Animal Models of Diabetic Complications Consortium

Annual Report
(2004)

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 currently are evaluating retinopathy in approximately 14 mouse models (provided by AMDCC members or generated ourselves) as well as pigs sent by the Gerrity and Michols labs.

At this point, we cannot yet focus on any particular models of retinopathy as being better than others. One genetically modified mouse model that we predict would progress on to proliferative retinopathy faster than other wildtype animals involves overexpression of ICAM (thus stimulating leukostasis and presumably capillary occlusion). This model seems not to be available, and we will have to generate it.

Major achievements have been:

Trypsin digest preparations have been made from retinas from 10 strains of diabetic mice. To date, we have not found evidence of a strain-difference in rate at which the microvasular lesions of retinopathy develop in these diabetic mice. To avoid subjective bias, all samples are coded, and the code not broken until all samples in the group have been analyzed. No evidence of retinal neovascularization has been found in any of the models. We have conducted validation studies with regard to how to quantitate neurodegeneration in the retina, and have evidence that some mouse strains are more susceptible to diabetes-induced retinal neurodegeneration than are other strains. Detailed studies of diabetes-induced retinal lesions have been conducted for C57Bl/6 mice and $Ins2^{Akita}$ mice, and these are ready for publication.

2. **Collaboration within your group:**

NA

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

We currently are evaluating retinopathy in approximately 14 mouse models (provided by AMDCC members or generated ourselves) as well as pigs sent by the Gerrity and Nichols labs. Mouse strains being evaluated include: DBA, MRC, KK, C57Bl/6, Mrl/MpJ, Mr36, CP/KK, bradykinen B2 receptor knockout, $Ins2^{Akita}$, and PDX.

4. **Pertinent non-AMDCC Collaboration:**

Estimates of neural apoptosis in the retina were validated in C57Bl/6 mice via collaboration with Dong F. Chen, PhD (Schepens Eye Research Institute, Harvard Medical School, Boston, MA) by comparing results from 4 different methods that focus on the number of ganglion cells: (1) counting the number of TUNEL-positive cells in the ganglion cell layer 2 weeks and 1, 2 and 6 months after diabetes induction,

(2) counting cells remaining in the ganglion cell layer (per linear length of retina) at 6 and 12 months of diabetes using retinal sections taken from diabetic and age-matched control mice, (3) counting the number of retinal ganglion cells stained by retrograde labeling from the brain after 3 months of diabetes, and (4) measuring caspase-3 activity in whole retina (largely due to neural cells). The results indicate that the neuroretina of C57Bl/6 mice exhibits a transient and small extent of cell apoptosis immediately after induction of diabetes, but there is no gross or sustained loss of ganglion cells in this strain. Thus, counts of number of ganglion cells remaining per linear length of retina yielded conclusions that were the same as those reached using several other independent tests.

In contast, quantitation of apoptosis of neural cells in the retina by counting the number of ganglion cells remaining per linear length of retina after in another strain of diabetic rat ($Ins2^{Akita}$), studied in collaboration with Drs Barber and Gardner (Hershey Medical Center, Hershey, PA), did reveal that diabetes caused a significant decrease in number of ganglion cells.

We contacted investigators who developed mice overexpressing IGF-1 that develop diabetic-like retinopathy (J Clin Invest. 2004 Apr;113(8):1149-57) to see if we could obtain some mice for collaborative studies, but to date, the investigators have been unwilling to make the animals available to me.

5. <u>Address previous EAC comments and responses:</u>

- Should work actively with other AMDCC investigators to identify the best rodent models for screening retinopathy.
  This is ongoing, and we should have analyses completed of all eyes sent to us by this summer.
- Establish and post validation criteria for retinopathy on the website. Perhaps you could prepare a video on eyeball removal/preparation to share with other AMDCC investigators.
  A written procedure drawings has been posted. Attempts to improve these instructions using a video have not been satisfactory.
- Methods used to detect and quantitate retinal neuronal loss must be validated.
  Validation of methods to assess retinal neuron loss are described above in this report.
- Encouraged to get pig eyeballs from Clemmons/Nichols laboratory at UNC.
  Done. Pig eyes have been obtained from the laboratories of Drs Nichols and Gerrity. These eyes currently are being analyzed.
- The DBA/2J strain may not be a good model for retinopathy due to altered IOP.
  We will continue to analyze all eyes sent to us. IOP has been invoked as a possible contributor to retinopathy severity also in patients, so its contribution remains to be discerned.