Francis Alenghat, M.D., Ph.D. – 2019 Awardee
University of Chicago

"Serum Amyloid A Inhibition of Macrophage Lipoprotein Lipase and its Impact on Synthesis of Specialized Pro-resolving Mediators"

Serum amyloid A (SAA) is a circulating acute-phase reactant that can rise ~1000-fold within hours of tissue injury or infection but is also elevated in chronic diseases with inflammatory components like coronary heart disease (CHD), and SAA is a strong independent predictor of CHD and future cardiovascular events. SAA's role has remained elusive—initially described in association with high density lipoprotein (HDL), SAA is also abundant in triglyceride-rich lipoproteins (TRLs), and its appearance in circulation during inflammatory responses coincides with a rise in plasma triglyceride levels. This hypertriglyceridemia is caused in part by inhibition of muscle and adipose lipoprotein lipase (LPL), but during macrophage polarization, LPL is also highly expressed on macrophages with regulatory phenotypes but not on inflammatory macrophages, likely due to the prominent role free fatty acids (FFA) play in regulatory macrophage physiology. We have recently discovered that SAA is an inhibitor of LPL activity. Therefore we suspect SAA impacts regulatory macrophage function by inhibiting LPL and limiting the supply of FFA required for energy production and biosynthesis of specialized inflammation-resolving second messengers. This mechanism is consistent with recent studies on ANGPTL3, ANGPTL4, and APOC3, all of which modulate LPL activity, showing that variants leading to increased LPL activity reduce cardiovascular events, and vice versa. All are now potential therapeutic targets, and defining SAA's impact on LPL would point to more.

Preliminary Data: By investigating LPL regulation using both the pure enzyme and live macrophages, we have discovered that SAA is a strong inhibitor of LPL activity. In parallel, we specialize in mechanisms of macrophage polarization and have shown that regulatory macrophages have high levels of LPL activity that can be inhibited by SAA.

Working Hypothesis: We hypothesize that TRLs enriched with SAA bind to macrophage LPL, allowing SAA to occupy a regulatory site and competitively inhibiting its enzymatic activity and preventing hydrolysis of TRLs. Furthermore, we hypothesize that LPL inhibition by SAA would support a pro-inflammatory phenotype while impairing the anti-inflammatory activities of LPL-expressing regulatory macrophages by blocking the supply of FFA precursors for the biosynthesis of specialized pro-resolving mediators (SPMs).

Objectives of this proposal: We aim to establish a physiological role for SAA in reducing TRL turnover rates through LPL inhibition which in turn blunts anti-inflammatory macrophage function important for preventing CHD. We wish to pursue two specific aims in parallel, the first focusing on SAA's influence on macrophage LPL and polarization, and the second focusing on the impact of LPL inhibition and SAA on regulatory SPM production.

Frank Brozovich, M.D., Ph.D. – 2019 Awardee
Mayo Clinic

"The Vascular Phenotype and Novel Targets for Heart Failure with Perserved Ejection Fraction (HFpEF)"

Heart failure affects ~5 million Americans and is classified as with reduced (HFrEF) or preserved ejection fraction (HFpEF). Despite the benefit of therapies for HFrEF, none benefit HFpEF. HFpEF represent 50% of patients with HF and has the same mortality as HFrEF. Thus, the pathophysiology of HFpEF requires further investigation to define novel targets for rational drug design to improve morbidity and mortality. Patients with HFpEF have hypertension and other comorbidities, including aging, obesity and diabetes, which are associated with an increase in TNFalpha. It has been hypothesized that when hypertension coexists with an inflammatory process, the milieu leads to the development of HFpEF, but the mechanism that produces HFpEF is unknown.

TNFalpha increases cytosolic Ca2+ by enhancing endoplasmic reticulum (ER) Ca2+ release, and in inflammatory diseases, an accumulation of unfolded proteins triggers an ER stress response, including phosphorylation of inositol-requiring enzyme 1 (IRE1) and alternative mRNA splicing of X-box protein 1 (XBP1), which is associated with an decrease in the expression of Mfn2, a downstream target of the pIRE1α-XBP1s ER stress pathway. Mfn2 tethers the mitochondria to the ER, which establishes the microdomain of higher cytosolic [Ca2+] necessary to activate the mitochondrial Ca2+ uniporter. Thus, mitochondrial Ca2+ influx, which is important for regulating mitochondrial function, depends on the proximity of mitochondria and ER, which is regulated by Mfn2-dependent ER-mitochondria tethering. Thus, an ER stress response will decrease ATP production by the mitochondria and results in an energy imbalance within cells.

HFpEF is also associated with abnormal contractility of both the myocardium and vasculature. In patients with HFpEF, we have demonstrated that the expression of the LZ+ isoform of MYPT1 is decreased. Importantly, LZ+ MYPT1 is a contractile protein, and in the vasculature, LZ+ MYPT1 expression determines both the sensitivity to NO mediated vasodilation and regulates vasoconstriction, both of which are abnormal in HFpEF. Further, TNFalpha has been demonstrated to alter Ca2+ signaling and contractile protein expression in both cardiac and smooth muscle, which suggest that the increase in TNFalpha associated with HFpEF could produce the contractile abnormalities in HFpEF.

We hypothesize that the increase in TNFalpha associated with the HFpEF, produces 1) an ER stress response, which alters myocardial energetics and 2) alters the contractility of both the myocardium and vasculature. We will use both swine and murine models of HFpEF to test this hypothesis and determine 1) if TNFalpha-induced ER stress (IRE1 phosphorylation and XBP1 mRNA splicing) reduces Mfn2 expression and decreases ATP production in vascular smooth and cardiac muscle, and 2) determine if the contractile abnormalities of cardiac and vascular smooth muscle are due to changes in Ca2+ signaling, Ca2+ sensitivity or changes in the expression or phosphorylation of the contractile proteins. Our results will define the molecular mechanism for the pathophysiology of HFpEF, identify new targets for rational drug design and lead to the development of novel treatment paradigms for HFpEF.

Junko (Junco) Warren, Ph.D. – 2018 Awardee
University of Utah

"Novel Approach of Mitochondrial Therapy in Heart Failure"

One fundamental cause of circulatory failure is the inability of the heart to efficiently pump blood throughout the body, with heart failure being a widespread cardiac disease leading to this condition. The prevalence of heart failure continues to increase with high rates of morbidity and mortality in developed countries. Despite the advances in heart failure therapies, 50% of patients still die within 5 years of diagnosis. Accumulating evidence from animal studies and clinical trials suggests that correcting cardiac metabolism may significantly alleviate cardiac dysfunction in the setting of heart failure. In the past years, several therapies targeting mitochondria have emerged with promising results in preclinical models. However, the development of drugs is challenging, mainly due to the lack of specific mitochondrial targets.

The heart undergoes metabolic remodeling during the development of heart failure, concurrent with global expression changes in genes involved in cardiac energetics. However, it remains unclear what commands this orchestra of metabolic remodeling in the heart. Our recent study using systems proteomics and metabolomics identified Smyd1, a muscle-specific histone methyltransferase, as a novel upstream regulator of mitochondrial energetics in the heart. We demonstrated that Smyd1 enhances cardiac energetics through transcriptional activation of PGC-1alpha, a key mitochondrial regulator.

However, a previous study showed that over-expression of PGC-1alpha could not rescue mitochondrial impairment and failed to prevent the progression of heart failure, suggesting that restoring only PGC-1alpha expression is not sufficient to maintain mitochondrial energetics under hemodynamic stress. Thus, to apply our findings for mitochondria targeted therapy in heart failure, it is crucial to delineate the mechanisms by Smyd1 regulates cardiac energetics. Our RNA-seq analysis of cardiomyocytes identified Perm1 as a potential downstream target of Smyd1. Perm1 is a novel muscle-specific regulator that enhances mitochondrial oxidative capacity and fatigue resistance in skeletal muscle. Thus, it is plausible that Perm1 is a critical player in cardiac energetics as part of the Smyd1/PGC-1alpha signaling pathway.

Our preliminary data shows that both Perm1 and PGC-1alpha were down-regulated in patients with heart failure as compared to donor hearts. Consistently, Perm1 and PGC-1alpha were down-regulated in the mouse failing heart. We also obtained the preliminary data showing that knockdown of either Perm1 or PGC-1alpha in cardiomyocytes led to reduced mitochondrial respiration capacity. These data suggest that simultaneous expression of Perm1 and PGC-1alpha is essential for mitochondrial energetics in the heart.

The overall hypothesis of this project is that normalizing both Perm1 and PGC-1alpha expression via Smyd1 is a novel approach of mitochondrial therapy in heart failure. The aims of this grant are to: 1) Delineate the Smyd1 signaling mechanisms that control mitochondrial energy production in cardiac muscle, and 2) Determine if over-expression of Smyd1 protects against mitochondrial dysfunction and the progression of heart failure under pressure overload through maintaining the expression levels of both Perm1 and PGC-1alpha. Success completion of this project may identify a new therapeutic target, relevant to the development of innovative strategies for the prevention of metabolic perturbations in heart failure.

Andreas Koenig, Ph.D. – 2018 Awardee
SUNY Upstate Medical University
(Originally at University of Vermont)

"Genomic Variation in the Lipid Anchor Biosynthesis Protein PIGC as Cardiovascular Risk Factor"

ORIGINAL PROJECT DESCRIPTION FROM APPLICATION

Lipopolysaccharides (LPS) are recognized by CD14, a surface receptor mainly expressed in monocytes and macrophages. CD14 exists in membrane-anchored (mCD14) and soluble (sCD14) forms. Increased plasma levels of sCD14 correlate with the differentiation of monocytes into pro-inflammatory macrophages, inflammatory disease activity, and risk of cardiovascular disease (CVD).

Atherosclerosis is a chronic inflammatory disease of the arterial wall. Chronic infections from Gram-negative bacteria, and the innate immune response that these bacteria provoke, may significantly contribute to inflammation that is characteristic of atherosclerosis. Although bacterial LPS are known to be pro-atherogenic, the exact cellular mechanisms and genes that accelerate plaque formation and the inflammation associated with bacterial infection are not known. It is not known whether the innate immune pathways that recognize bacteria can be deregulated in the absence of bacterial infection in atherosclerosis. Additionally, it is neither known whether monocytes are a source of increased sCD14 in the absence of infection, nor is the mechanism clear that generates high levels of sCD14 in certain individuals.

In this application we propose that a naturally occurring SNP in the PIGC gene determines the relative ratio of mCD14 to sCD14 in the absence of bacterial infection. We hypothesize that the P266S PIGC variant will result in an increased release of sCD14 from monocytes. sCD14 from monocytes acts then as an acute phase molecule and will correlate with an increased differentiation of monocytes into proinflammatory macrophages and foam cells. This hypothesis will be tested by 1) Determining the association between the P266S PIGC variant and CD14 expression, localization, and function in human monocytes, and 2) Determining the effect of the P266S PIGC variant on monocyte differentiation.

We anticipate that variations in the PIGC gene determine the plasma level of sCD14 in the absence of bacterial infection, and that these variations are associated with risk factors, such as a high waist-to-hip ratio (WHR), in patients with atherosclerosis. In the future we aim to develop novel assays to stratify patients by designer sCD14-targeted therapeutic attenuation of innate immune activation as a way to lower sCD14 levels and improve patient survival.

Padmini Sirish, Ph.D. – 2017 Awardee
University of California, Davis

"Improving Cell-Based Therapy For Coronary Heart Diseases"

ORIGINAL PROJECT DESCRIPTION FROM APPLICATION

Coronary Heart Disease remains the leading cause of morbidity and mortality in the US. After myocardial infarction (MI) due to coronary artery blockage, significant loss of cardiomyocytes results in a decrease in cardiac function, progressively leading to heart failure with lethal consequences. Stem cell-based therapy represents a new promising approach for the treatment of CHD since it aims at generating new functional myocardium and inducing neoangiogenesis. Stem cells possess the capacity for sustained proliferation, cardiac differentiation as well as trophic functions which enables myocardial repair. However, therapeutic strategies using cell-based therapy to combat ischemic cardiomyopathy have not produced full restorative functions. One of the main challenges of cardiac stem cell therapy is the survival and retention of transplanted cells in the hostile milieu. A high rate of transplanted stem-cell loss, ~ 40% within the first hour and ~90% within the first few days has been observed due to ischemic environment and inflammation. One of the prime causes of cell death is the acute and robust inflammatory response mediators. Another critical concern of cell-based therapies is the manifestation of post-cell therapy arrhythmias.

Here we propose a new therapeutic and potential clinical paradigm by using novel anti-inflammatory and anti-arrhythmic drug to improve the outcome of cardiac stem cell therapy. The key therapeutic target is the inhibitor of the enzyme soluble epoxide hydrolase (sEH). sEH converts the cytochrome P450 epoxygenase products, epoxyeicosatrienoic acids (EETs), which are major anti-inflammatory metabolites to corresponding diols, with diminished anti-inflammatory activities. Hence, we propose to use novel inhibitor of sEH (sEHI) to prevent the catalysis of EETs, thereby enhancing the cardioprotective activity of EETs.

Our central hypothesis is that novel sEH inhibitor with high potency and efficacy can be used as an anti-inflammatory and anti-arrhythmic drug in cardiac cell-based therapy to: i) increase the survival and retention of transplanted human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and ii) decrease the occurrence of post-cell therapy arrhythmias. The important innovations is this proposal are: 1) the use of orally active drug with no known side effects and no narcotic properties as both anti-inflammatory and anti-arrhythmic to enhance cardiac stem cell therapy, 2) to use in-house state-of-the-art techniques of metabolomics and ex vivo cardiac optical mapping to access drug-target engagement and cardiac structural and electrical remodeling respectively, and 3) to study the effects of cardiac cell-based therapy in a rabbit coronary injury model that can serve as a proof-of-concept study for future human studies.

This proposal tackles two critical setbacks from inflammation and arrhythmias that severely impede the success of cardiac cell-based therapies and the successful completion will pave way for future studies readily translatable to the clinic for precision medicine.

Scott Gordon, Ph.D. – 2018 Awardee
University of Kentucky

"Protease Activity in Atherosclerotic Lesions and Prevention by HDL-Targeting Protease Inhibitors"

ORIGINAL PROJECT DESCRIPTION FROM APPLICATION

Atherosclerosis is the most common cause of coronary artery disease (CAD) and the associated clinical symptoms that result from reduced blood flow to the heart. Inflammation within atherosclerotic plaques causes them to progress over time and become unstable lesions that are prone to thrombus (clot) formation. When a clot breaks free from the lesion and blocks blood flow to the heart, an acute cardiovascular event (e.g. heart attack) can occur. The inflammation in the artery wall that leads to lesion instability is promoted by protease activity. Proteases in the artery wall can degrade elastic connective tissue and activate signaling pathways that promote inflammation. Recent evidence indicates that high density lipoprotein (HDL) particles in the blood can carry protease inhibitor proteins. Previous studies have shown that HDL can be actively transported across the vascular endothelium. However, it is currently unknown if HDL can co-transport its protease inhibitor cargo to the atherosclerotic lesion. The global hypothesis of the proposed work is that HDL transports its protease inhibitor cargo across the vascular endothelium where suppression of proteolytic activity will prevent atherosclerosis progression. Additionally, we have developed HDL-targeting protease inhibitor peptides as a novel approach to reducing proteolytic activity in atherosclerotic lesions.

The aims utilize both in vitro and in vivo methods to address an important mechanistic question about inflammatory regulation in atherosclerosis and investigate a novel approach for suppression of this activity. The HDL-targeting protease inhibitor peptides bind to HDL when injected into the blood and confer protease inhibitor activity. In this proposal, these will be evaluated for their ability to prevent atherosclerosis in a mouse model. Successful completion of these studies could provide justification for advancement along the preclinical pipeline. In humans, it is anticipated that the action of these compounds would stabilize existing atherosclerotic lesions by maintaining the integrity of the endothelium and, therefore, prevent rupture and thrombus formation. The ultimate impact of this could be a reduction in cardiovascular events for high-risk patients.

Coeli Lopes, Ph.D. - 2016 Awardee
University of Rochester School of Medicine and Dentistry

"Effect of Statin therapy in combination with KCNH2 targeting drugs: identifying cardiac arrhythmia risk in coronary artery disease patients."

Statins are among the most commonly prescribed drug classes for prevention and treatment of coronary artery disease and their use is expected to increase due to recent changes in therapy guidelines. Statins are well known to reduce cardiovascular events and mortality in patients with coronary artery disease. Nonetheless, the effect of this drug class on cardiac electrophysiology, in particular for non heart-failure patients, has been incompletely studied. Indeed, some reports show statins decreasing repolarizing potassium channel currents in the heart, an effect expected to prolong QTc and increase the risk for arrhythmias. Our preliminary clinical data in Long QT syndrome patients suggest that statins may be detrimental to patients with mutations in the KCNH2 gene, increasing their cardiac risk, but may be protective to Long QT patients with mutations in other genes. The KCNH2 gene is the most common target for QT prolonging drugs. Our data indicates that statins inhibit KCNH2 channel trafficking to the plasma membrane and exacerbates the effect of drugs that block the channel. Thus, we propose that statins may serve as a pro-arrhythmic modulator of KCNH2-targeting drugs. The main goal of this project is to understand the molecular mechanism of action of statins in combination with commonly prescribed KCNH2-targetting drugs by studying the effect of statins at the cellular level. The ultimate goal of this project is to tailor statin therapy to the patient genetic profile and avoid detrimental drug-interactions. For that, we will first test the hypothesis that statins can increase the pro-arrhythmic effects of KCNH2 common genetic variants present in the general population and associated with increased risk of drug-induced arrhythmias. Second, we will investigate the molecular mechanism of channel regulation by statins by testing the hypothesis that statins increase channel ER-retention via the hERG chaperone, Hsp90. Finally, we will test the hypothesis that statins exacerbate the pro-arrhythmic effects of commonly used cardiovascular drugs, including anti-hypertensive, anti-arrhythmic and anti-anginal drugs, known to inhibit hERG function. Understanding the molecular effect of statins will allow tailoring of therapy to patients who would benefit the most and to avoid drug combinations or particular patient population for which statins may be harmful.

Javier Lopez, M.D. - 2016 Awardee
University of California, Davis School of Medicine

"Matching Stem Cell-derived Myocytes to Individual Patients' Heart Tissues: Personalizing Future Regenerative Treatments for Ischemic Heart Disease"

In the last decade, access to early revascularization, cardioverter-defibrillators and medications have lowered mortality rates from heart attacks. However, available interventions do not adequately address the myocardial insufficiency that stems from a lack of cardiac regeneration after infarction. In the future, patients suffering from progressive heart failure after a heart attack may instead have their myocardial insufficiency treated with regenerative strategies to recover the function of their native hearts.

The goal of this proposal is to develop, as a proof-of-concept, a novel in vitro 3D culture system of human tissue scaffolds that will allow future testing of new human derived myocytes for cardiac regenerative therapies. We propose that our new approach will help personalize the selection of human cell graft(s) from pluripotent stem cells to individual patients suffering of heart failure after a myocardial infarction. At the core of individualizing these therapies lies the testing of candidate cell grafts onto a patient's own cardiac tissue in the laboratory and before clinical use.

We hypothesize that the make-up of cardiac native tissues from patients and the size of implanted myocytes are critical determinants for the successful translation into the clinic of regenerative therapies. This project will apply a unique tissue-engineering approach developed at UC Davis to obtain extracellular tissue matrices from patient's own hearts. These matrices will serve as 3-dimensional scaffolds for testing the engraftment propensity of potential candidate cell grafts onto a patient's own heart. The key innovations of this proposal are: (1) the implementation of a tissue antigen-retrieval paradigm to generate cardiac tissue scaffolds that preserves the biophysical growth signals of an individual patient in vitro; 2) the testing of new small and mature human myocytes derived in Dr. Lopez's lab from pluripotent stem cells; and (3) a detail per-cell analysis of myocyte maturation in 3D cultures. This proposal aims to establish the early benchmarks of a future 3D culture system in a rat model first, and then, for the first time, using human tissues for future clinical translation.

Aim 1. To optimize 3D culture conditions for human myocytes derived from hiPSCs (hiPSC-CMs) grown over our unique adult rat cardiac 3D scaffolds (AR-ECM).

Aim 2. To produce human cardiac 3D scaffolds (AR-ECM) from surgical specimens and test their ability to engraft optimized human iPSC-CMs.

Annabelle Rodriguez Oquendo, M.D. - 2015 Awardee
University of Connecticut Health Center

"Immune Checkpoint Inhibition and Atherosclerosis"

Deficiency of the scavenger receptor class B type I (SR-BI) protein in mice is associated with significantly increased plasma HDL-C levels and paradoxically accelerated atherosclerosis. We were the first to report associations with these same phenotypes in humans with SR-BI gene (SCARB1) single nucleotide polymorphisms (SNPs). Among these SNPs, rs10846744, a noncoding SNP (G>C) within intron 1 located at a regulatory site, was significantly associated with increased subclinical atherosclerosis (SCA) and increased odds for cardiovascular disease (CVD) in Multi-Ethnic Study of Atherosclerosis (MESA) participants. Inclusion of traditional CVD risk factors in a multivariate regression model did not attenuate the association of rs10846744 with CVD, strongly suggesting that other pathway(s), including an inflammatory one, likely drive this important clinical association.

We used RNA-Seq to examine transcriptional differences in gene expression between lymphocytes isolated from hyperalphalipoproteinemic (HALP) carriers homozygous for the SCARB1 rs10846744 reference (GG) allele with carriers homozygous for the risk (CC) allele. We identified lymphocyte activation gene-3 (LAG-3), a gene that encodes the immune checkpoint inhibitor LAG-3 protein, and binds major histocompatibility complex II to suppress expansion of T effector cells, as a strong candidate in the SCARB1 rs10846744 CVD risk pathway. Additionally, carriers of the LAG-3 missense rs870849 SNP had significantly lower plasma LAG-3 protein levels and increased SCA. The hypothesis to be tested in this application is that LAG-3 protein deficiency exerts a major influence in the risk for SCA and CVD events. The two aims will be: (1) To determine LAG-3 protein as an independent predictor of SCA and CVD risk in MESA, and (2) To determine the mechanism of LAG-3 protein on HDL-cholesterol efflux in macrophages. The long term objective of this project is to unravel the novel intersection of the SCARBI rs10846744 variant and the immune checkpoint inhibitor LAG-3 protein in CVD risk.

William Wagner, Ph.D. - 2015 Awardee
Wake Forest University Health Sciences

"Biofabrication of a Novel Elastomeric Substitute for Autologous Veins in Coronary Artery Bypass Surgery"

Coronary artery disease is the main cause of death in the western world and is due primarily to atherosclerosis and thrombosis. Coronary artery bypass surgery utilizes autologous vein grafts; however due to compliance mismatch and intimal hyperplasia, the grafts typically fail due to atherosclerosis and thrombosis. We have synthesized and developed PFC, a novel viscoelastic composite polymeric material composed of poly (glycerol-sebacate), highly tensile silk fibroin, and Type I collagen. PFC was shown to have promising properties for use as a blood contacting material due to its non-thrombogenicity, tensile properties (2.3-5 Mpa elastic modulus), degradation (0.01% per week over 27 weeks), and cellular adhesion. This proposal details studies to develop PFC into a small diameter coronary artery vascular graft. The aims of the proposal include producing a PFC conduit by electrospinning and conducting in vitro mechanical property tests; fine tuning the nanofibrous conduit with extracellular matrix from cell sources; evaluating cell growth and performance on the conduit; and evaluating the performance of the conduit with and without arterial wall type cells through studies in a bioreactor. Completing these results should provide us with data to demonstrate the conduit facilitates cell growth, the cells function on the conduit, and the conduit is not subject to thrombosis and can service as a substitute for autologous veins in coronary artery bypass surgeries. These studies should demonstrate feasibility for future studies in an animal coronary artery bypass graft model.

Annarita Di Lorenzo, Ph.D. - 2014 Awardee
Weill Medical College of Cornell University

"Endothelial-derived Sphingolipids in Coronary Atherosclerosis"

The overall goal of this proposal is to explore novel ways of targeting endothelial cells to impact vascular inflammation in the pathogenesis of coronary artery disease (CAD) and heart failure. Despite multiple evidences linking the alteration of sphingolipid signaling to vascular inflammation, critical event in the development of cardiovascular diseases, specific molecular mechanisms are poorly understood.

The project focuses on our recent discovery that endothelial sphingolipid synthesis, and in particular sphingosine-1 phosphate (S1P) is negatively regulated by Nogo-B, a membrane protein of the endoplasmic reticulum. Our findings evidence a critical role of local endothelial sphingolipid production to regulate endothelial barrier functions in myocardial inflammation and reveal a novel regulation of endothelial sphingolipid synthesis by Nogo-B.

Thus, we hypothesize that Nogo-B governs the production of local sphingolipids to impact vascular inflammation and the development of CAD during pressure overload and hypercholesterolemia. As corollary to this hypothesis, we predict that modulating local sphingolipid synthesis to enhance homeostatic S1P signaling will protect the heart from CAD and failure.

To test this hypothesis we are proposing the following three specific aims:
1) To examine the role of endothelial specific Nogo-B in the pathogenesis of CAD by conditional deletion of Nogo-B in endothelial cells on Apo-E-/- background.
2) To correlate the expression and localization of Nogo-B with vascular inflammation in coronary plaque formation at different time point post-transverse aortic constriction (TAC).

Collectively, these studies will define the role of endothelial-derived sphingolipids, particularly S1P, in the pathogenesis of CAD and heart failure. Finally, the results of these proposed investigations may provide the foundation for novel approaches towards the treatment of CAD, in which vascular dysfunction and inflammation leads to or exacerbates this pathological state.

Timothy Fitzgibbons, M.D., Ph.D. - 2014 Awardee
University of Massachusetts Medical School

"Rationale and Discovery of A New Class of Anti-Atherosclerotic Compounds; Ligands for the Orphan Nuclear Receptor Nurr77/NR4A1"

Coronary artery disease (CAD) has surpassed infectious disease as the leading cause of death worldwide and it is imperative that we discover new cures. For at least 30 years CAD has been recognized as an inflammatory disorder. Arterial cholesterol deposition stimulates foam cell formation, growth of atherosclerotic plaque, and ultimately plaque rupture. Although traditionally considered detrimental, specific components of immunity are beneficial. For example, a recently described "intermediate class" of monocytes, are anti-inflammatory and protect the endothelium. We have found increased expression of genes required for the development of intermediate monocytes in the peri-coronary fat of patients without CAD. In mouse models, deletion of one of these genes (orphan nuclear hormone receptor, Nr4a1), results in progression of CAD. Our central hypothesis is that expression of Nr4a1 in macrophages is protective against CAD. We propose three specific aims to study this hypothesis.

First, we will create a transgenic mouse that overexpresses Nr4a1 in macrophages, and then cross transgenic mice with Apolipoprotein E (EKO) mice to determine if Nr4a1 overexpression protects against CAD. Second, using Nr4a1+/+ or Nr4a1-/- bone marrow transplants in EKO mice, we will test the hypothesis that the non-specific Nr4a1 ligand azathioprine prevents CAD, and whether or not this protection is dependent upon macrophage Nr4a1. Finally, in collaboration with the Broad Institute, we will perform a high-throughput screen for ligands of Nr4a1 in a human monocyte cell line (THP-1).

In summary, this is an innovative and ambitious proposal that will confirm the importance of Nr4a1 as a therapeutic target, determine the cellular hierarchy of Nr4a1 activity, and discover a heretofore un-described ligand for this receptor. Completion of this project may result in an entirely new class of medications to prevent disability and death from CAD by promoting activity of vasculo-protective monocytes.

Zahi Fayad, Ph.D. - 2013 Awardee
Icahn School of Medicine at Mount Sinai

"Nanoparticle Formulations of Cannabinoids to Treat Coronary Artery Disease"

Cardiovascular disease is the leading cause of morbidity and mortality in developed nations with an enormous societal and economic burden. Since the development of novel treatment strategies for atherosclerosis has stagnated in recent years there exists an urgent need to explore new and potent therapeutic approaches.

A major driving force in the progression to vulnerable plaques is a macrophage-driven maladaptive inflammatory response characterized by a defect in the resolution of inflammation (RI) phase. Cannabinoids are a class of hydrophobic compounds that can activate either the cannabinoid 1 (CB1) or CB2 receptor, or both. The main and most thoroughly investigated cannabinoid is tetrahydrocannabinol (THC). THC has been shown to exhibit interesting anti-inflammatory actions, while similar anti-inflammatory properties have been found for cannabidiol (CBD). Despite their enormous potential as an anti-inflammatory treatment their exploitation is hampered by poor bioavailability, water insolubility and, most notably, their psychotropic properties. Importantly, the latter and the widespread use of cannabinoids for (illegal) recreational use causes their medical application to be approached with great skepticism. Therefore, developing formulations with known and titratable amounts of cannabinoids that can improve bioavailability and reduce psychoactive effects may have a significant impact on their therapeutic and clinical potential as well as their acceptance as therapeutics.

The exploitation of nanotherapies in cardiovascular disease has been largely unexplored, but may have unprecedented benefits as they have the potential to help increase the efficacy of drugs with significantly reduced adverse side effects.

The research proposed in the application is aimed at improving the anti-inflammatory properties and specificity of cannabinoids for atherosclerotic plaques. We propose 1) a new therapeutic (and potential clinical) paradigm by developing nanoparticle formulations of cannabinoids and 2) applying and evaluating these in preclinical atherosclerosis. This application is highly innovative and is designed such that it facilitates clinical translation.

Margaret Doyle, Ph.D. - 2010 Awardee
University of Vermont

"The Role of Adaptive Immunity in Atherosclerosis and Coronary Disease"

Atherosclerosis directly contributes to coronary heart disease, heart failure, peripheral arterial disease, stroke and dementia. The adaptive immune response is a critical part, with Th1 and Th17 cells accelerating this process, and Th2 and Treg cells playing moderating roles. Many details are currently unknown about the adaptive response to atherogenic lipids, especially the degree of genetic control and the roles of the environment and health-related behaviors, indicating that epidemiological approaches are critically needed. Current methods for studying these cells are expensive and difficult to use, severely limiting progress. We propose to develop and apply improved tools for estimating Th bias, and Th17 and Treg cell responses in more readily available BioSample Repository samples. In Aim 1 we will use existing biomarkers to develop a panel that accurately estimate these adaptive response parameters. To optimize the number of candidate biomarkers we will determine the full set of cytokines expressed in cell culture supernatants from cells stimulated to yield optimal T Helper cell expression. And we will biochemically characterize several important panel candidates which are soluble forms of normally non-soluble transmembranous proteins. In Aim 2 we will determine if cryo-preserved cells are useful for flow cytometric analysis potentially increasing the number of subjects with cell-based measurements that might prove useful for correlation studies of our biomarker panels. In Aim 3 we will begin to utilize this biomarker panel of Th bias, and Th17 and Treg responses in epidemiological populations; specific applications will include determining age-, sex-, and ethnicity-related differences, determining the degree and nature of genetic control, and determining whether this panel improves CVD risk prediction beyond that observed with traditional CVD risk factors. This work will have a major impact by improving CVD risk prediction beyond that available from traditional CVD risk factors, and helping move towards immune modulation therapy for atherosclerosis.

Bernhard Kuhn, M.D. - 2010 Awardee
Boston Children's Hospital

"A Novel Strategy to Regenerate Heart Muscle after Myocardial Infarction and Ischemic Cardiomyopathy"

Myocardial infarction and ischemic cardiomyopathy are severe forms of coronary artery disease (CAD), leading via myocardial injury to heart failure and premature death. Injured human heart muscle regenerates very little: in fact, the current dogma is that human heart muscle cells (cardiomyocytes) cannot regenerate. Our finding of extracellular factors that stimulate cardiomyocyte regeneration presents a fundamental challenge to this belief. We have identified and characterized peptides of periostin (PN) and neuregulin1 (NRG1), and documented that they stimulate successful cardiomyocyte proliferation. Preliminary results indicate that PN is effective in rats and in pigs with myocardial infarction. Others have shown that NRG1 is safe and effective for treating patients with cardiomyopathy. These results raise the exciting possibility that these factors might also be effective regenerative factors in humans.

I propose translational research aimed at advancing a potentially curative strategy for treating heart failure. By stimulating controlled heart muscle regeneration, this strategy has the potential to prevent the development of heart failure, and greatly improve the quality of life and survival of patients with CAD.

My central hypothesis is that patients with CAD have altered cardiomyocyte proliferation and that specific molecular interventions stimulate cardiomyocyte proliferation. We take advantage of a unique international collaboration between my team and that of Cris dos Remedios, University of Sydney (Australia). We will contribute our established methodology for inducing cardiomyocyte regeneration, and our expertise in quantifying the proliferation of these cells. Dr. dos Remedios' team will contribute a large repository of human myocardial samples. Aim 1 is to quantify the activity of cardiomyocyte proliferation in CAD patients, relative to age-matched controls. Aim 2 is to determine the extent to which cardiomyocytes from CAD patients can be stimulated to proliferate with PN and NRG1. Collectively, our results should provide crucial pre-clinical data to support future clinical trials in humans.