Temple University

Annual Progress Report: 2009 Formula Grant

Reporting Period

January 1, 2010 – June 30, 2010

Formula Grant Overview

Temple University received $2,375,033 in formula funds for the grant award period January 1, 2010 through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

The Anabolic Role of Cannabinoid Receptors in Bone - The goal of the planned project is to define the anabolic role of cannabinoid receptors in bone. Recent studies have demonstrated the presence of endocannabinoids and their G protein-coupled cannabinoid receptors, CB1 and CB2, in the skeleton. Although it has become clear that this system is functional in bone, the precise mechanisms of action are only beginning to emerge. The planned studies will generate new information regarding the anabolic effects of cannabinoid receptors and mechanisms of action on osteoblast differentiation and function. Once we understand how cannabinoid receptors function to promote bone formation, this information will be helpful in developing new therapeutic strategies to selectively enhance bone formation in patients with clinically significant bone loss.

Anticipated Duration of Project

7/1/2010 – 6/30/2011

Project Overview

The goal of the project is to define the anabolic role of cannabinoid receptors in bone. Recent studies have demonstrated the presence of endocannabinoids and their G protein-coupled cannabinoid receptors CB1 and CB2 in the skeleton. Although it has become clear that this system is functional in bone, the precise mechanisms of action are only beginning to emerge. We have found that bone mass is significantly reduced in CB1/CB2 double receptor knockout (CB1/2 dKO) mice. To examine the functional significance of CB1 and CB2 receptors on osteoblast differentiation, we established primary osteoblast cultures utilizing newborn wild-type calvaria. Measurement of alkaline phosphatase (ALP) activity was assessed to evaluate the effects of specific CB receptor antagonists on osteoblast differentiation. Treatment with a CB1 antagonist or a CB2 antagonist caused a significant, dose-dependent inhibition of ALP activity. When cultures were treated with both CB receptor antagonists simultaneously, the inhibition of ALP activity was more pronounced compared to either antagonist alone. *Our results suggest that both CB1 and CB2 are functional receptors during osteoblast differentiation in vitro and in*

the regulation of bone mass in vivo. The observation that combined CB1 and CB2 receptor antagonism/deletion has a more dramatic effect compared to single receptor deletion warrants additional studies to evaluate intracellular signaling subsequent to ligand activation of CB1 and CB2 in osteoblasts.

In this project, we will characterize the mechanisms underlying this response by using a combination of pharmacological and genetic tools to identify the receptors involved. The effects of cannabinoid agonists, antagonists/inverse agonists and related compounds on osteoblast proliferation, apoptosis and differentiation will be compared in cultures prepared from WT, CB1 KO, CB2 KO and CB1/2 dKO mice. We will also identify the response of two key intracellular signaling pathways (ERK and Akt) to the most efficacious agents (using Western blot analysis and immunocytochemistry). Transcriptional activation of Osterix will be assessed by Runx2 activation of the Osterix gene promoter using CHIP assays. These experiments will provide insights into the role that the CB receptors play in regulating bone mass and into potential signaling mechanisms whereby CB receptors regulate osteoblast differentiation and function. Results from these studies could potentially be used to develop anabolic therapeutic agents for bone disease.

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Expected Research Outcomes and Benefits

It has recently been discovered that endocannabinoids and their G protein-coupled cannabinoid receptors type 1 (CB1) and type 2 (CB2) are present in the skeleton. In addition, polymorphisms in the CB2 gene are associated with osteoporosis. Although it has become clear that this system is functional in bone, the precise mechanisms of action are only beginning to emerge. We have found that both receptors are important for bone development in vivo and osteoblast differentiation in vitro; and furthermore, that there is a synergistic interaction between these two receptors. In this project, we expect to identify CB1- and CB2-mediated responses involved in osteoblast differentiation. Data from the studies will help explain the importance of signaling cascades from cannabinoid receptors (CB1 and CB2) during normal bone development and maintenance, as well as contribute to providing a mechanism for the CB1/2 double KO bone phenotype. The identification of cannabinoid compounds with an anabolic effect on bone could potentially be used to develop therapeutic strategies for treating various forms of osteopenia and

to accelerate bone formation in situations (e.g. fracture healing, distraction osteogenesis) where such intervention would have a favorable clinical outcome.

Summary of Research Completed

The start date has been delayed. There is no research to report.

Research Project 2: Project Title and Purpose

Effects of IL-19 on Endothelial Cell Activation and Angiogenesis - Cardiovascular disease is the number one killer of Americans. Vascular diseases such as atherosclerosis, and hypertension are inflammatory in nature. Very little has been reported on the potential protective effects of anti-inflammatory cytokines on development of vascular disease. We have novel preliminary data which shows that the naturally occurring anti-inflammatory compound, Interleukin-19 (IL-19) can have stimulatory effects on endothelial cells grown in culture. The purpose of this project is to determine if IL-19 can have pro-angiogenic effect in vivo.

Anticipated Duration of Project

7/1/2010 – 12/31/2013

Project Overview

Endothelial cell paracrine and autocrine activation can result in migration and proliferation, and is an essential component of multiple normal and pathophysiological processes including atherosclerosis, permeability, wound healing, and angiogenesis. Angiogenesis is the growth of new blood vessels and normally occurs in the process of healing wounds and restoring blood flow after injury or insult, and many inflammatory cytokines play an important role in angiogenesis. Although both inflammatory and anti-inflammatory cytokine expression are an essential part of wound healing, the role of anti-inflammatory interleukins in initiation and progression of angiogenesis are less clear. Many vascular diseases such as atherosclerosis and hypertension, are inflammatory in nature. Although a great deal of attention has been given to the role and effects of pro-inflammatory cytokines, very little has been reported on the potential protective effects of anti-inflammatory cytokines on the vascular response to injury, particularly with respect to direct effects on vascular smooth muscle cell (VSMC) pathophysiology. IL-19 is a newly described member of the IL-10 family of anti-inflammatory cytokines. We are the only laboratory to publish concerning the expression or presumed function of IL-19 in VSMC pathophysiology or a role in modulation of vascular diseases.

We have preliminary data which indicate that IL-19 is not present in normal endothelial cells (EC), but can be expressed in response to inflammation and injury. IL-19 expression is increased in neo vessels, and addition of IL-19 to cultured EC stimulates their growth and migration.

Despite this information NO studies on in vivo effects of IL-19 on development of angiogenesis have been reported. We have recently generated an IL-19 knock out mouse. We hypothesize that mice which lack IL-19 have reduced angiogenesis. In Aim 1 we will determine if there will be

decreased development of angiogenesis in IL-19 knock out mice subject to hind-limb ischemia. In Aim 2, we will determine if these mice have a reduced capacity to form microvessels toward matrigel plugs.

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Expected Research Outcomes and Benefits

Angiogenesis is the growth of new blood vessels and normally occurs in the process of healing wounds and restoring blood flow after injury. It is well known that many pro-inflammatory cytokines are pro-angiogenic. Very little is known about the effects of anti-inflammatory cytokines on the development of angiogenesis. IL-19 is a compound which occurs naturally in humans and functions to modify inflammatory cells and dampen the immune response. We have previously shown that IL-19 is induced in endothelial cells under inflammatory and pro-angiogenic conditions. Cultured EC in which IL-19 is added display pro-angiogenic effects, such as increased migration, proliferation, and tube formation. In this project, one outcome we expect is that mice, genetically deficient in IL-19, will have decreased angiogenesis. If these experiments are successful, then at least one major benefit of this research is that IL-19 could represent a new class of naturally occurring therapy to increase wound healing and improve outcome of ischemic vascular diseases.

Summary of Research Completed

The start date has been delayed. There is no research to report.

<u>Research Project 3: Project Title and Purpose</u>

A Targeted Drug Delivery System for Preventing Cardiac Remodeling after MI - Cardiac remodeling after myocardial infarction increases the stress on the surviving myocardium and predisposes viable myocardial cells to premature death leading to heart failure. Presence of an appropriate microvasculature is a prerequisite for preventing cardiac remodeling and improving cell survival resulting in maximal regeneration of a functional tissue after myocardial infarction. The goal of this project is to use targeted delivery of pro-angiogenic agents to enhance the morphology and function of post-infarct neovasculature, prior to scar formation, and to establish

the optimal time post-myocardial infarction when pro-angiogenic interventional strategies could prevent cardiac remodeling and congestive heart failure.

Anticipated Duration of Project

7/1/2010 – 6/30/2011

Project Overview

Myocardial infarction (MI) often leads to congestive heart failure (CHF) which is a leading cause of death in the United States and other industrialized countries. Cardiac remodeling after an MI increases the stress on the surviving myocardium and predisposes myocardial cells to premature death. Subsequent tissue repair does not involve a significant regeneration of the supporting microvasculature, which is a key component of the in vivo microenvironment necessary for survival of cells and the repair after injury and/or the engraftment of a functional engineered tissue. Recent data even suggest that the disappointing attempts at rebuilding the injured cardiac tissues using stem cells may be due to a lack of supporting microvasculature post-MI. However, concerns over possible side effects have hampered attempts at revascularizing the infarcted myocardium using systemic delivery of pro-angiogenic compounds such as *vascular endothelial growth factor* (VEGF) and/or *basic fibroblast growth factor* (bFGF). The goal of this project is to use targeted delivery of pro-angiogenic agents to enhance the morphology and function of post-infarct neovasculature, prior to scar formation, and to establish the optimal time post-myocardial infarction when pro-angiogenic interventional strategies could prevent cardiac remodeling and CHF. We hypothesize that targeted delivery of pro-angiogenic compounds after MI will result in the formation of new vessels in the immediate microenvironment, preventing or moderating cardiac remodeling. The specific aims of this project, using a rat model of MI, are to: 1) Develop immunoliposomes containing pro-angiogenic compounds and determine the mechanisms by which these drug carriers are incorporated into activated endothelium; and 2) In an infarct model in vivo, selectively deliver pro-angiogenic compounds to the infarct region using immunoliposomes, and determine if this targeted delivery approach results in significant improvements in cardiac morphology and function and prevention or moderation of cardiac remodeling. We will also study the efficacy of combining targeted delivery of pro-angiogenic compounds with stem cell therapy to determine if this combinational therapy can significantly improve vascularity, perfusion, and cardiac function when compared to targeted pro-angiogenic therapy alone. Development of this powerful technology will not only significantly prevent cardiac remodeling but will also improve survival and engraftment of engineered tissue.

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Expected Research Outcomes and Benefits

Expected research outcomes of the project are:

1) Development of immunoliposomes containing pro-angiogenic compounds and determination of the mechanisms by which these drug carriers are incorporated into activated endothelium.
2) Development of a system for selective delivery of pro-angiogenic compounds to the infarct region using immunoliposomes; and a methodology for significantly improving cardiac morphology and function, and preventing or moderating cardiac remodeling.

Pro-angiogenic therapy, especially if administered systemically, has many observed and potential side effects including unintended angiogenesis in distant tissue, increased microvascular permeability, development of new or enhanced growth of existing neoplasms, and increased atherosclerotic plaque mass and instability, among others. However, many of these side effects are circumvented through a targeted drug delivery approach in which the drugs/molecules/genes can be preferentially targeted to the infarct region via the upregulation of various adhesion molecules in the infarct region. Our study will use adjunctive targeted delivery of VEGF and/or bFGF to prevent cardiac remodeling and augment regeneration and myocardial angiogenesis after stem cell implantation.

Summary of Research Completed

There is no research to report.

Research Project 4: Project Title and Purpose

Mechanism(s) of Vascular Damage in Obesity - Cardiovascular disease accounts for much of the morbidity and mortality ultimately suffered by patients with diabetes mellitus. Adiponectin is a protein from fat cells that circulates in the bloodstream and has been shown to protect the blood vessels in diabetes. Interestingly, adiponectin opposes the adverse effects on blood vessels of several factors including glucose that is known to be elevated in Type 1 and/or Type 2 diabetes. In this project, we seek to identify the cellular mechanisms by which adiponectin exerts its salutary effects in the endothelial cells that line the blood vessels. These results will advance our understanding of the role of adiponectin in vascular protection in diabetes and in obesity with insulin resistance, conditions with a high risk of life-threatening cardiovascular complications.

Anticipated Duration of Project

7/1/2010 – 12/31/2010

Project Overview

The goal of this project is to define integrative mechanisms by which adiponectin preserves endothelial function in obesity-associated diabetes. The PI reported in the *JCI* in 2007 that adiponectin deficient mice exhibit a distinct inflammatory phenotype in both micro- and macrovascular beds with endothelial dysfunction characterized by impaired endothelial nitric oxide (eNO) generation and increased leukocyte-endothelium interactions due to increased expression of the endothelial cell adhesion molecules (eCAMs), E-selectin and VCAM-1. This inflammatory phenotype was reversed by Ad-replenishing therapy, which was shown to act via enhancing eNO bioavailability, a well-known regulator of eCAMs expression. In metabolic disorders such as obesity and diabetes, loss of physiological eNO levels is in part attributed to eNOS uncoupling by reactive oxygen species (ROS) and it is linked to endothelial dysfunction with inflammatory leukocyte-endothelium interactions. Very recent studies have also suggested that adiponectin reduces accumulation of myeloperoxidase (MPO) in inflamed tissues. MPO is a powerful oxidant released by activated leukocytes that is able to bind to the vascular endothelium where it drastically reduces eNO bioavailability by catalytic consumption. W*e hypothesize that the action of adiponectin to enhance eNO bioavailability and attenuate leukocyte-endothelium interaction in the vasculature largely occurs both via direct suppression of endothelial ROS generation and prevention of leukocyte-derived MPO binding to the endothelium, two novel integrated mechanisms of the vascular protective effects of adiponectin in obesity and diabetes*. Using a genetic mouse model of obesity-associated type II diabetes along with selected knockout mouse technology this project will test the following hypotheses according to these Specific Aims: *1)* To study the effect of adiponectin on eNO/ROS balance and endothelial function *in vivo*; *2)* To study the role of leukocyte-derived MPO in the endothelial protective action of adiponectin.

Summary: The results of these studies will provide important information on the intercellular mechanisms of the anti-inflammatory action of adiponectin in Type II diabetes. They will also provide new insight into the integrative mechanisms by which the endothelium and circulating leukocytes synergize to increase oxidative stress in the obese, diabetic vascular endothelium.

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Expected Research Outcomes and Benefits

We expect to uncover the mechanisms by which adiponectin protects vascular function. In particular we anticipate finding that reduction of oxidative stress in the mitochondria is one of the key mechanism of the vascular protective action of adiponectin. We will also define the mechanisms by which the various circulating forms of adiponectin exert vasculoprotective effects, with a focus on endothelial cell function *in vivo* using mouse models as well as cultured endothelial cells *in vitro*. Overall, this project will uncover novel mechanisms of vascular damage in obesity and diabetes and it will provide a framework for developing new therapeutic strategies to avert vascular and organ damage in obese, insulin resistant humans.

The developed world is currently experiencing an epidemic of cardiovascular disease that is caused by an ever-increasing prevalence of obesity with insulin resistance due to excess caloric intake and sedentary lifestyles. A key contributor to this increased vascular risk is endothelial dysfunction, a major component of the initiation of the complex process of atherogenesis. By studying the effects of adiponectin isoforms in vascular endothelial cells, we will contribute to our understanding of the mechanisms by which adiponectin exerts its salutary effects on vascular impairment induced by several major agonists that accompany obesity and diabetes. A greater understanding of these signaling pathways may ultimately lead to new targets for the prevention or treatment of the early vascular injury that accompanies obesity and diabetes.

Summary of Research Completed

The start date has been delayed. There is no research to report.