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ANIMAL MODELS

Specific Lipopolysaccharide Serotypes Induce Differential Maternal and Neonatal Inflammatory Responses in a Murine Model of Preterm Labor

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Intrauterine inflammation is recognized as a key mediator of both normal and preterm birth but is also associated with neonatal neurological injury. Lipopolysaccharide (LPS) is often used to stimulate inflammatory pathways in animal models of infection/inflammation-induced preterm labor; however, inconsistencies in maternal and neonatal responses to LPS are frequently reported. We hypothesized that LPS serotype-specific responses may account for a portion of these inconsistencies. Four different *Escherichia coli* LPS serotypes (O111:B4, O55:B5, O127:B8, and O128:B12) were administered to CD1 mice via intrauterine injection at gestational day 16. Although control animals delivered at term 60 ± 15 hours postinjection (p.i.), those administered with O111:B4 delivered 7 ± 2 hours p.i., O55:B5 delivered 10 ± 3 hours p.i., O127:B8 delivered 16 ± 10 hours p.i., and O128:B12 delivered 17 ± 2 hours p.i. (means ± SD). A correlation between the onset of preterm labor and myometrial activation of the inflammatory transcription factor, activator protein 1, but not NF-κB was observed. Specific LPS serotypes induced differential activation of downstream contractile and inflammatory pathways in myometrium and neonatal pup brain. Our findings demonstrate functional disparity in inflammatory pathway activation in response to differing LPS serotypes. Selective use of LPS serotypes may represent a useful tool for targeting specific inflammatory response mechanisms in these models. *(Am J Pathol 2015, 185: 2390–2401; http://dx.doi.org/10.1016/j.ajpath.2015.05.015)*

Preterm birth and its associated complications are now the leading cause of death among children <5 years.[1] A lack of knowledge of the basic molecular mechanisms orchestrating the onset of preterm and term labor has prevented advancements being made in early diagnosis and has inhibited the design of effective treatments. Evidence from both human and animal studies indicates that activation of inflammatory pathways in gestational tissues is a shared mechanism common to both normal and preterm birth.[2,3] Intrauterine inflammation is also associated with fetal brain injury, which may lead to long-term neurological disorders, such as cerebral palsy.[4,5] An increasing body of evidence supports the involvement of inflammation also, in the absence of overt infection, in preterm birth.[2] A key mediator of the inflammatory response in gestational tissues at the time of labor is NF-κ light chain enhancer of activated B cells (NF-κB); however, data have also implicated activator protein 1 (AP-1) and CCAAT/enhancer-binding proteins (C/EBPs) in the

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regulation of inflammatory pathways associated with the onset of labor.[6–9]

In various animal models of preterm labor (PTL), the Gram-negative bacterial cell wall component, lipopolysaccharide (LPS), is administered systemically to mimic bacterial infection during pregnancy or locally via an intrauterine injection to reflect ascending vaginal infection and/or chorioamnionitis.[10–21] Recognition of the LPS molecule by Toll-like receptors (TLRs), expressed on the surface of intrauterine cells,[15,21] activates an inflammatory cascade that drives proinflammatory cytokine production and, subsequently, the release of prostaglandins, cytokines, and chemokines promoting cervical ripening, uterine activation, and contractility.[14,22] Although the inflammatory response to LPS appears to be consistently achieved in mouse models of preterm birth, significant variation in the timing of preterm birth and neonatal survival outcomes is often reported, only a proportion of which can be attributed to differences in experimental design (eg, gestation age at time of injection, site of administration, animal species/strain, and LPS dose).

The LPS molecule is composed of a complex glycolipid containing a lipid A moiety (phosphorylated glucosamine disaccharide with multiple fatty acid chains), an oligosaccharide core, and an extending glycan polymer referred to as the O-antigen. The sugar composition of this polysaccharide side chain determines the serological specificity of the molecule, whereas the lipid A group typically confers toxicity.[23] Previous studies in rat models of hypothermia and albumin extravasation have reported functional differences caused by LPS serotype specificity.[24,25] Treatment of fever with the selective cyclooxygenase (COX)-2 inhibitor, 4-[5-(4-chlorophenyl)-3-(trifloromethyl)-1H-pyrazol-l-yl] benzenesulfonamide (SC-58236), is effective in the initial phase of animals administered with *Escherichia coli* O55:B5 LPS, but not those administered with *E. coli* O111:B4 LPS.[26,27] Moreover, different *E. coli* LPS serotypes can be associated with defined clinical syndromes of enteric/diarrheal disease.[28,29] Collectively, these data indicate serotype-specific activation of inflammatory pathways that translates into variable phenotypic responses. In the context of animal models of infection/inflammation-induced PTL, this could involve differential activation of the key inflammation-mediated pathways preceding labor, thus leading to differential maternal and neonatal outcomes. Therefore, we hypothesized that LPS serotypes elicit specific maternal and neonatal innate immune responses that involve differential activation of inflammatory pathways.

By using four different *E. coli* LPS serotypes (O111:B4, O55:B5, O127:B8, and O128:B12), we demonstrate functional disparity in LPS serotype activation of inflammatory pathways in the uterus and the pup brain. Our results indicate that the use of specific LPS serotypes may provide a useful tool for the selective activation of specific inflammatory response mechanisms in animal models of preterm birth.

Materials and Methods

Murine Studies and Ethics Statement

Animal studies were performed under UK Home Office License 70/6906, in accordance with the guidance to Animals Scientific Procedures Act of 1986, and with approval of the Imperial College London and University College London (London, UK) Ethical Review Committees. CD1 outbred virgin females were timed mated, and the presence of a copulatory plug was classified as embryonic day 0 (E0) of gestation. Mice were housed in open cages at 21°C ± 1°C with ad libitum access to standard rodent food and water and were exposed to a 12:12 light-dark cycle regimen. Unless otherwise stated, five biological replicates were collected for all experimental groups.

Murine Model of Inflammation-Induced PTL

Pregnant (E16) female dams were administered a s.c. injection of 2.5 mg/kg morphine 20 minutes before surgery. Animals were anesthetized by isoflurane, and a laparotomy was performed as previously described.[9,30,31] Briefly, both uterine horns were exteriorized and the number of live fetuses per horn was recorded. An intrauterine injection of 20 μg [25 μL total volume in phosphate-buffered saline (PBS)] of either *E. coli* LPS serotype O111:B4, O55:B5, O127:B8, or O128:B12 (Sigma Aldrich, Gillingham, UK) or sterile PBS was injected into the upper right uterine horn between the first and second sacs. Biochemical characteristics of the LPS serotypes were consistent [phenol extracted; protein content, ≤3%; solubility, 4.9 to 5.1 mg/mL; endotoxin level, ≥500,000 endotoxin units (EU)/mg]. Animals were continuously monitored after surgery remotely via an infrared closed circuit TV camera system during recovery until tissue collection or the onset of spontaneous delivery. The onset of labor was defined as delivery of the first pup.

Tissue Collection

For tissue collection, mice were anesthetized and sacrificed by cervical dislocation. A laparotomy was performed, uteri were immediately incised in the longitudinal direction, and pups were isolated and sacrificed by decapitation. Right and left horns of the uterus were snap frozen separately after removal of gestational membranes, placentas, and vasculature. Myometrium samples from the right uterine horns were used for mRNA and protein analyses. After decapitation, whole pup brains were isolated and snap frozen. Tissue was stored at −80°C until extraction. Before culling, pup viability was qualitatively assessed using a scoring system as follows: 3, pups displaying full body spontaneous movement when removed from the myometrium or amniotic sac; 2, pups with partial body movement (ie, lower half or full body movement when gently prodded with forceps); 1, pups exhibiting partial body movement or movement of