Animal Models of Diabetic Complications Consortium
(U01 DK060905-10)

Annual Report
(2009)

"Role and Mechanisms of Epithelial Injury in Diabetic Nephropathy"

Mount Sinai School of Medicine
Albert Einstein College of Medicine of Yeshiva University

Principal Investigator
Erwin P. Bottinger, MD

Address: One Gustave L. Levi Pl., Box 1003, New York, NY 10029
Phone: 212-241-0800
E-mail: Erwin.bottinger@mssm.edu

Table of Contents

EAC comments: Bottinger/Susztak

These two projects are essentially proceeding independently at Sinai and Einstein. Using in vivo and in vitro approaches, the Bottinger lab has focused on peroxisomal membrane Mpv-17 family proteins, focusing on the hypothesis that these molecules protect against oxidant injury in diabetes. Since the Mpv17 null mouse fails to develop significant hyperglycemia, original specific aim 2 is not feasible. The lab is now focusing on developing a conditionally targeted allele. Susztak's group continues work on CD36 overexpressing diabetic mice (a strategy based on whole genome expression analysis) and has found the CD36 overexpression in tubules of diabetic mice accelerates injury and cell loss. Future work focuses on mechanism of apoptosis using in vitro and in vivo approaches. Given the difficulty in recapitulating in mice the tubulointerstitial disease seen in human kidney biopsies, these experiments are particularly interesting.

PIs Response: We thank the EAC for their comments in support of our past and ongoing work focusing on epithelial cell injury pathways in tubular epithelia and podocytes. Of note, because the Specific Aim 2 of our original proposal (diabetes-induced renal lesions in Mpv17-/- mice) was not feasible due to lack of hyperglycemia in STZ-induced and Akita transgenic Mpv17-/- mice, the EAC previously advised that we should not spent efforts on understanding the mechanisms for the failure to develop type I diabetes in these mice. Instead, we introduced a new project (see new project 2), and are independently generating floxed Mpv17 alleles for conditional targeting of the Mpv17 locus.

Original published manuscripts:

Krick S , Shi S , Ju W, Faul C , Tsai S , Mundel P , and Bottinger EP. Mpv17l Protects against Mitochondrial Oxidative Stress and Apoptosis by Activation of Omi/HtrA2 protease. *Proc. Natl. Acad. Sci. U.S.A.*, 105:14206, 2008

Frank C. Brosius III, Charles E. Alpers, Erwin P. Bottinger, Matthew D. Breyer, Thomas M. Coffman, Susan B. Gurley, Raymond C. Harris, Masao Kakoki, Matthias Kretzler, Edward H. Leiter, Moshe Levi, Richard A. McIndoe, Kumar Sharma, Oliver Smithies, Katalin Susztak, Nobuyuki Takahashi, Takamune Takahashi for the Animal Models of Diabetic Complications

Consortium, Mouse Models of Diabetic Nephropathy, *J Am Soc Nephrol*, 2009 Sep 3. [Epub ahead of print]

Original manuscripts in revision:

Stefanie Krick[1], Liping Yu[1], Wenjun Ju [1], Shaolin Shi[1], Su-yi Tsai[1], Steven Dikman[4], Bernd Schröppel[1, 2] and Erwin P Bottinger[1,3]. Activation of HtrA2/Omi serine protease by Mpv17 in mitochondria protects podocytes against mitochondrial dysfunction and apoptosis . *J Clin Invest*, 2009, IN REVISION

Published Abstracts

Poster presentation at the 2008 Annual Scientific Meeting of the ASN, Philadelphia
Diabetes-induced loss of podocyte density is a novel phenotypic trait in C57BL/6J (resistant) and DBA2/J (susceptible) mouse strains
Haiying QI, Kremena Star, Erwin BOTTINGER
Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029

Poster TH-PO789, 2009 Annual Scientific Meeting of the ASN, San Diego
Activation of interferon and innate immunity pathways, followed by mitochondrial dysfunction and oxidative stress response are characteristic glomerular trancriptome profiles in inbred diabetic DBA/2J mice susceptible to diabetic nephropathy, compared with DN-resistant diabetic C57BL/6J mice
Haiying QI, Shaolin SHI, Yezhou SUN, Weijia ZHANG, Xiaoping LI, Jin CHEN, Taoran ZHANG, Erwin BOTTINGER
Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029

Animal Models of Diabetic Complications Consortium
(U01 DK060995-09)

Part B:

Update by Individual Project Leaders

Project 1: "Role of Mpv17-family members in DN"

Responsible Investigator: Bottinger, Erwin

1. Project Accomplishments:

A. Hypothesis

[Of note: the previously reported peroxisomal localization of Mpv17 and Mpv17l proteins has been revised in recent publications, including Spinazzola et al., Nat Genet. 2006 May;38(5):570-5. Epub 2006 Apr 2., and our group (Krick et al., Proc Natl Acad Sci U S A. 2008 Sep 16;105(37):14106-11. Epub 2008 Sep 4)].

Observations from the Bottinger lab provide a compelling rationale to focus our research program on investigating the emerging role and mechanisms of epithelial cell injury (podocyte and tubular) in diabetic nephropathy (DN). Specifically, we will test two novel hypotheses: 1. The mitochondrial membrane proteins Mpv17l and/or Mpv17 are essential regulators of antioxidant defenses in renal epithelial cells and protect against diabetes-induced epithelial cell injury and apoptosis by protecting against mitochondrial dysfunction (Bottinger, Mount Sinai)

B. Progress toward stated milestones

We have completed the in vitro work proposed under Aim 1.

Mpv17l: *Specifically we have reported our findings for proximal tubular Mpv17l in a manuscript published in the Proc. Natl. Acad. Sci. USA, 105:14106 by Krick et al.. The pertinent findings of this work are summarized in the manuscript abstract:*

Cellular localization determines whether the serine protease HtrA2 exerts pro- or anti-apoptotic functions. In contrast to the well characterized pro-apoptotic function of cytosolic HtrA2, mechanisms underlying the mitochondrial protective role are poorly understood. The Mpv17l is a transmembrane protein previously implicated in peroxisomal reactive oxygen species metabolism and a close homolog of the inner mitochondrial membrane protein Mpv17. Here we demonstrate for the first time a direct interaction between Mpv17l and HtrA2 in mitochondria. The interaction is mediated by a PDZ domain and induces protease activation of HtrA2. HtrA2 inhibits mitochondrial superoxide generation, stabilizes mitochondrial membrane potential, and prevents apoptosis at baseline and in response to extracellular inducers of mitochondrial stress. The physiological role of Mpv17l is underscored by the finding that oxidative stress-induced downregulation of Mpv17l is a consistant feature in renal injury models. In conclusion, our results identify Mpv17l as a novel interacting protein and regulator of HtrA2 protease mediating anti-oxidant and anti-apoptotic function in mitochondria.

Mpv17*: we had completed these studies and submitted a manuscript for publication to the J Clin Invest with Krick S as lead author. The manuscript is currently in revision for resubmission. The findings are summarized in the manuscript abstract:*

Mitochondria are involved in most degenerative disorders and glomerulosclerosis is a major renal complication of mitochondrial cytopathies. Mutations in the gene encoding the mitochondrial transmembrane protein *MPV17* underlie severe forms of hepatocerebral mitochondrial DNA depletion syndrome (MDS), characterized by progressive liver cirrhosis and early death. Mpv17 had previously been localized to peroxisomes in mouse podocytes. Mpv17-deficiency in mice caused proteinuria due to focal segemental glomerulosclerosis and hearing loss due with inner ear pathology. However, functional roles and regulation of MPV17 in mitochondria in renal cells remain unknown. Here we report that Mpv17 is expressed exclusively in mitochondria of podocytes in mice. Protein and mRNA are profoundly downregulated in murine experimental glomerulosclerosis and diabetic nephropathy (DN). Glomerular MPV17 staining was significantly reduced in biopsies of focal segmental glomerulosclerosis (FSGS) and DN, but not minimal change disease (MCD). In podocyte cultures, Mpv17 protein and mRNA expression was downregulated by high ambient glucose, transforming growth factor beta (TGF-β), and cobalt chloride ($CoCl_2$), a mimetic of hypoxia. Loss of Mpv17 protein induced by siRNA in podocytes was associated with mitochondrial (mt) DNA depletion, depolarization of the mitochondrial membrane potential, increased mitochondrial reactive oxygen species (ROS) generation, and apoptosis. In contrast, overexpression of Mpv17 prevented ROS-induced mitochondrial dysfunction and apoptosis. Mpv17 coimmunoprecipitated with the mitochondrial serine protease HtrA2/Omi, and Mpv17/HtrA2 interaction and HtrA2 protease function were essential for the mitoprotective activities of Mpv17. Together these results suggest that the podocyte-specific mitochondrial membrane protein Mpv17 interacts with and activates HtrA1/Omi protease to prevent mitochondrial dysfunction and apoptosis. Mpv17 protein expression is suppressed by classic mediators of glomerular injury in podocytes, and decreased in experimental and human glomerular disease, suggesting a novel pathomechanism for mitochondrial dysfunction and apoptosis in glomerular diseases.

<u>Work to generate conditional deletion model for Mpv17l by introducing loxP-flanked exon 3 allele in mouse for loxP-Cre mediated conditional deletion is ongoing.</u>

Progress: We have generated seven chimeric mice with >50% agouti coat color from three ES-cell lines with targeted insertion of heterozygous loxP-flanked exon3 allele. All chimeras are in mating protocols to test for germline transmission of the targeted allele. So far, three chimeric mice proved infertile. The other four chimeras produced offspring, three of those produced pups with agouti coat color. However, genotyping for the targeted allele in these pups has not proven the presence of the targeted allele to date. We are currently troubleshooting this surprising finding. First, we have considerably increased the frequency of matings to increase the number of offspring with agouti coat color. However, even after six generations of offspring from these four chimeras, we could consistently obtain agouti-coat color offspring, but all carried exclusively wildtype Mpv17l allele. Recent preliminary experiments indicate that the floxed Mpv17l allele may induce toxicity in haploid germ cells. We are currently intercrossing germline-active Cre mice with chimeras to attempt excision of loxP-flanked gene regions and/or the Neo-cassette in germ cells.

C. **Plans for the Upcoming Year**

The following milestones depend on successful completion of the previous milestone

- Matings with PTEC-selective Cre transgenic mice for targeted deletion of Mpv17 exon 3 in proximal tubuli in mice. For example, Ksp1-Cre (proximal and distal tubular promoter) and/or Pepck-Cre transgenic (proximal tubular promoter)
- Subject these mice to multiple low dose STZ protocol and other diabetes models
- Send $Mpv17^{flex3}$ mice to JAX lab

Priorities are:
1. Continue generation and characterization of mice carrying $Mpv17l^{flex3}$ alleles for conditional deletion of Mpv17l.
1.1. Use these mice to study the role of Mpv17l in tubular epithelial injury in diabetic nephropathy and experimental models of diabetes.
3. Identify the mitochondrial substrates for Htra2 protease.
4. Delineate the mechanisms and pathways by which Mpv17/HtrA2 complexes protect against mitochondrial dysfunction and impaired OXPHOS in renal epithelial cells in diabetes mellitus

2. Collaboration:

NA

3. Publications:

Krick S , Shi S , Ju W, Faul C , Tsai S , Mundel P , and Bottinger EP. Mpv17l Protects against Mitochondrial Oxidative Stress and Apoptosis by Activation of Omi/HtrA2 protease. *Proc. Natl. Acad. Sci. U.S.A.*, 105:14206, 2008

Stefanie Krick[1], Liping Yu[1], Wenjun Ju [1], Shaolin Shi[1], Su-yi Tsai[1], Steven Dikman[4], Bernd Schröppel[1, 2] and Erwin P Bottinger[1,3]. Activation of HtrA2/Omi serine protease by Mpv17 in mitochondria protects podocytes against mitochondrial dysfunction and apoptosis . *J Clin Invest*, 2009, IN REVISION

Project 2: "Genetics of glomerular disease susceptibility in murine diabetes models "

Responsible Investigator: Erwin Bottinger

1. Project Accomplishments:

A. Hypothesis

Recently, we treated inbred DBA/2J(D2) and C57BL/6J(B6) mice with multiple low dose STZ to induce diabetes, and found podocyte number was significantly decreased (22%) in diabetic D2 mice but not in diabetic B6 mice after 6 weeks of diabetes. This observation demonstrates that the podocytes of D2 and B6 mice respond to diabetic stress differently (lost or survive), and suggests the existence of genetic factors controlling the response of podocytes to diabetes. This work was presented at the 2008 Annual Scientific Meeting of the American Society of Nephrology in Philadelphia in poster format by Qi et al.. The published abstract is presented here:

[presented as poster at 2008 ASN]
Diabetes-induced loss of podocyte density is a novel phenotypic trait in C57BL/6J (resistant) and DBA2/J (susceptible) mouse strains
Haiying QI, Kremena Star, Erwin BOTTINGER
Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029

The onset of diabetes triggers a significant loss of podocytes in type I and type II diabetic patients [J Clin Invest 99:342; Kidney Int 59:2104], and type I and type II murine models of diabetes [Diabetes, 55:225]. Importanly, the number of podocytes is the strongest predictor of progression of diabetic nephropathy in a cohort of type II diabetic PIMA Indians [Diabetologia 42:1341]. Loss of podocytes in diabetic mice is associated with podocyte apoptosis and can be prevented by anti-oxidant scavengers [Diabetes 55:225]. Similar to differential genetic susceptibility in human diabetic nephropathy, diabetic inbred DBA2/J (D2) mice are more prone to albuminuria and mesangial expansion compared with diabetic C57BL/6J (B6) [Diabetes 54:2628]. We hypothesized that D2 mice are susceptible and B6 mice are resistant to podocyte loss after STZ-induced diabetes. 8-week-old D2 and B6 mice were subjected to multiple low-dose STZ injections for 5 constitutive days. The time of onset and the levels of hyperglycemia (>400mg/dl) were comparable between both strains. Control B6 (B6C) and D2 (D2C), and diabetic B6 (B6DM) and D2 (D2DM) mice were sacrificed after 3, 6, and 12 wks of diabetes, respectively (N=5 per group and time point). Frozen kidney sections were labeled with podocyte markers anti-WT1, anti-synaptopodin, and DAPI. WT1-positive (podocytes) and WT1-negative cells per glomerular surface area were determined digitally using Metamorph cell counting software in 50 glomerular cross-sections per mouse. Podocyte density (WT1-pos cells/μm^2 glomerular surface) decreased progressively in D2DM, but not in B6DM mice, compared to D2C and B6C controls, respectively, at 3 wks (-9.2% vs -4.8%), 6 wks (-25.5% vs +3.5%; P=0.001), and 12 wks (-32.2% vs -6.3%; P=0.003). In contrast, the density of WT1-negative cells did not change significantly. Urinary albumin excretion (UAE) was 10-fold increased in D2DM compared to D2C, compared to 4-fold increased in B6DM compared with B6C. The decline of

podocyte density in D2D mice was prevented by sustained insulin treatment. We conclude that inbred DBA2/J mice are susceptible and C57BL/6J mice are resistant to diabetes-induced loss of podocytes, suggesting that podocyte loss characteristic of diabetic nephropathy is a quantitative trait controlled by genetic factors. Ongoing work aims to identify the underlying quantitative trait loci and specific genetic variants using BxD recombinant inbred mouse strains.

Based on these observations, we proposed the following new studies to delineate the genetic and molecular basis predisposing to podocyte-loss characteristic of diabetic nephropathy in inbred mouse strains.

Aim 1: To map podocyte-loss-associated quantitative trait loci (QTL) using BXD recombinant inbred panel.
Aim 2: To determine molecular differences in glomeruli of C57BL/6J and DBA/2J inbred strains.

B. Progress toward stated milestones

We have completed Aim 2 and determined the genome-wide transcript profiles in glomeruli isolated from non-diabetic control and diabetic C57BL/6J and DBA/2J mice, respectively. We found a striking activation of interferon and innate immunity pathway at 3 weeks, as well as mitochondrial dysfunction and oxidative phosphorylation inhibition at 6 weeks specifically in diabetic DBA/2J, but not in diabetic C57BL/6J mice. The findings will be presented as poster at the 2009 ASN meeting in San Diego:
Session Title: Pathophysiology of Kidney Disease: Diabetes Mellitus: Basic I
Date: Thursday, October 29, 2009
Time: 10:00 am − 12:00 pm
Poster Board (Program Number): TH−PO789

[Abstract for poster TH-PO789, 2009 ASN meeting]
Activation of interferon and innate immunity pathways, followed by mitochondrial dysfunction and oxidative stress response are characteristic glomerular trancriptome profiles in inbred diabetic DBA/2J mice susceptible to diabetic nephropathy, compared with DN-resistant diabetic C57BL/6J mice
Haiying QI, Shaolin SHI, Yezhou SUN, Weijia ZHANG, Xiaoping LI, Jin CHEN, Taoran ZHANG, Erwin BOTTINGER
Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029
BACKGROUND: Similar to differential genetic susceptibility in human diabetic nephropathy, diabetic inbred DBA2/J (D2) mice are more prone to albuminuria and mesangial expansion compared with diabetic C57BL/6J (B6) [Diabetes 54:2628]. We previously presented results, demonstrating that D2 mice are also susceptible and that B6 mice are resistant to loss of podocytes associated with streptozotocin (STZ)-induced diabetes [ASN 2008].
OBJECTIVE: To characterize glomerular transcriptome profiles associated with differential susceptibility for glomerular manifestations of diabetic nephropathy (DN) in diabetic D2 mice in comparison with DN resistant diabetic B6 mice.

METHODS: Diabetes was induced in 8-week-old D2 and B6 mice by multiple low-dose STZ protocol (AMDCC protocol). Six experimental groups with 9 animals per group included: B6C and D2C (B6 and D2 controls), B6D and D2D (diabetic B6 and D2), B6DI and D2DI (insulin-treated diabetic B6 and D2), respectively. Time of onset and levels of hyperglycemia (>400mg/dl) were verified by blood glucose measurements. Animals were iron oxide perfused prior to sacrifice after 3 and 6 wks of diabetes, respectively, to enable magnetic separation of glomerular and tubular fractions. Genome-wide gene expression in glomerular fractions was examined using Affymetrix GeneChip platform.

RESULTS: Table 1 summarizes number of differentially-expressed genes (FDR) (false discovery rate <0.1; fold-change >1.5). Pathway analysis suggested highly significant abnormalities in interferon signaling, antigen presentation, innate immunity, and complement system after 3 wks, and mitochondrial dysfunction, oxidative phosphorylation, ubiquinone biosynthesis, and oxidative stress response after 6 wks of diabetes in DN susceptible diabetic D2 mice.

	Total	*Up*	*Down*
3-wk B6D vs B6C	16	1	15
6-wk B6D vs B6C	9	5	4
3-wk D2D vs D2C	93	79	14
6-wk D2D vs D2C	1612	584	1028

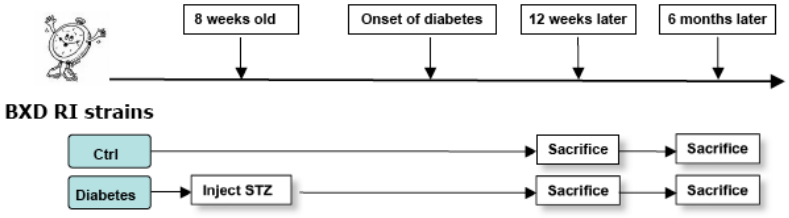
CONCLUSIONS: Our findings suggest that early activation of interferon and immune pathways, and subsequent mitochondrial dysfunction and oxidative stress response are characteristic glomerular transcriptome signatures in DN susceptible diabetic DBA/2J mice.

C. Plans for the Upcoming Year

Since D2 mice are susceptible to diabetes-induced podocyte loss while B6 mice are resistant as shown by our preliminary studies (see "Preliminary Results"), these two strains as well as the BXD panel are the ideal materials for us to map the QTL of the susceptibility of podocyte loss in diabetes. We have established a collaboration with Matthew Breyer from Eli Lily & Co, and Robert Williams from University of Tennessee Memphis to accomplish this.

- **Experimental design and methods.**

For each BXD strain, 7 diabetic mice were generated by STZ treatment and were sacrificed after 6 months of diabetes. UAE excretion was measured by the Breyer group at Eli Lily.
A summary of the experimental protocol is provided here:



Animals: 60 BXD RI strains, 7 diabetic mice/strain. 4-5 control mice/strain.

Induction of type 1 diabetes: eight-week old BXD male mice received low-dose streptozotocin (50 mg/kg) by intraperitoneal injection for five consecutive days according to AMDCC protocol and will be housed at Oak Ridge Laboratories.
Kidney preparation: vascular perfusion was performed through left ventricle with 4% paraformaldehyde(PFA) for 3 minutes and 18% sucrose for 5 minutes according to AMDCC protocol. This work was performed at under contract with Eli Lily & Co at Oak Ridge National Labs in collaboration with Dr. Matthew Breyer's group.

Our group received frozen and paraffin-embedded kidneys from all BXD mice (a total of 47 strains) to perform
Immunohistochemistry and Morphometry and QTL Computation: correlate phenotypic trait with genetic markers. Podocyte density of each BXD strain will be provided as the phenotypic trait.

Statistical analysis will be performed using the GeneNetwork database and applications.

2. Collaboration:

With other AMDCC Members: Matthew Breyer, Eli Lilly & Co

With other non-AMDCC PIs: Robert Williams, University of Tennessee, Memphis

3. Publications:

Poster presentation at the 2008 Annual Scientific Meeting of the ASN, Philadelphia
Diabetes-induced loss of podocyte density is a novel phenotypic trait in C57BL/6J (resistant) and DBA2/J (susceptible) mouse strains
Haiying QI, Kremena Star, Erwin BOTTINGER
Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029

Poster TH-PO789, 2009 Annual Scientific Meeting of the ASN, San Diego
Activation of interferon and innate immunity pathways, followed by mitochondrial dysfunction and oxidative stress response are characteristic glomerular trancriptome profiles in inbred diabetic DBA/2J mice susceptible to diabetic nephropathy, compared with DN-resistant diabetic C57BL/6J mice
Haiying QI, Shaolin SHI, Yezhou SUN, Weijia ZHANG, Xiaoping LI, Jin CHEN, Taoran ZHANG, Erwin BOTTINGER
Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029

Project 3: "Role of Cd36 in tubulo-interstitial injury of DN"

Responsible Investigator: Katalin Susztak

1. Project Accomplishments:

The specific aims of the proposal were to examine;
1. CD36-dependent intracellular pathways leading to tubular epithelial injury/apoptosis

3.1. Analyze whether there is a physical interaction between CD36 and Src-family tyrosine kinase (Fyn), and whether other downstream signaling mediators of CD36 including p42/44 MAPK, JNK, AKT kinase are activated following AGE and FFA treatment in human proximal tubular (HK-2) cells.

3.2. Examine whether FFA and AGE treatment of human proximal tubular cells leads to increased intracellular reactive oxygen species generation

3.3. Examine whether CD36 activation via FFA or AGE induce epithelial transdifferentiation of proximal tubular cells and whether there is a synergy between CD36 and RAGE

3.4. Examine, whether the activation of the src-, MAPK- and JNK-kinases play a role in the AGE and FFA induced CD36 mediated proximal tubular apoptosis

2. whether proximal tubular overexpression of AGE binding proteins CD36 and RAGE leads to increased tubular epithelial injury/apoptosis and tubulointerstitial progression of DN in mice

4.1. sglt2-CD36 and sglt2-RAGE transgenic strains will be submitted to AMDCC/MMPC model generation and phenotyping cores to generate new diabetic models of T2DM (sglt2CD36 xFVB$^{db/db}$) and T1DM (sglt2RAGExFVB$^{db/db}$) for standardized general and renal phenotyping

4.2. new models will be subjected to specialized phenotyping to determine tubular epithelial injury and tubulointerstitial progression of DN, including renal histopathology, ROS metabolism, epithelial apoptosis and epithelial dedifferentiation.

B. Rationale and Relevance

Our preliminary studies showed that as opposed to human diabetic nephropathy the currently examined murine diabetic animal models (db/db and STZ induced diabetes of C57B6J 129SvJ mice) do not develop significant tubulointerstitial fibrosis. We found that expression of a scavenger receptor; CD36 coincided with proximal tubular epithelial cell apoptosis and tubulointerstitial fibrosis in human DNP. CD36 expression was necessary and sufficient to mediate proximal tubular apoptosis induced by glycated albumins and free fatty acid palmitate through sequential activation of src kinase, and proapoptotic p38 MAPK and caspase3. In contrast, paucity of expression of Cd36 in PTEC in diabetic mice with diabetic glomerulopathy was associated with absence of tubular apoptosis and normal tubular epithelium. Mouse PTEC lacked Cd36 were resistant to glycated albumin induced apoptosis. Recombinant expression of CD36 in mouse proximal tubular epithelial cells conferred susceptibility to glycated albumin induced apoptosis. Our findings suggest that CD36 is as essential mediator of proximal tubular apoptosis in human DNP.

C. Summary of accomplishments

Earlier we cloned the human CD36 cDNA under the murine sglt2 (sodium/glucose co-transporter) promoter. The targeting vector was injected to FvB oocyte and transgenic animals were identified by tail genomic PCR analysis. Initial analysis indicated good transgene expression and renal phenotype consistent with tubular degeneration. However, unfortunately at the end of 2008 mouse parvovirus was identified in our animal room, specifically affecting the sglt2CD36 transgenic animal colony. MPV is a very resistant virus, which is very difficult to eliminate, therefore we needed to rederive our transgenic animal colony. This process took a bit longer than we expected, but now we have MPV free transgenic lines, that we are in the process of characterizing. As MPV can potentially interfere with the renal phenotype, therefore we are repeating the phenotype analysis in MPV free animals.
In addition, while we were waiting for the new MPV free colony we have also generated a new transgenic line where CD36 is under the tetracycline responsive promoter, using the tet-O-CD36 transgenic construct. Preliminary genotyping indicates that we have 2 animals that are positive for the transgene.
We already set-up a matings to cross the tet-O-CD36 animals with Pax8 rtTA mice. A recent publication from the group of Robert Koesters (Nature Medicine Sept 2008) indicates a very strong and widespread expression of rtTA in the renal tubules of these animals. After importing the animals we already performed some basic characterization of these animals already. After feeding the animals with doxycycline containing food we confirmed the strong expression of rtTA in the renal tubules and we were also able to use the animals to induce the expression of a different transgene.

4, Phenotype analysis of transgenic animals are in progress
A, No obvious phenotype was observed in control transgenic animals. Animals exhibit normal life-span and renal histology did not show obvious abnormalities.
B, In order to analyze the role of CD36 in diabetic nephropathy, animals were made diabetic by multiple low dose streptozotocin injection.
C, Studies determining the mechanism of CD36 mediated tubular degeneration are ongoing.
D, Additional experiments
The CD36 transgenic animals are on FvB genetic background. To analyze the role of CD36 in diabetic nephropathy development we will need to make them diabetic. There are many different diabetic animal models are available on FvB background. Thus we are in the process of performing head to head characterization of the different diabetic animal models on the FvB background. We have analyzed and sacrificed STZ induced diabetic FvB mice, FvB db/db mice, Ove26 transgenic mice on FVB background and we also back-crossed the Akita mutation into the FvB background. Our preliminary results indicate only minor albuminuria and glomerular injury in the FvBAkita and STZ induced FvB mice, db/db mice on FvB background show significantly increased albuminuria similar to the BKlS dbdb mice. We observed the most robust nephropathy in the Ove26 animals, but the degree of albuminuria and nephropathy still appears to be less severe in our facility compared to the Epstein group. One mouse cohort was sacrificed at 20 weeks of age and we plan to have a cohort that we will analyze at 9 months of age.

Progress toward stated milestones

First priority for the coming year is the completion of the characterization of the diabetic sglt2CD36 transgenic animals and crossing and characterization of the Pax8 rtTA/tet-O-CD36 animals.

Plans for the Upcoming Year

1, Crossing and characterization of the Pax8 rtTA/tet-O-CD36 animals and the (MPV-free) sglt2CD36 transgenic animals

2, Identify, which FvB diabetic mouse strain develops the most severe nephropathy and use these animals for further crossing.

3, Further in vitro studies analyzing the mechanism of CD36 mediated lipid uptake and cell damage in cultured tubular epithelial cells

4, Finish characterizing the different existing tubular epithelial cre mouse line (we imported the sglt2cre, PEPCKcre, Kspcre, GGTcre) animals. In addition we are also comparing the above mentioned cre lines with the Pax8rtTA animals.

2. Collaboration:

With other AMDCC PIs

With Jax

With the MMPCs

With other non-AMDCC PIs

3. Publications:

Increased tubular epithelial degeneration in sglt2CD36 transgenic diabetic mice Ana M Garcia, Antje Gruenwald, Velasco Cimica, Erwin Bottinger and Katalin Susztak Presented at the Annual Meeting of the American Society of Nephrology San Francisco 2007

Frank C. Brosius III, Charles E. Alpers, Erwin P. Bottinger, Matthew D. Breyer, Thomas M. Coffman, Susan B. Gurley, Raymond C. Harris, Masao Kakoki, Matthias Kretzler, Edward H. Leiter, Moshe Levi, Richard A. McIndoe, Kumar Sharma, Oliver Smithies, Katalin Susztak, Nobuyuki Takahashi, Takamune Takahashi for the Animal Models of Diabetic Complications Consortium, Mouse Models of Diabetic Nephropathy, J Am Soc Nephrol, in press, 2009.