Maternal Antepartum Administration of Lactoferrin Ameliorates Neonatal Infection by Bacteremia-Producing *Escherichia coli* in Mice

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Background

- *Escherichia coli* is the leading Gram-negative causing neonatal sepsis.
- Vaginal pathogenic *E. coli* strains ascend into the pregnant uterus infecting the offspring of colonized mothers.
- Lactoferrin (LF) is an antibacterial and immunomodulatory glycoprotein that has been given to preterm newborns to prevent late-onset sepsis.
- The effects of maternal LF administration to prevent neonatal *E. coli* invasive infection by clinical isolates that produce sepsis and meningitis have not been studied.

Objective

To determine the efficacy of vaginal lactoferrin administered prenatally to pregnant mice to prevent invasive *E. coli* disease in their embryos.

Methods

- Human lactoferrin (LF) 100 mcg/mL or placebo were administered vaginally to pregnant C57BL/6 mice twice daily on E16 and E17.
- Two hours after the 4th dose on E17, mice were infected vaginally with $1x10^5$ colony forming units (CFU) of the archetypal bacteremia/meningitis-producing clinical isolate RS218, which was modified by transposon mutagenesis to constitutively express chloramphenicol (Cam) resistance (RS218-CamR) as selection marker.
- On E18, maternal vaginal fluid samples were obtained, and placentas and embryo tissues were collected after humane euthanasia to determine bacterial loads by culture on Cam plates.

Results

Fig. 1. Automated growth curves in Luria Bertani broth showed no differences in growth capacity between wild-type RS218 and RS218-CamR.

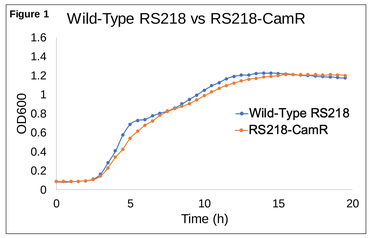
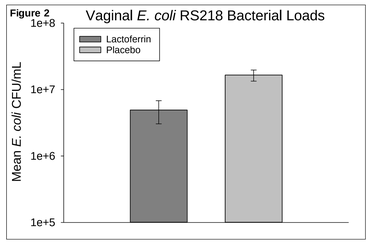
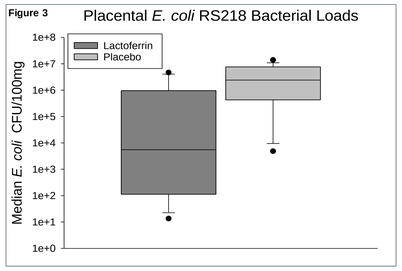


Fig. 2. Vaginal RS218-CamR loads were significantly lower in LF-pretreated pregnant mice compared to placebo, $4.9x10^6$ CFU/mL vs. $1.7x10^7$ CFU/mL, respectively (Welch's t-test P< 0.02).





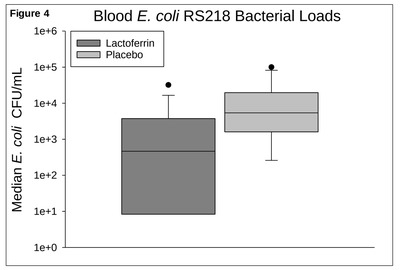
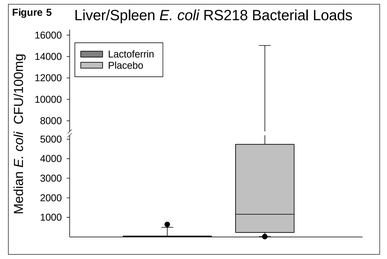


Fig. 3. Placental RS218-CamR loads were also lower in LF-pretreated mice compared to placebo, $5.5x10^3$ CFU/100 mg (IQR $1.1x10^2$-$9.6x10^5$,n=16) vs. $2.4x10^6$ CFU/100 mg (IQR $4.3x10^5$-$7.6x10^6$, n=17), respectively (Mann-Whitney P< 0.001).

Fig. 4. Bacterial loads in the blood of embryos of LF-pretreated dams were significantly lower compared to those of placebo-treated dams, 433 CFU/mL (IQR 0-3100, n=15) vs. 5400 CFU/mL (IQR 1600-19666, n=15), respectively (P<0.01).

Results



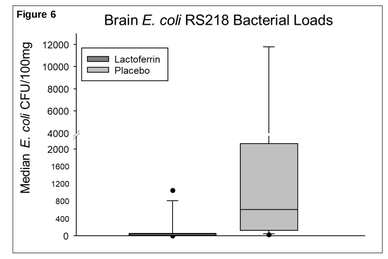


Fig. 5. Bacterial loads in the liver and spleen of embryos of LF-pretreated dams were significantly lower compared to those of placebo-treated dams, 6.6 CFU/100 mg (IQR 0-48, n=16) vs. 1160 CFU/100 mg (IQR 229-4736, n=18), respectively (P<0.001).

Fig. 6. Bacterial loads in the brain of embryos of LF-pretreated dams were significantly lower compared to those of placebo-treated dams, 3.3 CFU/100 mg (IQR 0-56, n=16) vs. 605 CFU/100 mg (IQR 11-2132, n=18), respectively (P<0.001).

- RS218-CamR was found in the brain tissue of 50% (total n=16) of embryos from LF-pretreated dams vs. 100% (n=18) of embryo brain tissue in the placebo-pretreated group (Fisher exact test, P< 0.001).

Conclusions and Future Studies

- Prenatal maternal vaginal administration of LF significantly decreased vaginal bacterial loads of a clinically significant neonatal invasive K1+ *E. coli* isolate.
- Placental bacterial loads, and burden of infection in the offspring were also significantly decreased.
- The mechanisms by which prenatal LF protects newborns from invasive *E. coli* disease need investigation.
- Prenatal lactoferrin is a potential preventative intervention against neonatal sepsis.

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