Animal Models of Diabetic Complications Consortium

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Retinopathy Core
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1. **Program Accomplishments:**

The overall goal is to identify the best rodent models for screening retinopathy.

Our main strategy is two-fold: (1) to analyze and quantitate retinal vascular lesions in eyes of diabetic rodent models generated by other members of the AMDCC, and (2) to evaluate retinopathy in other diabetic rodent models initiated by us and non-AMDCC collaborators.

We have or are evaluating retinopathy in mouse models (provided by AMDCC members or generated ourselves), as well as pigs sent by the Gerrity and Nichols labs. At this point, accelerated retinopathy development has not been detected in any mouse models, but accelerated formation of aneurismal-like structures has been detected in retinas of about 30% of pigs having both diabetes and elevated lipids (but not with high lipids alone).

Major achievements have been:

Trypsin digest preparations have been made from retinas from more than 15 strains of diabetic mice. To avoid subjective bias, all samples are coded, and the code not broken until all samples in the group have been analyzed. To date, we have not found evidence of a strain-difference in rate at which the microvascular lesions of retinopathy develop in these diabetic mice. No evidence of retinal neovascularization or microaneurysm formation has been found in any of the mouse models. Aneurismal-like structures have been detected in retinal vasculature from about 30% of pigs having both diabetes and elevated lipids (but not with high lipids alone). These structures seem different than the microaneurysms commonly seen in trypsin digests of diabetic humans or dogs, in that they are out of the focal plane of the rest of the vascular network, but several have been seen to connect to existing vasculature (and thus seem unlikely to be debris).

2. **Collaboration within your group:**

NA

3. **Collaboration with other AMDCC groups:**

In the past year, we have analyzed over 150 retinas provided by AMDCC members or generated ourselves. Mouse strains evaluated included:

Akita Bradykinin B2 $R^{-/-}$	Coffman
Akita	Kern
C57 Pdx x $LDL^{-/-}$	Feldman
DBA/2	Inman
GCLC dbdb with and without high fat diet	Feldman
Glut 1 Overexpressors x dbdb on high fat	Feldman
KLSdbdb with 3 different concentrations of resvervatrol	Feldman
$LDLR^{-/-}$ x $eNOS^{-/-}$ dbdb	Harris
$ApoE^{-/-}$	Harris
$COX2^{-/-}$	Harris

In addition, another approximately 40 retinas from DBA, KK and MRL strains were obtained from the Breyer lab. Extensive efforts to isolate the retinal vasculature from these samples were not successful due to improper fixation/storage before sending eyes to us.

Eyes from diabetic and hyperlipidemic pigs were sent to us by the Gerrity and Nichols labs. Isolation of retinal vessels from these pigs caused unanticipated problems (failure of the standard isolation technique to work in pig retina). Several weeks of concentrated work were required to revise suitable methods to make the trypsin digest technique work with formalin-fixed pig retinas. This modified procedure now requires the pig retina to be exposed to crude trypsin for 24 hours instead of the usual 2-3 hours. Since making this modification, retinal vessels have been isolated from all animals sent by the Nichols lab and Gerrity lab.

New samples: In the last 2 weeks, another 80 eyes were obtained from obob x $LDLR^{-/-}$ animals (Breslow lab), and these are now being prepared and evaluated. Eyes will be received (next week) and evaluated also from GLUT-1 overexpressing dbdb mice, as encouraged by the advisory group.

Problem: A problem with the existing system of having investigators send us eyes in a blinded manner so that we do not know group identifications until capillary degeneration has been evaluated is the variable number of eyes and durations of diabetes that we have received. We have gotten more eyes than are necessary to make a conclusion, and in other cases we have not received enough samples to reach a conclusion. For example, we received 80 eyes from one experiment, with some durations of diabetes being as little as 16 weeks (too many eyes and too little duration). In other cases, after breaking the code, we found that some groups only had 2 or 3 animals, or no controls, thus making it very unlikely that any conclusions could be drawn about those strains unless more animals are provided. To help minimize this problem, we have been corresponding with people who send eyes to make sure that a reasonable number of samples are sent and analyzed.

4. Pertinent non-AMDCC Collaboration:

A variety of mouse strains (MRL/MpJ, DBA, CP/KK, 129P3, KKCgAyJ) diabetic for 2-3 months were shipped to us by D. Chen (Schepens Eye Research Institute, Boston), maintained until diabetic a total of 6 months, and then evaluated for degeneration of retinal capillaries. Each of these strains showed a diabetes-induced increase in number of degenerate capillaries in retina, but none showed more accelerated or severe pathology than expected.

To determine if loss of sympathetic innervation alters contributes to the development of diabetes-induced degeneration of retinal capillaries are being carried out with J. Steinle (Southern Illinois University, Carbondale, IL). Sympathetic innervation to the eye was destroyed by surgical removal of the right superior cervical ganglion in rat, and the contralateral or left eye served as an intra-animal control. Sympathectomy produced a slight increase in the number of degenerate capillaries in nondiabetic animals, but caused a significant increase in capillary degeneration in animals diabetic for 4 months. Apparently, loss of sympathetic innervation to the eye accelerates

degeneration of retinal capillaries in diabetes, and diabetes-induced loss of sympathetic innervation contributes to the development of diabetic retinopathy.

5. Address previous EAC comments and responses:

1. Comment: Analyze retinopathy in the following promising models:

	Status
High priority:	
C57BL/6 tg GLUT, dbdb	**Dr. Heilig says that eyes will be sent to me in the next week.**
C57BL/6J LDLR-/- Akita/+, TgHuART	**Mice not yet made available**
Priority; Needs to be done:	
C57BL/6J ENU mutant #76, Akita	**Mice not yet made available**
C57BL/6J Akita, Flk-1 RAGE	**Mice not yet made available**
C57BL/6J B2R-/- Akita	**Done, but studies need to be redone since diabetics did not show expected increase in retinal capillary degeneration.**

2. Establish validation criteria for diabetic retinopathy. **Done and submitted to website**