Animal Models of Diabetic Complications Consortium
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Annual Report
(2007)

Project Title: Creating glucose responsive cardiovascular complications in the mouse

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Part A: Principal Investigator's Summary

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. In the 01 year of the grant, studies were performed to evaluate the effects of high fat versus high cholesterol diets on insulin actions and atherosclerosis in LDL receptor knockout (*Ldlr-/-*) mice. In addition, overexpression of aldose reductase, an atherosclerosis- exacerbating gene, was studied in this context.

Atherosclerosis and its pathophysiology is being evaluated in two ways. Dr. Fisher's laboratory has developed methods to study atherosclerosis regression in mice. As proposed in the application, the surgical artery transplant techniques are being complemented with methods to reverse hyperlipidemia in mice. Using adenoviral gene transfer, the Fisher laboratory is establishing techniques and the chronology of vascular change that occurs when LDL receptors are expressing in hypercholesterolemia *Ldlr-/-* mice that have established atherosclerosis.

To study the effects of the expression of aldose reductase, the investigators have made several constructs to create new transgenic mice. These include a tet-on-off system to express aldose reductase in specific tissues in a chronological manner.

To evaluate the effects of aldose reductase on heart function, a new line of transgenic mice in which aldose reductase is expressed in cardiomyocytes via the myosin heavy chain promoter was created. These mice are currently being bred and will be phenotyped in the coming year.

1. Project Accomplishments (2007)

Aim 1 creation of new mouse models of diabetic cardiovascular disease:

Production of an Inducible hAR mouse: We had proposed to extend the original model studied by the Goldberg lab (hAR transgenic/*Ldlr*-/-) by making an inducible version. This would allow us to mimic the clinical situation in which patients develop plaque in an insulin-sensitive state, then as they age and grow obese, develop insulin resistance, with attendant effects on existing plaques and on newly forming ones. The promoter for the hAR transgene is H2-K, which is generally expressed. We cloned the promoter into the first plasmid component of the "Tet-on" inducible system (i.e., the target gene, in this case hAR, will be induced by tetracycline in the drinking water), which regulates the production of the transactivating factor for the second plasmid. Into the second plasmid, we cloned hAR. In co-transfection experiments, we found that cells treated with tetracycline had a large induction of hAR enzymatic activity. We have now started working with the Jackson Lab MGHC to make mice transgenic for the two plasmids on the Ldlr-/- background. Based on Dr. Leitner's suggestion, the first attempt will be to use both plasmids at once in a C57BL6 background (instead of making 2 separate mice, which would have to be crossed). After confirming in vivo regulation of hAR, mice will be crossed to Ldlr-/- mice.

The second proposed mouse model was created. An α-myosin heavy chain (MHC) promoter was placed 5' to the hAR cDNA, injected into mice and two lines of transgenic animals were produced. These mice have a >20 fold increased in hAR expression and this increase is localized to the heart. The two lines are being characterized for hAR activity, effects on cardiac function as the mice age, and influence of diabetes. If, as expected, the animals develop cardiac pathology, they will be sent to the MGHC for breeding.

Aim 2 to study the development of vascular lesions in diabetic mice

Regression studies: As noted in the grant proposal, previous attempts to study the effects of hyperglycemia on regression of atherosclerosis were confounded by relatively low levels of plasma glucose in the PDX1+/- mice, as well as not having expression of hAR. In collaboration with the Breslow lab, we have started colonies of hAR, Ldlr-/-, Akita+/-,Ldlr-/-, and Akita+/-hAR mice to provide the necessary numbers of mice to perform a transplant study in which lesions will be allowed to develop in Ldlr-/- mice and then transplanted into recipients that are totally normal (wild type; WT), (WT/hAR), (Akita+/-,hAR), and Akita+/-. In this way, we can separately test the effects of hyperglycemia and hAR on the ability to regress plaques.

Non-surgical models of regression: In the first funding cycle, we were asked by the advisory board to consider developing a non-surgical approach to the study of regression. With that in mind, we were impressed by the recent results of Dr. Larry Chan (Baylor) in creating helper-dependent adenoviral vectors containing the LDL receptor, which led to long term expression in Ldlr-/- mice and correction of the their hyperlipidemia. We have received sufficient vector (and control vector) from Dr. Chan and will do a pilot study in the next few months in which Ldlr-/- mice will be fed an atherogenic diet for 16 weeks, then injected with either Ad-Ldlr-/- or Ad-control. Mice will be taken for plaque analysis just before injection and at

points up to 4 weeks after injection to get a time course of size and compositional changes. Based on these data to determine the best time point for analyses after transplantation, a larger group of mice will be studied for the effects of hyperglycemia on plaque regression. This will be accomplished by starting Ldlr-/- mice on the atherogenic diet. Then 16 weeks later, mice will be injected with Ad-Ldlr-/- or Ad-control, with half the animals in each sub-group treated with STZ (to cause hyperglycemia). At the chosen time point, mice will be sacrificed and the changes in the plaques analyzed.

Ongoing atherosclerosis progression studies have assessed the effects of diet, Akita, and aging on atherosclerosis progression in the hAR-Ldlr-/- background. Young Ldlr-/- mice ± hAR were begun on a high fat diet to create insulin resistance or a high cholesterol-only diet (1). The mice developed hypercholesterolemia, however, the levels of hyperglycemia and insulin resistance were modest. In this setting neither high fat/insulin resistance nor the introduction of hAR altered atherosclerosis progression. In a second study, hAR/Ldlr-/- was crossed onto the Akita background. The presence of Akita led to a 2 fold increase in plasma cholesterol levels which averaged ~1500 mg/dl. After 20 weeks, these mice have extensive lesions, the lesions were greater in the diabetic more hyperlipidemic mice, but hAR did not alter lesion size in these mice with very extensive atherosclerosis.

A third study is underway to test whether high fat insulin resistance is greater in older mice. As was reported to the AMDCC group by Dr. Hsueh, we found that feeding a high fat diet to mice that were greater than 6 months old led to much greater plasma cholesterol levels and more insulin resistance (2). We are testing whether hAR will alter lesion size in this model.

Finally, in an effort to understand how diabetes alters plasma lipids, we studied the effects of STZ-diabetes on parameters that affect the metabolism of plasma lipoproteins. Diabetic mice ate approximately 60% more diet than did non-diabetic controls on the same 0.15% cholesterol diet. However adjusting the diets by lowering the cholesterol to 0.075% thereby reducing the cholesterol intake of the STZ-treated mice, did not. Kinetic studies showed that STZ-treatment did not increase lipoprotein production. Rather initial decay, associated with lipoprotein trapping, was reduced.

Collaborations:

Within the AMDCC: The NYC 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, along with Dr. Abel studies are planned with the UTSW laboratory to develop methods to simultaneously assess the contributions of glucose and fatty acids to TCA cycle intermediates using labeled isolated perfused hearts.

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. In addition, his laboratory created the Akita/Ldlr-/- mice ± hAR and studies to assess the effects of diabetes on atherosclerosis and regression in the model are continuing.

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.

Publications:

1. Wu, L., Vikramadithyan, R., Yu, S., Pau, C., Hu, Y., Goldberg, I. J., and Dansky, H. M. (2006) *J Lipid Res* **47**(10), 2215-2222
2. Hsueh, W., Abel, E. D., Breslow, J. L., Maeda, N., Davis, R. C., Fisher, E. A., Dansky, H., McClain, D. A., McIndoe, R., Wassef, M. K., Rabadan-Diehl, C., and Goldberg, I. J. (2007) *Circ Res* **100**(10), 1415-1427