Chapter 22

Bacterial Mediated Gastrointestinal Inflammation

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Abstract

Mouse models have proven to be a key approach in our understanding of the etiology and physiology underlying bacterial mediated gastrointestinal inflammation. Generally, these models are based on the inoculation of genetically susceptible mice with either commensal or pathogenic bacteria to elicit an inflammatory response. Here, we describe models of acute and chronic gastrointestinal inflammation using interleukin 10-deficient (Il10\(^{-/-}\)) mice colonized with the pathogenic Campylobacter jejuni strain 81-176 or the commensal Escherichia coli strain NC 101.

Key words Gastrointestinal inflammation, Il10\(^{-/-}\) mouse, Campylobacter jejuni, Escherichia coli

1 Introduction

The human colon serves as a host for tens of thousands of bacterial species. It is estimated that these bacteria number into the hundreds of trillions [1, 2]. These diverse bacterial communities benefit by occupying their host who provides a source of nutrients. The host in turn derives physiological benefits provided by the bacteria [2]. Commensal bacteria have been found to play a direct role in biological processes including development of the mucosal immune system and nutrient metabolism [3]. Although we derive significant benefit from the presence of the gut microbiota, under certain circumstances the relationship between bacteria and human can go awry [4]. It is now well established that the gut microbiota plays a role in the etiology of chronic inflammatory bowel diseases, such as Crohn’s disease and ulcerative colitis [5], although the mechanisms underlying these processes remain to be elucidated.

In addition to understanding the role of commensal bacteria in physiology and chronic diseases of the gut, of significant importance is the investigation of mechanisms underlying acute gastrointestinal infection by pathogenic bacteria. Gastrointestinal enteritis induced by pathogenic bacteria is a major threat worldwide. Annually, millions of people are infected every year with food- and
waterborne bacterial pathogens, including *Campylobacter jejuni*, *Salmonella Typhimurium*, and certain strains of *Escherichia coli* [6, 7]. Billions of dollars are spent annually on efforts aimed at the prevention and treatment of these diseases. Despite the prevalence of pathogenic gastroenteritis worldwide, we still have a limited view as to the mechanisms underlying bacterial pathogenesis. This limited view is partly due to a lack of appropriate models with which to study these processes.

As part of ongoing research to identify mechanisms by which bacteria promote gastrointestinal disease, mouse models of bacterial induced colitis have been established [8, 9]. The development of technologies including germfree/gnotobiotic and gene knockout mice has been integral to this process [8]. The combination of these technologies has allowed us to model the behavior of the gut under conditions that closely recapitulate those found in patients with inflammatory bowel diseases, as well as acute conditions such as pathogen-induced gastroenteritis [9–11].

A predominant model used in the investigation of bacterial mediated gut inflammation is established by inoculating a genetically compromised host, commonly the germfree *Il10*−/− mouse, with either pathogenic or commensal (nonpathogenic) bacteria [12].

## 2 Materials

### 2.1 Animals
1. *Il10*−/−129/SvJ mice under germfree (GF) or specific pathogen-free (SPF) housing between 8 and 12 weeks of age (see Note 1).
2. Provide mice a standard chow diet and water *ad libitum*.

### 2.2 Bacteria
1. *C. jejuni*: strain 81-176.
2. *E. coli*: strain NC 101.

### 2.3 Culture Materials
2. *E. coli*: Lysogeny Broth (LB) agar and LB liquid broth.
3. GasPak Jar (GasPak EZ Campy container system).
4. Petri dishes.
5. L-shape cell spreaders.
6. Inoculation loops.

### 2.4 Antibiotics
1. Streptomycin.
2. Gentamicin.
4. Ciprofloxacin.
2.5 Gavage Instruments
1. PS20 gavage needles.
2. 1 ml syringes.

2.6 Other Materials
1. Spectrophotometer.
2. Cuvettes.
3. Pipettes: 20; 200; and 1,000 μl.
4. Pipette tips: 20; 200; and 1,000 μl.

3 Methods

3.1 Antibiotic Treatment for SPF Mice (Not Required for GF Mice)
1. Prepare the following antibiotic cocktail: Streptomycin 2 g/L, bacteriocin 1 g/L, gentamicin 0.5 g/L, and ciprofloxacin 0.125 g/L in distilled water.
2. Stir the solution at room temperature until all antibiotics dissolve.
3. Provide mice with antibiotic cocktail in place of their drinking water.
4. Replace the cocktail every 2 days with a freshly made stock.
5. After 7 days of antibiotic treatment, transfer the mice to clean cages.

3.2 C. jejuni Infection
1. Two days before C. jejuni infection, pipette 70 μl of C. jejuni stock on C. jejuni-selective blood plates and spread the bacteria using an L-shape cell spreader.
2. Place the plates into a GasPak EZ Campy container system at 37–42 °C for 2 days with 1 gas-generating sachet. C. jejuni grows optimally under microaerobic conditions, specifically 85 % N₂, 10 % CO₂, and 5 % O₂.
3. Collect the top layer of C. jejuni from the plates and transfer into Columbia broth using a cell scraper.
4. Measure the optical density (OD₆₀₀) of the C. jejuni in Columbia broth to generate an estimate of colony-forming units (CFU).
5. Hold mice firmly and orally gavage 200 μl of Columbia broth containing ~5 × 10⁹ CFU/ml of C. jejuni (10⁹ CFU) per mouse.

3.3 E. coli Infection
1. Streak E. coli NC 101 on an LB plate.
2. Place the plate at 37 °C in an aerobic incubator overnight.
3. After colonies have formed, pick one colony into LB broth and incubate at 37 °C with shaking overnight at 250–300 rpm.
4. After overnight incubation, spin down the culture at 200×g and resuspend the pellet in fresh LB broth.
5. Measure the OD$_{600}$ of the *E. coli* culture to estimate CFU (see Note 2).

6. Hold mice firmly and gavage 200 μl of $5 \times 10^8$ CFU/ml *E. coli* ($1 \times 10^8$) per mouse.

### 3.4 Evaluating Intestinal Inflammation

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<tr>
<th>Inflammation score</th>
<th>Histopathological features</th>
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| 0                  | No immune cell infiltration  
No epithelial hyperplasia  
Presence of goblet cells |
| 1                  | Infiltrating small number of immune cells into focal or partial lamina propria  
Minimal epithelial hyperplasia  
Presence of goblet cells |
| 2                  | Infiltrating extensive immune cells into partial lamina propria  
Obvious epithelial hyperplasia  
Mild loss of goblet cells |
| 3                  | Infiltrating profound immune cells into entire lamina propria  
Marked epithelial hyperplasia  
Moderate to marked loss of goblet cells  
Crypt architecture distortion |
| 4                  | Crypt abscesses  
Crypt ulcerations  
Transmural inflammation  
Absence of crypts |

1. *C. jejuni*-induced intestinal inflammation may be assessed 2 weeks post infection in germfree *Il10−/−* mice and in 2–4 weeks in antibiotic-treated SPF mice.

2. *E. coli*-induced intestinal inflammation may be evaluated 4 weeks post infection in germfree *Il10−/−* mice.

3. At the end of the experiment, euthanize mice using CO$_2$ asphyxiation (see Note 3).

4. Swiss roll preparation: Remove the colon, flush out the stool with PBS, and splay the colon longitudinally on a piece of Whatman filter paper. Roll the colon beginning from the distal to the proximal end so that it resembles a Swiss roll. Secure the roll with a 27–30 gauge needle to keep it from unraveling.

5. Fix the colon in 10 % buffered formalin overnight at 4 °C and process for H&E histological evaluation.

6. If desired, collect colon, mesenteric lymph node, and spleen tissues for further processing for RNA, protein, or bacterial culture.
7. If desired, process blank tissue slides to visualize bacterial invasion into the colon by fluorescence in situ hybridization.

8. Intestinal inflammation is assessed in H&E histology slides based on the degree of lamina propria immune cell infiltration, goblet cell depletion, architectural distortion, crypt hyperplasia, ulceration, and abscesses using a standard score from 0 to 4 (Table 1).

4 Notes

1. This model has been tested with C57BL/6 and 129/SvJ mice. C57BL/6 mice are more resistant to bacterial induced colitis using this model under most housing conditions; therefore, it is suggested that 129/SvJ mice be used in this model.

2. Generally, an OD\textsubscript{600} reading of 1 equals 2–8 \times 10^8 CFU/ml \textit{E. coli}.

3. It is necessary to seek permission from all relevant animal regulatory bodies (i.e., IACUC) prior to beginning experiments. Note that methods of euthanasia vary between institutions. Be sure to use the methods authorized by your institution.

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References


