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TITLE: Investigating Novel Approaches to Block Inflammation and Prevent Ischemia Reperfusion Injury During VCA Transplantation

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14. ABSTRACT

Ischemia reperfusion injury (IRI) contributes to inflammation, acute rejection, and negatively impacts VCA function and survival. Strategies to prevent IRI could decrease rejection episodes, improve VCA outcomes and facilitate immune tolerance induction strategies. Galectin-3 (Gal3), a beta-galactoside binding lectin, contributes to acute inflammation in response to tissue hypoxia, an unavoidable consequence of organ harvest and VCA transplantation. Blocking extracellular Gal3 using known Gal3 inhibitors including modified citrus pectin (MCP) has been shown in animal models to reduce inflammation and fibrosis. This proposal will elucidate the role of Gal3 in VCA IRI. We hypothesize that Gal3 significantly contributes to VCA IRI and that blocking extracellular Gal3 function using MCP can serve as a novel therapeutic approach to prevent or reduce IRI. We also hypothesize that circulating Gal3 levels can serve as a predictive biomarker of IRI and VCA function post transplantation.

15. SUBJECT TERMS
Vascular Composite Allograft (VCA) Transplantation; Ischemia Reperfusion Injury (IRI)

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1. Introduction

Over half of all combat related casualties in the US military since 2006 were sustained in IED related incidences. Up to 80% of those involved sustained wounds to the hands and or face. Victims of such attacks are often left permanently disfigured and in some cases with extremities or portions of their face missing.The reconstructive procedures used for major tissue loss are inadequate to reconstruct a lost hand or to treat significant injuries to the extremities or face. One solution for complex reconstruction is the use of hand and face vascularized composite allografts (VCA). VCA are potential surgical solutions for patients with traumatic or disfiguring injuries but are highly immunogenic and require elevated levels of immunosuppression. Although clinical VCA transplantation has resulted in successful outcomes, the high rates of acute rejection and increased requirements for immunosuppression have led to significant long-term complications. Because of their significant muscle components, VCAs are vulnerable to ischemia reperfusion injury (IRI). IRI is known to contribute to inflammation, acute rejection and poor VCA outcomes. Approaches that reduce inflammation associated with ischemic injury during VCA transplantation may permit reduction in immunosuppression with improved function. This study investigates a novel approach to reduce ischemia reperfusion injury (IRI) and the concomitant acute inflammatory responses associated with VCA transplantation. VCA are comprised of different tissue types and have a significant skeletal muscle component which makes them particularly susceptible to ischemic injury. IRI negatively impacts Vascularized Composite Allograft (VCA) function and survival. Strategies to prevent IRI could decrease rejection episodes, improve VCA outcomes and facilitate immune tolerance induction strategies. It is known that inflammation plays an important role in the pathogenesis of IRI. Reducing IRI may improve graft function and reduce the risk of chronic rejection. In addition, numerous approaches have been hypothesized to improve the graft survival by IRI reducing the inflammation.

Galectin-3 is a β-galactoside binding lectin found in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space. Pleiotropic biological functions of Gal3 have been reported depending on its subcellular or extracellular location. Numerous reports have demonstrated a strong association with high circulating levels of extracellular Gal3 and a transition to chronic inflammation and fibrosis in a wide range of inflammatory diseases including arthritis, cardiovascular disease, cancer and autoimmune diseases. Blocking Gal3 using known Gal3 inhibitors including modified citrus pectin (MCP) has been shown in animal models to reduce inflammation and fibrosis. The mechanism of action of MCP is through the binding of pectin-

derived galactose to Gal3 in the extracellular space. In this proposal we will elucidate the role of Gal3 in VCA IRI. We hypothesize that Gal3 significantly contributes to VCA IRI and that blocking Gal3 function using MCP can serve as a novel therapeutic approach to prevent or reduce IRI. We also hypothesize that circulating Gal3 levels can be used as a predictive biomarker of IRI and VCA function post transplantation.

Numerous reports have demonstrated a strong association with high circulating levels of extracellular galectin-3 (Gal3) and a transition to chronic inflammation and fibrosis in a wide range of inflammatory diseases. Blocking Gal3 using known Gal3 inhibitors including modified citrus pectin (MCP) has been shown in animal models to reduce inflammation and fibrosis. In this study we will elucidate the role of Gal3 in VCA IRI using two approaches. Gal3 blockade: Transplant recipients will be treated with MCP in the drinking water to block the carbohydrate binding domain of Gal3. Gal3 genetic depletion: A novel Gal3 knockout (KO) rat was established through a contract with GenOway, France to accomplish this goal. Gal3 KO rats will be used as VCA donors and/or recipients. We hypothesize that Gal3 significantly contributes to VCA IRI and that blocking Gal3 function can serve as a novel therapeutic approach to prevent or reduce IRI. We also hypothesize that circulating Gal3 levels can be used as a predictive biomarker of IRI and VCA function post transplantation.

2. Keywords

Ischemia reperfusion injury; Vascular Composite Allograft; Hind limb transplant; Inflammation; Galectin-3

3. Accomplishments

o What were the major goals of the project?

Specific Aim 1: Determine the role of donor and recipient galectin-3 in VCA ischemia reperfusion injury (IRI)

Major Task 1.1: Establish the optimal timing and temperature of hind limb ischemia to assess IRI

Major Task 1.2: Elucidate the role of donor and recipient galectin-3 in IRI and VCA function in an established rat hind limb VCA model with prolonged cold ischemia

Specific Aim 2: Determine whether blocking circulating galectin-3 function reduces graft failure following prolonged ischemia in the rat hind limb VCA model.

Major Task 2.1: Block galectin-3 function in recipients using modified citrus pectin

Specific Aim 3: Assess circulating levels of galectin-3 in response to IRI as a predictive biomarker of VCA graft outcome.

Major Task 3.1: Determine whether high levels of circulating galectin-3 correlate with graft failure following prolonged ischemia in the rat hind limb VCA model.

o What was accomplished under these goals?

Specific Aim 1: Determine the role of donor and recipient galectin-3 in VCA ischemia reperfusion injury (IRI)

Methods: The right hind limb of the Brown Norway (BN) donor is removed, the femoral artery is cannulated and flushed with heparinized lactated ringers, and the limb is wrapped in wet gauze and either immediately transplanted (~1 hr ischemia) or maintained at following conditions: 4 hours at room temperature (RT), 6 hours at RT, 6 hours at 4^{o}C and 24 hours at 4^{o}C prior to syngeneic orthotropic transplantation (Figure 1). Skin and muscle samples are obtained both pre-implantation and at post-operative day (POD) 6. The tissue is then examined using H&E and TUNEL stains to quantify the extent of injury and inflammation. Pre and post operation serum sample is collected for pro-inflammation cytokine evaluation.

Recipient rats were treated with Ringer's solution as hydration until POD 4, antibiotics until POD 3 and buprenorphine SR every 72 hours, for animal care. Elizabethan collar was applied after operation to prevent the rat from chewing the transplanted limb.

Results: All animals have shown histologic changes by POD 6. In donor limbs transplanted immediately (~1 hr room temperature normothermic ischemia) skin and muscle tissue showed minimal inflammatory infiltrates when looking at H&E and TUNEL staining. In donor limbs exposed to 6 or 24 hours of cold ischemia at 4 degree in Celsius, there was dermal and endomyosial inflammation with dermal edema and myocyte necrosis on POD 6 (Figure 2). Post-perfusion TUNEL staining revealed focal apoptosis in all limbs transplanted following 6 or 24 hours of cold ischemia.

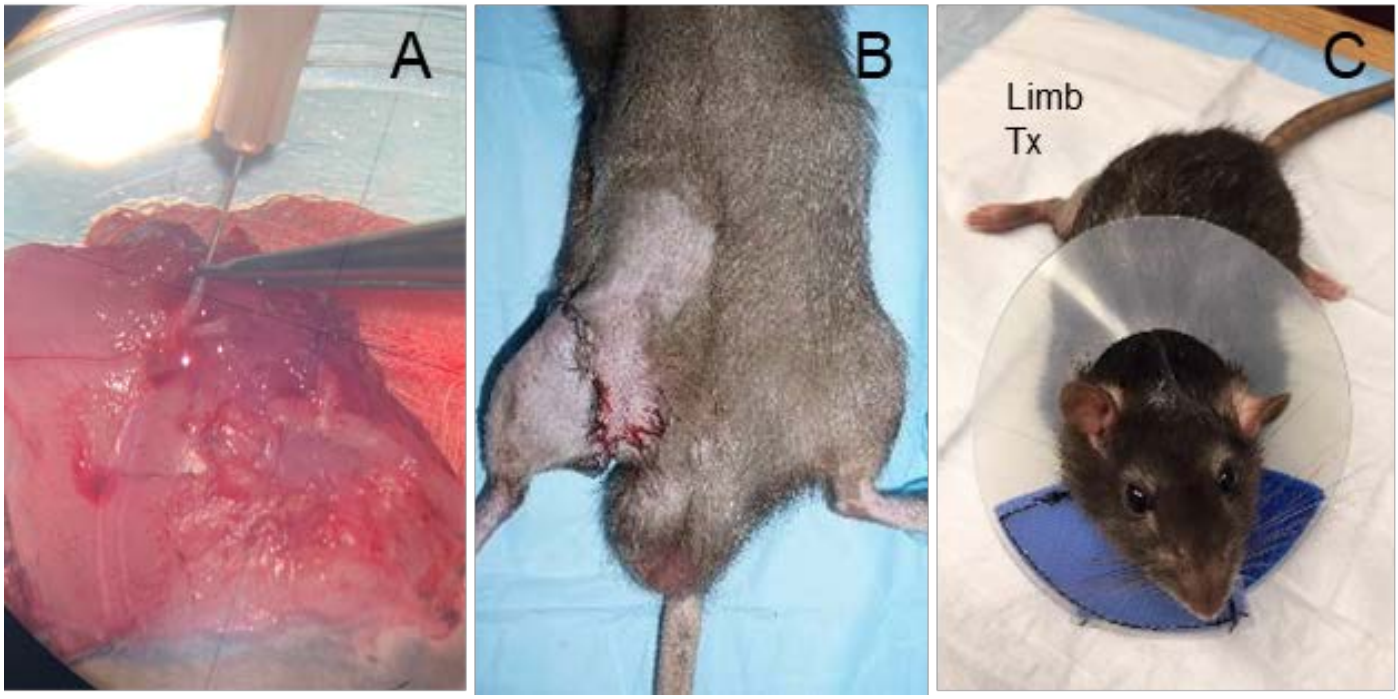

Figure 1: Successful completion of transplant procedure and post-surgical care: A) ex vivo hind limb flushing during ischemic period (1, 4 and 6 hours of ischemia are currently being evaluated; B) recipient rat immediately post-surgery; C) careful follow-up monitoring post transplantation**.**

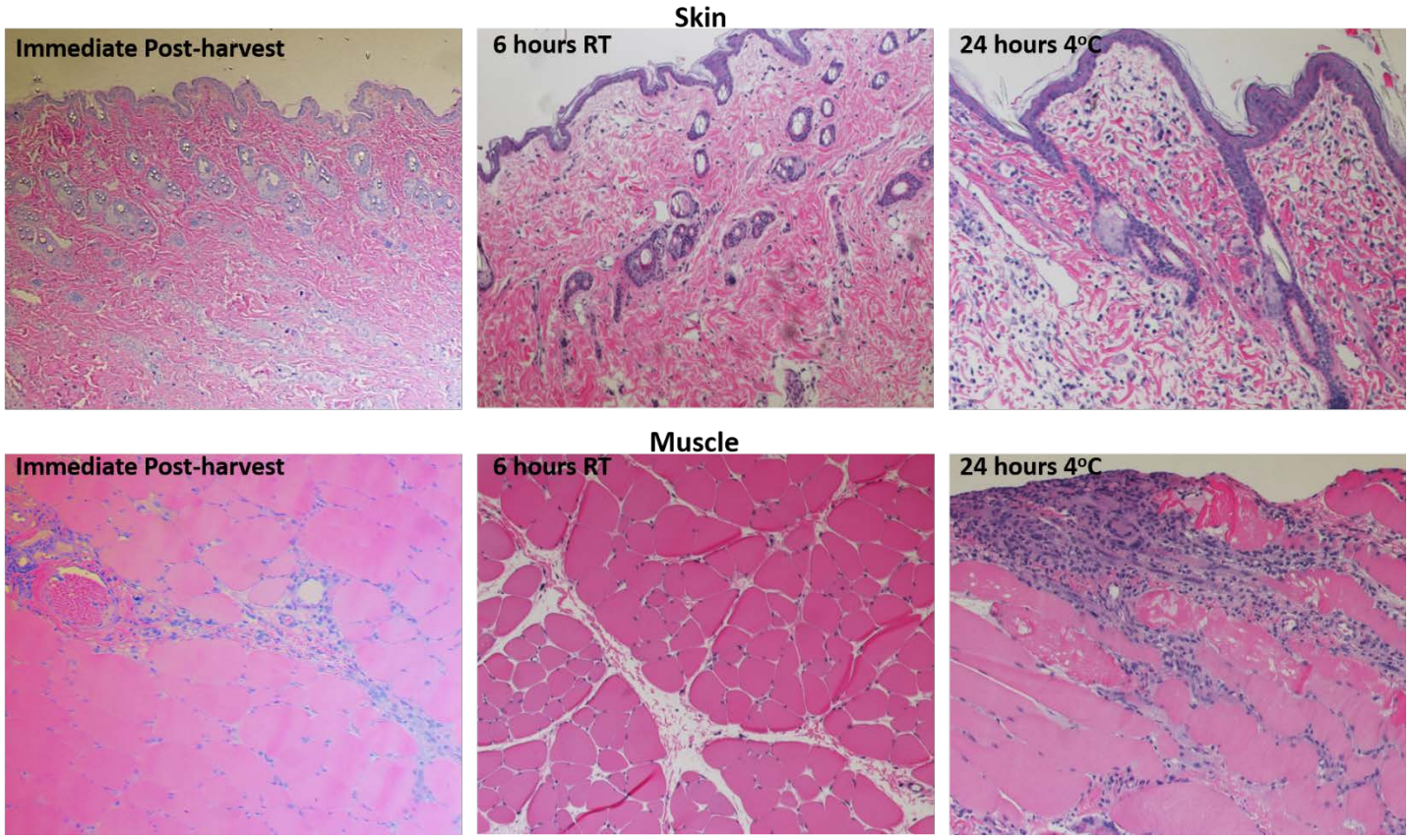

Figure 2. Histological analysis of skin (top row) and muscle (bottom row) harvested on POD 6 following transplantation of hind limbs that were subjected to different degrees ischemic insult. Left column: Hind limb transplanted immediately post-harvest from donor (minimal ischemia during transplantation ~1 hour at room temperature RT); Middle column: 6 hours RT; Right column: 24 hours cold ischemia at 4°C.

Conclusion and discussion: Using a syngeneic BN rat model of hind limb transplantation we have established a reliable platform to study VCA IRI and evaluate preventative strategies. In this model, the minimal 1 hour RT ischemia which occurs during the hind limb transplantation procedure itself results in minimal inflammation at POD 6, whereas 24 hours cold ischemia consistently yields extensive inflammation throughout the dermis and endomysium on POD 6. To assess inflammation due to ischemia reperfusion injury, IL6 and IL10 will be measured in the serum level on POD 6 by ELISA (in progress).

Specific Aim 2: Determine whether blocking circulating galectin-3 function reduces graft failure following prolonged ischemia in the rat hind limb VCA model.

Methods: Recipient rats were transplanted with prolonged ischemia limb (24 hours, at 4ºC). To achieve a parallel comparison, the recipients were grouped in pairs: one was treated with seven days of modified citrus pectin in drinking water (1% w/v) to block galctin-3 function following transplantation; the other without treatment as the control. Each pair of rats were trained with collar and water bottle since day -7. Water intake was monitored daily to make sure the recipient have sufficient water or MCP to block galectin-3.

Result: The prolonged ischemia model is stable and repeated. The water intake monitoring shows that the rat drinks enough water/MCP, and after the transplant, the intake volume doesn't decrease dramatically (Figure 3).

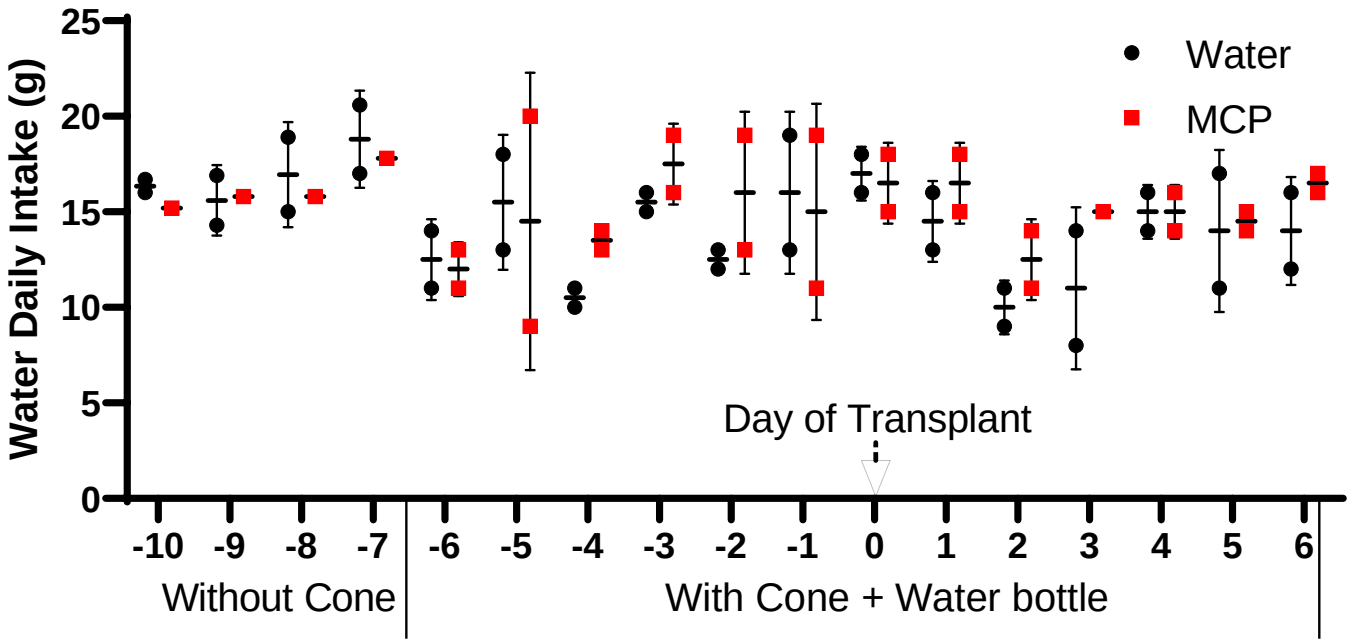

Figure 3. Rat daily water/MCP (MCP, 1% w/v) daily intake was monitored to make sure the rat take enough MCP for galectin-3 blockade.

Conclusion and discussion: the transplant model with prolonged ischemia is stable and repeatable. The successful rate > 60% (table 1) is achieved for either MCP or control group. More transplants are in plan to fill up the group number. Pathologic assessment would be used to evaluate the injury of skin and muscle tissue (in progress). The MCP treated rat drinks enough MCP to block the galectin-3 before and post-transplant. Circulation Galectin-3 level, as well as pro-inflammation cytokines including IL-6 and IL-10 could be measured by serum ELISA to assess the inflammation caused injury and to assess whether galectin-3 blockade is able to reduce IRI following VCA transplant.

Table 1. Summary of the hind limb transplant success rate.

Animal type	MCP Treatment	Ischemia Condition	Ischemia Time (h)	Success	Failure	Success rate	Transplants Needed
Gal+ to Gal+	W/O	RT	1	4	2	66.7%	8
		RT	4	2	0	100.0%	0
		RT	6	0	2	0.0%	0
		4 °C	6	0	1	0.0%	0
		4 °C	24	9	3	75.0%	3
Gal+ to Gal+	With MCP	RT	1				12
		4 °C	24	5	3	62.5%	7

Specific Aim 3: Assess circulating levels of galectin-3 in response to IRI as a predictive biomarker of VCA graft outcome.

ELISA assay is in progress to evaluate the galectin-3 level in serum from transplant recipients performed for the aims above.

o What opportunities for training and professional development has the project provided?

This project has allowed research staff in the laboratory to learn proper animal care of Brown Norway rat, knowledge of ischemia reperfusion injury, inflammation, function of the galectin-3 and basic concept of gene editing with CRISPR-Cas9. In addition, this project provided training in microsurgical skills for medical students and research residents involved in this project. Furthermore, lab trainees have had the opportunity to present this project at research seminars and medical conferences for knowledge development.

This project has provided trainees with a better understanding of the potential for vascularized composite allografts to restore limb function in military veterans; an understanding of the current limitations to vascularized composite allograft transplantation and associated risks of immunosuppression; and an appreciation of the impact of ischemia reperfusion injury on allograft outcomes and potential for galectin-3 blockade to mitigate ischemia reperfusion injury.

o How were the results disseminated to communities of interest?

Oral presentation at the 2020 Mountain West Society of Plastic Surgery, Snowmass, CO, Feb 27, 2020

Abstract accepted for the 2020 Virtual American Transplant Congress, May 30, 2020.

Abstract submitted and accepted for presentation at the 2020 Military Health System Research Symposium. Although the meeting was cancelled due to the global pandemic, the abstracts will be available on-line.

o What do you plan to do during the next reporting period to accomplish the goals?

Repeat more transplants to fill up the group number;

H&E and TUNEL staining for the skin and muscle tissue to evaluate injury; meanwhile to determine whether blocking galectin-3 can reduce tissue injury, necrosis or apoptosis.

Run ELISA for Galectin-3, IL-6 and IL-10 to assess whether blocking galectin-3 can reduce inflammation caused injury.

Do transplant with galectin-3 knock out rat to determine whether galectin-3 knock out can reduce inflammation caused injury.

4. Impact

o What was the impact on the development of the principal discipline(s) of the project?

The overall data from Operation Iraqi Freedom (OIF) indicate that there have been of a total 19,511 wounded soldiers. Injuries from improvised explosive devices (IED's) and other explosive devices differ markedly from those of gunshot wounds. The contamination and soft-tissue injury caused by these explosions require aggressive debridement that result in large complex wounds needing reconstruction. The high rate of vascular injuries and amputations reflect the substantial degree of severe soft-tissue injury suffered by our warfighters. There were 111 amputations with sixty-four of these amputations involving major limb loss proximal to the ankle or wrist. The clinical application of vascularized composite allograft (VCA) would allow for the reconstruction of the lost tissue with the exact tissue lost with no donor site issues. This could allow for the possibility to return to active duty after what previously were unreconstructible wounds, such as loss of an extremity or a devastating wound to the face.

The use of a VCA to reconstruct soldiers that have sustained severe injuries to their extremity and face would allow for the lost tissue to be replaced by the exact tissue lost. This transplant would enhance the tissue environment for healing by providing the missing bone, soft tissue, muscle and nerves. After the loss of a hand or a complex facial injury with extensive loss of bone, muscle and soft tissue, amputation is often the standard of care. In these cases a VCA could be harvested and enable limb salvage by providing the exact missing tissue and enabling the surgeon to achieve restoration of the bone, nerves and muscles. This could lead not only to a stable, healed wound, as is the goal currently, but a truly functional reconstruction, allowing injured military service members and veterans to continue to play a productive role in the armed services.

The overall scientific goal of this research is to evaluate galectin-3 as a therapeutic target to prevent the acute inflammatory responses associated with ischemia reperfusion injury (IRI). Approaches that reduce inflammation associated with ischemic injury during VCA transplantation may permit reduction in immunosuppression with improved function.

We have now optimized the rat hind limb transplantation model for assessing ischemia reperfusion injury with pharmacological galectin-3 blockade and have successfully engineered a novel galectin-3 knockout rat model in the Brown Norway background to determine the role of galectin-3 in VCA IRI.

o What was the impact on other disciplines?

The development of a novel rat galectin-3 knock-out line to accomplish the goals of this study will have a significant impact on research related to galectin-3 biology and how galectin-3 impacts transplant outcomes. Rats are preferred over mouse models for studies involving organ and tissue transplantation due to the vessel size.

o What was the impact on technology transfer?

Nothing to Report

5. Changes/Problems

o Changes in approach and reasons for change

Nothing to Report

o Actual or anticipated problems or delays and actions or plans to resolve them

We faced a couple of problems in establishing the model and now they are solved:

1): Several rat recipients chewed the suture/graft after transplant during the initiation of these studies:

Solution: Elizabethan Collar was applied to prevent chewing the graft with a 1 week period of acclimation prior to surgery.

2): Ischemia condition and time optimization:

Solution: we tried different ischemia time and condition including: 1 hour room temperature (minimal ischemic time during transplant surgery), 4 hours at room temperature, 6 hours at room temperature, 6 hours at 4 degrees Celsius, and 24 hours at 4 degrees Celsius. One hour room temperature ischemia will be used as the control for the minimal ischemic time that occurs during the hind limb transplant surgery and static cold storage for 24 hours at 4 degrees Celsius was chosen as the time period that results in consistent histological injury so we can be confident whether our treatment strategies are having a beneficial effect.

3): In order to improve the success rate of the transplant, the recipients were receiving collar and water bottle training since day -7. Daily water intake is monitored to make sure the rat take sufficient hydration or MCP.

4): Post transplant care optimization: antibiotics were given to the recipient to prevent infection; Ringer's solution was given to prevent dehydration and analgesic was given to relief pain and stay comfort.

5): A delay in shipment of the newly established heterozygous Gal3KO rat founders due to Coronavirus shutdown led to an almost 4 month delay in the initiation of the breeding of homozygous rats for experimental purposes. The rats are out of quarantine and currently breeding well. Experiments for Major Task 1.2 will be completed as soon as we have bred enough homozygous Gal3 KO Brown Norway rats to complete this task.

6) COVID19 Colorado state-wide restrictions required the shutdown of on-site research at our institution starting March 16 with a gradual phased-in approach to return to on-site research starting in June. This has resulted in a significant delay in the processing of pathology samples at our pathology research core facility as they were unable to process samples for over 3 months. In addition, given the limited staff allowed to return and the strict physical distancing requirements during this phase, there have been delays with scheduling and completing the rat hind limb surgeries, as only one microsurgeon can be in the animal facility operating room at a time. We are hopeful that COVID19 cases will start to drop in Colorado and that we will be allowed to progress to the next phase of research re-entry soon.

- **Changes that had a significant impact on expenditures**

Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to Report

- **Significant changes in use or care of human subjects**

Nothing to Report

- **Significant changes in use or care of vertebrate animals.**

Nothing to Report

- **Significant changes in use of biohazards and/or select agents**

Nothing to Report

6. Products

- **Publications, conference papers, and presentations**
  - **Journal publications**
    Nothing to Report
  - **Books or other non-periodical, one-time publications**
    Nothing to Report
  - **Other publications, conference papers, and presentations**

Wang Z*, Harrant AB*, Wang Y, Washington K, Farkash E, Huang CA. Rat Hind Limb Transplant Model to Assess Vascularized Composite Allograft (VCA) Inflammation and Ischemia Reperfusion Injury [abstract]. Am J Transplant. 2020; 20 (suppl 3). https://atcmeetingabstracts.com/abstract/rat-hind-limb-transplant-model-to-assess-vascularized-composite-allograft-vca-inflammation-and-ischemia-reperfusion-injury/. Accessed July 16, 2020.

Harrant AB*, Wang Z*, Anderson JB, Wang Y, Li B, Johnson AC, Washington K, Navarro-Alvarez N, Farkash EA, Huang CA. Rat Hind Limb Transplant Model to Assess Vascularized Composite Allograft (VCA) Ischemic Reperfusion Injury and Inflammation. Oral Presentation – Mountain West Society of Plastic Surgeons 2020 Annual Meeting, February 27-March 1, 2020; Snowmass Village, CO.

Harrant AB*, Wang Z*, Wang Y, Li B, Su A-J, Mathes DW, Washington K, Farkash EA, Huang CA. Investigating novel approaches to block inflammation and prevent ischemia reperfusion injury during vascularized composite allograft transplantation. 2020 Military Health System Research Symposium.

* Wang Z and Harrant AB contributed equally to this project

- **Website(s) or other Internet site(s)**

https://atcmeetingabstracts.com/abstract/rat-hind-limb-transplant-model-to-assess-vascularized-composite-allograft-vca-inflammation-and-ischemia-reperfusion-injury/

- **Technologies or techniques**

Established a novel galectin-3 knock-out model to complete these proposed studies

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name:	Christene A. Huang, PhD
Project Role:	Principal Investigator
Researcher Identifier:	ORCID: 0000-0001-9824-5716
Nearest person month worked:	1
Contribute to Projects:	Dr. Huang is the PI in this project and has been responsible for the overall direction of the project and interpretation of the data
Funding Support:	RT180168

Name:	David W. Mathes, MD
Project Role:	Collaborator
Researcher Identifier:	ORCID: 0000-0003-4388-1373
Nearest person month worked:	1

Contribute to Projects:	Dr. Mathes provides clinical advice on the IRI model
Funding Support:	RT180168/ Department of Surgery Funds

Name:	Kia Washington, MD
Project Role:	Collaborator
Researcher Identifier:	ORCID: 0000-0003-1803-5888
Nearest person month worked:	1
Contribute to Projects:	Dr. Washington provides surgical advice on the rat hind limb transplantation model
Funding Support:	RT180168/ Department of Surgery Funds

Name:	Yong Wang, MD
Project Role:	Research Staff
Researcher Identifier:	ORCID: 0000-0002-7002-6439
Nearest person month worked:	3
Contribute to Projects:	Dr. Wang is the primary surgeon performing the rat hind limb transplantation.
Funding Support:	RT180168

Name:	Zhaohui Wang, DVM
Project Role:	Research Associate
Researcher Identifier:	ORCID: 0000-0002-9035-3867
Nearest person month worked:	6
Contribute to Projects:	Dr. Wang is lead researcher responsible for this project. He is responsible for coordinating and assisting with the rat surgeries, providing post-operative animal care, harvesting tissues, designing assays and analyzing data.
Funding Support:	RT180168 /Dr. Huang's sundry funds

Name:	Alexander Harrant
Project Role:	Research Staff

Researcher Identifier:	ORCID: 0000-0002-5343-3462
Nearest person month worked:	6
Contribute to Projects:	Mr. Harrant assists with surgeries, harvesting tissue samples, performing pathologic analyses and providing animal care.
Funding Support:	Dr. Huang's sundry funds

Name:	Evan Farkash, MD, PhD
Project Role:	Research Staff
Researcher Identifier:	ORCID: 0000-0002-5136-079X
Nearest person month worked:	1
Contribute to Projects:	Dr. Farkash serves as pathology consultant and interprets histology
Funding Support:	University of Michigan Department of Pathology

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

- **What other organizations were involved as partners?**

University of Michigan Health System

Dr. Evan Farkash Assistant Professor, Dept. of Pathology, University of Michigan Health System, Ann Arbor, MI reviews the histology for the project. We have an MTA in place to be able to ship slides from UC Denver to University of Michigan for pathological assessment.

8. Special Reporting Requirements

Quad Chart

Investigating Novel Approaches to Block Inflammation and Prevent Ischemia Reperfusion Injury During VCA Transplantation

W81XWHI910163

PI: Christene A. Huang, PhD. **Org:** University of Colorado Anschutz Medical Campus **Award Amount:** $200,000

Study/Product Aim(s)

- Determine the role of donor and recipient galectin-3 in vascularized composite allograft (VCA) ischemia reperfusion injury (IRI).
- Determine whether blocking circulating galectin-3 function reduces graft failure following prolonged ischemia in the rat hind limb VCA model.
- Assess circulating levels of galectin-3 in response to ischemia reperfusion injury as a predictive biomarker of VCA graft outcome.

Approach

This proposal investigates a novel approach to reduce IRI and the concomitant acute inflammatory responses associated with VCA transplantation. IRI negatively impacts VCA function and survival. Galectin-3, a known inflammatory factor, is actively involved in ischemia-induced inflammation and fibrosis of various organs. This proposal will elucidate the role of galectin-3 in VCA IRI. As part of this proposal we will investigate whether galectin-3 can serve as a therapeutic target to prevent or reduce IRI and whether it can be used as a novel peripheral biomarker to predict the degree of ischemic damage and effect on graft function following VCA transplantation.

Animal type	MCP Treatment	Ischemia Condition	Ischemia Time (h)	Success	Failure	Success rate	Transplats Needed
Gal+ to Gal+	W/O	AT	1	4	2	66.7%	8
		AT	4	2	0	100.0%	0
		AT	6	2	0	100.0%	0
		4 °C	6	0	1	1.0%	0
		4 °C	24	9	3	75.0%	3
Gal+ to Gal+	With MCP	AT	1	0			12
		4 °C	24	5	3	62.5%	7

Accomplishments: Rat hind limb ischemia reperfusion injury model is stabilized and repeated; Daily water/modified citrus pectin (MCP, 1% w/v) daily intake was monitored to make sure the rat take enough MCP for galectin-3 blockade. Rat treated with MCP were in progress.

Activities (Start Date *May 1, 2019*) Month:	0-6	7-12	13-18
Obtain IACUC and ACURO approvals			
Development of reliable rat hind limb IRI Model			
Establish whether galectin-3 in the donor and/or recipient contributes to IRI and poor outcomes following VCA			
Evaluate galectin-3 blockade as a therapeutic approach to reduce IRI following VCA			
Establish whether galectin-3 can be used as a predictive biomarker of VCA graft Failure due to IRI			
Estimated Budget ($K)	$66,666	$66,666	$66,666

Updated: 07/22/2020

Goals/Milestones

Establish the optimal timing and temperature of hind limb ischemia to assess IRI

- [x] Milestone: Obtain IACUC and ACURO approvals
- [x] Milestone: Development of reliable rat hind limb IRI Model

Elucidate the role of donor and recipient galectin-3 in IRI and VCA function in an established rat hind limb VCA model with prolonged cold ischemia

- [x] Milestone: Establish whether galectin-3 in the donor and/or recipient contributes to IRI and poor outcomes following VCA ***(In Progress)***

Block galectin-3 function in recipients using modified citrus pectin

- [x] Milestone: Evaluate galectin-3 blockade as a therapeutic approach to reduce IRI following VCA***(In Progress)***

Determine whether high levels of circulating galectin-3 correlate with graft failure following prolonged ischemia in the rat hind limb VCA model

- [ ] Milestone: Establish whether galectin-3 can be used as a predictive biomarker of VCA graft Failure due to IRI

9. Appendices

Not Applicable