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Title: Functional Assessment of Intestinal Permeability and Neutrophil Transepithelial Migration in Mice Using a Standardized Intestinal Loop Model

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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. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 18
Number of Shots: 29

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Charles Parkos:** The intestinal loop model is a widely adaptable and physiological way to measure how leaky the gut is and how leukocytes migrate in the intestine in vivo in mice. This method helps to better understand mechanisms underlying increased intestinal permeability and pathologic intestinal inflammation.

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

1.2. **Anny-Claude Luissint:** This model employs a well-vascularized, exteriorized bowel segment, either of the ileum or proximal colon, for examination of intestinal barrier function or immune cell recruitment in mice.

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

OPTIONAL:

1.3. **Charles Parkos:** The technique can be employed to aid in our understanding of mechanisms regulating gut barrier function and pathological intestinal inflammation, as seen in ulcerative colitis and Crohn's disease.

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

1.4. **Anny-Claude Luissint:** With its broad applications, the intestinal loop model can provide insight into diseases resulting in or from a leaky gut barrier as well as the resulting inflammatory response.

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

1.5. **Kevin Boerner:** Struggles can be overcome by practicing the surgical steps. It is critical to gently exteriorize a fully vascularized intestinal segment and select appropriate locations for the ligations to avoid bleeding.

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

1.6. **Kevin Boerner:** The intestinal loop model is a microsurgical technique. Thus, as with other surgical procedures, it benefits greatly from visual presentation and a step-by-step demonstration of the method.

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

Ethics Title Card

1.7. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Michigan.

Protocol

2. Generation of the ileal loop

2.1. Begin by scrubbing the fur of the abdominal midline with alcohol swabs or a gauze sponge soaked with 70% ethanol **[1]**. Do not wet a wide area of fur with alcohol to prevent hypothermia **[2]**.

2.1.1. WIDE: Establishing shot of talent scrubbing the animal.

2.1.2. Appropriate area scrubbed with ethanol.

2.2. Using scissors, perform a midline laparotomy. Make a horizontal incision in the middle of the abdomen and expose the peritoneum, taking care to not injure intra-abdominal organs **[1]**. Place pre-cut wet cotton gauze over the exposed intra-abdominal cavity **[2]**.

2.2.1. Talent performing the laparotomy.

2.2.2. Talent placing the gauze over the intra-abdominal cavity.

2.3. Use wet cotton swabs to mobilize and exteriorize the caecum and carefully place it on wet cotton gauze **[1]**. Then, mobilize and gently exteriorize the ileum **[2]**.

2.3.1. Talent exteriorizing the caecum.

2.3.2. Talent exteriorizing the ileum.

2.4. Deploy at least 6 centimeters of terminal ileum on the wet cotton gauze without disrupting the mesenteric vessels and blood supply **[1]**. Keep the exposed tissues moist at all times with warm HBSS **[2]**.

2.4.1. Talent deploying the terminal ileum.

2.4.2. Talent wetting the exposed tissue.

2.5. Identify the major artery supplying the ileum in the mesentery, close to the caecum. Then, locate two ligation sites in the mesentery that are free of critical blood vessels **[1]**.

2.5.1. Major artery and two ligation sites.

2.6. Firmly grab the terminal ileum with blunt tissue forceps and use fine tip forceps to fenestrate the mesentery, avoiding blood vessels. Place silk suture across the perforation and tie a surgical knot to create the first ligation **[1]**. *Videographer: This step is important!*

2.6.1. Talent creating the first ligation.

2.7. Use the ruler to measure 4 centimeters away from the first ligature and create the second ligature **[1]**. Carefully cut next to each ligation with fine scissors to isolate the

4-centimeter ileal loop, keeping the blood supply and mesenteric membrane intact **[2]**. *Videographer: This step is difficult and important!*

2.7.1. Talent creating the second ligation.

2.7.2. Talent isolating the ileal loop.

2.8. Gently flush the content of the ileal loop segment with warm HBSS using a flexible yellow feeding tube attached to a 10-milliliter syringe **[1]**. Ligate the two cut ends of the flushed ileal loop with silk suture **[2]**. *Videographer: This step is important!*

2.8.1. Talent flushing the ileal loop segment with HBSS.

2.8.2. Talent ligating the ends of the ileal loop.

2.9. Use a 1-milliliter syringe with a 30-gauge needle to slowly inject 250 microliters of reagent such as FITC-dextrans or chemokine into the intestinal lumen. The ileal loop will inflate, causing a moderate distension of the mucosa **[1-TXT]**.

2.9.1. Talent injecting the reagent (in this case 4kDa FITC-dextran) into the intestinal lumen. **TEXT: Inject on the opposite side of the mesenteric artery**

2.10. Using wet cotton swabs, gently put back the ileal loop, proximal ileum, and caecum **[1]**. Close the abdominal wall using a needle holder, anatomical forceps, and 3.0 non-absorbable silk sutures with a reverse cutting needle **[2]**.

2.10.1. Talent putting back the organs.

2.10.2. Talent closing the abdominal wall.

2.11. Place the animal in a temperature-regulated anesthesia chamber for the incubation period **[1]**.

2.11.1. Talent placing the mouse in the anesthesia chamber.

3. Generation of the proximal colon loop (pcLoop)

3.1. Prepare the mouse for surgery and exteriorize the caecum as previously demonstrated **[1]**.

3.1.1. Prepared mouse with exteriorized caecum.

3.2. Using wet cotton swabs, exteriorize the entire ileum and place it on top of a wet cotton gauze **[1]**. Identify the proximal colon and the blood supply located in the mesocolon. Mobilize the proximal colon and create the first ligature in an area free of vessels in the mesocolon at about 0.5 centimeters distal from the caecum **[2]**. *Videographer: This step is important!*

3.2.1. Talent exteriorizing the ileum.

3.2.2. Talent creating the first ligature.

3.3. Measure 2 centimeters from the first ligature and create a second ligature at an area free of blood supply in the mesocolon **[1]**. Using fine scissors, carefully cut next to each ligation to isolate a 2-centimeter long pcLoop **[2]**. *Videographer: This step is difficult and important!*

3.3.1. Talent creating the second ligature.

3.3.2. Talent cutting next to the ligations.

3.4. Gently flush the pcLoop with warm HBSS to remove feces using a flexible yellow feeding tube attached to a 10-milliliter syringe **[1]**, then ligate the two cut ends of the flushed pcLoop using silk suture **[2]**. *Videographer: This step is important!*

3.4.1. Talent flushing the pcLoop.

3.4.2. Talent ligating the 2 cut ends of the loop.

3.5. Use a 1-milliliter syringe with a 30-gauge needle to slowly inject 200 microliters of reagent such as FITC-dextran or chemokine into the intestinal lumen. The pcLoop will inflate, causing a moderate distension of the mucosa **[1-TXT]**.

3.5.1. Talent injecting the reagent (in this case 4kDa FITC-dextran). **TEXT: Inject on the opposite side of the mesenteric artery**

3.6. Use wet cotton swabs to gently put back the ligated pcLoop, ileum, and caecum **[1]**. Close the abdominal wall using a needle holder, anatomical forceps, and 3.0 non-absorbable silk sutures with a reverse cutting needle **[2]**.

3.6.1. Talent putting back the organs.

3.6.2. Talent closing the abdominal wall.

3.7. Place the animal in a temperature-regulated anesthesia chamber for the incubation period **[1]**.

3.7.1. Talent placing the mouse in the anesthesia chamber.

Results

4. Results: JAM-A regulates intestinal permeability in vivo and promotes LTB_4-dependent recruitment of PMN into the lumen of the pcLoop

4.1. In order to verify the accuracy of the iLoop model for the assessment of intestinal permeability, a FITC-dextran pcLoop assay was performed to evaluate the role of TJ-associated protein JAM-A *(pronounce 'jam-A')* in the regulation of intestinal barrier function in vivo **[1]**.

4.1.1. LAB MEDIA: Figure 3.

4.2. With the pcLoop model, a 2.5-fold increase in FITC-dextran serum levels was quantified in *Jam*-a-null mice compared to controls **[1]**. Similar results were obtained with mice harboring selective loss of JAM-A on intestinal epithelial cells **[2]**.

4.2.1. LAB MEDIA: Figure 3 A.

4.2.2. LAB MEDIA: Figure 3 B.

4.3. The pcLoop model was used to study polymorphonuclear neutrophil, or PMN, recruitment into the intestinal mucosa and subsequent transepithelial migration in vivo. The number of PMN in the luminal content of the pcLoop was quantified by flow cytometry analysis **[1]**.

4.3.1. LAB MEDIA: Figure 4 A.

4.4. The number of PMN present in a segment of the proximal colon was low under physiological conditions **[1]**. Pretreatment with pro-inflammatory cytokines TNF-alpha and IFN-gamma prior to surgery resulted in augmented numbers of PMN recruited in the pcLoop lumen **[2]**.

4.4.1. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the black squares.*

4.4.2. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the white circles.*

4.5. Administration of the PMN chemoattractant LTB_4 *(pronounce 'L-T-B-4')* led to a dramatic increase in PMN counts **[1]**.

4.5.1. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the black circles.*

4.6. Immunohistochemical staining of PMN in the colonic mucosa corroborate the elevated recruitment of PMN following stimulation with cytokines and LTB_4 **[1]**.

4.6.1. LAB MEDIA: Figure 4 C.

4.7. The contribution of JAM-A to PMN transepithelial migration was studied using the pcLoop model on mice harboring selective loss of JAM-A on intestinal epithelial cells **[1]**. Loss of epithelial JAM-A led to a reduced number of transmigrated PMN in the colonic lumen compared to littermate controls **[2]**.

4.7.1. LAB MEDIA: Figure 4 D.

4.7.2. LAB MEDIA: Figure 4 D. *Video Editor: Emphasize the black circles.*

Conclusion

5. Conclusion Interview Statements

5.1. **Kevin Boerner:** A crucial aspect of this technique is to remember to preserve the blood supply to the exteriorized intestinal segment when performing the surgery.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.7, 3.3.*

5.2. **Kevin Boerner:** Following this protocol, other methods such as an intestinal permeability assay or a neutrophil transepithelial migration assay can be performed to investigate the loss of barrier integrity and intestinal inflammation in vivo.

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