

## PATHOGENICITY

### BIOMARKERS OF *LISTERIA MONOCYTOGENES* INFECTION AND TREATMENT WITH A SYNTHETIC ANTI-INTERNALIN PEPTIDE IN PREGNANT GUINEA PIGS

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*Listeria monocytogenes* is a bacterial pathogen known to cause spontaneous abortions and stillbirths. The mechanisms of *Listeria*-induced miscarriages and stillbirths are still largely unknown. Previously, pregnant primates and pregnant guinea pigs were used to develop a dose-response model for infection with *L. monocytogenes*. Our recent studies have focused on the maternal and fetal effects during the course of infection and biomarkers that might predict induction of a stillbirth. We hypothesize that after infection with *L. monocytogenes*, changes in the placental immunological status will occur. Additionally, we hypothesize that treatment with a synthetic peptide designed to bind to internalin A (anti-In1a) will decrease the invasiveness of *L. monocytogenes* in maternal and fetal tissues. Pregnant guinea pigs were treated on gd 35 with doses of  $10^4$  to  $10^8$  CFUs *L. monocytogenes* and sacrificed at post-treatment days 2, 6, 9, and 20 (gd 37, 41, 44, and 55). *Listeria* species were determined by plating enriched maternal and fetal tissues as well as maternal fecal samples on selective media. *L. monocytogenes* was confirmed using a chromogenic substrate test. The immunological response of the placenta was determined by qRT-PCR analysis of specific Th1 (IFN- $\lambda$ , TNF- $\alpha$ , IL-2) and Th2 (IL-5, IL-10) cytokines. Expression levels were determined using the comparative relative quantity method and are expressed as a fold-change compared to control levels. For the peptide study, three experimental groups were used and all treatments were administered orally. (A) Pregnant guinea pigs were treated with the peptide only, (B) 100 $\mu$ g/ml anti-In1a peptide was administered followed by an administration of  $10^8$  *L. monocytogenes* CFU/ml one hour later, or C) 100 $\mu$ g/ml anti-In1a peptide and  $10^8$  *L. monocytogenes* CFU/ml were pre-incubated for one hour prior to administration. Tissue infectivity was not affected by the duration of infection as *L. monocytogenes* was isolated from tissues samples at the earliest post-treatment day 2. However, cytokine expression levels were affected by the duration of infection with no changes occurring in placental cytokine expression levels from treated dams at post-treatment day 2. Th1 placental cytokine expression levels were significantly altered in treated dams at post-treatment days 6, 9, and 20. Th2 placental cytokine expression levels were not altered after maternal treatment with *L. monocytogenes*. For the anti-In1a study, of the guinea pigs that were exposed to *L. monocytogenes* 1 hour following the peptide (B), *L. monocytogenes* was isolated from maternal liver and spleen. Interestingly, *L. monocytogenes* could not be cultured from the placentas or fetuses of this group. Additionally, pre-incubation of the peptide with *L. monocytogenes* did not reduce infection of maternal tissues. Current data suggest that when administered to pregnant guinea pigs prior to *L. monocytogenes* exposure, anti-In1a peptide may reduce the occurrence of fetal infection and stillbirths.

### ENTEROBACTER SAKAZAKII VIRULENCE BASED ON INFECTION IN NEONATAL MICE

(A.N. Richardson and M.A. Smith)

*Enterobacter sakazakii* (*E. sakazakii*) has been associated with outbreaks of infection in neonatal intensive care units (NICUs) among premature or very-low-birth-weight infants fed contaminated powdered infant formula. *E. sakazakii* infection can result in severe illnesses such as septicemia, meningitis, hydrocephalus, or even death. The objective of the research project is to develop an animal model to assess the ability of *E. sakazakii* to infect and cause disease in premature human infants. We used four mouse strains (CD-1, C57BL/6, BALB/C, and A/J) and exposed them to *E. sakazakii* strain MNW2 to determine which was the most susceptible to the infection. Timed-pregnant mice were obtained, acclimatized, and allowed to give birth naturally. At postnatal day (PND) 3 or 4, the pups were orally gavaged with a single dose of vehicle or  $10^1$  -  $10^{11}$  CFU *E. sakazakii* per ml reconstituted powdered infant formula. Pups surviving to PND 10 or 11 were sacrificed and brains, livers, and ceca excised, pooled into groups within litters, and analyzed for the presence of *E. sakazakii*. The CD-1 mouse strain was the most susceptible showing the most infectivity and lowest infectious dose ( $10^2$  CFU). We continued using CD-1 mice to observe differences in virulence among *E. sakazakii* strains MNW2, SK81, and 3290 and to compare the susceptibilities of male and female neonatal mice. On treatment day, the mouse pups were sexed and randomized so that each dam had a litter of ten (5 females and 5 males). At PND 10 or 11, surviving pups were sacrificed and individual tissues were excised and analyzed for *E. sakazakii* infection. Comparing the invasion of

liver and brain tissues after treatment of pups with  $10^5$  CFU MNW2 and SK81 indicates that MNW2 is more invasive in both liver (43% versus 8%) and brain (71% versus 35%) than SK81. There appeared to be no significant difference in the susceptibilities of male and female CD-1 neonates administered *E. sakazakii* strain SK81, but the other strains have not been tested for sex differences. Testing of other strains of *E. sakazakii* is ongoing. Understanding and developing animal models for *E. sakazakii* infection will allow development of therapies to treat *E. sakazakii* infections. Comparisons of different strains of *E. sakazakii* will help determine which are more virulent and likely to cause morbidity and mortality in premature infants.

