Chapter 22

Bacterial Mediated Gastrointestinal Inflammation

Joshua Uronis and Xiaolun Sun

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

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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

1. *C. jejuni*: Mueller Hinton agar, *Campylobacter* selective blood plates (Remel), Columbia broth.
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.
7. Cell scrapers.

2.4 Antibiotics

1. Streptomycin.
2. Gentamicin.
3. Bacteriocin.
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 % N_2, 10 % CO_2, and 5 % O_2.
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 (OD_{600}) 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 ~5×10^9 CFU/ml of *C. jejuni* (10^9 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.

Table 1
Score system for evaluating colonic inflammation using H&E tissue slides

Inflammation score	Histopathological features
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

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 5×10^8 CFU/ml *E. coli* (1×10^8) per mouse.

3.4 Evaluating Intestinal Inflammation

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_{600} reading of 1 equals $2–8 \times 10^8$ CFU/ml *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

1. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 104(34):13780–13785
2. Neish AS (2009) Microbes in gastrointestinal health and disease. Gastroenterology 136(1):65–80
3. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007) The human microbiome project. Nature 449(7164):804–810
4. Haller D, Jobin C (2004) Interaction between resident luminal bacteria and the host: can a healthy relationship turn sour? J Pediatr Gastroenterol Nutr 38(2):123–136
5. Mantovani A (2005) Cancer: inflammation by remote control. Nature 435(7043):752–753
6. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011) Foodborne illness acquired in the United States–major pathogens. Emerg Infect Dis 17(1):7–15
7. Blaser MJ (1997) Epidemiologic and clinical features of Campylobacter jejuni infections. J Infect Dis 176(Suppl 2):S103–S105
8. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB (1998) Resident enteric bacteria are

necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 66(11):5224–5231
9. Lippert E, Karrasch T, Sun X, Allard B, Herfarth HH, Threadgill D, Jobin C (2009) Gnotobiotic IL-10; NF-kappaB mice develop rapid and severe colitis following Campylobacter jejuni infection. PLoS One 4(10):e7413
10. Sun X, Threadgill D, Jobin C (2012) Campylobacter jejuni induces colitis through activation of mammalian target of rapamycin signaling. Gastroenterology 142(1):86–95.e85
11. Patwa LG, Fan TJ, Tchaptchet S, Liu Y, Lussier YA, Sartor RB, Hansen JJ (2011) Chronic intestinal inflammation induces stress-response genes in commensal Escherichia coli. Gastroenterology 141(5):1842–1851, e1841–1810
12. Kim SC, Tonkonogy SL, Karrasch T, Jobin C, Sartor RB (2007) Dual-association of gnotobiotic IL-10$^{-/-}$ mice with 2 nonpathogenic commensal bacteria induces aggressive pancolitis. Inflamm Bowel Dis 13(12):1457–1466