Animal Models of Diabetic Complications Consortium
(U01 DK076131)

Annual Report
(2010)

"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 the development and progression of diabetic nephropathy, and to determine the role of mitochondrial mutations in diabetic complications.

Progress toward stated milestones

A. Model development and physiological assessment

- Phenotyping completed of whole body B1 and B2 receptor double null Akita diabetic mice compared with B2 null Akita mice (PNAS Epub in press). Diabetic complications occur in kidney, bone and nerves.
- Inhibition of tissue factor (coagulation factor III) abolished the increase in expression of genes responsible for inflammation and fibrosis caused by diabetes, lack of eNOS, and a high fat diet. Tissue factor is likely responsible for an exacerbation of diabetic nephropathy in eNOS null diabetic mice fed normal chow and a high fat diet (manuscript in revision).
- Detailed phenotyping of the diabetic nephropathy of F1 (C57BL/6 x129SvEV) eNOS-/- Akita/+ mice, eNOS+/- Akita/+ mice and eNOS+/+ Akita/+ mice has been finished. Remarkably, the eNOS +/- Akita mice have marked renal pathology even though they still make eNOS (at ~30% wt level) (manuscript in preparation).
- Detailed investigation completed of why the mitochondrial DNA polymerase gamma mutant (*Polg* D257A, a proof reading defect) has less severe diabetes relative to Akita/+ mice with wild type *Polg* (manuscript in preparation). The effect is largely due to appetite suppression via the testis.

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 achieved at Jackson Lab.
- Production of mice with conditional D257A *Polg*: New targeted ES cells generated, blastocyst injections completed, and new chimeras are currently breeding. Awaiting germline transmission.

Plans for the Upcoming Year

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

- To determine the effects 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 tissue factor in the whole body and in macrophages in the development of diabetic complications in eNOS null mice using bone marrow transplantation.
- To determine the effects on diabetic complications of homozygosity for the D257A mutation of *Polg* in a tissue or cell specific manner.

Preliminary Milestones for 2011 and Beyond

- Mice lacking both B1 and B2 receptors in specific tissues are expected to become available and their diabetic complications will be investigated.
- Heterozygous eNOS+/- Akita/+ mice show diabetic nephropathy similar in severity to that of homozygous eNOS-/-Akita/+ mice. They will be used to determine the effects of this reduced level of eNOS (comparable to that seen in ≥ 5% of humans) on diabetic complications in other systems.
- We will pursue our studies indicating that increased tissue factor is likely responsible for exacerbating diabetic complications in the eNOS+/- (or-/-) Akita/+ mice even in the absence of further obvious increases in oxidative stress.
- We will use the conditional D257A *Polg* mutant animals to see the effects of mt DNA mutations in fully diabetic mice.
- We have started and will continue transferring primary data to the AMDCC database.

1. Collaboration:

With other AMDCC PIs

- Diabetic mice lacking both the bradykinin B1 and B2 receptor genes have been sent to Drs. Eva Feldman, Philip Tsao, and Dale Abel for studies of diabetic neuropathy and cardiomyopathy.
- F1 (C57BL/6 x 129SvEv) heterozygous eNOS+/- Akita mice will be sent to Drs. Eva Feldman for diabetic neuropathy studies.
- Dr. Moshe Levi has found that the Farnesoid X Receptor regulates B2 receptor expression in the kidney, and will investigate whether the effect of FXR on diabetic nephropathy is mediated by B2R by testing the effects of an FXR agonist on B2R null Akita mice.

With Jax

- Bradykinin B2 receptor-/- Akita/+ mice: sent to Jax, and are being backcrossed to C57BL/6, and to the 129 SvEv and DBA/2J backgrounds.
- B1RB2R -/- mice (made in C57BL/6 ES cells): sent to Jax, being phenotyped and maintained on C57BL/6, and backcrossed to 129 SvEv and DBA/2J for distribution to other members of the consortium.
- Male eNOS-/- (129 SvEv) and female eNOS+/- Akita/+ (B6): sent to Jax, F1 eNOS+/- Akita mice are being generated for use by the consortium and are being phenotyped.

With the MMPCs N/A

With other non-AMDCC PIs N/A

2. Address previous EAC comments:

EAC comments: The Polg phenotype is surprising. Not sure proposed mechanisms for reduced diabetic phenotype make sense. Seems like Polg null mice should be malnourished if the responsible mechanism decreased intestinal transport, decreased food intake. The animals in the pictures look healthy. Not sure pursuit of tissue-specific deletion makes sense; no rationale given. This should be discussed by the Consortium.

The possibility that the KO of DNA polymerase gamma (*Polg*) has less injury due to lower food absorption is interesting but also points up the need for careful attention to factors known to favorably influence experimental renal disease in rodents e.g. dietary restriction. If defective gut function is the cause of the effects in this KO, further study of this model at least for renal effects seems of limited value.

Response: We have made considerable progress in understanding why the Polg mutation reduces the diabetic phenotype. Our data indicate that the improved diabetic phenotype is a result of a suppression of food intake caused by testis dysfunction. [The testes are critical for hyperphagia and necessary for the Akita diabetic phenotype, Toyoshima, et al in 2007.] However, even with a reduced diabetic phenotype, we observe increased urinary albumin and damage to the renal proximal tubular cells. A conditional knock-in of the D257A mutation will provide us with tissue specific control of mitochondrial DNA mutations and will allow us to evaluate the effects of both hyperglycemia and mitochondrial DNA mutations by bypassing the testicular dysfunction and maintaining a diabetic phenotype.

3. Publications:

Brosius FCIII, Alpers C, Bottinger E, Breyer MD, Coffman TM, Kretzler M, Gurley SB, Harris RC, Kakoki M, Leiter EH, Levi M, Quaggin SE, Sharma K, Smithies O, Susztak K,Takahashi N. Update on Mouse Models of Diabetic Nephropathy: A Midstream Analysis from the Animal Models of Diabetic Complications Consortium J Am Soc Nephrol 2009 20:2503-12.

Kakoki M, Sullivan KA, Backus C, Hayes JM, Oh SS, Hua K, Gasim AM, Tomita H, Grant R, Nossov SB, Kim HS, Jennette JC, Feldman EL, Smithies O. Lack of both bradykinin B1 and B2 receptors enhances nephropathy, neuropathy, and bone mineral loss in Akita diabetic mice. Proc Natl Acad Sci U S A. 2010 May 17. [Epub ahead of print]

Wende AR, Soto J, Olsen CD, Pires KMP, Schell JC, Litwin SE, Kakoki M, Takahashi N, Smithies O, and Abel ED. Cardiac Contractility and Mitochondrial Function Following Genetic Deletion of Bradykinin Receptors B1 and B2 in Type 1 Diabetic Akita Mice Endocrinology 2010 May 25. [Epub ahead of print]

Li F, Wang C-H, Wang J-G, Thai T, Boysen G, Xu L, Turner A, Wolberg AS, Mackman N, Maeda N, Takahashi N. Tissue factor and the exacerbation of diabetic nephropathy by lack of eNOS and a high fat diet. J Thromb Haemat (under revision)

Li F, Arbones-Mainar JM, Maeda N, Takahashi N. Nitric Oxide increases adiponectin through a guanylyl cyclase-dependent pathway. (in preparation)

Wang C-H, Hiller S, Li F, Maeda N, Smithies O, Takahashi N. Decrease in eNOS equivalent to *NOS3* polymorphism is sufficient to accelerate diabetic nephropathy (in preparation)

Fox RG, Kim HK, Reddick RL, Kujoth G, Smithies O, Maeda N. A mitochondrial DNA mutator (PolgD257A) ameliorates the hyperglycemia of Type 1 diabetic Akita mice while increasing renal proximal tubular cell damage (in preparation)