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# Genetic background of IL-10$^{-/-}$ mice alters host–pathogen interactions with *Campylobacter jejuni* and influences disease phenotype

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ABSTRACT

We hypothesized that particular genetic backgrounds enhance rates of colonization, increase severity of enteritis, and allow for extraintestinal spread when inbred IL-10$^{-/-}$ mice are infected with pathogenic *C. jejuni*. *Campylobacter jejuni* stably colonized C57BL/6 and NOD mice, while congenic strains lacking IL-10 developed typhlocolitis following colonization that mimicked human campylobacteriosis. However, IL-10 deficiency alone was not necessary for the presence of *C. jejuni* in extraintestinal sites. C3H/HeJ *tlr*4$^{-/-}$ mice that specifically express the *Cdcs1* allele showed colonization and limited extraintestinal spread without enteritis implicating this interval in the clinical presentation of *C. jejuni* infection. Furthermore, when the IL-10 gene is inactivated as in C3Bir *tlr*4$^{-/-}$ IL-10$^{-/-}$ mice, enteritis and intensive extraintestinal spread were observed, suggesting that clinical presentations of *C. jejuni* infection are controlled by a complex interplay of factors. These data demonstrate that lack of IL-10 had a greater effect on *C. jejuni* induced colitis than other immune elements such as TLR4 (C3H/HeJ, C3Bir IL-10$^{-/-}$), MHC H-2g7, diabetogenic genes, and CTLA-4 (NOD) and that host genetic background is in part responsible for disease phenotype. C3Bir IL-10$^{-/-}$ mice where *Cdcs1* impairs gut barrier function provide a new murine model of *C. jejuni* and can serve as surrogates for immunocompromised patients with extraintestinal spread.


## 1. Introduction

*Campylobacter jejuni* is a leading cause of bacterial diarrhea with cases in the US exceeding those of *Salmonella* and *Escherichia coli* [1]. Self-limiting gastroenteritis is the most common clinical syndrome [2], but infected patients show a spectrum of diseases including gastroenteritis, septicemia, meningitis, proctitis, abortion, and autoimmune diseases [3]. Immunocompromised patients have both a higher incidence of infection with *Campylobacter* and more severe disease manifestations with profuse diarrhea and persistence of the organism [4,5]. Less commonly, extremes of age or underlying immune compromise can predispose to bacteremia and subsequent spread to other organs [4,6]. Reactive inflammatory disorders can also occur as sequelae of *C. jejuni* infection. Transmural inflammatory changes in colon or small intestines sometimes occur that resemble inflammatory bowel disease (IBD) [7,8]. Antecedent *C. jejuni* has also been linked to Guillain Barré Syndrome (GBS), a debilitating inflammatory polyneuritis that often results in long term disability [9,10]. Reactive arthritis can also follow diarrheic episodes of [11] or asymptomatic exposure to [12] *C. jejuni* and is attributed to autoimmune response in joints [13,14]. Recently, the presence of *C. jejuni* in tissues was associated with mucosa-associated lymphoid-tissue (MALT) lymphoma, an immunoproliferative small intestinal disease [15].

In order to understand the basis of bacterial strain dependent disease manifestations, studies have been conducted to examine genetic diversity among *C. jejuni* strains. Microarray analysis of *C. jejuni* isolates has shown that both highly divergent and highly conserved gene classes exist among isolates from cases [16–20]; however, comparison of *C. jejuni* isolates implicated in GBS to isolates associated with mild enteritis showed that specific GBS genes or regions could not be identified [16,21]. Clinical evidence that *C. jejuni* strain variation does not account for all disease syndromes is shown by the fact that the same strains producing watery diarrhea in children in developing countries have been isolated from visitors

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with acute inflammatory disease [2]. In another study, there was no difference in serotypes isolated from symptomatic and asymptomatic children [22]. Although exploration of the significant diversity of *C. jejuni* genomes is ongoing, the outcome of infection likely depends on host, bacterial, and epidemiological factors, including bacterial strain variation, dose, number and timing of previous infections, host age and immune status, presence of other pathogens, or variation in the composition of the flora in the gastrointestinal (GI) tract. In this study we consider the allelic composition of the host genome as a determinant of disease after *C. jejuni* challenge.

Host genetic background plays a substantial role in susceptibility to infectious diseases [23]. Use of mice with altered immune systems has been crucial in eliciting specific clinical syndromes to model infection with GI pathogens, including *Campylobacter* [24] and *Helicobacter* [25]. Each inbred mouse strain has unique background alleles that can interact with and modify the expression of targeted mutations, genetic regions introduced from other strains, and transgenes [26]. Inbred mouse strains often vary in normal development and physiology due to the action of modifier genes that suppress or enhance gene expression by altering DNA transcription rates, altering mRNA stability, or inducing epigenetic effects such as DNA methylation [27]. Modifier genes can be normal constituents of the genome, arise by spontaneous mutation, or be discovered during construction of congenic mouse strains due to linkage to the locus of interest [27]. Modifier genes unique to particular genetic backgrounds were shown to have deleterious effects in exacerbating murine cecal hyperplasia associated with cytokine deficiency-induced colitis susceptibility 1 (*Cdcs1*) even in previously well-characterized inbred strains [28].

With such a complex interplay of factors, reliable animal infection models that reproduce human disease outcomes would facilitate research into mechanisms of *C. jejuni* pathogenesis and therapies. Our strategy was to develop murine models of enteritis induced by primary oral *C. jejuni* challenge using mice with a genetic background and immune bias expected to increase susceptibility and demonstrate the range of clinical syndromes observed in patients. Recently, we showed that C57BL/6 IL-$10^{+/+}$ and congenic IL-$10^{-/-}$ mice can be used as colonization and disease models, respectively [24]. *C. jejuni* 11168 colonized both C57BL/6J IL-$10^{+/+}$ and IL-$10^{-/-}$ mice and caused a high rate of typhlocolitis in IL-$10^{-/-}$ mice in time course and dose response experiments. A dose as small as $10^2$ cfu produced clinical disease and histological lesions in the GI tracts of the mice (unpublished results). By 28 days post infection, both C57BL/6 IL-$10^{+/+}$ and IL-$10^{-/-}$ mice developed robust T helper cell 1 (Th1) associated plasma immunoglobulin responses to *C. jejuni*. To extend these studies, we considered the possibility that background modifier genes may influence the development of *C. jejuni* enteritis due to IL-10 deficiency. We sought IL-$10^{-/-}$ mice of different genetic backgrounds that could be obtained and reared free of colitogenic bacteria with background genes, especially immune system defects, that might influence susceptibility and disease manifestations.

Our approach was to infect C57BL/6, non-obese diabetic (NOD), and C3H/HeJ wild-type (WT) mice and their corresponding IL-10 knockouts (B6.129P2-IL10$^{tm1Cgn}$/J (referred to below as C57BL/6 IL-$10^{-/-}$); NOD.Cg-*Il10*$^{tm1Cgn}$/LtJ (NOD IL-$10^{-/-}$); C3Bir.129P2(B6)-*Il10*$^{tm1Cgn}$/Lt (C3Bir IL-$10^{-/-}$)) with a primary oral challenge with *C. jejuni* 11168 and then to compare the outcomes by quantifying clinical signs, gross pathology, histopathology, immunohistochemical (IHC) staining, and anti-*C. jejuni* isotype specific antibody response. C57BL/6 mice have MHC H-2*b*, NOD mice have MHC H-2*g7*, a range of diabetes linked genes, and Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) that inhibits T cell function, and C3 mice have H-2*k*, a Toll-like receptor 4 deficiency (TLR4), and the *Cdcs1* allele. These experiments demonstrated that IL-$10^{-/-}$ mice of these genetic backgrounds have similar inflammatory disease processes in the colon when infected with virulent *C. jejuni*. All IL-$10^{-/-}$ and WT mice were colonized by *C. jejuni* at 35 days after infection. *C. jejuni* infected IL-$10^{-/-}$ mice of all three genetic backgrounds and a few NOD WT mice developed typhlocolitis that resembled IBD by 35–40 days post infection. For IL-$10^{+/+}$ mice of the C57BL/6 and NOD backgrounds, *C. jejuni* colonization was necessary but not sufficient for lesions. However, in the two experiments combined, 86% of C3Bir IL-$10^{-/-}$ uninfected control mice developed some degree of typhlocolitis despite an environment lacking previously identified colitis-causing bacteria compared to 61% in the *C. jejuni* infected group, although the latter had twice as many mice with the highest grade lesions. These results suggest the hypotheses (1) that TLR4 and IL-10 deficiency along with the *Cdcs1* allele [26] disrupt gut barrier function and (2) that *C. jejuni* is just one of many bacteria that can elicit colitis in these mice. Taken together, these results demonstrate that the lack of the cytokine IL-10 had the greatest impact on enhancing susceptibility to *C. jejuni* induced enteritis. Furthermore, mice of the C3 genetic background had significant extraintestinal spread of *C. jejuni* that was exacerbated by IL-10 deficiency. These results implicate TLR4, *Cdcs1*, and possibly other background modifier genes in extraintestinal spread of *C. jejuni* and suggest that C3Bir IL-$10^{-/-}$ mice can provide usable models for this aspect of infection.

## 2. Results

### 2.1. Monitoring for spontaneous colitis and diabetes in breeding colony mice

We recognized that the responses of IL-$10^{-/-}$ mice to *C. jejuni* infection could be skewed if environmental factors such as colitis-causing bacteria were present in either the mouse breeding colony or the containment challenge facility. Mice of C3 and NOD genetic backgrounds were most at risk because of their respective additional immune system deficiencies. Therefore, we monitored the background rate of colitis development in mice of the C57BL/6 and C3Bir genetic background and the background rate of both colitis and diabetes development in mice of the NOD genetic background in the breeding colony. Retired breeding mice of all genetic backgrounds and breeding colony mice euthanized due to evidence of spontaneous colitis were necropsied; each mouse was examined for colitis (enlargement and thickening of the wall of the proximal colon), enlargement of the spleen, and, in NOD mice, elevated urine glucose levels.

Breeding colony mice of the C57BL/6 and NOD genetic backgrounds had low rates of spontaneous colitis and splenic enlargement. Four of 88 (4%) C57BL/6 IL-$10^{-/-}$ mice and 0/33 C57BL/6 IL-$10^{+/+}$ mice developed spontaneous colitis; 2/88 (2%) C57BL/6 IL-$10^{-/-}$ mice and 0/33 C57Bl/6 IL-$10^{+/+}$ mice developed splenic enlargement, while 1/43 (2%) NOD IL-$10^{+/+}$ mice and 0/27 (0%) NOD IL-$10^{-/-}$ mice developed spontaneous colitis, and no NOD mice developed splenic enlargement. The single NOD IL-$10^{+/+}$ mouse that developed spontaneous colitis was also diabetic. Nineteen of 66 (29%) NOD IL-$10^{+/+}$ and $^{-/-}$ breeding colony mice in the age range of experimental mice developed blood glucose levels $\geq$100 mg/dL, but only one experimental NOD IL-$10^{+/+}$ mouse became diabetic during the course of the experiments. In contrast, 44/73 (60%) C3Bir IL-$10^{-/-}$ mice in the breeding colony in the same age range of experimental mice developed spontaneous colitis as evidenced by soft or liquid feces, enlargement of the colon, and thickening of the colon wall; 42/73 (57%) had enlarged spleens; and 48/73 (66%) had enlarged ileocecocolic/mesenteric lymph nodes. Thirty-one of 73 (42%) of these mice exhibited all three kinds of pathological change: colitis, lymph node enlargement, and splenic