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Title: Evaluating Therapeutic Interventions in the SHIP-deficient Mouse Model of Crohn Disease-like Ileitis and Fibrosis

Authors and Affiliations:

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   Locations required include the Sly Lab and the animal care facility. We estimate roughly 200 meters. An elevator will be taken from the fifth floor from the Sly Lab to access the animal care facility.

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length
Number of Steps: 26
Number of Shots: 49

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Maggie Ma:** We use SHIP-deficient mice to study the mechanisms driving intestinal inflammation and fibrosis in Crohn's disease and to evaluate new drugs or therapeutic strategies to treat Crohn's disease.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

What are the most recent developments in your field of research?

1.2. **Maggie Ma:** We have used the SHIP knockout mouse model of Crohn's Disease-like ileitis in pre-clinical studies to show the efficacy of "GlycoCaging" IBD drugs for targeted drug delivery.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Figure 1*

What advantages does your protocol offer compared to other techniques?

1.3. **Kwestan Safari:** Our protocol uses SHIP-deficient mice, which naturally develop ileal inflammation and fibrosis, providing a more physiologically relevant model than traditional chemically induced or injury-based models.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.2.*

How will your findings advance research in your field?

1.4. **Kwestan Safari:** Our findings establish SHIP-deficient mice as a robust model of ileal inflammation and fibrosis, enabling mechanistic studies and preclinical testing of therapies for Crohn's disease.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the University of British Columbia Animal Care Committee

Protocol

2. FITC-Dextran Assay to Measure Epithelial Barrier Permeability

Demonstrator: Wei Jen Ma

2.1. To begin, fast the mice for 4 hours before performing the assay **[1]**.

2.1.1. WIDE: Talent removing the food from the mouse cages.

2.2. Dissolve 4 kilodalton FITC-dextran *(Fit-Cee-Dextran)* in sterile PBS to reach a final concentration of 80 milligrams per milliliter **[1-TXT]**.

2.2.1. Talent adding FITC-dextran powder into a beaker and adding PBS with a pipette. **TXT: Prepare 150 µL/mouse + 50 µL for standard curve**

2.3. Next, gently restrain each mouse and administer 150 microliters of the FITC-dextran solution via oral gavage **[1]**. Then, keep the mice without food for another 4 hours after gavage **[2].**

2.3.1. Talent holding a mouse securely by the scruff and using a curved gavage needle to deliver the solution orally.

2.3.2. Talent places the mouse into the cage without food.

2.4. After euthanizing the mouse, perform cardiac puncture using a 25-gauge needle and a 1 milliliter syringe to collect blood **[1-TXT]**.

2.4.1. Talent performing cardiac puncture using a 25-gauge needle attached to a 1 milliliter syringe. **TXT: Euthanasia: 5% isoflurane and CO_2**

2.5. Immediately add 10 microliters of acid-citrate-dextrose solution to every 100 microliters of collected blood as an anticoagulant **[1]**. Mix thoroughly by inverting the tube several times **[2]**.

2.5.1. Talent pipetting acid-citrate-dextrose solution into the collected blood.

2.5.2. Talent inverting the tube gently to mix the contents thoroughly.

2.6. Centrifuge the collected blood samples at 1,500 *g* for 10 minutes at 4 degrees Celsius **[1]**. Carefully transfer the plasma to labeled 1.7-milliliter microcentrifuge tubes **[2]**. Keep all samples on ice and shielded from light until they are ready for analysis **[3]**.

2.6.1. Talent placing the blood collection tubes into a centrifuge set to 1,500 g and 4

degrees Celsius.

2.6.2. Talent using a pipette to carefully transfer the plasma into labeled microcentrifuge tubes.

2.6.3. Talent placing the labeled tubes into an ice bucket covered with foil or a dark lid.

2.7. Now, dilute each plasma sample at 1 to 2 and 1 to 10 ratios using PBS **[1]**. Add 100 microliters of each plasma dilution into an opaque 96-well plate, placing them in duplicate wells **[2]**.

2.7.1. Talent pipetting plasma into new tubes and adding PBS.

2.7.2. Talent pipetting 100 microliters of each dilution into the 96-well plate.

2.8. Perform 1 to 2 serial dilutions of the original 80 milligrams per milliliter FITC-dextran stock using PBS to prepare different concentrations of the solution **[1-TXT]**. Add 100 microliters of each dilution into duplicate wells of the 96-well plate **[2]**.

2.8.1. Talent adding FITC-dextran using a pipette and to labeled tubes. **TXT: 3,000, 1,500, 750; 375; 187.5; 93.8; 46.9; 23.4; and 0 ng/ml**

2.8.2. Talent pipetting 100 microliters from each concentration into the corresponding duplicate wells on the 96-well plate.

2.9. Then, measure fluorescence using a microplate reader set to 485 nanometers for excitation and 535 nanometers for emission **[1]**.

2.9.1. Show the microplate reader interface as the plate is inserted.

3. Gross Pathology Assessment of the Small Intestine

Demonstrators: Wei Jen Ma and Kwestan Safari

3.1. Following blood collection for the FITC-dextran assay, carefully excise the entire small intestine from the mouse **[1]**. Gently remove any attached fat and connective tissue from the intestine **[2]**.

3.1.1. Talent gently removing the entire small intestine.

3.1.2. Talent trimming away fat and connective tissue.

3.2. Lay the cleaned intestine flat on a sheet of blank white paper to enhance visual contrast **[1]**. Examine the tissue visually for any macroscopic signs of disease, paying particular

attention to the distal 10 centimeters in SHIP *(Ship)* knockout mice, where inflammation commonly occurs **[2]**.

3.2.1. Talent spreading out the intestine carefully on a sheet of white paper.

3.2.2. Close-up shot of the intestine for signs of inflammation, focusing on the distal portion.

3.3. Align the intestine alongside a ruler to provide scale **[1]** and photograph the entire specimen to document any gross pathological findings **[2]**.

3.3.1. Talent placing a ruler next to the intestine.

3.3.2. Talent capturing a photo.

4. Histological Assessment of the Small Intestine

Demonstrators: Wei Jen Ma and Kwestan Safari

4.1. Gently flush the intestinal lumen with PBS using a syringe to remove any intestinal contents while avoiding excessive force to prevent distortion of villus and crypt architecture **[1]**.

4.1.1. Talent inserting the tip of a syringe into the open end of the intestine and slowly flushing with PBS.

4.2. Identify the region of the distal ileum most representative of gross pathology and excise approximately 1 centimeter of tissue **[1-TXT]**. Lay the excised tissue flat in a histology cassette and sandwich it between two sponges to prevent folding during fixation **[2]**.

4.2.1. Talent cutting the distal ileum. **TXT: Include affected areas and match tissues anatomically across groups**

4.2.2. Talent carefully placing the tissue flat inside the cassette and positioning sponges above and below it before closing the cassette.

4.3. For hematoxylin and eosin staining and Masson's Trichrome staining, fix the tissue in 10 percent neutral buffered formalin using 10 to 20 times the tissue volume **[1]** overnight at 4 degrees Celsius **[2-TXT]**. After fixation, transfer the cassettes to 70 percent ethanol for storage and ensure they remain completely submerged until processing **[3]**.

4.3.1. Talent pouring 10 percent formalin into labeled containers and submerging cassettes in the solution. **TXT: Ensure all cassettes are fully submerged during fixation**

4.3.2. Talent places the containers at 4 °C

4.3.3. Talent transferring the fixed cassettes into a new container filled with 70 percent ethanol.

4.4. For Alcian blue and periodic acid–Schiff staining, fix the tissue in Carnoy's solution **[1-TXT]** overnight at 4 degrees Celsius **[2]**. After fixation, transfer the cassettes to 100 percent ethanol for storage until processing **[3]**.

4.4.1. Talent submerging the histology cassettes in Carnoy's solution. **TXT: Use 5 mL/cassette or 10-20 times the tissue volume**

4.4.2. Talent places the containers at 4 °C

4.4.3. Talent transferring the cassettes from Carnoy's solution to fresh containers containing 100 percent ethanol.

4.5. Then, embed the fixed tissues in paraffin **[1]** and section them into 5-micrometer slices for subsequent staining **[2]**.

4.5.1. Talent placing fixed tissue cassettes into a paraffin embedding machine.

4.5.2. Talent using a microtome to cut 5 micrometer sections.

4.6. Score the hematoxylin and eosin-stained ileal sections using a 16-point scale according to defined histopathological criteria **[1]**.

4.6.1. LAB MEDIA: Table 1

4.7. Evaluate the Masson's trichrome-stained cross-sections using a 6-point scale based on predefined criteria **[1]**. Calculate the final fibrosis score as the median of the scores assigned by two independent reviewers who are blinded to the experimental conditions **[2]**.

4.7.1. LAB MEDIA: Table 2.

4.7.2. Show the scoring table interface showing values from two reviewers and the calculated median fibrosis score. *Videographer: Please film the computer screen*

5. Determination of the Inflammatory State by Cytokine Assessment

Demonstrators: Wei Jen Ma and Kwestan Safari

5.1. Following dissection and removal of the histology sample, collect the remaining portion of the distal 10 centimeters of the ileum **[1]**.

5.1.1. WIDE: Talent shows the remaining distal ileum tissue from the mouse specimen.

5.2. Blot the collected tissue dry with a paper towel or lint-free wipe **[1]**, then record the tissue weight using a calibrated scale **[2]**. Flash-freeze the tissue in liquid nitrogen before storing it at minus 80 degrees Celsius for future processing **[3]**.

5.2.1. Talent dabbing the tissue gently with a paper towel to remove moisture.

5.2.2. Talent placing the tissue on a balance and recording the weight.

5.2.3. Talent submerging the tissue in liquid nitrogen.

5.3. When ready for processing, retrieve the frozen tissue and always maintain it on ice.

5.3.1. Talent placing the sample on ice in a chilled container.

5.4. Then, weigh the tissue samples again to confirm accurate mass **[1]**. Prepare the Homogenization Buffer by adding protease inhibitor to PBS **[2]**. Use 10 microliters of buffer per milligram of tissue **[3]**.

5.4.1. Talent placing the thawed tissue on a scale and noting the weight.

5.4.2. Talent pipetting potease inhibitor to PBS.

5.4.3. Talent adds the correct amount of buffer into sample tubes.

5.5. Using a benchtop homogenizer, homogenize the full-thickness ileal tissue in ice-cold Homogenization Buffer **[1]**. Continue until the homogenate is uniform and free of visible tissue fragments **[2]**. Rinse the homogenizer tip thoroughly with PBS between samples to prevent cross-contamination **[3]**.

5.5.1. Talent inserting a homogenizer probe into a sample tube placed on ice and turning on the homogenizer.

5.5.2. Close-up of the homogenized sample showing a smooth, fragment-free solution.

5.5.3. Talent rinsing the homogenizer tip under running phosphate-buffered saline between sample preparations.

5.6. Centrifuge the tissue homogenates at 10,000 *g* for 10 minutes at 4 degrees Celsius **[1]**.

5.6.1. Talent placing homogenized samples into a centrifuge set to 10,000 g and 4 degrees Celsius.

5.7. Aliquot the clarified supernatants into smaller volumes to prevent freeze-thaw cycles **[1]**, then store them at minus 80 degrees Celsius for future analysis **[2-TXT]**.

5.7.1. Talent pipetting clarified supernatant into labeled aliquot tubes.

5.7.2. Talent placing the aliquots into a -80 degree Celsius freezer. **TXT: Quantify cytokines in supernatants via ELISA per manufacturer's instructions**

Results

6. Results

6.1. Histological analysis revealed significantly greater intestinal damage in SHIP-deficient mice compared to SHIP controls **[1].** Dexamethasone treatment reduced histological damage in SHIP-deficient mice, although the decrease was not statistically significant **[2].**

6.1.1. LAB MEDIA: Figure 2A and B. *Video editor: Highlight the upper image panel labelled VC in 2A and the bar labeled "VC" in 2B*

6.1.2. LAB MEDIA: Figure 2A and B**.** *Video editor: Highlight the lower image panel labelled as "Dex" in figure 2A and the "Dex" bar in Figure 2B*

6.2. Masson's trichrome staining showed significantly increased collagen deposition in SHIP-deficient mice relative to SHIP controls, indicating higher fibrosis scores **[1].** Dexamethasone treatment markedly reduced fibrosis in SHIP-deficient mice, bringing scores closer to SHIP-positive controls **[2].**

6.2.1. LAB MEDIA: Figure 2C*. Video editor: Highlight the image panel labelled as VC and the bars labelled as VC.*

6.2.2. LAB MEDIA: Figure 2C**.** *Video editor: Highlight the image panel labelled as Dex and the bars labelled as Dex.*

6.3. Alcian blue and PAS staining confirmed goblet cell hyperplasia in SHIP-deficient mice **[1],** which was visibly reduced following dexamethasone treatment **[2].**

6.3.1. LAB MEDIA: Figure 2D. *Video editor: Zoom in on the dense, darkly stained goblet cells in the SHIP-/- VC image.*

6.3.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the SHIP-/- Dex image*

6.4. IL-1β *(I-L-One-Beta)* concentrations were significantly elevated in SHIP-deficient mice relative to SHIP controls **[1].** Dexamethasone significantly reduced IL-1β concentrations in SHIP-deficient mice **[2].**

6.4.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the bar labeled "VC" in the SHIP-/- group in the IL-1β panel, which is higher than the SHIP+/+ VC bar.*

6.4.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the "Dex" bar in the SHIP-/- group*

6.5. MPO *(M-P-O)* concentrations were significantly elevated in SHIP-deficient mice compared to SHIP controls **[1],** and this increase was reduced by dexamethasone

without reaching statistical significance **[2].**

6.5.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the "VC" bar in the SHIP-/- group in the MPO panel*

6.5.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the SHIP-/- "Dex" bar.*

6.6. LCN-2 *(L-C-N-Two)* concentrations were also elevated in SHIP-deficient mice, but high values were not consistently reduced by dexamethasone **[1].**

6.6.1. LAB MEDIA: Figure 3B**.** *Video editor: Highlight the LCN-2 panel*.

6.7. IL-1β concentrations positively correlated with histological damage scores and fibrosis scores, indicating a link between inflammation and tissue injury **[1].** Histological damage scores positively correlated with fibrosis scores, suggesting that tissue damage and fibrotic remodeling progressed in parallel **[2].**

6.7.1. LAB MEDIA: Figure 4A and B. Video editor: *Emphasize the upward-sloping trend of data points showing the correlation between IL-1ϐ and damage scores.*

6.7.2. LAB MEDIA: Figure 4C**.** *Video editor: Show the clear upward trend in the scatter plot*

Pronunciation Guide

1. **kilodalton**
   Pronunciation link: https://www.merriam-webster.com/dictionary/kilodalton
   IPA: /ˌkaɪloʊˈdæltən/
   Phonetic spelling: KY-loh-DAL-tuhn
2. **FITC-dextran**
   - *FITC* (as an acronym — "fluorescein isothiocyanate") – often spelled out as **F-I-T-C**
   - *dextran*
     Pronunciation link: https://www.merriam-webster.com/dictionary/dextran
     IPA: /ˈdɛkstrən/
     Phonetic spelling: DEK-struhn
3. **gavage**
   Pronunciation link: https://www.merriam-webster.com/dictionary/gavage
   IPA: /gəˈvɑʒ/
   Phonetic spelling: guh-VAHZH
4. **acid-citrate-dextrose**
   - *citrate*
     Pronunciation link: https://www.merriam-webster.com/dictionary/citrate

IPA: /ˈsɪtreɪt/
Phonetic spelling: SIT-rayt

- *dextrose*
  Pronunciation link: https://www.merriam-webster.com/dictionary/dextrose
  IPA: /ˈdɛkstroʊs/
  Phonetic spelling: DEK-strohs

5. **microcentrifuge**
   Pronunciation link: https://www.merriam-webster.com/dictionary/microcentrifuge
   IPA: /ˌmaɪkroʊˈsɛntrəˌfjuːdʒ/
   Phonetic spelling: MY-kroh-SEN-truh-fyooj
6. **opaque**
   Pronunciation link: https://www.merriam-webster.com/dictionary/opaque
   IPA: /oʊˈpeɪk/
   Phonetic spelling: oh-PAYK
7. **ileum**
   Pronunciation link: https://www.merriam-webster.com/dictionary/ileum
   IPA: /ˈiːliəm/
   Phonetic spelling: EE-lee-əm
8. **villus**
   Pronunciation link: https://www.merriam-webster.com/dictionary/villus
   IPA: /ˈvɪləs/
   Phonetic spelling: VIL-əs
9. **crypt (in crypt architecture)**
   Pronunciation link: https://www.merriam-webster.com/dictionary/crypt
   IPA: /krɪpt/
   Phonetic spelling: kript
10. **Carnoy's (as in Carnoy's solution)**
    No confirmed link found for "Carnoy's" as a proper noun in Merriam-Webster specifically.
    Approximated IPA: /kɑrˈnwɑɪz/
    Phonetic spelling: kar-NOYZ
11. **paraffin**
    Pronunciation link: https://www.merriam-webster.com/dictionary/paraffin
    IPA: /ˈpærəfɪn/
    Phonetic spelling: PAR-uh-fin
12. **histopathological**
    Pronunciation link: https://www.merriam-webster.com/dictionary/histopathological
    IPA: /ˌhɪstoʊˌpæθəˈlɑdʒɪkəl/
    Phonetic spelling: HIS-toh-path-uh-LOJ-i-kuhl
13. **median**
    Pronunciation link: https://www.merriam-webster.com/dictionary/median
    IPA: /ˈmiːdiən/
    Phonetic spelling: MEE-dee-uhn

14. **hyperplasia**
    Pronunciation link: https://www.merriam-webster.com/dictionary/hyperplasia
    IPA: /ˌhaɪpərˈpleɪʒə/
    Phonetic spelling: hy-PER-play-zhuh
15. **dexamethasone**
    Pronunciation link: https://www.merriam-webster.com/dictionary/dexamethasone
    IPA: /ˌdɛksəˈmɛθəzʌn/
    Phonetic spelling: dek-suh-METH-uh-sawn
16. **fibrosis**
    Pronunciation link: https://www.merriam-webster.com/dictionary/fibrosis
    IPA: /faɪˈbroʊsɪs/
    Phonetic spelling: fy-BROH-sis
17. **MPO (as acronym, often "M-P-O")**
    Typically spelled as separate letters: M-P-O
18. **LCN-2**
    Similarly, read as "L-C-N-two"
19. **interleukin**
    Pronunciation link: https://www.merriam-webster.com/dictionary/interleukin
    IPA: /ˌɪntərˈluːkɪn/
    Phonetic spelling: IN-tur-LOO-kin