OBSTETRICS

Longitudinal expression of Toll-like receptors on dendritic cells in uncomplicated pregnancy and postpartum

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OBJECTIVE: Toll-like receptors (TLRs) are integral parts of the innate immune system and have been implicated in complications of pregnancy. The longitudinal expression of TLRs on dendritic cells in the maternal circulation during uncomplicated pregnancies is unknown. The objective of this study was to prospectively evaluate TLRs 1-9 as expressed on dendritic cells in the maternal circulation at defined intervals throughout pregnancy and postpartum.

STUDY DESIGN: This was a prospective cohort of 30 pregnant women with uncomplicated pregnancies and 30 nonpregnant controls. TLRs and cytokine expression was measured in unstimulated dendritic cells at 4 defined intervals during pregnancy and postpartum. Basal expression of TLRs and cytokines was measured by multicolor flow cytometry. The percent-positive dendritic cells for each TLRs were compared with both nonpregnant and postpartum levels with multivariate linear regression.

RESULTS: TLRs 1, 7, and 9 were elevated compared with nonpregnant controls with persistent elevation of TLR 1 and interleukin-12 (IL-12) into the postpartum period. Concordantly, levels of IL-6, IL-12, interferon alpha, and tumor necrosis factor alpha increased during pregnancy and returned to levels similar to nonpregnant controls during the postpartum period. The elevated levels of TLR 1 and IL-12 were persistent postpartum, challenging notions that immunologic changes during pregnancy resolve after the prototypical postpartum period.

CONCLUSION: Normal pregnancy is associated with time-dependent changes in TLR expression compared with nonpregnant controls; these findings may help elucidate immunologic dysfunction in complicated pregnancies.

Key words: dendritic cells, innate immune system, pregnancy, toll-like receptors

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The maternal immune system plays an integral role at the maternal-fetal interface;[1-8] yet all the specific contributions of the immune system in normal and abnormal pregnancies remain to be elucidated. Toll-like receptors (TLRs) are an important aspect of the innate immune system that recognize both microbial and endogenous ligands as well as host products released during tissue damage.[1,9] Much evidence exists to support a role for TLRs in both uncomplicated pregnancies[2,8] and pregnancies complicated by preeclampsia,[6,10] and preterm labor.[11-13] However, there is limited information on whether expression of TLRs in the maternal circulation changes during uncomplicated pregnancies. By better understanding any time-dependent changes in TLR in normal pregnancies, comparisons can be made to TLR levels in pathologic conditions of pregnancy such as preterm labor, preeclampsia, and stillbirth.

TLRs are expressed on various antigen-presenting cells; dendritic cells are a main group of antigen-presenting cells that express 9 of the 10 TLR isoforms expressed in humans. There are 2 types of dendritic cells that differ in their TLR expression: myeloid dendritic cells typically express TLRs 1-6 and 8, whereas plasmacytoid dendritic cells express TLRs 7 and 9.[14] TLRs bind to highly conserved protein sequences known as pathogen-associated molecular patterns (PAMPs), which are expressed by and unique to specific microorganisms or endogenous ligands.[15] TLR 1 binds triacetylated lipoproteins, a component of gram-positive bacteria. TLR 2 recognizes bacterial lipoproteins, gram-positive bacterial peptidoglycan and lipoteichoic acid through the formation of heterodimers with TLR 1 or TLR 6.

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TLR 3 binds double-stranded RNA, and TLR 4 binds gram-negative bacterial lipopolysaccharide. TLR 5 recognizes bacterial flagellin; TLR 6 binds diacylated lipoprotein. TLR 7 and TLR 8 bind single-stranded RNA. TLR 9 binds nonmethylated CpG DNA, including fetal DNA.[16] In addition, TLRs interact with endogenous molecules called danger-associated molecular patterns (DAMPs) including reactive oxygen species and proteins released from dying cells under stress.[1,17] For example, TLR 4 and TLR 2 can bind DAMPs, such as heat shock protein 60, heat shock protein 70, and fibrinogen.[1]

Recent studies demonstrate a significant role of specific TLRs in both normal and complicated pregnancies at the maternal-fetal interface throughout gestation.[1-8] Elucidation of normative trends in these pregnancies may promote further understanding of altered TLR expression in women with complicated pregnancies.

In this current study, we sought to evaluate longitudinal TLR expression in dendritic cells during normal term pregnancies compared with the postpartum state and to nonpregnant controls. On the basis of prior studies demonstrating a proinflammatory state in the third trimester, we hypothesized that TLR and cytokine expression will increase toward the third trimester and return to baseline levels at the time of the postpartum collection.

Materials and Methods

Subject recruitment

After receiving institutional review board approval, we recruited patients receiving care at 2 teaching hospitals. For the pregnant cohort, nonobese women of any parity were included if they had singleton gestations and were without significant medical conditions including diabetes or chronic hypertension. Similarly, we selected control patients through recruitment in the gynecologic clinic after initial screening by the patient's physician. Women self-identified as free of medical problems and cigarette smoking. Women self-reported their last menstrual periods and whether there was possibility of an undiagnosed pregnancy. Women in both groups denied any use of immune-modulating medications or recent significant illnesses requiring antibiotics. Body mass index was based on self-reported height and scale-obtained weight at the time of the initial visit.

Blood sampling

Blood was obtained at 4 standardized collection times for the cohort of pregnant women: Collection 1 (first trimester) during routine initial prenatal laboratories or at the time of elective genetic screening; Collection 2 (mid-trimester) between 26-28 weeks at the time of routine gestational diabetes screening; Collection 3 (day of delivery) on presentation to labor/delivery before delivery of the infant; Collection 4 (postpartum) at the scheduled 6 week postpartum visit. Enrolled women had additional research blood obtained at the time of routine intravenous placement and standard admission laboratories on presentation to labor/delivery because of scheduled induction of labor, spontaneous labor, or scheduled cesarean delivery. Blood samples from nonpregnant control women were collected at clinic visits.

Isolation of human peripheral blood dendritic cells

Human peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Histopaque (Sigma-Aldrich, St. Louis, MO) gradient centrifugation.

Cell staining and flow cytometry

For fluorescence-activated cell sorting (FACS) assays, dendritic cells were identified in the PBMC preparations by staining with fluorescent antibodies to specific surface markers. PBMCs were suspended in RPMI 1640 medium plus 10% FBS and adjusted to a concentration of 2×10^6 cells/mL. Cells were then washed and frozen in 90% fetal bovine serum (FBS) containing 10% DMSO, and stored at -80°C. On the day of staining and analysis, cells were thawed, and cell surface labeling at 4°C was followed by washing in PBS containing 2% FBS and 0.05% sodium azide. For samples where intracellular staining was performed, they were fixed with BD Cytofix/Cytoperm buffer, then permeabilized in BD Perm/Wash buffer as described in the manufacturer's protocol.

For analyses of TLR expression, conventional and plasmacytoid dendritic cells were identified using antibodies against cell-surface markers (Anti-CD3 APC-Cy7, -CD14 APC-Cy7, -CD16 APC-Cy7, -CD19 APC-Cy7, -CD11c APC, -CD123 PE-Cy5, -HLA-DR PE-Cy7; BD Pharmingen, San Diego, CA). Cell-surface TLR expression was assessed using antibodies against TLR 1 (PE, clone GD2.F4; eBioscience, San Diego, CA), TLR 2 (Alexa 700, clone TL2.1; eBioscience), TLR 4 (Alexa700, HTA125; eBioscience), TLR 5 (FITC, 85B152.5; AbCam); intracellular TLR expression using antibodies against TLR 3 (FITC, 40C1285.6; AbCam), TLR 7 (FITC, 533707; R&D Systems), TLR 8 (PE, 44C143; AbCam), TLR 9 (PE, eB72-1665; BD Pharmingen); and finally, intracellular cytokine expression was assessed using interleukin-6 (IL-6) (PE, MQ2-6A3; BD Pharmingen), IL-12 (e450, Clone C8.6; eBioscience), tumor necrosis factor alpha (TNFα) (Alexa700, MAb11; BDPharmingen), interferon alpha (IFNα) (FITC, Clone FHC520; Chromaprobe, Maryland Heights, MO).

Approximately 0.2-0.5 million total events per sample were assessed using an LSR II flow cytometry instrument (BD Biosciences) with analysis using FlowJo software (Tree Star, Ashland, OR). Initial gating on parent dendritic cell subsets was further characterized by assessment of TLR and cytokine expression.

Statistical analysis

A sample size calculation was performed to detect a 20% difference in TLR protein expression between the pregnant and nonpregnant cohort with a power of 0.8. Therefore, we aimed to enroll 29 pregnant women and 29 nonpregnant controls. Assuming 30% loss to follow-up from miscarriage or missed collection because of logistical reasons, we aimed to enroll 40 pregnant women.