Animal Models of Diabetic Complications Consortium (U01 DK076131)

Annual Report (2008)

"Bradykinin, nitric oxide and mitochondrial damage in diabetic complications"

UNC

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Table of Contents

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Part A:

Principal Investigator's Summary

1. Program Accomplishments:

Hypothesis

Our long term objective is to unravel whether oxidative stress is required for development and progression of diabetic nephropathy, and to determine 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.
- 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.
- Mice lacking eNOS on 129 SvEv background have been generated and reposited to Jax May 2008.

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

- Production of mice allowing conditional deletion of B1 B2 receptors. Germline transmission awaited.
- Production of mice to conditionally mutate *Polg*. Germline transmission awaited.

C. Method development

- Development of a method for 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. This method is widely used by members of our consortium and by other investigators outside the consortium.

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 should be an excellent model of diabetic complications.
- Coagulation and inflammation are likely responsible for exacerbating diabetic complications even in the absence of further obvious increases in oxidative stress.

2. Collaboration:

With other AMDCC PIs

- Diabetic mice lacking both the bradykinin B1 and B2 receptor genes will be sent to Drs. Eva Feldman and Philip Tsao for diabetic retinopathy and cardiac studies.
- We are measuring creatinine clearance of mice from Dr. Moshe Levi using endogenous creatinine by mass spectrometry (LC-MS/MS).

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, for 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): sent to Jax for generating eNOS-/-Akita mice as the genetically homogeneous F1 progeny resulting 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

3. Address previous EAC comments:

EAC comments: Good progress on both general aims, role of bradykinin and mitochondrial mutation in generation and/or modulation of the DN phenotype. Your eNOS mice should be reposited with JAX by Spring 2008. Interesting comparison between your eNOS mice and those from Vanderbilt (BKS and db/db). Mice should be phenotyped for other vascular abnormalities (endothelial function, atherosclerosis) in addition to the ongoing myocardial studies. It would also be interesting to perform a whole battery of CV phenotyping with the bradykinin receptor double knockouts given the relationship between bradykinin and the renin-angiotensin system.

Response: eNOS mice on C57BL/6 and 129SvEv genetic background have been reposited to Jax. Determination of atherosclerosis in eNOS deficient diabetic mice and cardiac phenotyping of B1B2 double knockout mice are progressing.

4. Publications:

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: 17452647

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) PMID: 17149371

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)