Published in final edited form as:

J Perinat Med. 2020 September 25; 48(7): 700–722. doi:10.1515/jpm-2020-0159.

Pregnancy-specific transcriptional changes upon endotoxin exposure in mice

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Abstract

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Declaration of Interests Statement

The authors have no conflicts of interest to declare.

Background: Pregnant women are more susceptible to certain infections; however, this increased susceptibility is not fully understood. Herein, systems biology approaches were utilized to elucidate how pregnancy modulates tissue-specific host responses to a bacterial product, endotoxin.

Methods: Pregnant and non-pregnant mice were injected with endotoxin or saline on 16.5 days post coitum (n = 8 – 11 per group). The uterus, cervix, liver, adrenal glands, lung, and brain were collected 12 hours after injection and transcriptomes were measured using microarrays. Heatmaps and principal component analysis were used for visualization. Differentially expressed genes (DEGs) between groups were assessed using linear models that included interaction terms to determine whether the effect of infection differed with pregnancy status. Pathway analysis was conducted to interpret gene expression changes.

Results: We report herein a multi-organ atlas of the transcript perturbations in pregnant and non-pregnant mice in response to endotoxin. Pregnancy strongly modified the host responses to endotoxin in the uterus, cervix, and liver. In contrast, pregnancy had a milder effect in host response to endotoxin in the adrenal glands, lung, and kidney. However, pregnancy did not drastically affect the host response to endotoxin in the brain.

Conclusion: Pregnancy imprints organ-specific host immune responses upon endotoxin exposure. These findings provide insights into the host-response against microbes during pregnancy.

Keywords

Host immune response; Systemic inflammation; Preterm labor; Preterm birth; Inflammation; Infection; Acute chorioamnionitis; Funisitis; Fetal Inflammation; Maternal Inflammation

INTRODUCTION

Pregnancy was traditionally considered to be a state of immunosuppression [1–4]; however, it is now clear that this is not the case [5–13]. Yet, pregnant women are more susceptible to some infections [14–16] but not to others (e.g. COVID-19) [17–21]. For example, viral infections including influenza [14, 22–27], varicella [28–32], and measles [33, 34] have increased severity among pregnant women. In addition, malarial infection, a major cause of adverse birth outcomes in Africa [35, 36], is more severe in pregnant women than in non-pregnant women [16, 37, 38]. Although rare, bacterial pneumonia has also been reported to affect pregnant women more severely compared to other populations [39]. Furthermore, a proportion (~2%) of pregnant women develop acute pyelonephritis when asymptomatic urinary tract infections are untreated [40–42], which can lead to serious maternal inflammatory responses [43, 44]. Importantly, women with acute pyelonephritis [43, 45–49] can experience preterm labor and birth, the leading cause of neonatal morbidity and mortality worldwide [50–52]. Yet, the increased susceptibility to infection during pregnancy is not fully understood, and therefore, requires further investigation.

Pregnancy-specific susceptibility to infection has been attributed to the physiological changes required to accommodate the growth and development of the fetus [53, 54] and in preparation for parturition [55–57]. These changes include increased blood volume and

cardiovascular output [58–62] and the modulation of pulmonary [63–69], hepatic [70–74], renal [75–81], adrenal [82–86] functions as well as immunological adaptations [87–91]. Therefore, this increased susceptibility must involve multiple organ function. However, up to date, no studies have evaluated the global differences in host response to microbial products between pregnant and non-pregnant subjects.

To study the biological processes implicated in pregnancy-specific susceptibility to infection; herein, we propose the utilization of animal models. This concept is supported by the observation that pregnant rodents and rabbits develop a generalized Shwartzman reaction after a single injection of endotoxin, whereas non-pregnant animals require two injections [92–94]. Therefore, in the current study, pregnant and non-pregnant animals were exposed to an endotoxin dose capable of triggering preterm labor and birth [95], and systems biology approaches were utilized to gain new biological insights regarding how pregnancy modulates host response to bacterial products in a tissue-specific manner.

Materials and methods

Mice

C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and bred in the animal facility at the C. S. Mott Center for Human Growth and Development (Wayne State University, Detroit, MI, USA). Mice were housed under a circadian cycle (12 h light: 12 h dark). Females, 8–12 weeks old, were mated with male mice of proven fertility. Females were examined daily between 8:00 a.m. and 9:00 a.m. for the appearance of a vaginal plug, which was considered as 0.5 days *post coitum* (dpc). Females were then separated from the males, and their weights were monitored daily. Pregnancy was confirmed by a gain of 2 g by 12.5 dpc. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Wayne State University, Detroit, MI, USA (Protocol Number A 09-08-12).

Intraperitoneal injection of endotoxin

On 16.5 dpc, dams were intraperitoneally (i.p.) injected with either 15 µg of lipopolysaccharide (LPS) (*Escherichia coli* O111:B4; Sigma-Aldrich, St. Louis, MO, USA) in 200 µL of sterile 1X phosphate-buffered saline (PBS) or 200 µL of 1X PBS alone as a control (n = 10 per group). Non-pregnant female mice (8–12 weeks old) received the same i.p. injection of LPS or 1X PBS alone (n = 8 – 11 per group). The i.p. injection of 15 µg LPS consistently results in a 100% rate of preterm delivery between 17.0–17.5 dpc [96, 97]. Therefore, all injected mice were euthanized approximately 12 h post-injection. Animal dissection was then performed to obtain the maternal uterus, cervix, liver, adrenal glands, kidney, lung, and brain. Collected tissues were placed in RNA^{later} Stabilization Solution (Life Technologies, Grand Island, NY, USA) and stored at –80°C until use.

Isolation of RNA from murine tissues

Approximately 30–50 mg of each tissue was homogenized in 800 µL of Qiagen Buffer RLT Plus lysis reagent via bead-milling on the TissueLyser II (Qiagen, Hilden, Germany). Total RNA was purified following the large volume protocol for the QIASymphony RNA Kit on

the QIAasympyphony SP as described by the manufacturer (Qiagen). Purified total RNA was quantified by UV spectrophotometry using the DropSense 96 Microplate Spectrophotometer (PerkinElmer, Waltham, MA, USA) and the purity was assessed based on the A260/A280 and A260/A230 ratios. An aliquot of the RNA was assessed for quality by RNA ScreenTape on an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA).

Amplification and labeling of RNA

Total RNA was biotinylated and amplified using the Illumina TotalPrep™-96 RNA Amplification Kit (Ambion by Life Technologies, Carlsbad, CA, USA), following the manufacturer's protocol. Total RNA (250 ng) was reverse transcribed using a modified Eberwine method. The intermediate double-stranded complementary DNA served as the template for *in vitro* transcription generating hundreds to thousands of biotinylated, antisense RNA (cRNA) copies per mRNA transcript.

Microarray processing

Biotinylated cRNA (1500 ng) was hybridized to Illumina MouseWG-6 v2.0 arrays (Illumina, San Diego, CA, USA) and the arrays were washed, stained, and scanned as described in the Illumina WGGEX Direct Hybridization Assay Guide (Part# 11322355 A, Illumina, www.illumina.com). Briefly, biotinylated cRNA was mixed with hybridization buffer, denatured at 65°C, and introduced to the array. Hybridization was carried out at 58°C for 16–20 hours in a hybridization oven (Illumina). Next, the arrays were then removed, washed, and stained using streptavidin-Cy3. Finally, the stained arrays were dried and scanned using the Illumina iScan system (Illumina).

Microarray and statistical data analysis

Microarray data for each tissue was analyzed separately, although following the same strategy. Probe signal intensities were offset by adding a unit value to allow for subsequent log2 transformation to improve normality. The transformed data was then quantile normalized [98], using the preprocessCore [99] package in Bioconductor [100], to make the sample distributions identical. Only the probes that were detected (detection p-value < 0.1) in at least five samples were retained for further analysis. Principal component analysis (PCA) were conducted using data from all probes while heatmap representations were constructed using data from the top 200 genes with the highest variability across all samples for a given tissue. The goal of these unsupervised analyses was to visualize the transcriptomes and identify the sources of variability. Microarray probes were mapped to genes using annotation from the illuminaMousev2.db [101] package of Bioconductor [100]. Differentially expressed genes (DEGs) between groups were selected by fitting linear models, using the limma [102] package in Bioconductor [100], that included interaction terms to determine if the effect of infection differed by pregnancy status. Significance of the effects were inferred based on moderated t-test's false discovery rate (FDR) [103, 104] adjusted p-values (q-values) <0.1 and minimum fold change of 1.5 fold. The results were summarized with volcano plots and Venn diagrams [105] and UpSet [106] plots to assess overlap among gene lists.

The lists of genes with significant interactions between treatment and pregnancy status in each tissue were then mined to identify significantly enriched pathways using a hypergeometric test implemented in iPathwayGuide (ADVAITA Bioinformatics, Ann Arbor, MI, USA) [107–109]. The pathway annotation database was derived from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, release 90.0+/05–29 [110, 111]. Pathway enrichment significance was based on adjusted p-values <0.1, and up to 10 of the significant pathways are shown in Figures 1 to 7, section F.

RESULTS

Herein, we used a model of endotoxin-induced systemic inflammation that leads to preterm birth in pregnant mice [95–97, 112–115]. Non-pregnant mice were injected with the same dose of endotoxin. The aim of these experiments was to compare the transcriptome changes upon systemic inflammation induced by a bacterial product between pregnant and non-pregnant mice. Below, we describe the results in a tissue-specific manner.

The transcriptome of the uterine tissues in pregnant and non-pregnant mice in response to endotoxin

The uterus is considered the primary reproductive organ as is essential for a successful pregnancy. Therefore, we first examined the transcriptome of the pregnant and non-pregnant uterus in response to endotoxin-induced systemic inflammation (Figure 1A). PCA and heatmap visualization revealed that the uterine transcriptomes separated according to pregnancy status and the exposure to endotoxin (Figure 1B&C). DEG analysis showed that in pregnant mice, endotoxin induces the differential expression of 557 genes (312 upregulated, 245 downregulated) in the uterus (Figure 1D). DEG analysis showed that in non-pregnant mice, endotoxin induces the differential expression of 613 genes (391 upregulated, 222 downregulated) in the uterus (Figure 1D). Endotoxin-induced changes in the uterine transcriptome of pregnant mice were significantly different from those of non-pregnant mice (70 genes had a higher fold change, and 158 had a lower fold change, in response to endotoxin among pregnant compared to non-pregnant mice) (Figure 1E); the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 1). Pathway analysis of these pregnancy-specific genes identified multiple pathways, namely malaria, systemic lupus erythematosus, intestinal immune network for IgA production, antigen processing and presentation, leishmaniasis, extracellular matrix (ECM)-receptor interaction, focal adhesion, tuberculosis, and inflammatory bowel disease (IBD) (Figure 1F). These findings show that pregnancy modifies the host response to bacterial products in the uterus.

The transcriptome of the cervix in pregnant and non-pregnant mice in response to endotoxin

The cervix is the lower portion of the uterus and serves as the connection between the upper part of the vagina and the endometrial cavity [116]. The cervix plays a key role in the maintenance of the pregnancy and, as a result, abnormal or premature alterations of this organ (e.g. shortening or dilation) before term pregnancy can lead to preterm birth [117–123]. Therefore, we next investigated the transcriptome of the pregnant and non-pregnant

cervix in response to endotoxin-induced systemic inflammation (Figure 2A). PCA and heatmap visualization revealed that the cervical transcriptomes separated according to pregnancy status and the exposure to endotoxin (Figure 2B&C). DEG analysis showed that in pregnant mice, endotoxin induces the differential expression of 939 genes (525 upregulated, 414 downregulated) in the cervix (Figure 2D). DEG analysis showed that in non-pregnant mice, endotoxin induces the differential expression of 601 genes (386 upregulated, 215 downregulated) in the cervix (Figure 2D). Endotoxin-induced changes in the cervical transcriptome of pregnant mice were significantly different from those of non-pregnant mice (394 genes had a higher fold change, and 325 had a lower fold change, in response to endotoxin among pregnant compared to non-pregnant mice) (Figure 2E); the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 2). Pathway analysis of these pregnancy-specific genes identified multiple pathways, namely chemical carcinogenesis, c-type lectin receptor signaling pathway, drug metabolism – cytochrome P450, terpenoid backbone biosynthesis, phagosome, metabolism of xenobiotics by cytochrome P450, central carbon metabolism in cancer, and antigen processing and presentation (Figure 2F). These findings show that pregnancy strongly modifies the host response to bacterial products in the cervix.

The transcriptome of the liver in pregnant and non-pregnant mice in response to endotoxin

The liver is an essential metabolic center that detoxifies various metabolites and synthesizes proteins and digestive biochemicals [124–126]. The liver plays a crucial role in storing and processing fat, glucose, and amino acids from the gut to maintain homeostasis [124–126]; therefore, the functions of this organ are largely affected during pregnancy. The liver can also eliminate invading bacteria and bacterial products that have passed through the gut, preventing the spread of bacteria into the bloodstream (i.e. bacterial translocation) [127–129]. Hence, we examined the liver transcriptome of pregnant and non-pregnant mice upon injection with an endotoxin (Figure 3A). Heatmap visualization, and to a lesser degree PCA, revealed that the transcriptome of the liver separated according to the exposure to endotoxin and pregnancy status (Figure 3B&C). DEG analysis showed that in pregnant mice, endotoxin induces the differential expression of 1,193 genes (560 upregulated, 633 downregulated) in the liver (Figure 3D). DEG analysis showed that in non-pregnant mice, endotoxin induces the differential expression of 1,651 genes (789 upregulated, 862 downregulated) in the liver (Figure 3D). Endotoxin-induced changes in the liver transcriptome of pregnant mice were significantly different from those of non-pregnant mice (271 genes had a higher fold change, and 202 had a lower fold change, in response to endotoxin among pregnant compared to non-pregnant mice) (Figure 3E); the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 3). Pathway analysis of these pregnancy-specific genes identified multiple pathways, namely metabolic pathways, drug metabolism – cytochrome P450, chemical carcinogenesis, biosynthesis of amino acids, metabolism of xenobiotics by cytochrome P450, glycine, serine and threonine metabolism, retinol metabolism, tyrosine metabolism, carbon metabolism, and steroid hormone biosynthesis (Figure 3F). These findings show that pregnancy strongly modifies the host response to bacterial products in the liver.

The transcriptome of the adrenal glands in pregnant and non-pregnant mice in response to endotoxin

The adrenal glands (composed of cortex and medulla) are components of the endocrine system and are located on top of the kidneys (i.e. suprarenal glands). The adrenal glands produce several different types of hormones that help regulate metabolism, the immune system, and responses to stress, as well as contribute to pregnancy maintenance, fetal growth, and the process of parturition [55–57, 130]. Hence, we evaluated the transcriptome of the adrenal glands in pregnant and non-pregnant mice in response to endotoxin (Figure 4A). PCA and heatmap visualization revealed that the adrenal gland transcriptomes separated according to the exposure to endotoxin and pregnancy status (Figure 4B&C). DEG analysis showed that in pregnant mice, endotoxin induces the differential expression of 1,694 genes (761 upregulated, 933 downregulated) in the adrenal glands (Figure 4D). DEG analysis showed that in non-pregnant mice, endotoxin induces the differential expression of 1,462 genes (662 upregulated, 800 downregulated) in the adrenal glands (Figure 4D). Endotoxin-induced changes in the adrenal gland transcriptome of pregnant mice were significantly different from those of non-pregnant mice (80 genes had a higher fold change, and 87 had a lower fold change, in response to endotoxin among pregnant compared to non-pregnant mice) (Figure 4E); the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 4). Pathway analysis of these pregnancy-specific genes identified multiple pathways, namely influenza A, NOD-like receptor signaling pathway, cytokine-cytokine receptor interaction, steroid biosynthesis, herpes simplex virus 1 infection, chemokine signaling pathway, carbon metabolism, measles, graft-versus-host disease, and type I diabetes mellitus (Figure 4F). These findings show that pregnancy mildly modifies the host response to bacterial products in the adrenal glands.

The transcriptome of the kidney in pregnant and non-pregnant mice in response to endotoxin

The kidneys play a central role in the maintenance of homeostasis by regulating the body's fluid and ion balance, filtering waste products from the blood and forming urine, as well as secreting hormones [131–133]. Therefore, pregnancy modifies the physiological functions of the kidneys, and thus, we also evaluated the transcriptome of this organ in our study (Figure 5A). PCA and heatmap visualization revealed that the transcriptome of the kidney separated according to the exposure to endotoxin and pregnancy status (Figure 5B&C). DEG analysis showed that in pregnant mice, endotoxin induces the differential expression of 1,040 genes (497 upregulated, 543 downregulated) in the kidney (Figure 5D). DEG analysis showed that in non-pregnant mice, endotoxin induces the differential expression of 1,865 genes (858 upregulated, 1,007 downregulated) in the kidney (Figure 5D). Endotoxin-induced changes in the kidney transcriptome of pregnant mice were significantly different from those of non-pregnant mice (170 genes had a higher fold change, and 117 had a lower fold change, in response to endotoxin among pregnant compared to non-pregnant mice) (Figure 5E); the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 5). Pathway analysis of these pregnancy-specific genes identified multiple pathways, namely metabolic pathways, pentose and glucuronate interconversions, peroxisome proliferator-activated receptor (PPAR) signaling pathway, nitrogen metabolism, butanoate metabolism, phenylalanine metabolism, 2-oxocarboxylic acid metabolism, and

proximal tubule bicarbonate reclamation (Figure 5F). These findings show that pregnancy modifies the host response to bacterial products in the kidney.

The transcriptome of the lung in pregnant and non-pregnant mice in response to endotoxin

The primary function of the lungs is to facilitate gas exchange between the atmosphere and the bloodstream [134–136]. Pregnant women are more likely to develop pulmonary complications (e.g. acute respiratory distress syndrome) in the context of infection [137, 138]; therefore, we also examined the transcriptome of the lung in pregnant and non-pregnant animals injected with endotoxin (Figure 6A). PCA and heatmap visualization revealed that, unlike the uterine and cervical transcriptomes, the lung transcriptomes largely separated according to the exposure to endotoxin although among untreated animals there were slight changes with pregnancy status (Figure 6B&C). DEG analysis showed that in pregnant mice, endotoxin induces the differential expression of 911 genes (515 upregulated, 396 downregulated) in the lung (Figure 6D). DEG analysis showed that in non-pregnant mice, endotoxin induces the differential expression of 1,374 genes (711 upregulated, 663 downregulated) in the lung (Figure 6D). Endotoxin-induced changes in the lung transcriptome of pregnant mice were significantly different from those of non-pregnant mice (37 genes had a higher fold change, and 60 had a lower fold change, in response to endotoxin among pregnant compared to non-pregnant mice) (Figure 6E); the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 6). Pathway analysis of these pregnancy-specific genes identified multiple pathways, namely biosynthesis of unsaturated fatty acids, PPAR signaling pathway, complement and coagulation cascades, phagosome, human papillomavirus infection, systemic lupus erythematosus, *Staphylococcus aureus* infection, leishmaniasis, pertussis, ECM-receptor interaction, and fatty acid elongation (Figure 6F). These findings show that pregnancy mildly modifies the host response to bacterial products in the lung.

The transcriptome of the brain in pregnant and non-pregnant mice in response to endotoxin

The brain is the center of the nervous system and controls all neural activities, including cognition, movement, and behavior in vertebrates; yet, pregnancy may not influence the brain transcriptome. Therefore, we utilized this organ as a control tissue (Figure 7A). As expected, PCA and heatmap visualization showed that the brain transcriptomes were not clearly separated according to pregnancy status nor the exposure to endotoxin (Figure 7B&C). DEG analysis showed that in pregnant mice, endotoxin induces the differential expression of 96 genes (85 upregulated, 11 downregulated) in the brain (Figure 7D). DEG analysis showed that in non-pregnant mice, endotoxin induces the differential expression of 221 genes (160 upregulated, 61 downregulated) in the brain (Figure 7D). Endotoxin-induced changes in the brain transcriptome of pregnant mice were significantly different from those of non-pregnant mice (20 genes had a higher fold change, and 43 had a lower fold change, in response to endotoxin among pregnant compared to non-pregnant mice) (Figure 7E); the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 7). Pathway analysis of these pregnancy-specific genes identified multiple pathways, namely cocaine addiction, purine metabolism, renin secretion, morphine

addiction, gap junction, circadian entrainment, dopaminergic synapse, alcoholism, and chemical carcinogenesis (Figure 7F). These findings show that pregnancy does not strongly modify the host response to bacterial products in the brain.

Overall analysis of the transcriptome in pregnant mice

A comprehensive overview of the DEGs in the seven organs evaluated in pregnant mice is provided in Figure 8. Figures 8A and 8B display the UpSet plots of the upregulated and downregulated differentially expressed genes in pregnant mice based on the transcriptome of the seven organs. The horizontal bars show the number of differentially expressed genes identified in each organ. The vertical bars display the amount of overlap among the genes identified in each organ. Connected dots signify shared genes among tissues. This analysis revealed that the response to endotoxin in the adrenal glands was the most unique. In addition, most of the up- and down-regulated genes upon injection of endotoxin were shared among organs, except the brain. Figures 8C and 8D show the Venn diagrams of the shared and non-shared genes among the seven tissues. This analysis revealed that although several up- and down-regulated genes were shared among organs, each organ displayed unique gene expression changes. Therefore, pregnancy changes the transcriptome of multiple organs in response to endotoxin.

DISCUSSION

In the study herein, we provide a multi-organ atlas of the genes that are differentially expressed in pregnant and non-pregnant mice in response to endotoxin. Overall, pregnancy strongly modifies the host response to endotoxin in the uterus, cervix, and liver. In contrast, pregnancy has a milder effect in host response to endotoxin in the adrenal glands, lung, and kidney. However, pregnancy does not drastically affect the host response to endotoxin in the brain. Collectively, these findings show that pregnancy imprints specific host responses against microbial products, mainly in the uterus, cervix, and liver.

The uterine transcriptome in the pregnant and non-pregnant state

As pregnancy progresses, the uterus undergoes dramatic anatomical and physiological modifications in order to accommodate the growing fetus and placenta [139]. These changes comprise an increase in the size of the uterus, transformation of the thickness of the uterine wall, and enlargement of the blood vessels, among others [140–144]. At the cellular level, pregnancy induces hypertrophy of myocytes and accumulation of fibrous and elastic tissue [143, 145, 146]. Additionally, the recruitment of leukocytes from the maternal circulation into the uterus throughout pregnancy drives local and specific cellular immune responses [147, 148]. These physiological alterations can also be detected at the gene expression level [149]. For example, the transcriptome of the uterine tissues from women at term (without labor) is different from that of non-pregnant women [149]. The differentially expressed genes coding proteins implicated in several physiological functions, such as growth and differentiation, signal transduction, cell adhesion, molecular recognition and immunity, and structure and contractility [149]. Consistently, in the current study, we report that the uterine transcriptome between pregnant and non-pregnant mice is strikingly different. Therefore, pregnancy alone changes the transcriptome of the human uterine tissues.

The onset of labor at term is associated with dramatic modifications in the transcriptome of the uterine tissues [150–163]. These transcriptional changes involve various biological pathways, including inflammatory signaling pathways such as cytokine signaling, chemotaxis, and immune response, as well as non-inflammatory pathways such as apoptosis, cell proliferation, and differentiation [163]. Such findings are in line with the concept that the physiological process of term labor comprises a complex transformation from a quiescent to a highly contractile stage [55, 56, 164] and is inflammatory in nature [56, 165–173]. The pathophysiological process of preterm labor leading to preterm birth is also associated with changes in the uterine transcriptome [174–176]. Yet, a mouse study found that there are differences in the uterine transcriptome between the physiologic process of labor (term labor) and two different subsets of preterm labor/birth (i.e. endotoxin- and RU486-induced) [162]. In the study herein, we extend these observations by showing that pregnant mice respond differently than non-pregnant mice to endotoxin exposure. Interestingly, we report that specific signaling pathways are altered solely in pregnant mice, namely malaria, systemic lupus erythematosus, intestinal immune network for IgA production, antigen processing and presentation, leishmaniasis, ECM-receptor interaction, focal adhesion, tuberculosis, and IBD. Collectively, these results suggest the pregnant uterus mounts a specific immune response during microbial infection.

The cervical transcriptome in the pregnant and non-pregnant state

The uterine cervix is a central organ which acts as a structural and immunological barrier in both pregnant and non-pregnant women [177–181]. The cervical epithelial and stromal cells are capable of generating a host immune response against pathogens [177–181]. In non-pregnant women, this immunological function changes in accordance with the phase of the menstrual cycle and in the post-menopausal period [177, 178, 180]. The cervix also serves as a gatekeeper of pregnancy and has a central role in the physiological process of parturition [181–183]. Throughout pregnancy, the cervix undergoes remodeling, permitting the transformation from closed and rigid to a distensible structure that stretches to allow the passage of the fetus [181–188]. The premature process of cervical remodeling can lead to preterm birth in a subset of women [119, 123, 189–195]. At the cellular level, the process of cervical remodeling comprises inflammatory processes characterized by an influx of immune cells into the stroma [181, 183, 196–199], upregulation of cytokines and chemokines [168, 172, 200], and degradation of cross-linked collagen by collagenases such as metalloproteinases [201–204]. An inflammatory milieu is also required during the repair phase in the post-partum period [181, 198, 205, 206].

In line with the aforementioned concepts, the transcriptome of the cervix during pregnancy is dynamic. For example, transcriptome analysis of the rat cervix identified major gene differences between the prepartum period and the non-pregnant state, including canonical pathways for cell signaling and inflammation [207]. Interestingly, in women without labor at term, there are major differences between the transcriptome of the unripe and ripe cervix, including several biological processes (e.g. cell adhesion, regulation, and anatomical structure) but not inflammation [208]. Nevertheless, the cervical transcriptome of women who underwent spontaneous labor at term, including cervical shortening, is enriched in biological processes related to the immune response [209, 210].

Yet, the transcriptomic signature of the cervix of women who underwent the pathological process of preterm labor is different to that of the physiological process of labor at term [211]. This concept is supported by a mouse study showing that several genes related to immunity and inflammation were upregulated in the cervix of mice prior to inflammation-induced preterm birth, but not prior to labor at term [212]. Additionally, a subsequent study showed a distinct transcriptome signature between cervical ripening at term and that induced in preterm gestations by inflammation [213]. Importantly, the process of preterm cervical ripening involved genes coding for inflammasome components, a central pathway in the pathophysiology of preterm birth [214–221].

Consistent with the abovementioned literature, herein, we report that the cervical transcriptome of pregnant dams is different from that of non-pregnant mice and that the administration of an endotoxin induces the upregulation of inflammatory genes. Moreover, for the first time, we show that the pregnant cervix responds differently to endotoxin exposure than in a non-pregnant state. Specifically, we found that distinct signaling pathways are solely altered in pregnant mice, namely chemical carcinogenesis, c-type lectin receptor signaling pathway, drug metabolism – cytochrome P450, terpenoid backbone biosynthesis, phagosome, metabolism of xenobiotics by cytochrome P450, central carbon metabolism in cancer, and antigen processing and presentation. Collectively, these results suggest that the pregnant cervix mounts a unique immune response against bacterial infections.

The liver transcriptome in the pregnant and non-pregnant state

During pregnancy, women undergo significant metabolic and physiological alterations. Therefore, the physiological and biochemical profile of the liver is also altered during pregnancy, which is largely driven by the sex hormones (estrogens and progesterone) [222]. Specifically, the blood flow of the hepatic artery and portal vein greatly increases [71], and the serum concentrations of the liver enzymes, albumin, fibrinogen, and lipids vary [73] during pregnancy. In addition, the size of the liver increases during pregnancy, which is explained by the progressive increase in cell proliferation and inhibition of apoptosis signaling [223]. A subsequent study showed that the transcriptome of the liver changes across pregnancy, involving several metabolic pathways such as citrate cycle, retinol metabolism, bile acid synthesis, and steroid bile synthesis [224]. In addition to the role as a primary metabolic organ, there is solid evidence suggesting that the liver also plays an important function in immune defense [225]. Indeed, Kupffer cells (liver-specific macrophages) have the potential to eliminate circulating endotoxin [226]. Also, the liver synthesizes numerous soluble pathogen-recognition receptors and complement components, which are critical for controlling systemic innate immunity [227] and are pathways associated with pregnancy complications [228–233].

In line with previous studies, in the current study, we showed that the transcriptome of the liver of pregnant mice is different from that of non-pregnant mice. Specifically, we found that distinct signaling pathways are solely altered in pregnant mice, namely metabolic pathways, drug metabolism – cytochrome P450, chemical carcinogenesis, biosynthesis of amino acids, metabolism of xenobiotics by cytochrome P450, glycine, serine and threonine

metabolism, retinol metabolism, tyrosine metabolism, carbon metabolism, and steroid hormone biosynthesis. Interestingly, the liver of pregnant and non-pregnant mice responds differently to endotoxin exposure, implying that bacterial infections trigger distinct metabolic and immune processes in the liver during pregnancy.

Conclusion

In the current study, systems biology approaches revealed the tissue-specific responses to endotoxin in pregnant and non-pregnant animals. Overall, pregnancy strongly modifies the host response to endotoxin in the uterus, cervix, and liver. In contrast, pregnancy has a milder effect in host response to endotoxin in the adrenal glands, lung, and kidney. However, pregnancy does not drastically affect the host response to endotoxin in the brain. Collectively, these data show that pregnancy imprints organ-specific and unique host responses upon endotoxin exposure. These findings provide insight into the host-response against microbes during pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This research was supported, in part, by the Perinatology Research Branch (PRB), Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, U. S. Department of Health and Human Services (NICHD/NIH/DHHS), and, in part, with federal funds from the NICHD/NIH/DHHS under Contract No. HHSN275201300006C. This research was also supported by the Wayne State University Perinatal Initiative in Maternal, Perinatal and Child Health. The authors are grateful to Daniel Lott for conducting the RNA extraction and the microarray experiments at the Applied Genomics Technology Center of Wayne State University in Detroit, Michigan.

List of Abbreviations

COVID-19	Coronavirus disease 2019
IACUC	Institutional Animal Care and Use Committee
i.p.	Intraperitoneally
LPS	Lipopolysaccharide
PBS	Phosphate-buffered saline
RNA	Ribonucleic acid
cRNA	Antisense ribonucleic acid
DNA	Deoxyribonucleic acid
PCA	Principal component analysis
DEG	Differentially expressed gene
FDR	False discovery rate

KEGG	Kyoto Encyclopedia of Genes and Genomes
ECM	Extracellular matrix
IBD	Inflammatory bowel disease
PPAR	Peroxisome proliferator-activated receptor

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Figure 1. The transcriptome of the uterine tissues in pregnant and non-pregnant mice in response to endotoxin

(A) Mice were injected with either 15 μ g of lipopolysaccharide (LPS) (Pregnant n = 9, non-pregnant n = 8) or sterile 1X phosphate-buffered saline (PBS) (Pregnant n = 10, non-pregnant n = 11). (B) An unsupervised Principal Component Analysis based on the expression of all genes on the array. (C) A clustered heatmap based on the top 200 most varying genes. (D) A Venn diagram representing overlap between differentially expressed genes (DEGs) as detected by comparison between LPS- and PBS-injected pregnant mice (pink) and non-pregnant mice (blue). (E) A volcano plot showing the interaction between the pregnant and non-pregnant states when comparing DEGs in the uterus from LPS- or PBS-injected mice. (F) The list of pathways enriched by the analysis of pregnancy-specific genes.

Figure 2. The transcriptome of the cervix in pregnant and non-pregnant mice in response to endotoxin
 (A) Mice were injected with either 15 µg of lipopolysaccharide (LPS) (Pregnant n = 10, non-pregnant n = 9) or sterile 1X phosphate-buffered saline (PBS) (Pregnant n = 10, non-pregnant n = 11). (B) An unsupervised Principal Component Analysis based on the expression of all genes on the array. (C) A clustered heatmap based on the top 200 most varying genes. (D) A Venn diagram representing overlap between differentially expressed genes (DEGs) as detected by the comparison between LPS- and PBS-injected pregnant mice (pink) and non-pregnant mice (blue). (E) A volcano plot showing the interaction between the pregnant and non-pregnant states when comparing DEGs in the cervix from LPS- or PBS-injected mice. (F) The list of pathways enriched by the analysis of pregnancy-specific genes.

Figure 3. The transcriptome of the liver in pregnant and non-pregnant mice in response to endotoxin

(A) Mice were injected with either 15 μ g of lipopolysaccharide (LPS) (Pregnant $n = 10$, non-pregnant $n = 9$) or sterile 1X phosphate-buffered saline (PBS) (Pregnant $n = 10$, non-pregnant $n = 11$). (B) An unsupervised Principal Component Analysis based on the expression of all genes on the array. (C) A clustered heatmap based on the top 200 most varying genes. (D) A Venn diagram representing overlap between differentially expressed genes (DEGs) as detected by the comparison between LPS- and PBS-injected pregnant mice (pink) and non-pregnant mice (blue). (E) A volcano plot showing the interaction between the pregnant and non-pregnant states when comparing DEGs in the liver from LPS- or PBS-injected mice. (F) The list of pathways enriched by the analysis of pregnancy-specific genes.

Figure 4. The transcriptome of the adrenal glands in pregnant and non-pregnant mice in response to endotoxin

(A) Mice were injected with either 15 μ g of lipopolysaccharide (LPS) (Pregnant $n = 10$, non-pregnant $n = 9$) or sterile 1X phosphate-buffered saline (PBS) (Pregnant $n = 10$, non-pregnant $n = 11$). (B) An unsupervised Principal Component Analysis based on the expression of all genes on the array. (C) A clustered heatmap based on the top 200 most varying genes. (D) A Venn diagram representing overlap between differentially expressed genes (DEGs) as detected by the comparison between LPS- and PBS-injected pregnant mice (pink) and non-pregnant mice (blue). (E) A volcano plot showing the interaction between the pregnant and non-pregnant states when comparing DEGs in the adrenal glands from LPS- or PBS-injected mice. (F) The list of pathways enriched by the analysis of pregnancy-specific genes.

Figure 5. The transcriptome of the kidney in pregnant and non-pregnant mice in response to endotoxin

(A) Mice were injected with either 15 µg of lipopolysaccharide (LPS) (Pregnant n = 10, non-pregnant n = 9) or sterile 1X phosphate-buffered saline (PBS) (Pregnant n = 10, non-pregnant n = 11). (B) An unsupervised Principal Component Analysis based on the expression of all genes on the array. (C) A clustered heatmap based on the top 200 most varying genes. (D) A Venn diagram representing overlap between differentially expressed genes (DEGs) as detected by the comparison between LPS- and PBS-injected pregnant mice (pink) and non-pregnant mice (blue). (E) A volcano plot showing the interaction between the pregnant and non-pregnant states when comparing DEGs in the kidney from LPS- or PBS-injected mice. (F) The list of pathways enriched by the analysis of pregnancy-specific genes.

Figure 6. The transcriptome of the lung in pregnant and non-pregnant mice in response to endotoxin

(A) Mice were injected with either 15 μ g of lipopolysaccharide (LPS) (Pregnant $n = 10$, non-pregnant $n = 9$) or sterile 1X phosphate-buffered saline (PBS) (Pregnant $n = 10$, non-pregnant $n = 11$). (B) An unsupervised Principal Component Analysis based on the expression of all genes on the array. (C) A clustered heatmap based on the top 200 most varying genes. (D) A Venn diagram representing overlap between differentially expressed genes (DEGs) as detected by the comparison between LPS- and PBS-injected pregnant mice (pink) and non-pregnant mice (blue). (E) A volcano plot showing the interaction between the pregnant and non-pregnant states when comparing DEGs in the lung from LPS- or PBS-injected mice. (F) The list of pathways enriched by the analysis of pregnancy-specific genes.

Figure 7. The transcriptome of the brain in pregnant and non-pregnant mice in response to endotoxin

(A) Mice were injected with either 15 μ g of lipopolysaccharide (LPS) (Pregnant n = 10, non-pregnant n = 9) or sterile 1X phosphate-buffered saline (PBS) (Pregnant n = 10, non-pregnant n = 11). (B) An unsupervised Principal Component Analysis based on the expression of all genes on the array. (C) A clustered heatmap based on the top 200 most varying genes. (D) A Venn diagram representing overlap between differentially expressed genes (DEGs) as detected by the comparison between LPS- and PBS-injected pregnant mice (pink) and non-pregnant mice (blue). (E) A volcano plot showing the interaction between the pregnant and non-pregnant states when comparing DEGs in the brain from LPS- or PBS-injected mice. (F) The list of pathways enriched by the analysis of pregnancy-specific genes.

Figure 8. Overall analysis of the transcriptome in pregnant mice

(A, B) UpSet plots of (A) upregulated and (B) downregulated differentially expressed genes (DEGs) in pregnant mice based on the transcriptome of the uterus, cervix, lung, liver, kidney, adrenal glands, and brain. (C, D) Venn diagrams representing the shared (C) upregulated and (D) downregulated DEGs in pregnant mice based on the transcriptome of the uterus, cervix, liver, adrenal glands, kidney, lung, and brain.