Animal Models of Diabetic Complications Consortium

(UO1HL0879450-03)

Annual Report
(2010)

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 Co-PIs and their laboratories. This has included meetings approximately every 6 weeks and weekly phone communications. Both major projects are proceeding and being critiqued by the PIs and an outside reviewer, Dr. Jan Breslow (Rockefeller University). In addition, we have collaborated with Dr. Abel (U Utah) to study models of cardiomyopathy

The Project includes methods to produce and evaluate mouse models of two major cardiovascular complications of diabetes: atherosclerosis and heart failure. Because diabetes leads to major effects on plasma lipoprotein concentrations, our efforts have focused on isolating effects due to diabetes from those that are secondary to hyperlipidemia. We have studied why diabetes leads to such severe hyperlipidemia. We then developed a model to study atherosclerosis biology in mice that have similar levels of cholesterol; this entailed creating mice in which cholesterol was markedly reduced to allow for atherosclerosis regression. In addition, we have pursued studies of the effects of aldose reductase (AR) the initial enzyme in the polyol pathway and showed that this enzyme increases fructose production in vivo and leads to more atherosclerosis in several mouse diabetic/hyperlipidemic mice. Finally, we have studied AR effects in the heart and have collaborated with Dr. Abel to characterize a lipotoxic heart model.

1. Project Accomplishments (2010)

New model development: We proposed to create models in which to study the effects of the enzyme aldose reductase (AR) in two complication of diabetes: atherosclerosis and heart failure. In addition, we planned to develop an animal model to assess how diabetes affects atherosclerosis regression, defined as the loss of CD68+ cells from atherosclerotic plaques after reduction of circulating cholesterol.

AR and atherosclerosis: We have completed studies of the effects of generalized expression of human AR (hAR) on atherosclerosis in the mouse. The animals were crossed onto the apoE knockout (apoE0) background. We used this model because diabetic vascular disease can be studied while the mice are consuming a chow rather than high fat or high cholesterol diet. These later diets in the LDL receptor knockout model sometimes cause extremely high levels of circulating cholesterol that obscures any effects of diabetes. As in the LDL receptor knockout model, hAR/apoE0 diabetic mice developed a marked increased in atherosclerosis lesion area compared to diabetic apoE0 mice; non-diabetic mice show no effects due to expression of the hAR transgene. These studies were performed in collaboration with R. Ramasamy (Columbia).

Creation of methods to alter hAR in various cells: We created and sent to Jackson Labs vectors to allow a tet-on expression of hAR in mice. The two vectors included a tet-responsive hAR and a tet-on promoter element associated with a major histocompatibility gene, the same promoter used to create the hAR transgenic line that we have used in the past. These vectors were used by the Jackson Labs to create mice and we were able to show doxycycline induction of hAR expression in cells from the mice. The 3 highest expressing founder lines (which had AR levels as high as the transgenic mouse model) are being bred to LDL receptor knockout mice in order to provide a model of atherosclerosis with inducible expression of AR. These mice can then be used to study the effects of AR expression after plaques are allowed to develop.

MHC-hAR mice: A line of mice in which hAR was expressed in cardiomyocytes was studied. These mice develop heart dysfunction, i.e. reduced ejection fraction at greater than 10 months. This is associated with defective recovery from ischemia/reperfusion injury. The reasons for these defects are being explored.

Development of generalizable models of plaque regression: One objective was to develop models exclusive of surgical transplant to allow study of atherosclerosis regression with diabetes. We have done this in two ways. 1) The Reversa mouse model allows for the development of hypercholesterolemia due to knockout of the LDL receptor. This model has an inducible hepatic deletion of microsomal triglyceride transport protein (MTTP) that then prevents apoB production by the liver, leads to a marked reduction in plasma cholesterol levels, and causes plaque regression. We have used this model to study atherosclerosis regression in the setting of streptozotocin diabetes and with expression of hAR. A manuscript describing the basic Revesa model of regression was submitted to the journal Circulation. The reviews were encouraging and we were invited to submit a revised manuscript. We are in the process of finishing these studies. A manuscript summarizing the effects of diabetes on

regression has also been prepared and we are submitting it to the journal Diabetes. Very briefly, the diabetic state impaired the regression process in spite of dramatic reductions in the plasma levels of non-HDL lipoproteins.

A second model proposed in the original grant has been validated. We have created plaque regression in LDL receptor knockout mice by introduction of an adeno-associated virus expressing LDL receptors. Replacement of the receptor and a switched to chow diets from the western diet reduced plasma cholesterol from >700 to ~150 and was associated with reduced plaque CD68+ cell (mostly macrophages) content.

2. Collaborations:

Within the AMDCC: The Goldberg/Fisher Project has developed an on-going collaboration with Dr. Abel to assist with the evaluation of 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.

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

Outside the AMDCC: An ongoing collaboration has continued with Dr. Breslow, Rockefeller U. 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. Drs. Goldberg and Fisher have collaborated with R. Ramasamy (Columbia but moving to NYU) to study the effects of hAR in atherosclerosis and in regression.

3. Address previous EAC comments:

Comments of EAC are in bold

The goals of this project are to generate and characterize mouse models of atherosclerosis and heart failure in the setting of diabetes, with a focus on the hyperglycemic-mediated causes of these complications. The PIs have made significant progress in the past year. Specifically, the Goldberg group has generated (with JAX) a tissue-specific human aldose reductase mouse. They have also discovered that the human aldose reductase (hAR) mouse expressing hAR using the MHC promoter causes heart failure. The Fisher group has studied atherosclerosis regression in diabetic mice. Their findings suggest that diabetic mice have less atherosclerosis regression and the regression is associated with loss of macrophages and reduced collagen content in the lesions, which actually may make the plaque more unstable. Their findings suggest that hyperglycemia (in the absence of hyperlipidemia) impacts macrophage function. This is an important finding. This is also reflected in their comments to the last review, where the panel asked them to comment on 'how to mimic human lipidology in animal models of diabetes'. Their response is that we should focus on separating hyperglycemic vs. hyperlipidemic effects on vascular complications, such as atherosclerosis, which this reviewer completely agrees with. One question to pose to these investigators though is a methodologic one: can these investigators aid the panel in determining how best to separate hyperglycemic vs. hyperlipidemic effects on atherosclerosis in mice? Can it successfully be done in BKS, or other inbred strain? Use of ldlr or apoe may be necessary for the hyperlipidemic component to initiate lesion development. Of course, there is the hAR model itself, as well as the RIP mouse that will aid in this regard. Additional insights on this topic by these leaders in the field will be helpful to many.

As noted above, we have developed methods to study regression, a process that occurs in the setting of cholesterol reduction, that eliminates as a factor diabetes-mediated hyperlipidemia. This is an advantage because diabetes leads to marked exacerbation of hyperlipidemia in the usual mouse models of atherosclerosis, LDL receptor knockout and apoE knockout. We are currently also exploring a new method to assess some diabetes-mediated processes from others. We have treated LDL receptor and control mice with STZ-followed by phlorizin and successfully lowered circulating glucose levels in these mice. We hope to segregate the effects on the arterial wall of hyperlipidemia from hyperglycemia and defective insulin actions.

The hypothesis that aldose reductase plays an important role in cardiovascular complications continues to be investigated. The investigators have exploited the fact that the mouse does not have AR and are producing both general and tissue-specific transgenics. AR-Tgs have increased ventricular size and evidence of cardiac dysfunction. Moreover, there is more damage to the heart after ischemia/reperfusion. Studies have also focused on different PPAR isoforms and their effects upon cardiac lipotoxicity. Interestingly, the three isoforms appear to have independent and interacting effects upon gene expression and lipid handling in the heart. Animals were sent to Dr. Abel to investigate mitochondrial function, again highlighting an important collaborative aspect of the Consortium. These types of studies should be continued.
We appreciate the comments of the AEC. Dr. Abel's expertise has been invaluable in our analysis of cardiac dysfunction in our lipotoxic mice. During the past year, he has performed a detailed examination of the mitochondria of our cured lipotoxic MHC-PPARγ/PPARα mice; the mitochondria appear not to be the reason for the reduced toxicity. Nonetheless, we, like the AEC, realize the valuable information that the Utah group brings to these studies of cardiac dysfunction.

Dr. Fisher's group has incorporated the Reversa mouse (in which hyperlipidemia can be conditionally reversed) to their studies of vascular lesion regression. Using AMDCC recommended procedures, they have found no differences in either plaque size or collagen content of lesions. However, they demonstrate that the lesions found in STZ-Reversa animals have higher levels of macrophages despite not having any effect upon LDL levels. Studies to delineate effects upon macrophage influx or egress are underway. They have begun to look at macrophage-specific gene expression by laser capture microdissection. These studies take advantage of strengths within the PI's lab as well as Consortium resources. While difficult, laser dissection of other cell types may add insight into how/why macrophages are attracted to lesions.
During the past year we have demonstrated the lesional CD68+ cells from regressed diabetic mice have more inflammatory markers and less expression of markers associated with the alternatively activated (M2) phenotype. We are currently attempting to determine why this occurs.

We agree with the reviewers that studies of gene expression of other vascular cells during regression would be revealing. The cells that we are specifically interested in are the endothelial cells. However, because these cells are so thin, techniques to obtain RNA using LCM are limited. So, we have studied protein expression, e.g. of ICAM and VCAM, by immunohistology. As suggested by the AEC it might be useful to isolate these cells, perhaps by FACS of digested aorta and we will attempt this.

4. Publications:

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, 328:496-503, 2009

Ravichandran R, **IJ Goldberg**. Aldose reductase and cardiovascular diseases, creating human-like diabetic complications in an experimental model. Circ Res, in press.