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Title: Targeted Ex vivo Nanotherapy for use in Cardiac Transplantation
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Project Summary:

Cardiac allograft vasculopathy (CAV) is the hallmark pathology of chronic rejection. It is the result of an accumulation of various injuries to an allograft, including insults to endothelial and smooth muscle cells. CAV remains a major cause of long term graft loss and mortality, with up to 50% of allografts demonstrating some amount of CAV by 10 years post-transplantation. While improvements in immunosuppressive agents have greatly improved short term survival, long term survival has remained essentially unchanged, largely due to CAV. Rapamycin, as an immunosuppressant, has demonstrated tremendous immuno-modulatory ability and potential for conferring tolerogenic phenotypes on cardiac allografts in animal models. However, clinically, the use of rapamycin has been severely limited by a host of systemic side effects. The ability to target the drug to an allograft would be beneficial in abrogating effects of CAV as well as systemic sequelae. In fact, previous studies have demonstrated that the hydrophobic immunosuppressant rapamycin is more readily taken-up by antigen presenting cells, when packaged into a PLGA nanoparticle, allowing for an avenue of targeted drug delivery. Further, packaging and intracellular delivery are associated with improved inhibition of DC/T cell co-stimulatory functions. To that end, we have designed, developed, and characterized a novel non-toxic integrin receptor Targeted Rapamycin Micelle (TRaM) and have shown improved cellular and graft penetration, as compared to untargeted rapamycin micelles (RaM). Further, our preliminary data demonstrates that TRaMs inhibit EC cytokine release and significantly reduces MHC expression, as compared to free drug or RaM.
In this proposal we seek to develop a means to effectively deliver rapamycin to donor allografts prior to transplantation in a clinically relevant paradigm in an effort to ameliorate CAV. The overall goal of this grant is to test and develop strategies to deliver rapamycin to the donor organ during the cold ischemic time prior to transplantation. Standard immunosuppressive regimens take time to quell the immune system, and therefore the aim of this proposal is to design treatment strategies that will not only protect during cold storage, but also inhibit the early damage caused by ischemia reperfusion injury and memory T cells.

Specific Aims:

Cardiac allograft vasculopathy (CAV) is the hallmark pathology of chronic rejection. It is the result of an accumulation of various injuries to an allograft, including insults to endothelial and smooth muscle cells. CAV remains a major cause of long term graft loss and mortality, with up to 50% of grafts demonstrating some amount of CAV by ten years posttransplantation 2, 3. While improvements in immunosuppressive agents have greatly improved short term survival, long term survival has remained essentially unchanged, largely due to CAV2-4. In this proposal, we seek to develop a means to effectively deliver rapamycin to donor cardiac allografts prior to transplantation, in a clinically relevant paradigm, in an effort to ameliorate CAV. The overall goal of this grant is to test and develop strategies to deliver rapamycin to the donor organ during the cold ischemic preservation stage prior to transplantation. Standard immunosuppressive regimens take time to quell the immune system, and therefore the aim of this proposal is to design treatment strategy that will not only protect during cold storage, but also inhibit the early damage caused by ischemia reperfusion injury and memory T cells. To that end, we have designed, developed, and characterized a novel non-toxic integrin receptor Targeted Rapamycin Micelle (TRaM), and have shown improved cellular and graft penetration, as compared to untargeted rapamycin micelles (RaM). Further, our preliminary data demonstrates that TRaMs inhibit EC cytokine release and significantly reduces MHC expression, as compared to free drug or RaM5. We hypothesize that organ pretreatment with TRaMs will improve rapamycin uptake and ultimately lead to the amelioration of EC activation, cytokine release, and impair costimulation, thereby reducing allograft injury.

Aim 1: Elucidate the mechanism of TRaM conditioning on endothelial cell antigen presentation, co-stimulatory molecule expression, and response to injury in an in-vitro model of transplantation using both mouse and human cell lines in normoxic and hypoxic conditions. We will utilize mouse cardiac endothelial cells (MCEC) as well as human umbilical vein endothelial cells (HUVEC) in isolation and in co-culture systems of normal oxygen tension and hypoxic conditions with

allogeneic T cells to interrogate the impact of TRaMs on EC activation, antigen presentation capacity, and inflammation. We will additionally utilize memory T cell co-cultures to dissect the effect of TRaM on sensitized memory T cell populations in-vitro.

Aim 2: Determine the uptake and immunologic effect of perioperative TRaM conditioning during hypothermic preservation in an in-vivo model of allograft vasculopathy. We will utilize the established mouse orthotopic aortic allograft model to ascertain the nanoparticle bio-distribution and uptake kinetics as well as the impact of ex-vivo delivered TRaMs in standard University of Wisconsin (UW) preservation solution on allograft vasculopathy, EC activation and graft-specific T cell infiltration. Molecular and biochemical markers of injury will be assessed in the in-vivo setting both at the graft level as well as in the serum of mice.

Success will be determined by demonstrating that preoperative TRaM therapy reduces EC activation, co-stimulation, antigen presentation capacity, and memory T cell activation.

Lay Summary:

1. What is the major problem being addressed by this study?

Almost 6 million adults in the U.S. have heart failure, and with realistically no current medical treatment options, 50% of those patients will die within 5 years of diagnosis. Consequently, cardiac transplantation represents their only viable option for survival, however this is also fraught with complications including long term immunosuppression, which is very difficult to tolerate, and ultimately a median survival of only 11 years. Additionally, due to a shortage of quality organs, only a small percentage of heart failure patients will ever receive their life-saving transplant. Our research aims to make more organs available to end-stage heart failure patients, and then make living with their new organs more tolerable.

2. What specific questions are you asking and how will you attempt to answer them?

In transplantation, organs spends time stored on ice outside the body, where they incur injuries from lack of nutrients and oxygen. This damage results in priming the immune system to attack post-transplantation. We have designed a novel targeted immunosuppressant nano-molecule which has demonstrated an ability to protect donor organs prior to transplantation without the need for further administration of immune system depleting drugs in the post-transplantation period. The goal of our studies is to characterize what our nanotherapy is doing at a cellular level, and more definitively demonstrate how our treated organs are better able to function and survive while minimizing the toxicity of whole-body drug administration.

3. Overall, what is the potential impact of this work to the mission of the AHA?

If successful, our drug nanotherapy has the potential to decrease the toxicity associated with current transplant-related immunosuppressive medications, while simultaneously improving long term heart transplant outcomes. That is, heart transplant patients would live longer, healthier lives while requiring less intensive maintenance regimens to remain free from transplant-specific cardiovascular disease. Lastly, the prospect also exists that this pre-treatment of hearts prior to transplant could demonstrate an ability to recover marginal or sub-optimal organs that would otherwise go untransplanted, thereby increasing the donor pool and ultimately allowing more heart failure patients to receive the lifeline they so desperately need.