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Short communication

Decidual expression and localization of human surfactant protein SP-A and SP-D, and complement protein C1q

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ABSTRACT

Surfactant proteins SP-A and SP-D, and complement protein C1q are soluble innate immune pattern recognizing molecules. SP-A, SP-D and C1q have an overall similar structure composed of an N-terminal triple-helical collagen region that is followed by a trimeric globular domain. While SP-A and SP-D belong to the collectin family (collagen containing lectin), C1q is the first recognition subcomponent of the classical pathway of the complement system. Recently, SP-A, SP-D and C1q have been considered to play important roles in early and late pregnancy. However, their expression in early human decidua has not been examined. Here, we investigated whether SP-A, SP-D and C1q are expressed within first trimester decidua in humans and their expression is associated with trophoblasts and decidual stromal cells. Decidual samples from women undergoing elective vaginal termination of pregnancy during first trimester were obtained from 25 subjects. Immunohistochemical studies using anti-human SP-A, anti-human SP-D and anti-human C1q antibodies were performed on decidual tissue sections along with anti-vimentin and cytokeratin-7 antibodies to identify stromal cells and trophoblasts. The expression was also examined by immunostaining and PCR using decidual and stromal cells. C1q expression was significantly higher when compared to SP-A and SP-D in the first trimester human decidua. Double immunostaining revealed that all stromal cells and trophoblasts expressed SP-A, SP-D and C1q, while only few invasive trophoblasts expressed C1q. Thus, expression of SP-A, SP-D and C1q in human decidua during first trimester suggests potential role of SP-A, SP-D and C1q during the early stages of pregnancy including implantation, trophoblast invasion and placental development.

1. Introduction

Surfactant protein SP-A and SP-D belong to a group of carbohydrate binding proteins called collectins that are characterized by an N-terminal triple-helical collagen region, an α-helical coiled-coil trimerizing neck region, and a homotrimeric C-type lectin or carbohydrate recognition domain (CRD) (Thiel and Reid, 1989). These soluble multimeric lectins resemble the complement protein, C1q and share overall structure, mode of assembly, and interaction with receptors (Malhotra et al., 1992; Uemura et al., 2006).

Previous studies have demonstrated SP-A and SP-D to be lung-specific (alveolar type II cells) (Kishore et al., 2006; Madsen et al., 2000, 2003), while C1q is known to be secreted by tissue macrophages and monocytes derived dendritic cells, endothelial cells, keratinocytes derived from hematopoietic origin in addition to predominantly produced in the liver (Loos et al., 1989; Langeggen et al., 2000; Reis et al., 2007). These innate immune molecules act as the first line of host defense playing an important role in recognizing and clearing the invading pathogens (Lawson and Reid, 2000; Wright, 2005; Thiel and Reid, 1989; Hoppe et al., 1994), and in regulating innate and adaptive immune responses (Epstein et al., 1996; Vandivier et al., 2002).

Besides their immunoprotective role against pathogens, SP-A and SP-D have versatile tissue-specific immunomodulatory roles in host defense and maintenance of physiological conditions in several extra-pulmonary tissues and organs such as nasal cavity (Schicht et al., 2013), salivary system (Bräuer et al., 2012, 2009),

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brain (Schob et al., 2013), and digestive system (Bourbon and Chailley-Heu, 2001). C1q is also synthesized by mesenchymal cells (Morris et al., 1978), epithelial cells (Colten, 1976), dendritic cells (Castellano et al., 2004), fibroblasts (Gulati et al., 1993), microglial cells (Farber et al., 2009), endothelial cells (Bossi et al., 2011) where they modulate cell-specific immune response. More recently, these innate immune molecules have been found to be localized in the reproductive tracts. Their expression, distribution and function have been reported in the uterus, ovaries, oviducts, vagina, cervix, amniotic fluid, decidua and placenta (Akiyama et al., 2002; Agostinis et al., 2010; Bulla et al., 2008; Kankavi et al., 2007; Oberley et al., 2004; Pellis et al., 2005).

During early stages of pregnancy, developing fetal lungs synthesize and release surfactant proteins into circulation. Thus, amniotic fluid represents the major source of circulating SP-A and SP-D. In 1994, Miyamura and co-workers demonstrated the presence of both SP-A and SP-D in human amniotic fluid during the 26th week of gestation. SP-A is dramatically upregulated and remains high till the term demonstrating SP-A to be a crucial early indicator of fetal lung maturity (Miyamura et al., 1994). In agreement with the previous study, SP-A in murine pregnancy has been reported to upregulate pro-inflammatory cytokines, crucial for initiating the onset of labor (Condon et al., 2004). Placenta samples with attached fetal membranes from normal term singleton pregnancies after cesarean section, showed increased expression of SP-A in amnion by western blot and immunohistochemistry. Further SP-A was observed to induce stress fibers that maintains the integrity of the endothelial cells by regulating F-actin filament organization in human myometrial cells. In contrast, the expression of SP-D was low in chorion and in the decidual cells, and faint staining was seen in the amniotic epithelial cells (Breuiller-Fouché et al., 2010). Recently, a critical role played by SP-A and SP-D in spontaneous labor has been reported. SP-D appears to increase the expression of pro-inflammatory response within the feto-maternal interface that triggers cervical ripening leading to labor (Yadav et al., 2014).

The finding that SP-A and SP-D gene-deficient mice have a delay in the timing of labor/parturition suggests that both these proteins play a regulatory role in parturition (Montalbano et al., 2013). This is in line with another study which has shown SP-A to suppress preterm delivery via toll like receptor 2 thus preventing preterm labor and associated inflammatory responses (Agrawal et al., 2013). Complement protein C1q, which is widely distributed in the trophoblasts, has been proposed to be involved in the early stages of pregnancy, which promotes endovascular trophoblast invasion, spiral artery remodeling and normal placentation (Agostinis et al., 2010; Bulla et al., 2008). C1q gene deficiency in mice is associated with abnormal placentation with impaired labyrinth formation, decreased placental vascular endothelial growth factor and increased fetal resorption. Deficiency of decidual C1q is likely to play a pivotal role in the pathogenesis of abnormal placental development during implantation (Singh et al., 2011). These results suggest that C1q is a key component for trophoblast invasion during normal placentation.

Decidua is a highly specialized, but temporary, organ that lines the uterus. It offers an intimate contact between the fetal-maternal interfaces. The maternal innate and adaptive immune system co-exists within the decidua to protect the fetus against the invading pathogen, and tolerates the semi-allogenic fetus (Bulmer and Sunderland, 1984; Hsu and Nanan, 2014). Immunohistochemical assessment of human immune cells from normal early pregnancy decidua reveals 50% of the major resident deciudal cells at the fetal-maternal interface; out of which approximately 40% immune cell population are predominantly NK cells (70–80%), macrophages (20–25%) and dendritic cells (2%) (Bulmer et al., 1988; Trundley and Moffett, 2004).

The human decidua also consists of non-immune cells such as decidual stromal cells (DSCs) and trophoblasts. Proliferation and differentiation of endometrial fibroblast-like stromal cell precursors (decidualization) gives rise to unique non-immune stromal cells (75% of the decidual tissue) that exhibits several immune functions such as antigen presentation and production of cytokines. They are the major cellular component of the decidua during early pregnancy (van Rijn et al., 2008). During embryogenesis, proliferation and differentiation of extra-embryonic trophoblastic cells derived from stem cells are the first fetal cell type to be seen invading the maternal uterus leading to placental development (Golos et al., 2013). As the cytotrophoblasts invade, they differentiate to form villous and extravillous trophoblasts (EVT) until placenta becomes fully developed. This process involves interaction of fetal trophoblasts with the maternal stromal cells in the decidua, thereby controlling the establishment and maintenance of successful pregnancy (Giakoumopoulos and Golos, 2013; Douglas et al., 2009).

However, very little is known about SP-A, SP-D and C1q in human decidua. Previous reports using immunohistochemistry analysis have demonstrated the expression of SP-A in endometrial stromal cells from non-pregnant women (Snegovskikh et al., 2011) and term decidua (Yadav et al., 2014) while C1q has been observed in DSCs and EVT (Bulla et al., 2008; Agostinis et al., 2010). We wondered whether SP-A and SP-D may be present at the feto-maternal interface since no clear evidence for the expression of SP-A and SP-D has been reported in first trimester human decidua. Thus, the present study was designed to clarify and evaluate the expression, distribution and localization of SP-A, SP-D and C1q in early human decidua at the fetal-maternal interface, as well as their expression in DSCs and trophoblasts. To the best of our knowledge, this is the first study to examine the expression of SP-A and SP-D in early human decidua. Our data provide a new insight into the potential role of SP-A and SP-D in early pregnancy events.

2. Materials and methods

2.1. Tissues samples

All human decidual tissue samples collected were obtained from healthy women undergoing elective termination of pregnancy during first trimester, 8–12 week of gestation. Informed and written consent were obtained from each patient providing the tissue samples. The research protocol was approved by the Domain Specific Review Board of National University Health System by National University Hospital affiliated to the National University of Singapore. Gestation was determined by ultrasound before collection of tissues. Samples collected from different subjects were not mixed to avoid cross-reaction and were transported to the laboratory in ice-cold heparinized 0.9% NaCl during the first 30 min after curettage. A total of twenty-five decidual tissues were obtained from women undergoing elective termination of pregnancy during first trimester. Small portion of all the tissues were embedded in paraffin wax and 4–5 μm sections were cut for immunohistochemistry while rest of the sample was used for other experiments.

2.2. Immunohistochemistry

Decidual tissues of approximately 1 cm^2 were rinsed generously in PBS to remove any blood clots. Tissues were fixed in 4% PFA at room temperature for 2–3 days and embedded in paraffin. Immunohistochemistry was performed by an indirect horse peroxidase-conjugated (HRP) method. Serial sections of 5 μm thick were cut and mounted on lysine-coated slides, dried at 45 °C for 24 h. Slides were deparafinized, rehydrated and endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide for 10 min