Animal Models of Diabetic Complications Consortium
(U01 DK076131)

Annual Report
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“Bradykinin, nitric oxide and mitochondrial damage in diabetic complications**”**

UNC

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Part A:

Principal Investigator's Summary

Program Accomplishments:

Hypothesis

Our long term objective is to unravel whether oxidative stress is required for development and progression of diabetic nephropathy, and the role of mitochondrial mutations in diabetic complications.

Recent Progress and Major Accomplishments:

A. Model development and physiological assessment

- Detailed phenotyping of diabetic nephropathy in whole body B1 and B2 receptor double null Akita diabetic mice compared with B2 null Akita mice.
- Determining ischemia/reperfusion injury in whole body B1 and B2 receptor double null mice compared with that in B2 null mice (published in Proc Natl Acad Sci U S A. 2007).
- Detailed phenotyping of diabetic nephropathy in eNOS null STZ diabetic mice fed normal chow and a high fat diet (manuscript submitted).
- Generation of mitochondrial DNA polymerase gamma *Polg* mutant (a proof reading defect) Akita/+ mice by mating.

B. Production of mice with mutations in candidate susceptibility genes for diabetic complications

- Generation of mice lacking both the bradykinin B1 and B2 receptor genes by single targeting event.
- Construction of a targeting vector to conditionally delete B1 B2 receptors.
- Construction of a targeting vector to conditionally mutate *Polg*, obtaining 5 targeted C57BL/6 ES cells, and injecting these ES cells into blastocysts.

C. Method development

- Development of a method of repeatedly measuring glomerular filtration rate in mice using endogenous creatinine in plasma and urine with less than 10 ul of samples (published in Kidney Int 2007). This method uses mass spectrometry and stable isotope ^{3}H-creatinine as an internal standard, and is useful for detecting subtle changes in GFR.

Plans for the Upcoming Year

Because our projects are progressing very well, we do not anticipate any changes in our plans.

- To determine the effect on diabetic complications of eliminating both bradykinin receptors in a tissue or cell specific manner, and the effects of reducing oxidative stress in these mice.
- To investigate the role of coagulation in the development of diabetic complications in eNOS null mice.
- To test the hypothesis that increasing the frequency of mtDNA mutations by introducing a proof reading defect into *Polg* will exacerbate the complications in Akita diabetic mice even though oxidative stress is not further increased over that due to the diabetes alone.

Preliminary Milestones for 2009 and Beyond

- Mice lacking both B1 and B2 receptors could be an excellent model of diabetic complications.

- Coagulation and inflammation are likely responsible for exacerbating diabetic complications even without further increase in oxidative stress.

1. Collaboration:

With other AMDCC PIs

- Diabetic mice lacking both the bradykinin B1 and B2 receptor genes will be sent to Dr. Eva Feldman for diabetic retinopathy studies.

With Jax

- Bradykinin B2 receptor-/- Akita/+ mice: sent to Jax for continuation of backcrossing to C57BL/6, and for moving them to the 129 SvEv and DBA/2J backgrounds. [B2R-/- was made in 129 ES cells and has been backcrossed 6 times to B6.]
- B1RB2R -/- mice (made in C57BL/6 ES cells and so pure C57BL/6): sent to Jax for maintaining on C57BL/6, backcrossing to 129 SvEv and DBA/2J, and for distribution to other members of the consortium.
- Male eNOS-/- (pure 129 SvEv) and female eNOS+/- Akita/+ (pure B6): will be sent to Jax for generating eNOS-/-Akita mice as F1 progeny from a cross between 129 SvEv and C57BL/6 parents for our use and for others in the consortium.

With the MMPCs N/A

With other non-AMDCC PIs N/A

2. Address previous EAC comments:

NOT APPLICABLE THIS YEAR

3. Publications:

PMID: 17431507
Kakoki M, Kizer CM, Yi X, Takahashi N, Kim HS, Bagnell CR, Edgell CJ, Maeda N, Jennette JC, Smithies O. Senescence-associated phenotypes in Akita diabetic mice are enhanced by absence of bradykinin B2 receptors. J Clin Invest. 116:1302-9 (2006).

PMID: 17452647
Kakoki M, McGarrah RW, Kim HS and Smithies O. Bradykinin B1 and B2 receptors both have protective roles in renal ischemia-reperfusion injury. J. Clin. Invest. 104:7576-81 (2007).

PMID: 17149371
Takahashi N, Boysen G, Li F, Li Y, Swenberg JA. Tandem mass spectrometry measurements of creatinine in mouse plasma and urine for determining glomerular filtration rate. Kidney Int. advanced on-line publication Kidney Int. 71: 266-271 (2007)

Li F, Wang C-H, Thai T, Boysen G, Xu L, Wolberg AS, Maeda N, Takahashi N. Elevated tissue factor activity and hypercoagulability in severe glomerulosclerosis of diabetic mice lacking eNOS. (submitted)