Animal Models of Diabetic Complications Consortium

(UO1HL0879450-03)

Annual Report
(2009)

Project Title: Creating glucose responsive cardiovascular complications in the mouse

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Part A:

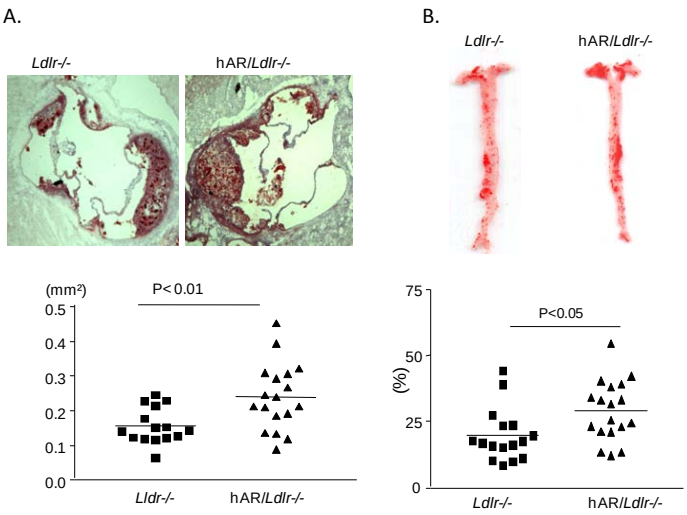
Principal Investigator's Summary

Introduction: This Project has led to a continued intellectual and experimental collaboration between the two PIs and their laboratories. This has included meetings approximately every 6 weeks and weekly phone communications. Thus, both major projects are proceeding and being critiqued by the PIs and an outside reviewer, Dr. Jan Breslow (Rockefeller University).

The Project includes methods to produce and evaluate mouse models of two major cardiovascular complications of diabetes: atherosclerosis and heart failure.

Atherosclerosis Studies: Two objectives of this project were to assess the effects of aldose reductase in several models of atherosclerosis and to create mice that would allow tissue specific expression of the human aldose reductase (hAR) gene. We reported that young mice with hAR on the LDL receptor knockout (Ldlr-/-) background put on a high cholesterol high fat (western) diet had only minor changes in blood glucose and insulin levels. The hAR transgene led to no change in atherosclerosis. However, when the same genotype mice were put on the western diet at age 6 months, atherosclerosis was increased (Figure 1).

Figure 1



Studies to assess hAR expression in individual tissues will use a new mouse line. The constructs to create the tissue specific hAR were sent to Jackson Laboratory and mice expressing the constructs have been screened.

Atherosclerosis and its pathophysiology is also being evaluated by assessing whether diabetes affects lesion regression. The surgical artery transplant techniques initially developed in the Fisher laboratory have been augmented. Two methods were used to create regression in Ldlr-/- mice. Reversa mice (in which hyperlipidemia can be conditionally reversed) and injection of an adenovirus expressing the LDL receptor were used to reduce hypercholesterolemia to levels that allow lipid and macrophage reduction in established lesions. In the presence of diabetes created with streptozotocin loss of macrophages from the lesions is reduced as is development of collagen in both of these models. This suggests the creation of more unstable plaques in the diabetic mice.

Heart failure: Mice expression hAR via the MHC promoter have been followed for a year. At that time the hAR mice have a reduction in cardiac ejection fraction, so hAR expression leads to heart failure.

Project Accomplishments (2009)

Aim 1 creation of new mouse models of diabetic cardiovascular disease:
Production of an Inducible hAR mouse: Jackson laboratory has used the two plasmids supplied by this project to make a transgenic mouse in which AR expression can be induced by adding tetracycline to the drinking water ("tet-on" system). The separate plasmids are called rtTA (which produces the transcription factor that activates the second plasmid) and TRE/hAR (which will express human AR when activated). We are currently screening cross-bred mice to determine if tetracycline leads to hAR expression in these mice. Very recent preliminary data have shown that in some of the mice shipped by Jackson, there was little expression of hAR in the absence of tetracycline, but 10-50X more when tetracycline was added to the drinking water. If this is confirmed, then expression at the protein and enzymatic levels will be assessed, and appropriate mice bred to supply the animals for the experiments described in the original application.

MHC-hAR mice: These mice have reduced cardiac function. Whether this is increased with diabetes is to be determined. MHC-hAR mice did not show changes in plasma lipids, glucose and heart to body ratio changes compared with normal control mice at ages three and thirteen months. Young MHC-hAR mice had normal cardiac function. Thirteen month old mice, however, showed obvious cardiac dysfunction with a significant decrease in cardiac fractional shortening and increase of left ventricular systolic dimension. Gomori's trichrome stained heart tissue section showed a large increase of fibrosis in 13-month old MHC-hAR mice. There was increased cardiac aldose reductase downstream gene expression: sorbitol dehydrogenase (SDH), fructokinase (KHK), aldose A and aldose B. Glut4 and iNOS mRNAs were also significantly increased in MHC-hAR mice.

Aim 2 to study the development of vascular lesions in diabetic mice
Non-surgical model of regression: We are completing a manuscript describing the reduced regression that occurs in diabetic mice, Reversa model. Meanwhile we have also performed a similar study treating *Ldlr-/-* mice with a helper virus-dependent adenovirus containing an expression cassette for the LDL receptor (obtained from Dr. Larry Chan, Baylor College of Medicine). As noted above, in both cases, with hyperglycemia, there is reduced regression after lipid levels were normalized, with plaques relatively enriched in macrophages and poor in collagen.

The reasons for the reduced regression in diabetes are being studied. The major possibilities are that hyperglycemia, hypoinsulinemia or elevated fatty acids alter macrophage function. Most tissue culture studies of macrophage-like cells utilize culture medium containing 25 mM glucose. Both mouse bone marrow derived macrophages and a cell line model of immature dendritic cells (D2.4) have a strikingly different phenotype when grown in 5 versus 25 mM glucose. In low glucose the cells are less inflammatory and have reduced adhesion to the culture dish. Expression of the inflammatory cytokine S100a is markedly increased in 25 mM. Thus, hyperglycemia alone appears to affect macrophage biology and might prevent lesion regression. In the ongoing experiments, we are studying the plaque macrophages by laser-capture microdissection and RT-PCR, as well as cell culture models to determine the molecular effects of hyperglycemia.

2. Collaborations:

Within the AMDCC: The Goldberg/Fisher Project has developed a on-going collaboration with Dr. Abel to assist with the evaluation of diabetic cardiomyopathic mice. Animals with lipid-induced cardiomyopathy have been sent to the University of Utah and glucose and fatty acid oxidation in isolated perfused hearts have been studied. When additional data are obtained on the MHC-hAR mice, these animals will also be studied as part of this collaboration.

With Jax: Vectors to produce the inducible hAR mice have been sent to Jackson Laboratories.

With the MMPCs: Although we have had no formal use of the MMPCs, studies in other models associated with altered lipid uptake into hearts were designed with Dr. Brunengraber (CWR). We plan to send mice to Cleveland to assess heart function in mice with defective fatty acid uptake and determine whether reduced heart function in these mice can be improved by inclusion of acetate in the perfusate.

Outside the AMDCC: An ongoing collaboration has continued with Dr. Breslow, Rockefeller U. Dr. Breslow attends the joint data presentation meeting with Drs. Goldberg and Fisher. Dr. Fisher has established a collaboration with Dr. L. Chan (Baylor) to utilize helper dependent adenoviral infection to reverse hypercholesterolemia and atherosclerosis in Ldlr-/- mice.

3. Address previous EAC comments:

Comments of EAC are in bold

Comments to the cardiovascular consortium
The CV break-out group discussed the need to mimic human lipidology in animal models of diabetic complications, especially when trying to model vascular disease. All agreed that for atherosclerosis to occur, lipids in general must be high and that diabetes should exacerbate lipid abnormalities. The question of validation criteria was discussed and it was decided that there should be both an acceleration of atherosclerosis as well as lipid changes. Again, the issue of lesion complexity was raised. There was general agreement that cross-sectional quantitation of BCA lesions was fraught with variability. Should coronal sectioning be standard?
The issue is to segregate the effects of diabetes from the secondary effects of diabetes induced hyperlipidemia; showing more atherosclerosis associated with more hypercholesterolemia does not take us very far. Moreover, if the hyperlipidemia is too severe, then the specific pathophysiology is likely to be obscured. In an effort to do this, we have studied the reasons for the greater hyperlipidemia with diabetes and shown that in Ldlr-/- mice this is associated with defective liver uptake of lipoproteins (see publications). In addition, we have studied the Tie2-hAR transgenic mice on both Ldlr-/- and apoE-/- backgrounds. ApoE-/- mice will develop atherosclerosis on chow and with diabetes have a less severe hypercholesterolemia. In the regression studies, it should be noted, we are studying the hyperglycemia independent of hyperlipidemia, since the lipids are either normalized by transplantation of aortas into normolipdemic mice or reversed by a genetic switch..

The CV group has had continued discussions of methodologies. Our site has elected to show in situ lesions that include the BCA, but along with the MMPC lab in Seattle we are not satisfied with the quantification of these sectioned lesions.

Further investigation of the effect of age on insulin resistance and atherosclerosis
We have studied some of our models at both 3 and 6 months. The effects of diet on lipids and insulin resistance, and the development of atherosclerosis are more marked in older mice. Reasons for this were recently reported by Dr. Hsueh who noted that older mice had upregulation of inflammatory genes in the aorta.

Further defining the initiating mechanisms of vascular disease
The initiating factor in most models is hyperlipidemia.

Producing a "menu" of different diets that result in disease-specific phenotypes.
The effects of different diets on lipoprotein profiles and atherosclerosis in Ldlr-/- and apoE-/- mice are reported in the literature.

Specific comments on the Goldberg/Fisher project
This group has completed construction of the two plasmid constructs necessary for tet-on expression of human aldose reductase, validated in cultured cells, and sent to JAX for production. Correct and an update is included above.

A cardiac-specific transgenic mouse has also been attempted (MHC-hAR)—2 lines have been produced. Mice express >20 fold increased hAR expression. Initial results indicate enhanced expression of ANF and BNP, indicating cardiac stress. Other functional measures would be helpful. As indicated by the investigators, the effects of STZ-induced diabetes on the background will be interesting.
The MHC-AR mice have reduced fractional shortening at age 8 months. So, they develop a cardiomyopathy. We have enlisted a collaborator to study the effects of MHC-hAR in ischemia/reperfusion and are studying the effects if STZ diabetes in this model.

Studies by Dr. Fisher's group are aimed at investigating mechanisms of vascular lesion regression. Non-surgical methods include adenovirus-aided expression of the LDL receptor in LDLr-/- mice to reduce LDL cholesterol levels. A second "genetic" switch model is also being pursued that reduces VLDL secretion and LDL levels to normal over the course of about a week. Both are being combined with STZ hyperglycemia to investigate the effects of elevated glucose levels. Interpretation of these experiments may be challenging as it may be difficult to discern a proposed reduction in the "loss of macrophages" as compared to accelerated macrophage accumulation.
Fortunately, the changes were significant enough that, we have found that diabetes leads to less loss of macrophages from lesions during regression. As noted by the EAC the next challenge is to determine whether this is due to reduced egress or increased influx of cells. We are attempting to use labeled macrophages to determine this, a technique Dr. Fisher has established in other studies of mouse atherosclerosis.

4. Publications:

Goldberg IJ, Y Hu, HL Noh, J Wei, LA Huggins, MG. Rackmill, H Hamai, BN Reid, WS Blaner L-S Huang. Decreased lipoprotein clearance is responsible for increased cholesterol in streptozotocin treated LDL receptor knockout mice, Diabetes, 57:1674-82, 2008

Park T-S, Y Hu, H-L Noh, K Drosatos, K Okajima, J Buchanan, J Tuinei, S Homma, X-C Jiang, ED Abel, Ira J. Goldberg. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy/ J Lipid Res, 49:2101-12, 2008

Noh H-L, Y Hu, T-S Park, T DiCioccio, AJ Nichols, K Okajima, S Homma, IJ Goldberg. Regulation of plasma fructose and mortality in mice by the aldose reductase inhibitor lidorestat. J Pharmacol Exp Ther, accepted