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TITLE: Efficient and Rapid Development of Transgenic Hamster Models of TSEs Using a Radical New Technology

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14. ABSTRACT

The development of blood-based or other diagnostic tests for the TSE diseases should be greatly facilitated by an integrated set of compatible resources: 1) a susceptible large animal (sheep) to provide blood or other substrates, 2) susceptible transgenic rodents that can be used as a sensitive bioassay, 3) standard reference samples of brain, blood, and plasma of sufficient volume to be used by the TSE community. We are breeding a flock of 50 genotyped sheep to select for the high scrapie susceptibility genotype VVRRQQ. All three lines of the mouse transgenics carrying sheep, human, and elk PrP have been now re-derived. We have observed the first transmission of the disease from our standard scrapie-infected sheep brain inoculum to the transgenic mice with sheep PrP and have completed an end-point dilution titration. We have pooled and aliquoted the infected sheep whole blood, and separately, plasma (100 liters total). The pooled whole blood and plasma have been inoculated into transgenic mice for limiting dilution titrations that will quantify their exact level of infectivity, thus increasing their value to researchers. A protocol for sharing or sale of these resources has been prepared and samples have already been distributed.

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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 to use a 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 and to use the new transgenic animals to measure the titer of blood-associated infectivity in naturally infected hosts to establish the relevance of rodent studies on blood infectivity. As explained in earlier reports, the TOSK transposon technology never materialized and to date no hamster transgenic has been produced either by conventional or transposon element-based techniques. Once it became clear that the new technology was not going to produce a transgenic within the time frame of this award, we reverted to the fall back position that we had proposed in the original submission basing our transmission attempts on conventional mouse transgenics. A revised statement of work was approved and this report is based on that revision. We have now completed end point dilution titrations of our standard sheep brain inoculum in our sheep transgenic, all three of the original WHO human reference samples for CJD and vCJD in our humanized transgenic and have titrations underway of the new WHO familial reference and CWD inocula prepared from elk and mule deer. Our sheep result was strong enough to encouraged us to proceed with a limiting dilution titration of naturally infected whole blood and plasma from scrapie infected sheep. If we get transmissions from blood, we will have achieved the most important goal 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. Moreover, 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.

BODY

Revised item 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.

The following subsection for Aim 1 is taken from a report prepared by

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OBJECTIVE: To maintain a flock of 50 genotyped sheep at high risk of developing scrapie from natural exposure for the purpose of providing samples requested by BREF researchers as source material for TSE diagnostic assay development.

RESULTS: Fifty sheep were selected from the Caine scrapie flock based on positive IHC test results, clinical symptoms and genotype. Twenty-six of those sheep died or were euthanized because of clinical scrapie during the year. Blood totaling over 48 liters from those 26 sheep was sent to BREF. No tissues have been sent to BREF to date. In order to maintain the required 50 sheep, more animals from the Caine flock were assigned to the BREF flock, based on positive IHC results, clinical symptoms and/or genotype. Requests were made for urine, bladder and kidney from positive IHC and/or clinical sheep. These samples have been collected, stored and are awaiting shipment.

The National Animal Disease Center has requested eyeballs from scrapie and from non-scrapie control sheep. We have sent them one shipment to date, with another one ready as soon as the IHC results are completed.

DISCUSSION: We have received the genotyping results for the 2006 lamb crop and are going to inoculate at least 5 of the VVRRQQ (codons 136, 154 and 171, respectively) lambs with the 20% scrapie brain homogenate. We continue to receive requests from various prion researchers and are working to accommodate their requests while still fulfilling the mandate of this contract.

PROBLEMS ENCOUNTERED: The very clinical sheep do not handle stress. At least one has died from the stress of being in a metabolism crate to collect urine. One researcher wants fresh (not frozen) urine so we are trying to get the volume needed from a sheep during or just after euthanasia for immediate shipment.

Revised item of work 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.

This aim has been accomplished. We have pooled, and brought to homogeneity the sheep plasma and separately the frozen whole blood that we have collected over the last five years and redistributed each into aliquots of 100, 500 and 1000 ml. We have 56 liters of the naturally infected sheep plasma and 32.8 liters of plasma from experimentally (orally) infected sheep that can serve as a reference material for assay development and validation and for comparison of blood-based diagnostics for TSEs. There is no equivalent resource. Some of this material has already been distributed to the research community.

Revised item of work 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.

This aim has been accomplished. The protocol and price list have been prepared and samples have already been distributed. A publication is being prepared giving the characteristics of these materials which will also serve to advertise the availability of this resource.

Revised item of work 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 using limiting dilution titration. This will accomplish one of the ultimate goals of the original proposal.

The rederivation of all three lines of the mouse transgenics carrying sheep PrP, human PrP, and elk PrP have been completed and all lines are now under production by Harlan. We have completed end point dilution titrations of the same stock of natural sheep scrapie that is producing very short incubation times in sheep inoculated by the oral route in the sheep transgenic. We have also completed titrations of the first three WHO sporadic and variant CJD samples and the new familial standard has been inoculated. The results are summarized below and suggest by incubation time and titer relatively high efficiencies of transmission (Table 1). However, it will never be possible to know the real efficiency of transmission with out same species transmissions into the same

Table 1 Titration of natural infections in mice with same-species PrP transgene.

Titration Method: End-Point Dilution					
Sample:	WHO sCJD I	WHO sCJD II	WHO vCJD	WHO fCJD	Natural Sheep Scrapie
Transgenic	Human	Human	Human	Human	Sheep
Days pi	393	387	386	274	550
Dilution[1]	Infected/inoculated				
10^{-1}	2/2	toxic	0/2	0/5	3/3
10^{-2}	4/4	5/5	0/4	0/5	5/5
10^{-3}	-	5/5	0/4	0/5	5/5
10^{-4}	4/4	5/5	0/5	0/5	4/4
10^{-5}	5/5	0/3	0/4	0/5	4/4
10^{-6}	0/5	04	0/5	0/4	0/2
10^{-7}	0/5	04	0/4	0/5	0/2
ID/g[1]	1.0×10^7	1.0×10^6	none	?	1.0×10^7
Uninoculated control	0/5	0/6	0/2	0/2	0/5

*Does not include animals that died of intercurrent causes, which may or may not have developed scrapie had they lived longer. Five animals were originally inoculated at each dilution.

[1]Dilution relative to whole brain. ID/g of brain

species hosts. This would be possible with sheep but has not been done for lack of funding. It will never be possible with human strains. One indicator of primary passage transmission efficiency is the incubation time relative to secondary transmission. However, this comparison is usually not valid because the incubation time should be compared at the same titer for each transmission. We will have this information because we are conducting a second end point dilution titration of homogenates prepared from infected brain from each primary transmission. If the incubation times at the same relative titer of inoculum at each passage are the same it would indicate a minimal species barrier. It will take another year to obtain this information. In the mean time, the titers, 10^7ID/ml, and incubation times, six to eight months, for the natural scrapie transmission are sufficiently encouraging that we have undertaken limiting dilution titrations of scrapie infected sheep whole blood and the plasma pool that we have made.

To compensate for the potential reduced efficiency of a cross species transmission 300 mice were inoculated intracranially with whole blood. Plasma from the pool is being inoculated as we receive the mice. Due to the very low titer of TSE infectivity in blood, we do not expect to see until much later than for brain.

Problems: It is highly desirable to have our transgenic lines maintained by Harlan, both because it saves us the effort of maintaining breeding colonies but most importantly because, as a commercial supplier, the health status of mice raised by Harlan is not an issue when supplying the mice to other institutions. However, we no longer have the funds to support the Harlan contracts and so will be moving the breeding colonies to the VA in the near future. We have sent breeding stock to Jackson laboratories for freezing preservation under an NIH program, and have sent breeders to the Institute for basic research, Albert Einstein, and Richard Rubenstein at Downstate New York for local breeding.

Revised item of work 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.

Marie Bulgin has now identified a ram in the high susceptibility flock with the VVRRQQ (codons 136, 154 and 171, respectively)genotype that can be used to increase the production of VVRRQQ animals until he succumbs to the disease himself. Marie has used the genotyping results for the 2006 lamb crop to identify and inoculate another 5 VVRRQQ lambs with the short incubation scrapie brain homogenate.

KEY RESEARCH ACCOMPLISHMENTS

We have titered the scrapie-infected sheep brain inoculum from naturally infected sheep by intracranial inoculation into transgenic mice carrying the sheep PrP gene. By this assay, the infected sheep brain used to make the stock inoculum had 10^7 ID_{50}/g.

The limiting dilution titrations of infected sheep whole blood and plasma are incubating. No infections have been seen yet (as expected for the short time that has passed since inoculation).

Through our sheep breeding program, we have produced new lambs with the high scrapie susceptibility genotype VVRRQQ that can be used in experiments with the new short incubation time scrapie strain.

REPORTABLE OUTCOMES

Three publications have now been submitted for publication that have been supported by this funding. One by the Bulgin laboratory on use of lymph nodes for preclinical diagnosis of scrapie. Another is by Richard Rubenstein on the transgenic CWD mouse. A third is by Juergen Richt on the activity of the sheep brain inoculum. Other publication are being prepared on the humanized mouse transmission and sheep transmissions.

CONCLUSIONS

Work toward all of the specific aims in the revised statement of work is proceeding well. A new strain of sheep scrapie with a short incubation time (six months) is being characterized. Transgenic mice with the sheep PrP gene are being used to develop a bioassay for sheep scrapie infectivity. Large pools of infected sheep blood and plasma have been created and aliquoted for use by the TSE research community. The infectivity in the pools is being measured by limiting dilution titration in transgenic mice. The breeding of sheep highly susceptible to scrapie is proceeding by selecting for the VVRRQQ genotype that is being bred out in commercial flocks. The development of blood-based or other diagnostic tests should be greatly facilitated by this assemblage of compatible resources: 1) a large animal (sheep) to provide blood or other samples, 2) susceptible transgenic rodents that can be used to bioassay infectivity, 3) standard reference samples of brain, blood, and plasma of sufficient volume to be used by the many TSE researchers, 4) monoclonal antibodies to sheep prion protein developed earlier.