Journal of Reproductive Immunology 128 (2018) 30–37

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

Innate immune responses to toll-like receptor stimulation are altered during the course of pregnancy

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ARTICLE INFO

Keywords:
Toll-like receptors
Plasmacytoid dendritic cells
Interferon α
Progesterone
Estradiol
Pregnancy
Hormone
Human chorionic gonadotropin
Tumor-necrosis factor α
Monocytes
Monocyte subsets
Prospective study

ABSTRACT

During pregnancy the maternal immune system has to develop tolerance towards the developing fetus. These changes in maternal immunity can result in increased severity of certain infections, but also in amelioration of autoimmune diseases. Pregnancy-related hormones have been suggested to play a central role in the adaptation of the maternal immune system, but their specific effects on innate immune function is not well understood. In a longitudinal study of pregnant women, we investigated innate immune cell function in response to toll-like receptors (TLR) 4 and 7 stimulation, two TLR pathways playing a critical role in early innate immune recognition of bacteria and viruses. IFNα production by TLR7-stimulated pDCs was decreased in early pregnancy, and increased towards the end of pregnancy. In contrast, pro-inflammatory TLR4-induced TNFα production by monocytes was increased during early pregnancy, but declined after the first trimester. Changes in cytokine production were associated with changes in pregnancy-related hormones and monocyte subpopulations over the course of pregnancy. These data demonstrating a significant association between pregnancy-related hormones and modulation of innate immune responses mediated by TLRs provide novel insights into the immunological adaptations occurring during pregnancy.

1. Introduction

Successful pregnancy relies on the development of tolerance by the maternal immune system towards the semi-allogeneic fetus. This adaptation of the maternal immune system influences disease pathogenesis during pregnancy. The severity of diseases that are aggravated by inflammatory immune responses, including autoimmune diseases such as multiple sclerosis or rheumatoid arthritis, is reduced during pregnancy (Confavreux et al., 1998); (Ostensen and Villiger, 2002). In contrast, an increased susceptibility but also severity for a variety of bacterial and viral infections, including Listeriosis (Gellin et al., 1991); (Krishnan et al., 1996); (Baud and Greub, 2011), HIV-1 (Palacios et al., 2009); (Gray et al., 2005) and Influenza (Neuzil et al., 1998); (Jamieson et al., 2009), has been reported for pregnant women.

The immune adaptation towards the semi-allogeneic fetus occurs on multiple levels regulated by a cross-talk between pregnancy-related hormones and cells of the immune system (Veenstra van Nieuwenhoven et al., 2003a). Besides effects on sexual differentiation and reproduction, sex hormones have been shown to modulate the immune system, resulting in sexual dimorphism between women and men, as well as in changes in immune function during the menstrual cycle and the course of pregnancy (Oertelt-Prigione, 2012); (Robinson and Klein, 2012); (Klein and Flanagan, 2016). Pregnancy-related hormones can exert differential effects on immune cells regulated not only by the concentration of the hormones, but also the density, distribution and types of steroid hormone receptors (Robinson and Klein, 2012), which influence the outcome of hormone-mediated signaling on immune cell functions.

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https://doi.org/10.1016/j.jri.2018.05.009
Received 15 January 2018; Received in revised form 20 April 2018; Accepted 24 May 2018

The hormone human chorionic gonadotropin (hCG) signals the fetus' presence to the maternal organism and contributes to the endocrine-immune cross-talk (Tsampalas et al., 2010). The activity of hCG can be mediated via the mannose receptor CD206 that is expressed on cells of the immune system (Gazi and Martinez-Pomares, 2009). Progesterone and estrogens are produced by the corpus luteum in early pregnancy, and later by the placenta. Progesterone and estrogen receptors are expressed by several immune cell types (Kovats, 2015), and elevated levels of estrogen and progesterone during pregnancy have been implicated in a number of widespread effects on cells of the immune system (Robinson and Klein, 2012); (Kovats, 2015); (Pennell et al., 2012).

Toll-like receptors (TLRs) are type I transmembrane glycoproteins that can be expressed either on the cell surface or intracellularly. Recognition of microbial products by TLRs is a central event in the host response to pathogens. Cell surface TLRs (TLR1, TLR2, TLR4, TLR5 and TLR6) mainly recognize microbial membrane components such as lipopolysaccharide, whereas TLR3, TLR7, TLR8 and TLR9 are expressed on intracellular compartments and recognize nucleic acids, including viral genomes and replication products. Monocytes and pDCs play a central role in the early recognition of bacterial and viral infections. Monocytes can recognize bacterial components via TLR4, triggering the release of pro-inflammatory cytokines, including TNFα. Recently, a new classification of monocyte subpopulations has been suggested (Ziegler-Heitbrock et al., 2010). The three different monocyte populations (classical, intermediate and non-classical) have been described to have distinct phenotypic and functional characteristics (Gordon and Taylor, 2005). Monocytes play a central role in the inflammatory response that is thought to play a role in the pathogenesis of pre-eclampsia, one of the leading complication of pregnancy (Faas et al., 2014a). pDCs represent the principal source of IFNα production in human blood and their expression of endosomal TLRs makes them the first line of defense against viral infections. Beside of recognizing specific molecular patterns that are associated with different types of pathogens, TLRs may also detect a number of self-proteins and endogenous nucleic acids making them key players in autoimmune diseases (Mohammad Hosseini et al., 2015); (Duffy and O'Reilly, 2016).

Published data on the modulation TLR signaling pathways during pregnancy have largely focused on local, tissue-specific responses to date (Amirchaghmaghi et al., 2013); (Koga and Mor, 2010); (Gonzalez et al., 2007). Several reports revealed an altered tissue-specific expression of certain TLRs in the female reproductive tract during the menstrual cycle and pregnancy, suggesting that estrogen and progesterone can modulate TLR expression levels (Amirchaghmaghi et al., 2013); (Aflatoonian and Fazeli, 2008). Steroid hormones have been reported to modulate TLR-mediated cytokine secretion by immune cells (Griesbeck et al., 2015); (Laffont et al., 2014); (Seillet et al., 2012); (Jones et al., 2008), suggesting that hormones that change during pregnancy can modulate innate immune responses to TLR ligands, but no longitudinal assessment of changes in TLR responsiveness against viral and bacterial pathogen-associated molecular patterns (PAMPs) has been conducted to date. In the present study, we examined innate immune cell distributions, their TLR responsiveness and resulting cytokine production in a cohort of 30 pregnant women at three specifically defined time points during the course of pregnancy and in non-pregnant controls. Our data show changes in TLR4 and TLR7 signaling pathways and subsequent cytokine production throughout the course of healthy human pregnancy, which were associated with changes in cell subset composition and pregnancy-related hormones.

2. Materials and methods

2.1. Study subjects

Pregnant women were enrolled in an ongoing prospective cohort study, the PRINCE (Prenatal Identification of Children's Health) study (Diemert et al., 2016a); (Diemert et al., 2016b). Adult healthy women with singleton pregnancies were enrolled at the University Medical Center Hamburg-Eppendorf (UKE), Germany and followed at three time points during pregnancy. Once per trimester (gestational week 12 + 0 to 14 + 6, 22 + 0 to 24 + 6, and 34 + 0 to 36 + 6, respectively) participants presented for maternal blood draw and detailed examination (Diemert et al., 2016a); (Diemert et al., 2016b). For the present study, 30 newly recruited participating women, during a pre-defined time period, participated. Additionally to the regular study protocol, blood samples of these women were analyzed as described below. From initially enrolled 30 pregnant women, one woman experienced a preterm birth, one woman missed the last study visit, and one experiment failed due to a technical error, leading to the inclusion of longitudinal data from 27 pregnant women. 21 non-pregnant women and 17 male age-matched control participants were cross-sectionally recruited at the University Medical Center Hamburg-Eppendorf (UKE), Germany and had a single blood draw for this study. The studies were approved by the ethical commission of the Ärztekammer Hamburg. Each participant gave informed consent prior to enrollment. EDTA-blood was processed within 2 h after venipuncture to prevent loss of responsiveness to stimulation (Meier et al., 2008). EDTA-blood was initially centrifuged 600 x g for 7 min for the extraction of plasma. PBMCs were extracted from whole blood using Biocoll (Biochrome/Merck, Darmstadt, Germany) gradient centrifugation. Peripheral blood mononuclear cells (PBMCs) were maintained in RPMI1640 supplemented with 10% heat-inactivated FBS.

2.2. Measurement of pregnancy-associated hormones in plasma

Hormone levels in EDTA plasma samples of a subset of pregnant females were assessed in the Central Laboratory of the University Center Hamburg-Eppendorf (UKE), Germany. Briefly, 17ß-estradiol and progesterone were quantified by chemiluminescence-immunoassay using ADVIA Centaur XP system (Siemens). Total ß-hCG (the intact hCG heterodimer and the free ß subunit) levels were quantified by homogenous, sandwich chemiluminescent immunoassay based on LOCI® technology using Dimension Vista 1500 analyzer (Siemens). All procedures were performed according to the manufacturer's protocols and the standard operation procedures of the laboratory.

2.3. Flow cytometric analysis of CD14 and CD16 distribution on monocytes

Monocyte surface expression of CD14 and CD16 was assessed by flow cytometry using a BD LSRFortessa™ (Becton Dickison, Franklin Lakes, New Jersey, US). Monocytes were defined as following: $CD3^{neg}CD19^{neg}CD56^{neg}$HLA-$DR^{pos}CD11c^{pos}CD14^{pos}$. Antibodies used are listed in Supplemental Table 2.

2.4. In vitro stimulation *of PBMCs with TLR ligands*

Two million PBMCs/mL were incubated for 17 h with the following: 1 µg/mL TLR7/8 agonist CL097 (Invivogen, San Diego, CA, US) or 100 ng/mL TLR4 agonist lipopolysaccharide (LPS) (Sigma, St Louis, Missouri, US) or left unstimulated in the presence or absence of Brefeldin A (5 µg/mL; Sigma), as previously described (Meier et al., 2009); (Simmons et al., 2013).

2.5. Measurement of single-cell cytokine production by multiparameter flow cytometry

Intracellular cytokine staining was carried out following 17 h of stimulation as previously described (Meier et al., 2009); (Simmons et al., 2013) on a subset of pregnant women. Cell populations were defined as following: pDCs: $CD3^{neg}CD19^{neg}CD56^{neg}$HLA-$DR^{pos}CD14^{neg}CD11c^{neg}CD123^{bright}$ cells.