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Studies and Results

The goal of this project is to test the hypothesis that TLR-mediated inflammatory signaling contributes to the pathogenesis of diabetic cardiac dysfunction. The Specific Aims are unchanged.

Aim 1: To probe the role TLR-mediated inflammation in the development and severity of metabolic cardiomyopathy using the transgenic MHC-ACS cardiac lipotoxicity model.

We have made significant progress on this aim during the funding period with regards to characterizing the inflammatory response in MHC-ACS mice. First, using quantitative real-time PCR (qRT-PCR) and western blotting we have defined the kinetics and composition of the inflammatory mediators that are expressed in MHC-ACS transgenic mice. By four weeks of age, prior to the onset of cardiomyopathy, we observed significant upregulation of the inflammatory cytokine interleukin(IL)-6 as well as osteopontin (OPN) and macrophage chemotactic protein (MCP)-2 in the hearts of MHC-ACS mice compared with littermate controls. Interestingly, upregulation of OPN expression has been associated with diabetic complications in other organ systems. Second, we have characterized the immune cell infiltrate that occurs in MHC-ACS mice. Given the significant upregulation of macrophage chemotractants in the MHC-ACS mice, we assessed macrophage recruitment to heart using MAC-3 immunohistochemistry. This analysis revealed a marked increased in the number of MAC-3^+ cells in the myocardium of transgenic mice compared to WT mice, even at a very early stage of disease. In a third approach, we developed a flow cytometry technique to phenotype leukocytes in single cell suspensions of dissociated hearts. We determined that macrophages in the hearts of MHC-ACS animals belong to a unique subtype that express CD11c ($CD11b^{+/}CD11c^+$ / $F4/80^+$). This finding is particularly provocative given that $CD11c^+$ macrophages have been shown to infiltrate adipose tissue in diet-induced obesity prior to the onset of insulin resistance. Thus, lipotoxic injury may promote signals that trigger the recruitment of this specific macrophage subset.

To address the role of TLR4 signaling in the recruitment and activation of macrophages in lipotoxic cardiomyopathy, we have begun crossing the MHC-ACS mouse with TLR4 KO animals. These two models are in the FVB and C57 backgrounds, respectively. We have now completed a backcross the TLR4 knockout allele into the FVB genetic background for eight generations. In the FVB background, we are now crossing the MHC-ACS and TLR4 KO animals, from which we anticipated having animals for analysis in the next 3-4 months. We have also crossed the MHC-ACS mice into the C57 background for 8 generations. These mice will now be bred with C57 TLR4 KO mice.

Future directions:

1. Once we have completed the breeding of ACS mice into the TLR4 knockout background, we will analyze cardiac function, inflammation, and macrophage recruitment. Based on these findings, bone marrow transplant experiments and/or additional genetic crosses with mice deficient in TLR4-signaling molecules or OPN will be conducted.

2. To elucidate the role of macrophages in lipotoxic cardiomyopathy we will inject MHC-ACS mice with liposomal clodronate (clodrolip) to deplete macrophages. The effect of macrophage depletion on cardiac dysfunction and inflammation will be assessed.

Aim 2: To define the contribution of TLR-mediated signaling pathways in diabetic cardiomyopathy.

To address this question we first induced diabetes using streptozotocin and a high fat diet in 3 different strains of mice beginning at 10 weeks of age (2 independent lines of C57BL/6 and FVB). Cardiac function was assessed by echocardiography at 2 and 3 months after the induction of diabetes.

Unfortunately, despite appropriate induction of diabetes and an increase in myocardial triglyceride, no significant changes in cardiac function, inflammation, or ER stress could be demonstrated in these mouse strains. It is possible that a more prolonged duration of diabetes or a secondary stress (such as ischemia or hypertension) may be necessary to unmask the cardiac dysfunction linked to diabetes. Alternatively, it may be necessary to move to a different strain or begin with more aged animals.

Future Directions:

1. Since previous studies have suggested that older mice are more susceptible to diabetes-induced end-organ dysfunction, we plan to induce diabetes in aged mice to determine if there is a more robust inflammatory response and heart failure.

2. We plan to obtain myocardial tissue samples from patients with diabetes and heart failure to look for stigmata of myocardial inflammation and macrophage recruitment. One analysis will use tissue from patients with end-stage cardiomyopathy who are undergoing left ventricular support device placement. Inflammatory gene expression and histology will be compared between diabetic and non- diabetic tissues. A second analysis will focus on diabetics undergoing CABG who have mild to moderate LV dysfunction. Again a similar analysis will be performed.

Significance

Cardiovascular disease is the leading cause of death in diabetics who suffer from aggressive atherosclerosis and cardiomyopathy. Inflammation is now recognized as a central feature of obesity, insulin resistance, and diabetes. This work probes the role of inflammatory pathways in the pathogenesis of diabetic cardiac dysfunction and has the potential to identify targets for development of novel therapeutics.

Publications

None as yet

Project generated resources

None as yet