using limiting dilution titration. This will accomplish one of the ultimate goals of the original proposal.

5) Continue to develop the short incubation time model of sheep scrapie by breeding for VVQQ genotype and infecting with inocula prepared from the first passage transmissions.

KEY RESEARCH ACCOMPLISHMENTS

1. We have a transgenic mouse with the sheep PrP transgene.
2. We currently have more than 40 liters of scrapie infected plasma.
3. Dr. Bulgin currently maintains a naturally scrapie-infected sheep flock.
4. We have access to Dr. Detwiler's scrapie-free certified flock for use as control animals.

REPORTABLE OUTCOMES

Since we have just recently received approval for our newly revised statement of work, we have limited number of reportable outcomes. Under our NIH contract, we began an end-point dilution titration of infected sheep brain in November of 2004. In June of 2005, animals in the 10^{-1} to 10^{-3} dilutions began showing symptoms of scrapie. A collaborator of ours, Dr. Pedro Piccardo, has performed histopathology on the brains and determined that the animals did contract scrapie. Although this titration was done with brain material, not plasma as outlined in the statement of work, the animals do demonstrate sensitivity to scrapie and we hope that the upcoming plasma titration will be equally successful.

CONCLUSIONS

Due to the time lost with TOSK Inc., we have recently received permission for a one year no-cost extension to accomplish the newly approved statement of work. We feel confident that the new re-direction of funds and the one year no-cost extension will allow us to accomplish the newly approved statement of work.