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**HUMAN BREAST MILK PROTECTS AGAINST NECROTIZING ENTEROCOLITIS THROUGH REDUCED TLR4 EXPRESSION IN THE INTESTINAL EPITHELIUM IN A “NEC IN A DISH” MOUSE ENTEROID MODEL**

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**Introduction:** Necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal disease in premature infants and is characterized by exuberant TLR4 signaling in the premature intestinal epithelium. Breast milk is protective against NEC, but the underlying mechanisms for this protection remain unknown, and the role of breast milk on the intestinal mucosa versus peripheral cells remains unknown. To study the effect of breast milk on the intestinal epithelium in NEC directly, we developed a novel ex-vivo model of the disease, termed “NEC in a Dish”, utilizing intestinal enteroids incubated with stool bacteria from NEC patients. We now hypothesize that breast milk protects against NEC specifically through its effects on the intestinal epithelium and seek to discover the mechanisms involved.

**Methods:** Enteroids derived from primary stem cells of neonatal mouse intestines were subjected to the “NEC in a Dish” model which includes treatment with NEC bacteria and periods of hypoxia. To determine the effects of breast milk on the intestinal epithelium in NEC, enteroids were either subjected to NEC (eNEC, n=6) alone, or with the addition of 50μL of human breast milk (eNEC+BM, n=6). Control enteroids (eCTRL) were grown in standard media. Model severity was evaluated by structural integrity, PI staining, and gene expression of TNFa by qRT-PCR.

**Results:** eNEC enteroids showed structural damage and increased PI staining indicating cell death, and significantly elevated expression of TNFa (eNEC: 7.14, eCTRL: 1.47, p<0.05). In contrast, treatment with breast milk restored structural integrity and decreased PI staining, and reduced TNFa levels (eNEC+BM: 3.2, p<0.05). In seeking the mechanism involved, breast milk treatment significantly reduced expression of TLR4 in enteroids subjected to NEC (eNEC: 34.8, eNEC+BM: 27.0, p<0.05).

**Conclusion:** This study suggests that human breast milk protects against NEC through a TLR4 dependent pathway directly on intestinal epithelium, thus establishing a novel discovery platform for this devastating disease.