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Expression of surfactant proteins SP-A and SP-D in murine decidua and immunomodulatory effects on decidual macrophages

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ABSTRACT

Surfactant proteins SP-A and SP-D are pattern recognition innate immune molecules that belong to the C-type lectin family. In lungs, they play an important role in the clearance of pathogens and control of inflammation. SP-A and SP-D are also expressed in the female reproductive tract where they play an important role in pregnancy and parturition. However, the role of SP-A and SP-D expressed at the feto-maternal interface (decidua) remains unclear. Here, we have examined the expression of SP-A and SP-D in the murine decidua at 17.5 (pre-parturition) and 19.5 dpc (near parturition) and their effect on lipopolysaccharide (LPS)-treated decidual macrophages. SP-A and SP-D were localized to stromal cells in the murine decidua at 17.5 and 19.5 dpc in addition to cells lining the maternal spiral artery. Purified pre-parturition decidual cells were challenged with LPS with and without SP-A or SP-D, and expression of F4/80 and TNF-α were measured by flow cytometry. On their own, SP-A or SP-D did not affect the percentage of F4/80 positive cells while they suppressed the percentage of TNF-α positive cells. However, simultaneous addition of SP-A or SP-D, together with LPS, reduced TNF-α secreting F4/80 positive cells. It is likely that exogenous administration of SP-A and SP-D in decidua can potentially control infection and inflammation mediators during spontaneous term labor and infection-induced preterm labor. Thus, the presence of SP-A and SP-D in the murine decidua is likely to play a protective role against intrauterine infection during pregnancy.

1. Introduction

Intrauterine infection and chorioamnionitis are very common complications of pregnancy leading to stillbirth, premature birth, and neonatal sepsis. Chorioamnionitis complicates as many as 40–70% of preterm births due to premature membrane rupture or spontaneous labor and up to 13% of term births (Chang et al., 2013). Understanding immunological mechanisms that initiate parturition while offering a defense shield at the feto-maternal interface can help devise strategies to reduce preterm birth.

Surfactant proteins, SP-A and SP-D are collagenous C-type lectins (also called collectins) which perform a range of innate immune functions in the lungs, including clearance of pathogens and apoptotic/necrotic cells, regulation of inflammation, and priming of adaptive immunity (Kishore et al., 2005, 2006; Sano and Kuroki, 2005; Nayak et al., 2012). SP-A and SP-D are 26–36 KDa and 43 KDa proteins in size that assemble further to form high molecular weight oligomeric structure of 630 KDa and 520 KDa, respectively (Holmskov et al., 2003). SP-A differs from SP-D in the gene organization, structure, ligand binding and function (Holmskov, 2000). Their primary structure comprises of an N-terminal domain with cysteine residues for interchain disulphide bond formation, a C-terminal carbohydrate recognition domain (CRD), the alpha-helical coiled neck with amphipathic helix, a collagen-like domain with repeating Gly-X-Y and hydroxyproline residues (Holmskov and Jensenius, 1993; Haagsman and Diemel, 2001; Kuroki and Sano, 1999). SP-A and SP-D bind their targets mostly via CRDs while the triple-helical collagen region can interact with CD91- calreticulin complex on the cell surface of phagocytic

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cells, leading to effector mechanisms such as phagocytosis, superoxide radical generation, and cytokine production (Gardai et al., 2003; Wright, 2005).

In lungs, SP-A and SP-D are synthesized and secreted by alveolar type II and Clara cells at the air-liquid interface of the surfactant (Crouch et al., 1992; Voorhout et al., 1992). Expression of SP-A and SP-D has also been reported in extra pulmonary tissues such as brain, salivary glands, lachrymal glands, heart, trachea, kidney, pancreas, thymus, spleen, gall bladder, esophagus, small intestine, large intestine, testis, prostate and urinary tract (Madsen et al., 2003; Herías et al., 2007; Breuiller-Fouché et al., 2010; Nayak et al., 2012; Schicht et al., 2015). In addition, reproductive tissues have also been shown to express both SP-A and SP-D (Sati et al., 2009; Condon et al., 2004; Yadav et al., 2011).

SP-A and SP-D can be localized within the fetal membranes (amniotic epithelium and chorionic membrane); the choriodecidual layer of the late pregnant uterus; cytotrophoblast, intermediate trophoblast and syncytiotrophoblast of early gestation; and trophoblast of late normal placental villi (Miyamura et al., 1994; Leth-Larsen et al., 2004). SP-D level in the amniotic fluid increases gradually from 0.11 μg/ml (14–16th week of gestation) to 26.3 μg/ml (38–42nd week of gestation) (Miyamura et al., 1994; Leth-Larsen et al., 2004). SP-A shows a rise from 3 μg/ml (30–31st week of gestation) to 24 μg/ml (40–41st week of gestation) near the term (Miyamura et al., 1994). Expression of SP-A in pre- and post-menopausal vaginal stratified squamous epithelium and vaginal lavage fluid has also been demonstrated (MacNeill et al., 2004). Human deciduaat term as well as first trimester show presence of SP-A and SP-D (Snegovskikh et al., 2011; Madhukaran et al. 2015). In mouse, SP-D mRNA and protein are mainly expressed in the vagina, uterus, ovary, cervix, and oviduct (Akiyama et al., 2002). SP-D expression in the mouse uterus is hormonally regulated, increasing toward estrus and decreasing near diestrus (Oberley et al., 2007; Kay et al., 2015). Human term placental tissues express both SP-A and SP-D and their levels alter significantly during spontaneous labor (Yadav et al., 2014).

Decidua is an immunologically privileged site that bridges the maternal and fetal immune mechanisms at the maternal-fetal interface, offering protection to the semi-allogenic fetus (Taglauer et al., 2010; Hsu and Nanan, 2014). Within decidua, there are two distinct regions; decidua basalis and decidua parietalis. Decidua basalis is embedded into the placental bed invading the interstitial trophoblast while decidua parietalis remains in contact with the fetal membrane (Gomez-Lopez et al., 2010). Decidua is enriched with terminally differentiated macrophages that are immunosuppressive (Houser et al., 2011). Macrophages are the second most predominant leukocyte population (20–25%) in decidua with several functions from early until late gestation (Trundley and Moffett, 2004; Leonard et al., 2006; Gomez-Lopez et al., 2010). Their number increases during the first trimester and remains constant until the third trimester; however, it decreases significantly prior to labor, during labor and postpartum (Mackler et al., 1999; Shynlova et al., 2013). In human, early pregnancy decidua has ~50%, while term pregnancy decidua has ~20–30% of $CD14^+$ decidual macrophages (DMs) (Gomez-Lopez et al., 2014). DMs isolated from the human term placenta produce a considerable amount of TNF-α when stimulated with LPS (Singh et al., 2005; Gomez et al., 1997). Thus, infiltration of DMs in the mouse decidua has been considered important for labor cascade (Hamilton et al., 2012). Infection in the decidua is a significant threat to the mother and the fetus during pregnancy (Mogensen, 2009). Intrauterine infection and consequent inflammatory response have been associated with preterm labor (Burdet et al., 2014). Infection during pregnancy, as in chorioamnionitis, pyelonephritis, and chronic deciduitis, activates DMs via LPS, which in turn generates pro-inflammatory TNF-α and prostaglandin $F_{2\alpha}$ in the decidua, leading to preterm labor (Casey et al., 1989; Snegovskikh et al., 2011).

Condon et al. have proposed that SP-A secreted from fetal lungs can activate fetal macrophages (Condon et al., 2004), which get infiltrated into the maternal tissues and provoke pro-inflammatory response via increased expression of IL-1β and NF-κB that initiates labor (Condon et al., 2004). This highlights the importance of SP-A in the cervical ripening and uterine contraction leading to parturition. SP-A can modulate LPS-induced signaling via TLRs (Sano et al., 1999; Sano et al., 2000; Agrawal et al., 2013). Recently, we have shown that SP-A and SP-D are expressed by stromal cells and trophoblasts in early human decidua (Madhukaran et al., 2015).Here, we show the expression of SP-A and SP-D in the murine decidua pre (17.5 dpc) and near parturition (19.5 dpc) and their immunomodulatory effects on DMs when challenged with LPS.

2. Materials and methods

2.1. Ethics statement

The study was approved by the institutional animal ethics committee (IAEC no: 78/1999) at the National Institute for Research in Reproductive Health, Mumbai, India. All procedures were carried out in accordance with the institutional guidelines for the care and use of experimental animals.

2.2. Animal models

Inbred strains of C57BL/6 female and male mice were housed in a humidity-controlled animal facility under standard environmental conditions (12 h, light/dark cycle) and fed ad libitum. Female virgin mice (8–12 week old) were housed overnight with males and checked for the presence of vaginal plugs the next morning to obtain accurately timed pregnant mice. The day of the plug formation was counted as 0.5 post coitus (dpc). Pregnant mice delivered between day 19 and 21. Decidual tissues were collected on gestational days 17.5 dpc and 19.5 dpc. Similarly, mouse fetal lung sample was collected for PCR.

2.3. Isolation of murine decidua and fetal lungs at 17.5 and 19.5 dpc

A vertical incision was made in the abdomen of euthanized (via cervical dislocation) female mice under sterile conditions. The uterine horns with embryos were then carefully removed and washed with PBS (Fig. 1A). The embryo was separated from the uterine membrane with intact placenta (Fig 1B). The top layer of the embryo, the yolk sac, was opened along the anti-mesometrial side. Each embryo was then detached from its placenta and rinsed in cold PBS (Fig 1C). Decidua parietalis, was identified by their smooth, grayish solid appearance (Fig 1C). Decidua was gently removed leaving behind the placenta and decidua spongiosa which is dark brown in color with spongy appearance (Fig 1D) (Dudley et al., 1993). The decidua was washed several times with ice-cold PBS to minimize blood contamination and weighed (Fig 1E). Decidual tissues at 17.5 dpc and 19.5 dpc were cut into 4–5 μm thick sections, fixed, embedded in paraffin wax, stained with hematoxylin and eosin for histological examination (Fig 1F), or processed for immunohistochemistry. In all cases, decidualized endometrial stromal cells with abundant cytoplasm and vesicular nuclei were observed. Decidual cells were round to polygonal with sharply defined cell borders and single nucleus containing small but prominent nucleolus (The observations made are not visible from the Fig. 3, it requires a higher magnification to show nucleus and