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Pathogenesis of *Escherichia coli* O157:H7 strain 86-24 following oral infection of BALB/c mice with an intact commensal flora

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ABSTRACT

Escherichia coli O157:H7 is a food-borne pathogen that can cause hemorrhagic colitis and, occasionally, hemolytic uremic syndrome, a sequela of infection that can result in renal failure and death. Here we sought to model the pathogenesis of orally-administered *E. coli* O157:H7 in BALB/c mice with an intact intestinal flora. First, we defined the optimal dose that permitted sustained fecal shedding of *E. coli* O157:H7 over 7 days ($\sim 10^9$ colony forming units). Next, we monitored the load of *E. coli* O157:H7 in intestinal sections over time and observed that the cecum was consistently the tissue with the highest *E. coli* O157:H7 recovery. We then followed the expression of two key *E. coli* O157:H7 virulence factors, the adhesin intimin and Shiga toxin type 2, and detected both proteins early in infection when bacterial burdens were highest. Additionally, we noted that during infection, animals lost weight and ~30% died. Moribund animals also exhibited elevated levels of blood urea nitrogen, and, on necropsy, showed evidence of renal tubular damage. We conclude that conventional mice inoculated orally with high doses of *E. coli* O157:H7 can be used to model both intestinal colonization and subsequent development of certain extraintestinal manifestations of *E. coli* O157:H7 disease.

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1. Introduction

Escherichia coli O157:H7 is a food-borne pathogen that results in an estimated annual incidence of 73,000 cases of diarrheal illness each year in the United States [1]. Hemolytic uremic syndrome, or HUS, is the most serious sequela of *E. coli* O157:H7 infection that occurs on average in 4% of those infected [2]. However, the recent 2006 spinach-associated *E. coli* O157:H7 outbreak in the United States had a higher rate of HUS (16%) that was perhaps attributable to the emergence of a more virulent clade of *E. coli* O157:H7 [3, 4]. HUS consists of a triad of symptoms that include microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure [5]. Although most people recover from HUS, late complications and even death can result [6].

The sequence of events by which *E. coli* O157:H7 establishes infection and causes disease is imperfectly understood. It is known that *E. coli* O157:H7 can cause illness in a person who has ingested as few as 100 organisms [7]. Therefore, at least a portion of that inoculum must be capable of surviving the acidic conditions of the stomach to colonize the intestine (reviewed in Ref. [8]). Intimate adherence of *E. coli* O157:H7 to the intestinal mucosa is considered to require expression of the bacterial outer membrane protein intimin and other factors secreted through the type III secretion system (TTSS) (reviewed in Ref. [9]).

The development of HUS is associated with the production of Shiga toxin (Stx) by *E. coli* O157:H7 and other Stx-producing *E. coli* (STEC) [10–12]. This toxin is produced by the infecting bacteria in the gut, as evidenced by the presence of Stx in the feces of *E. coli* O157:H7-infected individuals [11–13]. *E. coli* O157:H7 can produce two types of Stxs, Stx1 and/or Stx2, as well as variants of these toxin types. Stxs are AB_5 toxins that bind to cells through the pentameric B subunits via a glycosphingolipid receptor known as globo-triaosylceramide, or Gb_3. Stx is an N-glycosidase that cleaves a purine residue in ribosomal 28S RNA. This catalytic event leads to inhibition of protein synthesis in the target cell and, subsequently, cell death by apoptosis (reviewed in Ref. [14]). The primary targets for Stx *in vivo* are small vessel vascular endothelial cells that express Gb_3 [15–17], such as those within the renal glomerulus [17,18]. It is not known how Stx crosses the intestinal barrier to target small vessel endothelial cells in the lamina propria of the gut (thought to be a primary event in the manifestation of hemorrhagic colitis), nor the mechanism by which the toxin enters the blood stream and travels through the circulatory system to the Gb_3-rich small vessel endothelia in the kidney.

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Mouse infection models that mirror various aspects of STEC pathogenesis or disease (such as intestinal colonization, renal impairment, central nervous system damage, and death) have been developed (reviewed in Ref. [19]). While these animal systems are helpful for assessing features of STEC disease, many require that mice undergo artificial manipulations such as prolonged dietary restriction to promote colonization, mitomycin C injection to facilitate Stx expression, or antibiotic treatment to reduce the normal flora that can inhibit the establishment of an exogenous infection [20–25]. Furthermore, in antibiotic-treated or axenic animal model systems the STEC strain is likely to have a pathogenic advantage as it is no longer competing with the entire normal flora microbiome for resources. On the other hand, in a mouse model in which the normal gut flora is suppressed, the infecting *E. coli* O157:H7 strain may not receive molecular signals made by commensal bacteria that lead, through a quorum-sensing regulatory network, to TTSS expression by *E. coli* O157 [26]. Thus, to better reflect the typical gastrointestinal environment to which *E. coli* O157:H7 is exposed following ingestion, we sought to develop an oral model of *E. coli* O157:H7 infection in mice with an intact commensal flora (ICF).

2. Results

2.1. Dose–response studies

As a first step in the development of a mouse model of *E. coli* O157:H7 infection in BALB/c mice with an ICF, we conducted dose–response experiments to determine whether inoculum size correlated with the percent of animals colonized or the level of colonization over time. For this purpose, groups of 5–10 mice were intragastrically administered approximately 10^5, 10^6, 10^7, 10^8, or 10^9 CFU of *E. coli* O157:H7 strain 86-24Nal^R and the degree of bacterial colonization measured as defined by the number of 86-24Nal^R bacteria present per gram [colony forming units (CFU)/g] feces.

The percent of animals colonized on day 1 following infection with strain 86-24Nal^R varied by challenge dose (Fig. 1A). At an inoculum of 10^5 or 10^6 CFU, 40% or 80% of the mice, respectively, had detectable bacterial counts within their feces on day 1 post-infection. All animals challenged with 10^7 CFU or greater shed detectable bacteria on day 1 of infection. The relative proportion of mice that remained colonized after day 1 was also dose-dependent (Fig. 1A). Groups of animals given $\leq 10^7$ CFU of *E. coli* O157:H7 strain 86-24Nal^R had fewer infected mice on day 2 than on day 1, with 60% or less of the mice in those groups infected throughout the remainder of the study. Of the animals that received 10^8 CFU, 7 of 8 mice remained colonized for the first 5 days after infection (2 of the original 10 animals died during the study and, thus, were no longer included in this analysis). At the highest inoculum of 10^9 CFU, mice were both consistently (% of mice colonized) and persistently (prolonged, high-level colonization) infected for the 7 days that followed challenge (2 of 5 mice, both highly colonized, died on day 4 post-infection). In summary, as the inoculum dose increased there was a statistically significant increase in the percent of mice that were colonized over time ($p = 0.005$ in a trend analysis).

Next, we assessed the differences in overall levels of colonization among the inocula groups (Fig. 1B). We found that as the challenge dose went up, the number of bacteria shed into the feces increased ($p = 0.001$). At a low inoculum (10^5 CFU), animals shed *E. coli* O157:H7 with a geometric mean (GM) less than 10^3 CFU/g feces over the course of the experiment. The recoverable bacteria from mice infected with 10^6 or 10^7 CFU, while somewhat higher, still remained relatively low (GM at or below 10^4 CFU/g feces). In comparison, animals that received an inoculum of 10^8 or 10^9 CFU had more recoverable bacteria on the day after infection (GMs of 6.5×10^5 and 1.9×10^6 CFU/g feces, respectively). Mice that received 10^8 CFU had a statistically higher colonization level than animals challenged with 10^5 CFU, irrespective of time ($p = 0.001$). At the largest inoculum of 10^9 CFU, statistically higher levels of colonization were measured compared to doses of 10^5, 10^6, or 10^7 CFU ($p \leq 0.01$), again irrespective of time. However, as the overall colonization levels declined during the study these variations by inoculum dose in colonization loads eventually disappeared (except at the highest inoculum when comparing CFU/g feces on day 7 post-infection, Fig. 1B).

We next evaluated the impact on colonization of administration of 10^8 or 10^9 CFU of strain 86-24Nal^R to the mice by intragastric administration versus pipette feeding (orally through a micropipette tip). We observed a more consistent infection after intragastric administration at the highest inoculum of 10^9 CFU ($p = 0.023$, Fig. 1C). When assessing the colonization levels in the various groups (Fig. 1D), significantly higher colonization levels were achieved at higher inocula ($p = 0.008$), as was noted previously (Fig. 1B), regardless of method used to introduce the bacteria. Additionally, as infection progressed, colonization levels generally declined in all groups ($p < 0.001$). When the data were averaged across all days, there was no overall difference between the procedures used to infect the animals at either dose. However, there was an indication that providing the inoculum by gavage resulted in increased levels of colonization on certain days post-infection, and this was most pronounced on day 3 ($p = 0.004$) (Fig. 1D). Nevertheless, the most consistent and persistent colonization levels in mice were achieved with an inoculum of 10^9 CFU regardless of the route of bacterial challenge used. Therefore, for all subsequent experiments, we orally infected mice either intragastrically by gavage or by pipette feeding with approximately 10^9 CFU of the microbe. The inoculum method applied was largely dictated by the question under study in a particular experiment.

2.2. Sites of 86-24Nal^R colonization in the mouse intestinal tract

To determine the site of *E. coli* O157:H7 colonization within the intestines of the ICF mice after challenge with 86-24Nal^R, animals were orally inoculated with about 10^9 CFU of the bacterium by pipette feeding. We elected to use the pipette feeding method for this study because it is more like the natural route of exposure to the organism, ingestion, than is gavage. At various times following infection, groups of animals ($n = 8$–17) were sacrificed, and the numbers of 86-24Nal^R bacteria associated with the tissues of the small intestine, cecum, and large intestine, as well as in the luminal contents of those intestinal segments were determined.

When the 86-24Nal^R counts of the organ tissues were compared, we found that the bacterial load was the highest in the cecum, followed by the large intestine, while the small intestine had the fewest recoverable *E. coli* O157:H7 (Fig. 2A). The number of *E. coli* O157:H7 bacteria harvested from each of the tissues dropped from 6 h to 24 h, and then leveled off for the remainder of the study. The number of bacteria recovered from the luminal contents showed a similar pattern to that seen for the organs themselves, with the exception that the contents of the large intestines remained high throughout the study (Fig. 2B). We also observed that the large intestine luminal contents had significantly more recoverable *E. coli* O157:H7 than did the large intestinal tissue (compare Fig. 2B line with open diamonds to Fig. 2A line with solid diamonds; $p < 0.001$). In fact, the large intestine luminal contents had equivalent levels of *E. coli* O157:H7 to those found in the cecum, the cecal contents, or both, depending on the time-point assessed. As was seen for the small intestinal tissue, the small intestine luminal contents had significantly fewer recoverable *E. coli*