Animal Models of Diabetic Complications Consortium
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"Dislipidemia, Lipoic Acid and Diabetic Vascular Complications in Humanized Mice"

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Part A:

Principal Investigator's Summary

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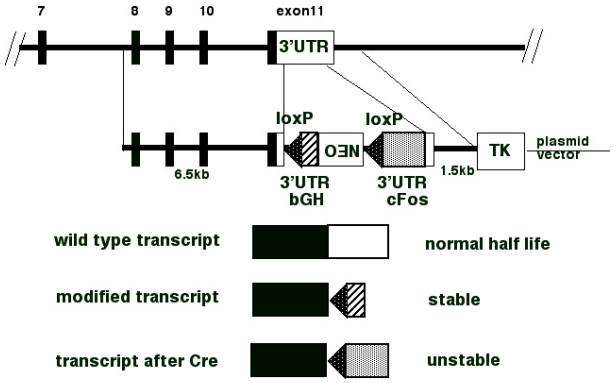
Hypothesis

1. Mice humanized lipoprotein emtabolism system will devcelop a more human-like diabetic dyslipidemia and cardiovascular problems.
2. Genetically determined differences in eh levels of endogenous antioxidants affect the development of cardiovascular complications of diabetes.

Recent Progress and Major Accomplishments

1. We previously showed that diabetes increases markers of oxidative stress in tissues and in plasma, and enhances the development of atherosclerosis in mice lacking apoE (Apoe-/- mice). We also showed that a dietary supplement of lipoic acid (LA), a strong natural antioxidant, effectively prevents the enhancement of atherosclerosis by diabetes. We have now examined the effects of supplementing the diet with another natural antioxidant, vitamin C, on atherosclerotic plaque development using Gulo-/-Apoe-/- mice. Gulo-/- mice carry a null mutation in the gene coding for gulonolactone-γ-oxidase, a key enzyme for the endogenous synthesis of ascorbic acid. Humans lack this enzyme and are dependent on dietary vitamin C supplement for life, as are the Gulo-/- mice. The Gulo-/-Apoe-/- mice were treated with STZ and separated into two groups; one group was given vitamin C at 330mg/L (a supra-survival level-10X) in their drinking water for 5 months and the second group at 33 mg/L (a survival level-1x). The liver vitamin C levels after 5 months of diabetes in Gulo-/-Apoe-/- mice that received 330mg/L supplement (161±20 μg/g) was similar to the levels in non-diabetic wild type mice. In contrast, liver vitaimin C levels in mice that received 33 mg/L vitamin C supplement were approximately a quarter normal (42±5 μg/g, $P<0.0001$), suggesting that these mice are marginally vitamin C deficient. However, erythrocyte GSH and plasma glucose levels were not significantly different in the two groups. In addition, while the diabetes increased the mean atherosclerotic plaque areas measured at the aortic root by about 50% compared to non-diabetic mice, the amount of vitamin C supplement did not affect the mean areas ($318x10^3 \mu m^2$ in 330 mg/L mice vs $334x10^3 \mu m^2$ in 33 mg/L mice, n=17 each, not significant). We conclude that the level of vitamin C deficiency from survival to 10X does not influence the enhancement of atherosclerosis caused by diabetes, and that athero-protective effects of antioxidants depend on the nature of the antioxidant.



2. LA is naturally synthesized in all the cells in the body, and mice lacking the key enzyme for LA synthesis (lipoic acid synthase, Lias) die in utero. Towards the generation of a new model that combines diabetes with conditionally decreased antioxidant defense by reduction of Lias, we have made a DNA construct to modify the 3'UTR of the *Lias* gene in ES cells. The top line of figure illustrates the 3' half of the mouse *Lias* gene. The targeting construct (second line) is designed so that the targeted *Lias* gene will initially produce stabilized transcripts of the gene using the 3'UTR sequence of bovine growth hormone (bGH). The resulting mice (Lias-H mice) are expected to express higher than normal levels of Lias. After Cre-mediated recombination has been induced between the two loxP sites (triangles), the 3'UTR sequence will change to that from the

cFos gene which results in unstable transcripts. Cre-recombination can be applied in a tissue specific fashion. The resulting mice (Lias-L) will have lower than normal Lias gene expression and a reduced production of endogenous LA. They will allow us to study the effects of reduced LA production on diabetic complications, which we predict will be enhanced. The DNA construct, together with the oligonucleotide primers for screening correctly targeted ES cells and a probe fragment for the subsequent confirmation, were sent to JAX earlier this spring; they tell us that they have been confirming the *Lias* gene structure in the B6 ES-cell line they are going to use for this experiment, and will let us know when they will start gene targeting experiments.

3. While waiting for the new Lias mouse models described in 2 above, we have begun to investigate consequences of a 50% reduction in Lias gene expression on the development atherosclerosis and its enhancement by diabetes. To carry out this experiment, we first produced Lias+/- Apoe-/- mice on a 129/SvEv background by breeding our existing mutants. The plasma levels of cholesterol, triglycerides and glucose in the Lias+/-Apoe-/- mice do not differ from those in Lias+/+Apoe-/- mice, but the Lias+/-Apoe-/- mice develop significantly larger atherosclerotic plaques at the aortic root ($114\pm8 \times 10^3 \mu m^2$, n=25) than the Lias+/+Apoe-/- mice ($79\pm9 \times 10^3 \mu m^2$, n=13, $P<0.01$). Thus even this modest genetic decrease in Lias expression affects atherogenesis.

4. Towards our Specific Aim 1 we treated with STZ mice expressing the human apoE isoforms (apoE2, apoE3 or apoE4) in place of mouse apoE. The effects of STZ treatment were rather variable, as judged by plasma glucose levels of animals, and only half of them became significantly diabetic with sustained high plasma glucose levels. Nevertheless, in the diabetic mice with plasma glucose over 300 mg/dl, we observed an apoE isoform-dependent increase in plasma cholesterol levels. The increase of plasma cholesterol was more pronounced in mice expressing apoE4 than in mice expressing apoE3, while STZ treatment did not alter the levels significantly in mice expressing apoE2. Interestingly, the effects of diabetes are inversely related to the non-diabetic cholesterol levels of the apoE-isoform mice. We are repeating the experiment with a larger number of mice, and are also testing mice and expressing increased levels of LDL receptor, or no LDL receptor, in addition to the human apoE isoforms. Since atherosclerotic plaque development in individual animals varies, the added variability in the diabetes induced by STZ treatment makes it very difficult to study subtle changes in atherosclerosis. We have therefore decided to introduce *Ins2-C96Y* mutation of Akita diabetic mice into mice expressing human apoE2 or apoE4 to obtain more uniform hyperglycemia than is possible with STZ. The first group of experimental mice expressing human apoE2/E2 and diabetic due to the Akita mutation will become available shortly.

Plans for the Upcoming Year

1. We will continue to study the diabetes-induced dyslipidemia and atherosclerosis in mice with the human apoE isoforms that express different levels of LDLR. Diabetes due to STZ treatment and to the Akita diabetic mice will both be used, but we will move more towards the latter.
2. The effects of heterozygous reduction of the *Lias* gene on diabetes-induced atherosclerosis will be examined in the STZ-treated Lias+/-Apoe-/- mice.
3. We expect to receive the Lias-H mice carrying a modified Lias gene from JAX during the coming year. We will then test whether our overall scheme works as planned by crossing the mice with mice that express Cre-recombinase in germ-line and consequently will produce Lias-L mice. Expression of the Lias gene will be assessed by RT-PCR. Although mice lacking LA completely die early during the embryonic development, we expect that the Lias-L mice will survive but will be highly susceptible to oxidative tissue damage. These mice will be crossed with Akita diabetic mice to make them diabetic.

Preliminary Milestones for 2009 and Beyond

2009: Assessment of the effects of apoE-isoforms on the lipid metabolism in Akita-type I diabetes will be completed.
2010: Effects of type I diabetes on the atherosclerosis development in Akita mice expressing human apoE2 or human apoE4 will be determined. Assessment of the effects of reduced endogenous lipoic acid synthesis in diabetic atherosclerosis will be completed in the STZ-treated Lias+/-Apoe-/- mice.
2011:

1. Collaboration:

With other AMDCC PIs

None at this moment.

With Jax

In early March of 2007, we sent to JAX the DNA construct to be used for the modification of the Lias locus in ES cells. PCR primers to be used for ES cell screening and a DNA fragment to be used as a probe for Southern blot analysis to confirm correct modification of the locus have also been provided to them. We understand that the experiment is on the production line of the JAX facility, and expected to begin sometime late in August.

With the MMPCs

Not at this moment.

With other non-AMDCC Pis

none

2. Address previous EAC comments:

NOT APPLICABLE THIS YEAR

3. Publications:

none