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Expression and localization of collectins in feto-maternal tissues of human first trimester spontaneous abortion and abortion prone mouse model

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ABSTRACT

Dysregulation of immune response at the feto-maternal interface during first trimester of pregnancy is one of the leading causes of spontaneous abortion. Previously, we reported differential expression of collectins, soluble pattern recognition molecules involved in immunoregulation, in placental and decidual tissues during spontaneous labor. In the present pilot study, the expression of collectins was analyzed in the inflamed human gestational tissues of spontaneous abortion ('SA') and in 13.5 dpc placental tissues from resorption survived embryos of murine model (CBA/J X DBA/2J). Transcripts of SP-A were significantly down-regulated and SP-D were significantly up-regulated in placental and decidual tissues of 'SA' group compared to that of 'normal' group. Immunostaining for SP-D and MBL proteins was positive in placental and decidual tissues. However, levels of SP-D and MBL proteins were not significantly altered in placental as well as in decidual tissues of 'SA' group in comparison to the 'normal' group. Placental tissues of viable embryos from the abortion prone mouse model showed significantly enhanced expression of mSP-A and mSP-D transcripts at 13.5 day post coitus (dpc) and 14.5 dpc compared to the control group (CBA/J X Balb/c). Mouse collectins were localized in placental tissues (13.5 dpc), with increased staining in murine model compared to control. Human and murine data together indicate that SP-A, SP-D and MBL are synthesised in early gestational tissues, and may contribute to regulation of immune response at the feto-maternal interface during pregnancy.

1. Introduction

First trimester spontaneous abortions affect 15% of pregnancies and majority of them are attributed to a breach in immune tolerance at the feto-maternal interface (Weeks and Danielsson, 2006; Hemberger, 2013). For a successful pregnancy, mother's immune system has to be modulated to tolerate the allogenic fetus. A regulated immunomodulation is orchestrated at the feto-maternal interface by placental trophoblast cells, maternal decidual stromal cells and immune cells. Implantation of the blastocyst (a stage of developing embryo) into the decidualised maternal endometrium (tissue adjacent to the uterine lumen) results in initiation of development of placenta from the outer layer of blastocyst. Placental tissue is composed of two distinct cell layers; the underlying cytotrophoblast layer and the overlying syncytiotrophoblast layer. The syncytiotrophoblast is a multinucleated continuous cell layer that forms as a result of differentiation and fusion of the underlying cytotrophoblast cells and secretes immunomodulatory molecules.

Besides development of a tolerant phenotype by maternal immune cells in the endometrium during decidualisation, maternal immune response to the placental antigens is further regulated by an array of immune-modulatory molecules produced by placenta and decidua (Hemberger, 2013). Increased levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, LIF) lead to spontaneous abortion in first trimester (Wilczynski, 2006; Christiansen, 2006). Significantly decreased levels of anti-inflammatory cytokines IL-4 and IL-10 have also been associated with early recurrent pregnancy losses (Chatterjee et al., 2014).

The semi-allogeneic pregnancy between CBA/J females ($H2^k$) and DBA/2J males (H^{2d}) (abortion prone mouse model) produces high rate of embryo resorptions/abortions (Bonney and Brown, 2014). Systemic maternal immune inflammation, increased lymphocyte trafficking, increased complement deposition, increased

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co-stimulatory molecules and activation of NK cell and T cells in the feto-maternal tissues are causal factors for embryo resorption in this model and are evident between 10 and 12 dpc (day post coitus) of pregnancy (Bonney and Brown, 2014). Thus, pro-inflammatory response is the common signature of immune rejection of fetus in spontaneous abortion and in abortion prone mouse model (Weeks and Danielsson, 2006; Hemberger, 2013; Wilczynski, 2006; Christiansen et al., 2006; Chatterjee et al., 2014; Bonney and Brown, 2014). Importantly, treatment with anti-inflammatory molecules such as IL-10 or blocking TNF-α activity rescued fetal death in rat models emphasizing the involvement of pro-inflammatory response in pregnancy loss (Renaud et al., 2011).

Expression of molecules regulating inflammation at the feto-maternal interface, thus, could be critical for preventing spontaneous abortions. Various members of Collectins (Collagenous domain containing C-type lectins), a family of pattern recognition proteins, have been localized at the feto-maternal interface and regulate both innate and adaptive immune responses (Yadav et al., 2011). Genes encoding the three classical collectins namely, secretory surfactant protein A (SP-A), surfactant protein D (SP-D) and serum mannan binding lectin (MBL) are present in human and mouse genome on chromosome 10 and chromosome 14 respectively. SP-A,SP-D and MBL are involved in various immune cell functions like phagocytosis, antigen presentation, oxidative burst, apoptotic cell clearance, and cytokine secretion besides pathogen recognition and agglutination (Nayak et al., 2012; Kerrigan and Brown, 2009). MBL is also known for its function in activation of lectin mediated complement pathway (Ip et al., 2009).

SP-A, SP-D and MBL are present in the human female reproductive tract and protect against reproductive tract infections (MacNeill et al., 2004; Oberley et al., 2004; Bulla et al., 2010). SP-D protein was localized in human uterine luminal and glandular epithelial cells (Leth-Larsen et al., 2004). Mouse SP-D transcripts, but not SP-A, were observed in mouse uterus and are regulated by ovarian hormones (Akiyama et al., 2002; Oberley et al., 2007; Kay et al., 2015). SP-A was observed in human cervico-vaginal lavage and its levels increased during the early follicular phase in comparison to the luteal phase (Macneill et al., 2012).

SP-A and SP-D proteins were localized in trophoblast and stromal Hofbauer cells (macrophages) of the 4th to 8th week gestational human normal placenta (Sati et al., 2010). SP-D mRNA, but not SP-A, were detectable in the 16 dpc murine placenta (Salminen et al., 2008). We recently reported increased SP-D and decreased levels of SP-A in term feto-maternal tissues during spontaneous labor (Yadav et al., 2014). SP-D significantly augmented the secretion of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-8 in term placental explants (Yadav et al., 2014).

In view of the immunomodulatory roles of collectins at feto-maternal interface, we hypothesize that they could be relevant in spontaneous abortion. Low maternal serum levels of MBL were observed in patients with unexplained recurrent miscarriage (Christiansen et al., 1999). Presently, there are no reports on association of SP-A and SP-D with spontaneous abortion. Henceforth, we investigated differential expression of collectins and their localization in feto-maternal tissues of human first trimester spontaneous abortion. Mouse SP-A (mSP-A), SP-D (mSP-D) and MBL (mMBL-1), were similarly analyzed in placental tissues of viable embryos of the mouse model of embryo resorption (CBA/J X DBA/2J).

2. Materials and methods

2.1. Human feto-maternal tissues

First trimester human feto-maternal tissues (from 6 to 12 weeks of gestation) were collected from two groups of participants in the normal fertility period (20–40 years), who visited the clinic at the Department of Obstetrics and Gynaecology, Seth Gordhandas Sunderdas Medical College and King Edwards Memorial (KEM) Hospital and provided written informed consent prior to sample collection. First group of the pilot study composed of pregnant women who opted for elective termination of pregnancy ('normal' group, $n = 5$), and the second group comprised of women who underwent spontaneous abortion and visited the clinic for dilatation and curettage treatment ('SA' group, $n = 5$). The study was approved by the Institutional Ethics Committee for Clinical Research (152/2009-NIRRH), NIRRH (ICMR) and Ethics Committee for Research on Human Subjects (EC/GOVT-7/2009), Seth Gordhandas Sunderdas Medical College and KEM Hospital. Participants included in the study neither reported nor showed any reproductive tract infections, chronic diseases, or pathophysiological conditions of pregnancy (e.g. endometriosis), on clinical examination. None of the study participants were being treated with hormones. The feto-maternal samples were collected in sterile PBS/saline and dissected under stereomicroscope to separate the placenta and decidua basalis tissues (Murugappan et al., 2014). Hematoxylin and Eosin staining was performed on the tissue sections to examine the morphology of decidual tissues and chorionic villi. Cytokeratin 7 staining was used to confirm appropriate separation (Snegovskikh et al., 2011), of placental and decidual tissues (data not shown). The placental or decidual samples were divided into three parts, (a) one part was treated with 10% neutral formaldehyde buffer for immune-histochemical analysis, (b) second part was stored in trizol at −80 °C for real time RT-PCR analysis and (c) third part was stored in lysis buffer at −80 °C for Western blot analysis.

2.2. Human term amniotic fluid

Human term amniotic fluid (AF) was collected and pooled from women at term undergoing C-section ($n = 20$) at the Department of Obstetrics and Gynaecology, Seth G.S. Medical College & KEM Hospital, Parel, Mumbai, with written informed consent from the participants obtained prior to the sample collection. The pooled amniotic fluid was stored at −20 °C until further use. The study was approved by the Institutional Ethics Committee for Clinical Research (153/2009-NIRRH), NIRRH (ICMR) and Ethics Committee for Research on Human Subjects (EC/GOVT-6/2009-KEM), Department of Obstetrics and Gynaecology, Seth G.S. Medical College & KEM Hospital, Mumbai. Briefly, the pooled AF was thawed and centrifuged at 11,000 rpm for 30 min. Supernatant was collected and total protein was precipitated in chilled acetone. Precipitated proteins were dissolved in PBS and protein concentration was estimated using the Micro BCA kit (Thermoscientific, USA).

2.3. Abortion prone mouse model

Breeding pairs of CBA/J mice were obtained from National Institute of Immunology, New Delhi and DBA/2J mice were procured from Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Mumbai, India. The mouse colonies were maintained in the animal housing facility of NIRRH, Mumbai under pathogen-free conditions. Abortion prone mouse model was produced by age matched mating of CBA/J females ($H2^k$) with DBA/2J males ($H2^d$). CBA/J females ($H2^k$) mated with Balb/cJ ($H2^d$) males produced normal pregnancy and served as the study control group. Study was approved by the Institutional Animal Ethics Committee, NIRRH (ICMR), Mumbai [Project No. 12/09]. Pregnant CBA/J females from both the mating combinations were sacrificed at 13.5 dpc ($n = 5$) and 14.5 dpc ($n = 3$). Placental discs from viable embryos of the abortion prone model and the control group were obtained and dissected in chilled sterile PBS under stereomicroscope to remove