Probiotic Bacterium Outcompetes Salmonella enterica serovar Typhimurium for Iron Acquisition in the Mammalian Intestines

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ABSTRACT
Microbiota play a critical role in providing a physical barrier in mammalian intestines. Intestines are mainly colonized by commensal microbes such as Bacteroides and Firmicutes. A host prevents pathogenic growth by sequestering iron by using various host proteins such as transferrin, ferritin, heme and lactoferrin. A strain of Escherichia coli (E.coli), E.coli Nissle 1917 was found to avert a variety of intestinal disorders. In an experiment done at the University of California, Irvine, mice were infected with the inflammatory bacterium Salmonella enterica serovar Typhimurium (S. Typhimurium), both wild type and mutant E. coli Nissle to determine the beneficial effects of this probiotic. The researchers found that S. Typhimurium and E. coli Nissle compete to survive in the inflamed gut by utilizing various iron chelators also known as siderophores.

INTRODUCTION
Iron is critical for cellular functions, however, high concentrations are toxic. (Kortman). To counterbalance the high concentrations of iron in the gut, high affinity proteins such as lactoferrin, transferrin and ferritin bind to iron and lower the concentrations (Kortman). These high affinity proteins also act as an innate microorganism defense system that compete with pathogens when uptaking iron. When E. coli infects the gut and causes inflammation, the host will secrete peptides lipocalins to sequester ferric enterohematin to limit the growth of E. coli (Singh). E. coli needs iron to use it as a coenzyme that activates ribonucleotide, a key enzyme for the synthesis of DNA (Symeonidis). In addition, when the gut is inflamed, E. coli and salmonella export siderophores (Singh). Siderophores are high-affinity chelating compounds released by bacteria and fungi as a mechanism to aid in their quest for iron (Kortman). These varying siderophores from different bacteria create a competitive environment between the host and pathogen (Kortman).

MATERIALS & METHODS
Fecal iron was measured by inductively coupled plasma mass spectrometry (ICP-MS). The mice model that were used for the acute and chronic infections were C57Bl/6 and 129X1/SvJ respectively. To ensure chronic Salmonella coliitls infection all the mice were treated with streptomycin before infected with S. Typhimurium by oral gavage. A 1:1 ratio of E.coli Nissle and S. Typhimurium were used for co-infection and fecal pellets were collected at various time points (24, 48, 60 hours). To determine colony forming units (CFU) the fecal pellets were mixed with one milliliter of sterile phosphate buffer saline and serially diluted on Luria Broth agar. The inflammation score was determined by evaluating tissues samples that were stained with hematoxylin and eosin for the following: cystitis, surface erosions, inflammatory exudates, neutrophils, mononuclear infiltrate and submucosal edema.

RESULTS
Mice infected with S. Typhimurium had lower amounts of iron in their feces than mice that were not infected (Figure 1). Three weeks after 129X1/SvJ mice were infected with S. Typhimurium, the mice had high levels of inflammation and fecal shedding of the bacteria. A single dose of wild type E.coli Nissle significantly decreased the amount of S. Typhimurium and intestinal inflammation (Figure 2, 3). A E.coli Nissle that had a mutated tonB gene was able to colonize the gut of the mice but was not able to reduce the colonization of S. Typhimurium. A second mutated E.coli Nissle Δg, that lacked four iron transport system, also could not reduce the S. Typhimurium infection. Only the wild type E.coli Nissle was able to reduce the colonization of S. Typhimurium when the two bacteria were co-administered in C57BL/6 mice (Figure 4). Mice that were precolonized with wild type E.coli Nissle three days before infected with S. Typhimurium had reduced colonization of S. Typhimurium.

CONCLUSION
E. coli Nissle has both proinflammatory and anti-inflammatory effects, which causes an additional layer to the mucosal barrier (Deriu). S. Typhimurium infection causes gastrointestinal symptoms such as nausea, vomiting, abdominal cramps, diarrhea, fever, chills, headache, and blood in the stool. If left untreated it can potentially cause life-threatening complications such as dehydration, bacteremia, and arthritis (Xu Lin). In this study E.coli Nissle was shown to outcompete S. Typhimurium, therefore reducing the inflammation and function as an probiotic. E. coli Nissle therefore can be used as a noninvasive treatment to prevent the growth of S. Typhimurium (Verna). A possible direction for future investigation is to determine what other gastrointestinal pathogens that E.coli Nissle can defend against.

REFERENCES

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