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TITLE: Hemodynamic Changes and Pancreatitis

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CONTRACTING ORGANIZATION: Yale University, New Haven, CT

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b>  The proposed project relates to the FY17 PRMRP topic area on pancreatitis. The project will explore a previously undescribed mechanism of acute pancreatitis in which pathological hemodynamic changes in the pancreas could induce acute pancreatitis responses. Two Aims are proposed. Aim 1 will determine the mechanism by which pathological hemodynamic changes cause acute pancreatitis. In Aim 2, we will determine the role of lymphangiogenesis in the development and/or the resolution of acute pancreatitis. During the reporting period, we have established tissue clearing method with a immune-labeling technique for 3-dimensional imaging of lymphatic vessels in the pancreas. The method will be useful for analysis of not only lymphatic vessels, but also other cells in the pancreas. We also showed that macrophages are a key player for acute pancreatitis and pancreatic lymphangiogenesis in mice with portal hypertension.					
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## 1. INTRODUCTION:

The proposed project relates to the FY17 PRMRP topic area on pancreatitis. The causes of acute pancreatitis are not fully elucidated. Further, the role of the lymphatic system is little understood in acute pancreatitis in particular and in the study of the pancreas in general. The development of simple and reproducible experimental models of acute pancreatitis that are relevant to human disease are urgently needed. This project addresses these critical problems with innovative ideas. First, the project will define a new etiology of acute pancreatitis and explore a previously undescribed mechanism in which pathological blood flow changes in the pancreas could induce acute pancreatitis. Second, it will examine the role of the pancreatic lymphatic system in acute pancreatitis, representing the first step toward understanding biology of the pancreatic lymphatic system. Third, a new and simple experimental model of acute pancreatitis, which can also be used for the study of the pancreatic lymphatic system, will be established. Addressing a new etiology and a new area of study, the project will significantly contribute to our understanding of the etiology and mechanism of acute pancreatitis and could lead to the identification of new risk factors as well as new therapeutic strategies for this disease.

**2. KEYWORDS:** *(limit to 20 words).*

Acute pancreatitis, lymphatic system, lymphangiogenesis, etiology, blood flow, experimental model, risk factors, macrophages, T-cells, pancreatic stellate cells, inflammation, edema, VEGF-C

### 3. ACCOMPLISHMENTS:

#### ▪ What were the major goals (Specific Aims) of the project?

**Specific Aims 1:** Determine the mechanism by which pathological hemodynamic changes cause acute pancreatitis.

1. Determine the role of mechano- signaling in the development of acute pancreatitis (AP)  
Exp 1-1 (in progress)
2. Determine the role of immune cells in the development of AP (Exp 1-2 to 1-4).  
Exp 1-2 (completed)
3. Assess a sensitizing effect of pancreatic hemodynamic changes on AP (Exp 1-5).  
In progress

**Specific Aim 2:** Determine the role of lymphangiogenesis in the development and/or the resolution of acute pancreatitis.

4. Establish a 3-D imaging method for lymphatic vessels in the pancreas (Exp 2-1).  
Completed
5. Determine the role of lymphangiogenesis in the development of AP (Exp 2-2 & 2-3).  
Exp 2-2 (completed), Exp2-3 (in progress)
6. Meeting presentation and manuscript preparation.  
\* Two abstracts related to this project were presented during the 1<sup>st</sup> year of funding.  
\* A manuscript is in preparation and will be submitted by the end of 2021.

#### • What was accomplished under these goals (Specific Aims)?

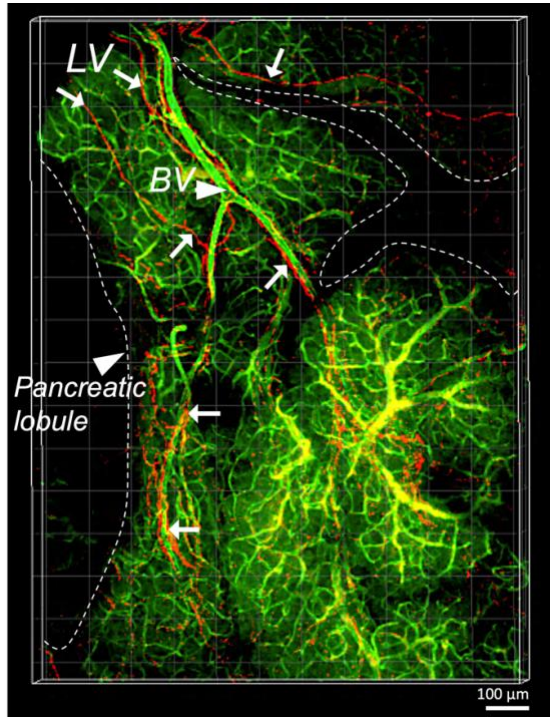
Major accomplishments during this period are listed under each goal above [in blue](#). Below are summaries of major activities, specific objectives and significant results/key outcomes related to these accomplishments in each Aim (Goal).

**Specific Aims 1:** Determine the mechanism by which pathological hemodynamic changes cause acute pancreatitis.

Experiments to assess whether pancreatic hemodynamic changes increase sensitivities to acute pancreatitis are in progress (Exp 1-5).

**Specific Aim 2:** Determine the role of lymphangiogenesis in the development and/or the resolution of acute pancreatitis.

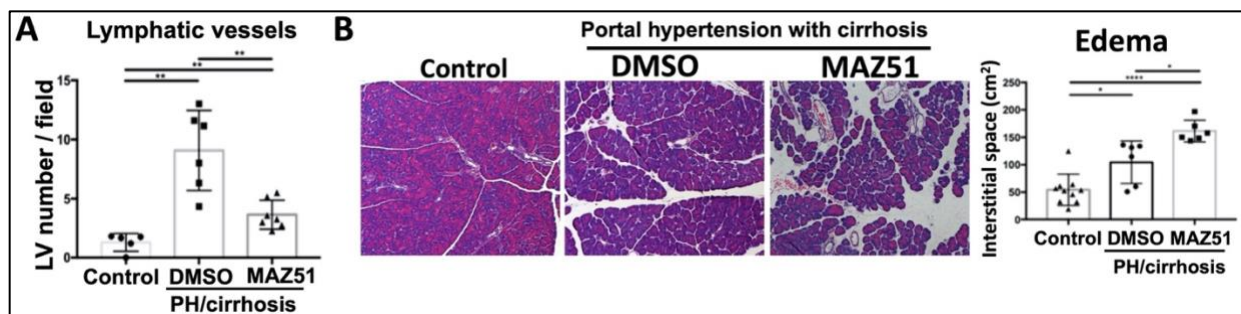
The objective of this Aim is to determine whether lymphangiogenesis facilitates or mitigates acute pancreatitis. To accomplish this objective, first, we proposed to develop a protocol for efficient tissue clearing and immunolabeling of pancreatic lymphatic vessels to visualize them in 3 dimension (Exp 2-1). We have significantly improved the imaging quality by adjusting the protocol. Figure 1 shows lymphatic vessels (red, arrows) immunolabeled with Lyve-1 (a lymphatic vessel marker) and all vasculatures (green, arrow heads, VE-cadherin-positive endothelial cell).



**Figure 1. 3D image of lymphatic vessels (LV; red; arrows) in relation to all vasculatures (BV; green; arrow heads) in the pancreas.**

SunJin Lab Optical Clearing solution (Cedarlane Corporation, Burlington, NC) was used for tissue clearing. *Cdh5-cre mTmG* reporter mice, which express GFP in endothelial cells, were used to visualize all endothelial cells (all vasculatures) in the pancreas. Lyve-1 (red) was used for immunolabeling lymphatic endothelial cells.

Next, we proposed to determine the effect of inhibition of lymphangiogenesis on pancreatitis (Exp 2-3). Pancreatic lymphatic vessels play a critical role in clearing edema. Blocking VEGF-C/VEGFR3 signaling reduced lymphangiogenesis and increased edema in the pancreas (Figure 2). Given that sustained edema could lead to severe tissue injury, pancreatic lymphangiogenesis may be essential for efficient clearing of edema, inflammatory immune cells and tissue waste materials, thus critical for resolving pancreatitis.



**Figure 2. A decrease in lymphatic vessels increases pancreatic edema in cirrhosis with portal hypertension.** Rats with liver cirrhosis and portal venous congestion were injected with MAZ51, a VEGFR-3 kinase inhibitor for 3 times a week for 3 weeks. **A.** Lymphatic vessel (LV) numbers in the rat pancreas. Pancreas sections were co-stained with LYVE-1 and podoplanin antibodies to identify lymphatic vessels. **\*\*** $p < 0.01$ . **B.** H&E staining was used to evaluate the interstitial space of the pancreas (an indicator of edema). **\*** $p < 0.05$ , **\*\*\*\*** $p < 0.001$ .

- **What opportunities for training and professional development has the project provided?**  
Nothing to report.
- **How were the results disseminated to communities of interest?**  
Nothing to Report.
- **What do you plan to do during the next reporting period to accomplish the goals?**  
Following experiments will be performed for the rest of this funding period.

**Specific Aims 1:** Determine the mechanism by which pathological hemodynamic changes cause acute pancreatitis (AP).

1. Determine the role of mechano- signaling in the development of AP (Exp 1-1).  
**Plan:** We will generate a sufficient number of VE-cad-TMD mice and their WT control, perform PPVL surgery and determine effects of mechano-sensing defect on AP and pancreatic fibrosis.
2. Determine the role of immune cells in the development of AP (Exp 1-3 and 1-4).  
**Plan:** First, we assess effects of macrophage depletion on AP and pancreatic fibrosis induced by PPVL. Second, we will perform PPVL surgery on immune deficient mice (nude mice) and assess AP and pancreatic fibrosis. Third, we will isolate macrophages and T-cells from pancreas of mice given PPVL or sham operation and perform RNA sequencing to determine genes responsible for the development of AP and pancreatic fibrosis.
3. Assess a sensitizing effect of pancreatic hemodynamic changes on AP (Exp 1-5).  
**Plan:** We will administer cerulein intraperitoneally to mice with sham or PPVL surgery and AP and pancreatic fibrosis.

**Specific Aim 2:** Determine the role of lymphangiogenesis in the development and/or the resolution of acute pancreatitis (AP).

4. Determine the role of lymphangiogenesis in the development of AP (Exp 2-3).  
**Plan:** We will perform PPVL surgery on mice with control or adoviral-sVEGFR3 delivery to block lymphangiogenesis, then assess AP and pancreatic fibrosis.

I would like to thank the Department of Defense for supporting our research on acute pancreatitis, which was new to us. This grant provided us to generate numerous preliminary data and tools to study the role of lymphatics in acute pancreatitis. Using data generated from this grant, we will apply R01 application in 2022.

#### 4. IMPACT:

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

We have established tissue clearing method and immune-labeling protocol for 3-D imaging of lymphatic vessels in the pancreas. The method can be used for visualizing not only lymphatic vessels, but also other cells in the pancreas. Pathological hemodynamic changes in the pancreas caused by portal venous congestion represent a new etiology of acute pancreatitis. Other conditions, such as transarterial chemoembolization for hepatocellular carcinoma and hypercoagulation states (acquired or inherited), may cause similar hemodynamic changes in the pancreas, indicating broader implications of this study.

- **What was the impact on other disciplines?**

Nothing to report.

- **What was the impact on technology transfer?**

Nothing to report.

- **What was the impact on society beyond science and technology?**

Nothing to report.

**5. CHANGES/PROBLEMS:**

Due to the COVID19 pandemic, the hiring process for a research personnel for this study has been delayed. We also had limited access to some instruments and a slower service in core facilities. Since labs are fully opened recently, we are hopeful to complete proposed experiments during the funding period.

## 6. PRODUCTS:

- **Publications, conference papers, and presentations**

1. **Journal publications.**

Nothing to report.

2. **Books or other non-periodical, one-time publications.**

Nothing to report.

3. **Other publications, conference papers, and presentations.**

Nothing to report.

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

Nothing to report.

- **Technologies or techniques**

We have established a tissue clearing method with immune-labeling techniques for 3-D imaging of lymphatic vessels in the pancreas. The method can be used for visualizing not only lymphatic vessels, but also other cells in the pancreas. We will share this technique in our future publication.

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

Nothing to report

- **Other Products**

This project generated a new animal model of acute pancreatitis, namely partial portal vein ligation (PPVL).

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

Name:	Jain Jeong
Project Role:	<i>Postdoc associate</i>
Researcher Identifier (e.g. ORCID ID):	<i>None</i>
Nearest person month worked:	3 months
Contribution to Project:	<i>Dr. Jeong has worked on the experimental protocol to establish immune-labeling and 3-D imaging methods for pancreatic tissues and analysis of lymphangiogenesis and edema</i>
Funding Support:	DOD and NIAAA

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

None.

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

Nothing to report.

## 8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**

Nothing to report.

- **QUAD CHARTS:**

Nothing to report

**9. APPENDICES:**

None.