Interleukin-10 Knockout Mice Do Not Reliably Exhibit Macroscopic Inflammation: A Natural History Endoscopic Surveillance Study

Seung Young Kim1 · Jae Ho Park2 · Gabriela Leite3 · Mark Pimentel3,4 · Ali Rezaie3,4

Received: 7 March 2022 / Accepted: 6 February 2023 / Published online: 17 March 2023
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2023

Abstract
Background Interleukin (IL)-10 knockout (KO) mice, a model for inflammatory bowel disease (IBD), develop chronic enterocolitis due to an aberrant immune response to enteric antigens. Endoscopy, the gold standard for evaluation of human mucosal health, is not widely available for murine models.

Aims To assess the natural history of left-sided colitis in IL-10 KO mice via serial endoscopies.

Methods BALB/cJ IL-10 KO mice underwent regular endoscopic assessments from 2 up to 8 months of age. Procedures were recorded and blindly evaluated using a 4-component endoscopic score: mucosal wall transparency, intestinal bleeding, focal lesions and perianal lesions (0–3 points each). An endoscopic score ≥ 1 point was considered as the presence of colitis/flare.

Results IL-10 KO mice (N = 40, 9 female) were assessed. Mean age at first endoscopy was 62.5 ± 2.5 days; average number of procedures per mouse was 6.0 ± 1.3. A total of 238 endoscopies were conducted every 24.8 ± 8.3 days, corresponding to 124.1 ± 45.2 days of surveillance per mouse. Thirty-three endoscopies in 24 mice (60%) detected colitis, mean endoscopy score 2.5 ± 1.3 (range: 1–6.3). Nineteen mice (47.5%) had one episode of colitis and 5 (12.5%) had 2–3 episodes. All exhibited complete spontaneous healing on subsequent endoscopies.

Conclusions In this large-scale endoscopic surveillance study of IL-10 KO mice, 40% of mice did not develop endoscopic left-sided colitis. Furthermore, IL-10 KO mice did not exhibit persistent colitis and universally exhibited complete spontaneous healing without treatment. The natural history of colitis in IL-10 KO mice may not be comparable with that of IBD in humans and requires careful consideration.

Keywords Colitis · IL-10 knockout mouse model · Endoscopy

Introduction

Interleukin-10 (IL-10) is an immunoregulatory cytokine that is essential for the maintenance of intestinal homeostasis, and is secreted by a wide range of immune cells including macrophages, dendritic cells, and T cells [1]. IL-10 targets both innate and adaptive immune responses and helps to preserve tissue integrity and promote tissue homeostasis through its anti-inflammatory, anti-apoptotic, and tissue-regenerating properties [2].

The IL-10 knockout (KO) mouse is a well-studied model of inflammatory bowel disease (IBD) that develops spontaneous Crohn’s disease-like intestinal inflammation, which is mediated by a Th-1 type cytokine profile and requires an intestinal bacterial microbiota to develop [3]. Colitis in IL-10-deficient mice is characterized by histological findings similar to those in human IBD [4, 5]. In general, IL-10 KO mice develop colitis, which is not present in the neonatal...
period, by 2–3 months of age under specific pathogen-free (SPF) conditions [6]. The discontinuous and transmural inflammatory lesions are characterized by inflammatory cell infiltrates into the lamina propria and submucosa, epithelial hyperplasia, mucin depletion, crypt abscess, ulcers, and thickening of the intestinal wall [5, 7].

In this murine colitis model, the severity of intestinal inflammation is mainly determined by histological examination. However, longitudinal assessment via histological examination is not possible as it requires colon harvest after euthanasia. Endoscopic examination in mice has proven a useful research tool and provides for objective assessment of colonic inflammation as well as repeated evaluations of individual animals over time [8, 9]. We have shown that murine colonoscopy has substantial interobserver reliability [10]. Therefore, direct serial examination with endoscopy represents a useful tool to elucidate the natural history of colitis in the IL-10 KO mouse model, and considering that animals can be scoped safely and repeatedly without a need for euthanasia, the number of animals needed per study can be potentially reduced. In this study, we assessed the course of the development of left-sided colitis in BALB/cJ IL-10 KO mice via serial endoscopies.

### Methods

#### Animals

Adult male and female homozygous BALB/cJ IL-10−/− mice (C.129P2(B6)-IL10tm1Cgn/J, Jackson Laboratories, Bar Harbor, ME) were housed in a vivarium in an accredited animal facility. Animals were maintained in a specific pathogen free (SPF) environment, where immune competent rodents (4 weeks old) were used as sentinels for biological agent screening for mouse hepatitis virus (MHV), murine norovirus (MNV), Theiler's murine encephalomyelitis virus (TMEV), mouse rotavirus (EDIM), Sendai virus, Mycoplasma pulmonis, pneumonia virus of mice (PVM), reovirus (REO3), lymphocytic choriomeningitis virus (LCMV), Ectromelia virus, K virus, Lactate Dehydrogenase Elevating Virus (LDV), mouse adenovirus (MAV 1, MAV 2), murine cytomegalovirus (MCMV), mouse thymic virus (MTV), Polyomavirus, Encephalitozoon cuniculi, Hantaan virus, and Clostridium perfringens type A. Rodentolepis nana, Helicobacter spp. (bilis, ganmani, hepaticus, mastomyrurus rodentium, typhlonius), Syphacia obvelata, Syphacia muris, and Aspiculuris tetraptera by fecal PCR; mouse parvovirus (MPV 1–5), minute virus of mice (MVM) by spleen PCR; Streptobacillus moniliformis by oral swab PCR; and Mycopetes, Radfordia/Myobia by fur swab PCR. Each side of the mouse racks had a dedicated sentinel. Quarterly PCR panels were also performed in swabs collected from exhaust air dust plenums for biological screening of MHV, MVM, MPV1-5, TMEV, EDIM, Sendai, M. pulmonis, PVM, REO3, LCMV, Ectromelia, C. piliforme, Helicobacter spp. (bilis, ganmani, hepaticus, mastomyrurus rodentium, typhlonius), S. obvelata, S. muris, A. tetraptera, Mycopetes and Radfordia/Myobia.

Animals were maintained under barrier-protected conditions with a 12 h/12 h light/dark cycle, and bedding was changed weekly. To ensure a homogeneous microbial environment, dirty cage bedding was transferred between cages. Mice were housed at a maximum of 5 animals per cage and consumed a standard chow diet (24.65% calories from protein, 13.2% from fat and 62.14 from carbohydrates) (PicoLab® Rodent Diet 20 5053, LabDiet, MO) and water ad libitum. The study was approved by the Cedars-Sinai Institutional Animal Care and Use Committee (IACUC #7304).

### Endoscopic Examination

Mice underwent regular endoscopic assessments from 2 months of age to up to 8 months of age. No bowel preparation was used prior to endoscopies. To avoid the presence of excess fecal content during endoscopic examination, bowel movements were induced via stimulation of the anus under manual restraint before anesthesia. An isoflurane anesthetic gas (1–5%) mixture (carrier gas: compressed oxygen) was used to induce anesthesia in a chamber, and once the respiratory rate had slowed to approximately one breath per second, the animals were removed from the induction chamber and maintained under sedation using a nose cone anesthesia. A rigid pediatric cystoscope (Olympus A37027A) was used to assess the intestinal mucosa up to the splenic flexure. During the endoscopic examination, room air (approximately 1 cc) was insufflated through the cystoscope using a 5 mL syringe attached to one of the channels. No water irrigation was performed. All endoscopies were recorded and interpreted blindly by two gastroenterologists with expertise in animal model endoscopies.

Endoscopies were scored using a mouse endoscopic scoring system devised by Kodani et al. [9] (Supplemental Table 1). In this scoring system, two major components are used: (1) assessment of the extent and severity of colorectal inflammation (based on perianal findings, transparency of the wall, mucosal bleeding, and focal lesions), and (2) numerical sorting of clinical cases by their pathological and research relevance based on decimal units with assigned categories of observed lesions and endoscopic complications (decimal identifiers).

The score for each component of colorectal inflammation varied from 0 to 3 points. An endoscopic score ≥ 1 point was considered to indicate the presence of colitis/flare.
Endoscopic surveillance data was recorded for each mouse throughout the study.

**Results**

**Endoscopic Examination of IL-10 KO mice**

A total of 40 IL-10 KO mice (31 male, 9 female) were assessed via serial endoscopies. Mean age at the time of first endoscopy was 62.5 ± 2.5 days and the average number of endoscopic examinations per animal was 6.0 ± 1.3. A total of 238 endoscopies were conducted every 24.8 ± 8.3 days, cumulatively corresponding to 4,964 days (13.6 years) of endoscopic surveillance. During surveillance periods (124.1 ± 45.2 days per mouse), 16/40 mice never developed colitis within reach of the endoscope (40%), and 24/40 mice (60%) exhibited at least one episode of colitis (colitis score ≥ 1) with a mean endoscopy score of 2.5 ± 1.3 (range: 1–6.3).

The most common finding was erosion or erythema. Mean age at first episode of colitis was 83.7 ± 37.5 days. The prevalence of endoscopic colitis appeared to decrease with age as 22.7% (20/88) of endoscopies exhibited colitis in mice less than 80 days old, as compared to 10.1% (7/69) in mice between 81 and 120 days old, and 7.6% (6/79) in mice older than 120 days.

**Pattern of Colitis in IL-10 KO Mice**

Nineteen mice (19/40, 47.5%) showed a single flare and 5 mice (5/40, 12.5%) showed 2 or 3 flares. The colitis pattern was similar between males and females with 45.2% (14/31) and 55.6% (5/9) having a single colitis episode and 12.9% (4/31) and 11.1% (1/9) having multiple episodes of colitis, respectively (Supplemental Table 2). There was no difference in distribution of colitis pattern between the two groups. All mice with colitis exhibited complete spontaneous healing on subsequent endoscopies (Fig. 1). Three distinct endoscopic phenotypes were observed among IL-10 KO mice (Fig. 2). No mice exhibited perianal disease.

![Fig. 1 Successive endoscopic images of a mouse colon (mouse age is shown in days). Erosions, loss of vascular markings and mucosal bleeding/friability on day 71 were found to have spontaneously resolved without treatment or intervention on follow-up endoscopies. Some fecal matter is visible in the images from days 71 and 113 (yellow material).](image)

![Fig. 2 Schematic graph showing three distinct phenotypes of BALB/cJ IL-10 KO mice during endoscopic surveillance.](image)
Discussion

Repeated blind endoscopic assessments of 40 BALB/cJ IL-10 KO mice, which corresponded to a total of 13.9 years of surveillance, revealed that only 60% of mice developed transient left-sided colitis with a variable and unpredictable recurrence pattern. However, the most striking finding of our study is the universal spontaneous healing of colitis in IL-10 KO mice in the absence of any treatment. To our knowledge, this phenomenon has not been previously reported.

The IL-10 KO model (C57BL/6 × 129 Ola) devised by Kuhn et al. [4] is widely used as a colitis model for IBD. The authors noted that when these IL-10-deficient mice were raised under “conventional conditions”, they exhibited weight loss, anemia and colitis at the age of 4–8 weeks [4]. In a later study, the rate of spontaneous histologic colitis in IL-10 KO mice (C57BL/6 × 129 Ola) at the age of 3 months was nearly 100%, with an average colonic disease score of 6.6 ± 0.8, and persisted up to 6 months, suggesting that this model is useful as a model of spontaneous progressive colitis [7]. The rate of spontaneous colitis in IL-10 KO mice on a BALB/c background was also 100% at 3 months, with an average colonic disease score higher than that in IL-10 KO mice on a C57BL/6 background (8.6 ± 1.2) [7]. In this study, we found that 60% of BALB/cj IL-10 KO mice developed transient endoscopic left-sided colitis, while 40% never developed any endoscopic left-sided lesions. This difference could be due to a disconnect in the endoscopic presentation of IL-10 KO mice in relation to presence of mural inflammation. Although other studies also observed that IL-10 KO mice do not always develop histologically detectable colitis [3], outright disconnect between histology and endoscopic findings is uncommon in human IBD. While the genetic background of IL-10 KO mice plays a role in incidence rate of colitis, considering the high incidence and severity of colitis reported in the IL-10 KO BALB/c strain [7], genetic background is unlikely to explain the low rate of endoscopic colitis in our study. The prevalence of endoscopic colitis appeared to decrease with age as 22.7% of endoscopies exhibited colitis in mice less than 80 days old, as compared to 10.1% in mice between 81 and 120 days old, and 7.6% in mice older than 120 days. This decreasing rate of endoscopic colitis after 80 days in our study is an interesting finding, as it has been observed that IL-10 KO mice develop colitis by 2–3 months of age under specific pathogen-free (SPF) conditions [6]. What is new in our study is potential lack of persistence of colitis beyond the onset at 2–3 months. It should be noted that spontaneous resolution of extensive colitis in humans is an unlikely scenario [11]. The IL-10 KO mouse can be described as a multi-hit model where a colitogenic trigger initiates the inflammatory process, strain-specific genetic factors determine the dysregulation of the mucosal immune response, and the gut microbiota modify these susceptibilities and responses [1]. In the study by Kuhn et al. discussed above, most mice exhibited colitis when kept under “conventional conditions”, whereas IL-10 KO mice kept under SPF conditions developed mild-to-moderate colitis confined to the proximal colon [4]. Individual IL-10 KO mice in the same colony and even in the same cage develop spontaneous colitis at a nonuniform rate [12]. IL-10 deficient C3H mice from the same parental breeding stocks but maintained in two different facilities had significant differences in histopathological scores at the same age. These differences have been attributed to differences in dietary sources and ingredients, and to the water consumed (autoclaved or not) [13]. To avoid these potential confounding effects, mice in this study were housed under the same barrier-protected conditions and bedding was changed weekly. Furthermore, all mice were fed the same diet and to ensure a homogeneous microbial environment, dirty cage bedding was transferred between cages.

Our study has several limitations. We did not measure mouse body weights, levels of inflammatory markers in stool or blood samples, or perform histologic assessments, so we could not determine the disease activity index. We also did not take biopsy samples during endoscopies, due high risk of perforation. In clinical practice, the severity of IBD is usually determined through a combination of endoscopic, histologic and radiological findings, and serologic or fecal biomarkers are often used as noninvasive and inexpensive supporting methods. Along with C-reactive protein and erythrocyte sedimentation rate, fecal calprotectin and fecal lactoferrin have become part of the current battery of laboratory tests performed during the clinical management of IBD [14]. Fecal Lipocalin-2 has been reported to serve as a potential biomarker of intestinal inflammation in dextran sulfate sodium-induced colitis and IL-10 KO mouse models [15]. Further longitudinal studies on animal models of colitis are warranted to correlate endoscopic findings with inflammatory markers and histology. Specifically, measurement of inflammatory cytokines related to known pathways implicated in the development of colitis in IL-10 KO mice (e.g. IL-12, IL-17 and interferon gamma) can shed more light on development of endoscopic colitis [1]. Given the sharp angulation of splenic flexure in mice, we could not assess the endoscopic appearance of the proximal colon using a rigid scope. IL-10 KO mice tend to develop histopathologic pancolitis with predominance of distal colon [16], hence it is unlikely that we missed isolated proximal colitis in our mice. Depending on the research question and the cost and potential procedural complications of flexible...
colonoscopy in mice, rigid endoscopy can be a safe and reproducible method for repetitive endoscopic assessment in mice.

In conclusion, in this largest endoscopic surveillance study of IL-10 KO mice to date, only 60% of mice developed transient left-sided colitis with a variable recurrence pattern. Other studies have also reported mixed outcomes in IL-10 KO mice under SPF conditions, including no development of histologically detectable colitis [3], or development of colitis with lesser intensity [4]. However in our study, we surprisingly observed a resolution of colitis over time without any intervention. This natural history of colitis in IL-10 KO mice may not be comparable to human IBD, and requires careful consideration when drawing conclusions in translational research. Other strains of IL-10 KO mice should be assessed to further delineate the natural history of colitis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10620-023-07871-y.

Acknowledgments This study was supported in part by a grant to AR from the Kenneth Rainin Foundation.

Author’s contribution MP and AR: conceived and designed the study. SYK, JHP, GL, and AR: performed the experiments. SYK, JHP, GL, and AR: wrote the manuscript, and SYK, JHP, GL, AR and MP: revised and edited the manuscript. All authors read and approved the final manuscript.

Funding This study was supported in part by a Grant to AR from the Kenneth Rainin Foundation.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Keubler LM, Buettner M, Hager C, Bleich A. A Multihit Model: Colitis Lessons from the Interleukin-10-deficient Mouse. Inflamm Bowel Dis. 2015;21(8):1967–1975.
2. Ouyang W, O’Garra A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. Immunity. 2019;50(4):871–891.
3. Sellon RK, Tonkonogy S, Schultz M, Dielamant LA, Grenteher W, Balish E et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. 1998;66(11):5224–5231.
4. Kuhn R, Lohler J, Renick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993;75(2):263–274.
5. Bleich A, Mahler M, Most C, Leiter EH, Liebler-Tenorio E, Elson CO et al. Refined histopathologic scoring system improves power to detect colitis QTL in mice. Mamm Genome. 2004;15(11):865–871.
6. Rennick DM, Fort MM, Davidson NJ. Studies with IL-10--/- mice: an overview. J Leukoc Biol. 1997;61(4):389–396.
7. Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G et al. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. J Clin Invest. 1996;98:1010–1020.
8. Huang EH, Carter JJ, Whelan RL, Liu YH, Rosenberg JO, Rotterdam H et al. Colonoscopy in mice. Surg Endosc. 2002;16:22–24.
9. Kodani T, Rodriguez-Palacios A, Corridoni D, Lopetuso L, Di Martino L, Marks B et al. Flexible colonoscopy in mice to evaluate the severity of colitis and colorectal tumors using a validated endoscopic scoring system. Journal of visualized experiments : JoVE. 2013;80:e50843.
10. Park J, Kim SY, Leite G, Pimentel M, Rezaie A. Sa1742 Interleukin-10 Knockout Mice Do Not Exhibit Persistent Leftsided Colitis: A Natural History Endoscopic Surveillance Study. Gastroenterology. 2019;156:383.
11. Fumery M, Singh S, Dulai PS, Gower-Rousseau C, Peyrin-Biroulet L, Sandborn WJ. Natural History of Adult Ulcerative Colitis in Population-based Cohorts: A Systematic Review. Clin Gastroenterol Hepatol. 2018;16:343–345.
12. Elliott DE, Setiawan T, Metwali A, Blum A, Urban JF Jr, Weinstock JV. Heligmosomoides polygyrus inhibits established colitis in IL-10-deficient mice. Eur J Immunol. 2004;34:2690–2698.
13. Mähler M, Leiter EH. Genetic and environmental context determines the course of colitis developing in IL-10-deficient mice. Inflamm Bowel Dis. 2002;8:347–355.
14. Di Ruscio M, Verna F, Ciccone A, Frieri G, Latella G. Surrogate Fecal Biomarkers in Inflammatory Bowel Disease: Rivals or Complementary Tools of Fecal Calprotectin? Inflamm Bowel Dis. 2017;24:78–92.
15. Chassaing B, Srinivasan G, Delgado MA, Young AN, Gewirtz AT, Vijay-Kumar M. Fecal lipocalin 2, a sensitive and broadly dynamic non-invasive biomarker for intestinal inflammation. PLoS One. 2012;7:e44328.
16. Kim SC, Tonkonogy SL, Karrasch T, Jobin C, Sartor RB. Dual-association of gnotobiotic IL-10--/- mice with 2 nonpathogenic commensal bacteria induces aggressive pancolitis. Inflamm Bowel Dis. 2007;13:1457–1466.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.