Animal Models of Diabetic Complications Consortium
(U01 DK61018)

Annual Report
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"Generating Mouse Mutants With Diabetic Nephropathy"
Vanderbilt University School

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Part A:

Principal Investigator's Summary

The goal of AMDCC is to develop animal models that faithfully reproduce diabetic complications observed in humans. The realization that microalbuminuria is a strong predictor of death from cardiovascular disease has drawn attention to the endothelium as a common site of injury in both microvascular and macrovascular disease in humans. Accumulating evidence implicates endothelial dysfunction in the pathogenesis of diabetic complications, particularly nephropathy, retinopathy, neuropathy, and macrovascular disease. The Vanderbilt component of the AMDCC is focusing on eNOS and prostacyclin synthase (PGIS), two endothelial genes encoding biochemically interrelated enzymes and in which polymorphisms associated with altered enzyme activity have been implicated in human diabetic nephropathy.

Both PGIS and eNOS activity are critical for the maintenance of normal endothelial function. COX2 appears to be the major source of urinary prostacyclin excretion in man, and prolonged COX2 inhibition is associated not only with reduction of PGIS but also with excess cardiovascular mortality from thrombotic events. This is consistent with a cardioprotective action of prostacyclin. Functionally significant polymorphisms in eNOS and PGIS have been identified in humans. ENOS and PGIS activity are not only topographically linked but also biochemically linked through oxidative stress, which not only uncouples eNOS, but also results in increased peroxynitrite levels, which directly reacts with and inactivates prostacyclin synthase . Both eNOS uncoupling and peroxynitrite-induced inactivation of prostacyclin synthase have been demonstrated to be direct consequences of hyperglycemia. It has been hypothesized that as a result of this, diabetics exhibit impaired endothelial dependent acetylcholine induced vasodilation and glomerular barrier function which is reflected as albuminuria. This may also be associated with the global cardiovascular disease associated with diabetic nephropathy.

Responsible Investigator: Raymond C. Harris, M.D.

1. Project Accomplishments:

Hypothesis: The goal of AMDCC is to develop animal models that faithfully reproduce diabetic complications observed in humans. The realization that microalbuminuria is a strong predictor of death from cardiovascular disease has drawn attention to the endothelium as a common site of injury in both microvascular and macrovascular disease in humans. Accumulating evidence implicates endothelial dysfunction in the pathogenesis of diabetic complications, particularly nephropathy, retinopathy, neuropathy, and macrovascular disease. The Vanderbilt component of the AMDCC is focusing on eNOS and prostacyclin synthase (PGIS), two endothelial genes encoding biochemically interrelated enzymes and in which polymorphisms associated with altered enzyme activity have been implicated in human diabetic nephropathy.

Recent Progress and Major Accomplishments The goal of **Aim 1** is to determine the role of endothelial eNOS activity in the progression of diabetic nephropathy by generating floxed eNOS mice and studying them in the DN susceptible DBA2/J Akita mouse. In this regard, we have completed the targeting construct and have initiated planning with Jackson Labs for transfer and phenotyping. Because the Jackson Lab ES facility is not able to undertake the electroporation and screening of ES cells at present, we decided that we would undertake the initial generation and genotyping of the floxed mice here at Vanderbilt and they would then be shipped to JAX for further characterization and breeding. Therefore, the Vanderbilt transgenic core has now electroporated the construct into 29S6/SvEvTac ES cells. We are in the process of performing the appropriate Southern blots.

The goal of **Aim 2** is to determine the role of endothelial prostacyclin synthase in the progression of diabetic nephropathy nephropathy by generating floxed PGIS mice and studying them in the DN susceptible DBA2/J Akita. In this regard, we have completed the construct and have electroporated 129P3ES cells with the floxed PGIS targeting vector. The original screen of ES cells was negative. The construct has been re-electroporated into ES cells and we will screen for positives.

Plans for the Upcoming Year: Our goal is to have the floxed mice available by early 2008. We then hope to send them to Jackson Labs for speed congeneic backcrossing to 129/sv and DBA2/J Akita backgrounds. In the meantime, we also will make the Tie-2-Cre mice available to Jackson Labs for backcrossing to DBA2/J background.

Preliminary Milestones for 2009 and Beyond: Our goal is to be able to make the appropriate crosses to produce endothelial-specific deletion of either eNOS or PGI synthase during 2009 and determine the effects on development of diabetic nephropathy.

2. Collaboration:

With Jax: As indicated above, when our floxed mice are available, we have made arrangements with Jackson Labs to undertake the appropriate backcrosses onto the strains of interest.

With the MMPCs: We will continue to utilize the Phenotyping facilities at the Vanderbilt MMPC for functional characterization of the mice generated in this project.

3. Publications:

1. Kanetsuna, Y., Hirano, K., Nagata, M., Gannon, M.A., Takahashi, K. Harris, R.C., Breyer, M.D. and Takahashi, T. Characterization of Diabetic Nephropathy in a Transgenic Model of Nonobese Diabetes Am. J. Physiol Renal 291:F1315-22, 2006.

2. Zhao, H J, Wang, S, Cheng, H, Zhang, M-Z, Takahashi, T, Fogo, AB, Breyer, MD, and Harris, RC. Endothelial nitric oxide sythase deficiency produces accelerated nephropathy in diabetic mice. JASN 17:2664-9, 2006.

3. Tchekneva EE, Rinchik EM, Polosukhina D, Davis LS, Kadkina V, Mohamed Y, Dunn SR, Sharma K, Qi Z, Fogo AB, Breyer MD. A sensitized screen of N-ethyl-N-nitrosourea-mutagenized mice identifies dominant mutants predisposed to diabetic nephropathy. JASN 18:103-12, 2007.

4. Kanetsuna, Y, Takahashi, K, Nagata, M, Gannon, MA, Breyer, MD, Harris, RC and Takahashi, T. eNOS Deficiency Confers Susceptibility to Diabetic Nephropathy in Nephropathy Resistant Inbred Mice. Am. J. Pathology 170:1473-84, 2007

5. Breyer MD, Tchekneva E, Qi Z, Takahashi T, Fogo AB, Zhao HJ and Harris RC. Genetics of diabetic nephropathy: lessons from mice. Semin Nephrol. 27:237-47, 2007.

6. Breyer, MD and Harris, RC Diabetic Nephropathy, in The Molecular and Genetic Basis of Kidney Disease, D Mount and M Pollak, ed. 2007.