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Surfactant proteins A and D in pregnancy and parturition

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1. ABSTRACT

Surfactant proteins A and D have extra-pulmonary expression at various mucosal sites including the reproductive tract. Reproductive tissues require a fine immune balance, strong enough to keep infection at a bay and at the same time, subtle enough to support an allogeneic fetus throughout the pregnancy. Roles of SP-A and SP-D have been studied in depth and include immunoregulatory function, besides strengthening the innate immune system against various pathogens in the lungs. Interestingly, levels of SP-A and SP-D in the amniotic fluid increase progressively in pregnancy. SP-A has been implicated in the induction of parturition. The present review elaborates the plausible roles of SP-A and SP-D in pregnancy maintenance and future applications.

2. INTRODUCTION

Of those mucosal surfaces in the body, the female reproductive tract has unique immune mechanisms to protect against pathogens and adapt to a spectrum of physiological events that includes fertilization, implantation, pregnancy, and parturition. These processes are sex hormone driven which, in turn, affect the local mucosal immune system such as expression of various immune molecules including cytokines and chemokines, the distribution of various cell populations, and antigen presentation of the female genital tract. An immuno-homeostasis is maintained throughout the menstrual cycle and pregnancy to respond to the challenges of pathogens and to provide the conducive immune milieu for important reproduction associated events. The earlier paradigm of a

Table 1. SP-A and SP-D with respect to chromosomal location, size, expression pattern in the female reproductive tract and carbohydrate preferences

Gene Names	Chromosomal Location	Molecular weight (kDa)	Receptors	Expression pattern in female reproductive tract tissues	Carbohydrate preferences
SFTPA	10q22.3-q23.1	26.242	SPR210, C1qR, TLR-2, TLR-4, SIRPα, Calreticulin/CD91	pre- and postmenopausal vaginal epithelium, vaginal lavage fluid, chorioamniotic membranes and amniotic fluid	ManNac>Fuc,Mal>Glc>Man> Gal,GlcNac
SFTPD	10q22.2-q23.1	37.728	C1qR, TLR-2, TLR-4, SIRPα, Calreticulin/CD91	non pregnant uterus, ovaries and oviduct of human female reproductive tract, amniotic fluid, cytotrophoblast, intermediate trophoblast and syncytiotrophoblast of early gestation, trophoblast of late normal placental villi	Mal>Man,Glc>Lac, Gal>GlcNac>Fuc

Abbreviations : ManNac: N-acetyl mannosamine; Fuc: Fucose; Mal: Maltose; Glc: Glucose; Man: Mannose; Gal: Galactose; GlcNac: N-acetyl glucosamine

passive role of the immune system to resist attacking the allogeneic sperm and fetus has shifted to a more active player supporting reproductive functions.

The Immune system has been conventionally classified as innate and adaptive based on the specificity of their response to a challenge. The innate immune system, the first line of defense, has now been assigned a new role wherein it creates the immune milieu that decides the type and outcome of adaptive immune response in pathogen mediated or autoimmune diseases. Very few studies have investigated the presence and function of the innate immune molecules in the female reproductive tract. What is becoming clearer is that the innate immune system is present throughout the reproductive tract and functions in synchrony with the adaptive immune system.

The innate immune system involves a cellular component, comprising macrophages, dendritic cells (DCs), neutrophils, natural killer (NK) cells, epithelial cells, and a secretory component comprising immune molecules, cytokines and chemokines. Recognition of non-self by the innate immune system relies on conserved germline- encoded receptors or the pattern recognition receptor (PRRs) of the host. Surfactant protein A (SP-A) and surfactant protein D (SP-D) belong to an important class of PRRs, called collectins. Collectins are C-type lectins (Ca^{2+} dependent carbohydrate recognition) containing collagen domains, mostly secreted, and recognize non-self based on carbohydrate structures. Besides SP-A and SP-D, there are several other member proteins in the collectin family such as, mannan binding lectin (MBL), scavenger receptor collectin placenta-1 (CL-P1), collectin liver-1 (CL-L1), collectin kidney-1 (CL-K1), conglutinin, collectin of 43 kD (CL-43), and collectin of 46 kD (CL-46).

Surfactant proteins A and D, are secreted into the pulmonary alveolar and airway lining fluid by type II alveolar cells. Several studies have emphasized the role of SP-A and SP-D in host defense. Upon recognition of the infectious agents, they put into action effector mechanisms like direct opsonization, neutralization, agglutination, complement activation and phagocytes to curb the microbial growth. High-affinity interactions between collectins and microorganisms depend, on the one hand, on the high density of the carbohydrate ligands on the microbial surface, and on the other, on the degree of oligomerization of the collectin. Apart from binding to microorganisms, SP-A and SP-D can interact with receptors on host cells. Thus, it can modulate inflammatory and allergic responses, affect apoptotic cell clearance and modulate the adaptive immune system (1-5).

Recent studies have shown that SP-A and SP-D are localized in various mucosal epithelial cells from the skin, eyes, gastric tract and both male and female reproductive tracts. The review discusses various studies localizing SP-A and SP-D, in the female reproductive tract and highlights their plausible roles in pregnancy and parturition.

3. STRUCTURE OF SP-A AND SP-D

The collectin family proteins are characterized by the presence of four distinct regions in their polypeptide chains: a cysteine-rich N-terminal domain, a collagen-like region, an alpha-helical coiled-coil neck domain and a C-terminal lectin or carbohydrate-recognition domain (CRD). These polypeptide chains form trimers that may assemble into larger oligomers. Table 1 summarises the information on SP-A and SP-D with respect to chromosomal location, size, expression pattern in the female reproductive tract and carbohydrate preferences.

4. LOCALISATION OF SURFACTANT PROTEINS IN FEMALE REPRODUCTIVE TRACT

Immunoreactive SP-D protein has been localized in the non pregnant uterus, ovaries and oviduct of human female reproductive tract (6). SP-D immunoblot analysis of cervical tissue revealed more protein content than control lung tissue. The same study also demonstrated the presence of SP-D mRNA in Hela, Hec1b and KLE endocervical cell lines. SP-D protein was localized in the apical portion of reproductive epithelial cells (6). Furthermore, proliferative and secretory phases of endometrium are SP-D negative and positive respectively, suggesting that expression of SP

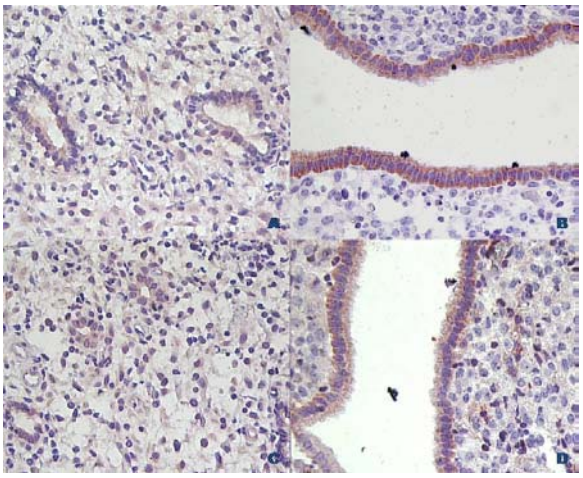

Figure 1. Immuno-localisation of SP-A (C and D) and SP-D (A and B) in human endometrium of follicular phase (A and C) and luteal phase (B and D).

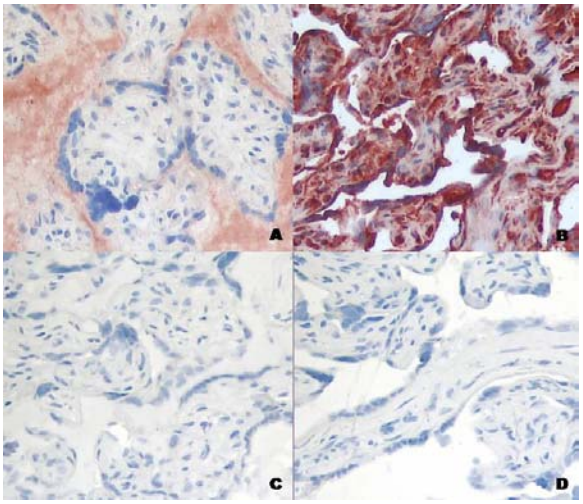

Figure 2. Immunohistochemistry of human term placental sections probed with mouse monoclonal antibody against SP-A or SP-D (upper panel) while lower panels represent respective controls. All the pictures shown are in 40X magnification.

D in endometrium is hormonally regulated (7*)*. SP-D has been localised in the villous and extra villous trophoblast of the human placenta (6-10). Specifically, cytotrophoblast, intermediate trophoblast and syncytiotrophoblast of early gestation and trophoblast of late normal placental villi are SP-D positive while SP-D is absent in the stromal cells of the villous core and amniotic cells (7).

SP-D mRNA and protein have been also detected in the vagina, uterus, ovary, cervix and oviduct of the mouse (11). SP-D protein is primarily localized to epithelial cells lining the genital tract and is also present in secretory material within the lumen of the uterus and cervix. The levels of SP-D mRNA in the uterus vary by a factor of 10 during the estrous cycle with peak levels present at estrus and the lowest levels at diestrus (10), again confirming hormonal regulation. In another study, presence of the SP-A and SP-D has been reported in the genital tract of the mare (12).

A detailed study on the localization of SP-A in the human female reproductive tract, reported that it is expressed in the pre- as well as postmenopausal vaginal stratified squamous epithelium, and is present in vaginal lavage fluid (13). By immunocytochemistry, this group identified SP-A in two layers of the pre-menopausal vaginal epithelium: the basal portion of metabolically active cells of the intermediate layer (the site of newly differentiated epithelial cells); and the superficial layer (comprising dead epithelial cells), where SP-A is probably extracellular and associated with the glycocalyx. In the same study, northern blot analysis and RT-PCR demonstrated the presence of two closely related SP-A genes, SP-A1 and SP-A2 in the vaginal mucosa. Unlike SP-D, immunoreactivity of SP-A in the premenopausal vaginal epithelium does not show any cyclic changes and is also present in the post-menopausal vaginal epithelium. Together these findings suggest that SP-A is constitutively expressed in the vaginal epithelium and unaffected by ovarian hormones.

Our recent studies show that SP-A is also present in human endometrium and placenta akin to the localizations observed for SP-D (Unpublished data; Kay *et al*, 2009; Unpublished data; Yadav *et al*, 2009) (Figure 1 and Figure 2).

5. SP-A AND SP-D IN AMNIOTIC FLUID AND AMNIOTIC EPITHELIUM

A progressive change in the levels of several proteins in amniotic fluid with advancing gestational age has been reported. These mostly include innate immune proteins such as cytokines, chemokines and complement proteins. Similarly, such a change has been reported in the levels of SP-A and SP-D, in amniotic fluid with the gestational age. SP-A concentration in amniotic fluid increases as from less than 3 micrograms/ml at 30-31 weeks to 24 micrograms/ml at 40-41 weeks (17). However, levels of SP-D in amniotic fluid rise moderately in comparisons to SP-A in the same samples. Maximum observed values of SP-A and SP-D were 4978 micrograms /l (in the 39th week) and 793 micrograms /l (in the 39th week), respectively when measured in the same amniotic fluid samples (18).

Immuno-histochemistry of paraffin sections of fetal membranes, revealed the presence of both SP-A and SP-D in the amniotic epithelium and chorio-decidual layers (17). Malhotra *et al*, 2004, reported non-uniform cell surface staining of the amniotic epithelium with anti-collectin receptor serum (18). Partial intracellular staining of the epithelium was also observed, suggesting an intracellular pool of the collectin receptor, as has been demonstrated in U937 cells. A protein of 56,000 MW was isolated from the amnion cell line (FL) and was shown to have identical characteristics to collectin receptor from other sources (18).

Moreover the presence of both SP-A and SP-B has been reported in micelles (lamellar bodies) isolated from human term amniotic fluid (19).

It is interesting to note that the levels of SP-A and SP-D increased sharply towards term, at a time when total protein levels of serum proteins such as albumin or transferrin present in the amniotic fluid are reported to decrease significantly. Variation in SP-A and SP-D levels with gestational time in the samples reflect the development of the fetal lung, as SP-A and SP-D are secreted into the amniotic fluid by the fetal lung. However, observation of intracellular SP-A and SP-D in the amniotic epithelium and decidua may indicate synthesis of these proteins, rather than uptake. Later studies reported that SP-A can be also synthesized locally by chorioamniotic membranes (20, 21). Han *et al*, 2007 reported that monocytic cell lines (THP-1 and U937) and peripheral blood monocytes (CD14+/CD115+) obtained from pregnant women also expressed SP-A1 mRNA and protein, suggesting the presence of autocrine/paracrine activation *in vivo*. Interestingly, a mid-trimester amniotic fluid sample obtained from a case of tracheal atresia contained SP-A (3.1 microgram/ml), indicating the presence of SP-A of extrapulmonary origin (21).

6. ROLE OF SP-A AND SP-D IN HOST DEFENSE OF FEMALE REPRODUCTIVE TRACT

The role of collectins in combating infection and inflammation associated with bacterial, fungal and viral micro-organisms is well documented. Hence, a definite role of SP-A and SP-D in the female reproductive tract is to protect the fetus from intra-amniotic infections. Intraperitoneal administration of lipopolysaccharide to pregnant mice, injected at 16 or 17 day post coitum , led to a 3-4 fold increase in SP-A in the uterus (22). This study also reported that, in contrast, SP-D didn't show any significant change at the protein or transcript level in uterus or placenta after LPS administration (22).

Chlamydiae are intracellular bacterial pathogens that infect mucosal surfaces, i.e., the epithelium of the lung, genital tract, and conjunctiva of the eye. Oberley *et al*, 2004, showed that SP-A and SP-D lung collectins enhanced the phagocytosis of *Chlamydia pneumoniae* and *Chlamydia trachomatis* by THP-1 cells, a human monocyte/macrophage cell line. SP-A aggregated both *C. trachomatis* and *C. pneumoniae* but SP-D aggregates *C. pneumoniae* only. After phagocytosis in the presence of SP-A, the number of viable *C. trachomatis* pathogens in the THP-1 cells 48 h later was increased approximately 3.5-fold (23).

Chlamydia trachomatis genital tract infections are a major cause of fallopian tube occlusion and primary site of infection is the cervix. Untreated chlamydial infections of the female reproductive tract often result in sterility of the infected woman. Since SP-D protein is produced in the cervical glands, Oberley *et al*, 2004, examined the effect of SP-D on chlamydial infection of cervical epithelial cells *in vitro* (6). They reported that SP-D protein, through its lectin-binding domain, inhibits the infection of HeLa cells (an endocervical epithelial cell line) by *C. trachomatis* in a dose-dependent manner. Oberley *et al*, 2007, reported an increase in the SP-D protein content of reproductive tract epithelial cells on *Chlamydia muridarum* infection in mice (10). These data are suggestive that SP-D may play a role in innate immunity in the female reproductive tract *in vivo*.

However, women with intra amniotic infections (IAI) surprisingly had no significant differences in amniotic fluid concentrations of SP-A or SP-D compared to controls (24). The study analysed data from four groups, namely steroid treated with or without IAI and steroid untreated with or without IAI. Mean SP-A levels showed an increase by four fold in steroid untreated with IAI in comparison with control group, but were not found to be significant as the number of cases compared were 11 (without IAI) and 6 (with IAI). Mean SP-D levels showed a two fold decrease in patients with IAI in comparison with control group, but the range of SP-D levels actually increased in patients with IAI (0-52.4 ng/ml) in comparison with control group (0-24.4 ng/ml). This report emphasized that amniotic fluid surfactant proteins may have other roles besides the host defense.

7. SP-A AND SP-D IN PREGNANCY MAINTENANCE

A progressive increase in the levels of collectins in amniotic fluid with advancing gestation implicates the relevance of collectins for pregnancy maintenance and plausibly, in the onset of labor. There is a correlation between early spontaneous preterm labor and the presence of infection or inflammation in the placental membranes and decidua (chorioamnionitis or deciduitis); however idiopathic preterm labor can often occur, for unknown reasons, in the absence of infection/ inflammation. The inflammation of the fetal membranes would result in the release of prostaglandins from decidua and fetal membranes, thus triggering labor. We hypothesize that within a physiological range of concentration, SP-A and SP-D, help in continuation of pregnancy by preventing an infection, down-regulating inflammation and regulating the immune milieu that is conducive for pregnancy maintenance. An increase in SP-A and SP-D levels or an alteration (increase/decrease) of their physiological concentrations, due to advance in gestation or an infection/ inflammation, may lead to initiation of term or preterm labor, respectively.

7.1. Role of SP-A in parturition

Parturition is timed to begin only after the developing embryo is sufficiently mature to survive outside the womb. Current dogma suggests functional regionalization of the pregnant human uterus occurs with the lower segment displaying a contractile phenotype throughout gestation changing to a relaxatory phenotype at labor to allow passage of the fetal head whereas the upper segment has a relaxatory phenotype throughout most of gestation to accommodate the growing fetus and adopts a contractile phenotype for expulsion at labor. Several studies

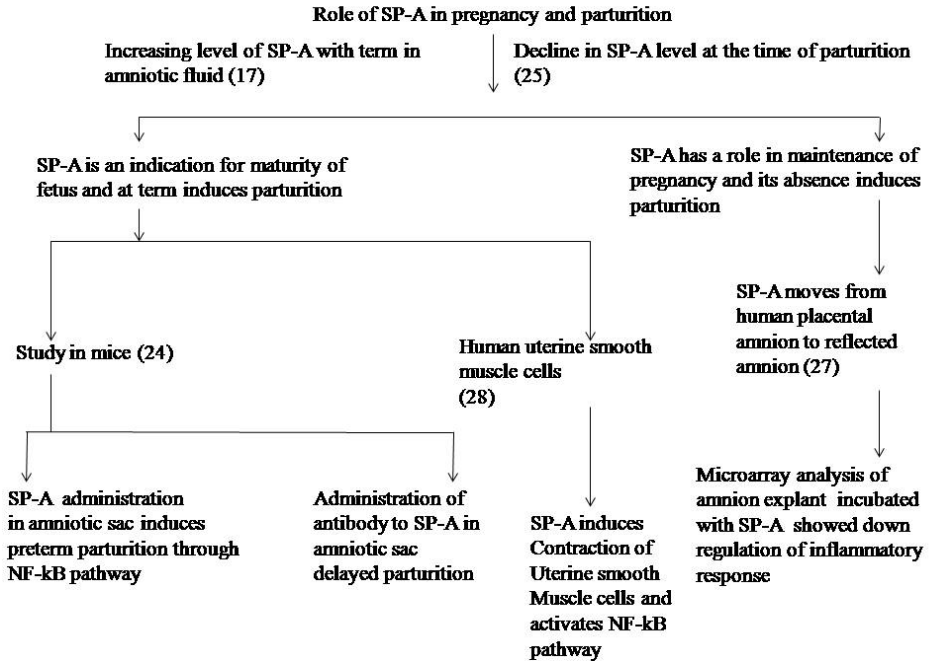

Figure 3. Role of SP-A in pregnancy and parturition.

to understand the role of various proteins, implicated in this phenomenon are currently underway. It has been postulated that the signal for the initiation of parturition arises from the fetus although the nature and source of this signal remain obscure.

Intraamniotic injection of SP-A in mice caused preterm delivery of the fetuses within 6-24 h and administration of an SP-A antibody or NF-kappaB inhibitor into the amniotic fluid delayed labor by >24 h (24). Due to its suggested role in mice parturition, the amniotic fluid SP-A concentration in women at term in labor and not in labor was compared. SP-A level was observed to be significantly lower in the amniotic fluid of women at term in labor than in women not in labor (25). In the same study, concentration of SP-B did not show any significant difference in the two groups of women.

Han *et al*, 2007, carried out a study to determine whether parturition at term, gestational age and chorioamnionitis in preterm delivery (PTD) are associated with changes in the expression of SP-A in the fetal membranes (21). Chorioamniotic membranes were obtained from women at term and women with PTD (n=58). Quantitative real-time reverse transcription-PCR demonstrated predominant expression of SP-A1 mRNA, whose expression was 17.4-fold higher in patients with PTD with chorioamnionitis (n=15) than in those without (n=13) (p=0.018). However, they did not observe any difference in SP-A1 mRNA expression in the chorioamniotic membranes of women at term not in labor (n=16) compared to those in labor (n=14) (p=0.87). But the expression in term membranes was higher than that in membranes from women with PTD without chorioamnionitis (p=0.003). The study suggested that SP-A may not be essential for the onset of pre-term labor or labor at term. Accumulating evidence indicates that a positive feedback loop involving glucocorticoids, proinflammatory cytokines, prostaglandins, surfactant protein-A (SP-A) and 11-beta-hydroxysteroid dehydrogenase-1 is formed locally in human fetal membranes towards term or in preterm labor. This positive feedback loop would produce abundant biologically active glucocorticoids and prostaglandins in the fetal membranes or amniotic fluid, which would ultimately promote fetal organ maturation and initiate parturition (26).

In a recent study, Lee *et al*, 2010, investigated a potential role for SP-A in human pregnancy and parturition by examining SP-A expression patterns in AF and amnion (27). High molecular mass (>250 kDa) oligomeric SP-A was increased in AF with advancing gestation. Interestingly, these oligomers were more abundant in placental amnion before labor at term, while they increased primarily in reflected amnion during labor ($p < 0.05$). Thus, corroborating earlier observation that SP-A levels decrease in placental amnion at labor. These findings related to roles of SP-A in pregnancy and parturition are summarized in a flow chart (Figure 3).

7.2. Mechanisms underlying SP-A mediated induction of parturition

In vitro studies show that human myometrial cells express functional SP-A binding sites and respond to SP-A to initiate activation of signaling events related to human parturition (28, 29). These studies provided

evidence that activation of myometrial cells by SP-A leads to nuclear translocation of RELA (also known as NF-kappaB p65 subunit). SP-A rapidly activated mitogen-activated protein kinase 1/3 (MAPK1/3) and protein kinase C zeta (PRKCZ). The prolonged treatment of myometrial cells with SP-A upregulated PTGS2 (COX2) protein levels. Since PRKCZ is reported as essential upstream mediator of NFKBIA/NF-kappaB it also suggested NF-kappaB signaling regulation by SP-A (30). A recent investigation has shown the up-regulation of MAPKs activity in myometrium of women at term and immediately after parturition compared to non-pregnant women at term (31). MAPKs are also known to phosphorylate some proteins such as caldesmon (32) as well as GJA1 (connexin 43), implicated in the modulation of gap junctions, an intercellular communication network that permits the uterus to develop contractions in a synchronous fashion (33). SP-A is also reported to interact with the intermediate filaments desmin and vimentin and prevents polymerization of desmin monomers in myometrial cells (29). It also enhances the filamentous-actin pool in myometrial cells (29). A recent study reported that SP-A and not SP-D released by human fetal membranes, induces actin stress fiber formation and, thus is able to exert a paracrine regulation of F-actin filament organization in myometrial cells (34). However, the role of this interaction in the parturition process remains unclear.

Since cervical ripening resembles an inflammatory reaction, studies have been carried out to understand the effect of inflammatory cytokines on myometrial cells. Inflammatory cytokine IL-1beta treated human myometrial cells showed induction of PTGS2 and consequent prostaglandin production via MAPK pathway (35) and thus, supported inflammatory action of SP-A on myometrium through myometrial SPR-210 (28, 29). Cortisol (10^{-7}M and 10^{-6} M, 24 h) induced SP-A expression in cultured chorionic trophoblasts, which could be blocked by the glucocorticoid receptor antagonist RU486. Treatment of chorionic trophoblasts with SP-A (10-100 micrograms/ml, 24 h) caused a dose-dependent increase of prostaglandin E2 release and an induction of cyclooxygenase type 2 (PTGS2)(20).

However, there is evidence suggesting that SP-A inhibits LPS induced NF-kappaB signaling. The carbohydrate recognition domain (CRD) of SP-A binds to the recombinant soluble form of the extracellular TLR4 domain (sTLR4) and the endotoxin pathway myeloid differentiation protein-2 (MD-2) in a Ca^{2+}-dependent manner, leading to attenuation of cell surface binding of smooth LPS and smooth LPS-induced NF-kappaB activation in TLR4/MD-2-expressing cells (36-38).

In order to look for the role of SP-A in parturition, Lee *et al*, 2010, carried out a microarray analysis of human amnion explants incubated with SP-A (27). The data revealed a molecular signature of inhibited cytokine-cytokine receptor interaction with down-regulation of IL-1beta, CXCL2, and CXCL5 mRNA expression. The findings suggest that SP-A signals amniotic anti-inflammatory response via AF during pregnancy. They propose that movement of SP-A from placental amnion to reflected amnion leads to signaling of pro-inflammatory cascade leading to induction of labor. This is in disagreement with several studies that have implicated SP-A directly in induction of NF-kappaB pathway and pro-inflammatory cytokines in the uterine myometrium and thus induction of labor. This dilemma is not new and has been highlighted by Gardai *et al*, 2003, where they showed that both SP-A and SP-D can mediate pro-inflammatory and anti-inflammatory signaling through two distinct membrane receptors namely calreticulin and SIR-p alpha (39). It was also shown that the CRD domain interacts with SIR-p alpha while collagen domain interacts with calreticulin. The study hypothesized that in presence of a pathogen, the CRD domain interacts with the glycoproteins present on the surface or secreted by the pathogen and thus, is not available to interact with SIR-p alpha to signal for anti-inflammatory cascade. The collagen domain interacts with calreticulin to signal induction of pro-inflammatory cascade.

7.3. Inhibition of SP-A to delay preterm labor?

Various observations suggest that SP-A can induce an inflammatory response in myometrium even in the absence of any microbial infection in the uterus and thus may adversely affect pregnancy maintenance. It is important to understand the regulation of expression of SP-A in the uterus. The uterus may express some inhibitors of the SP-A or their receptors to avoid the inflammatory signaling during the gestation. Whether inhibition of SP-A in the uterine myometrium can delay pre-term labor, is worth exploring. If the hypothesis of SP-A being an anti-inflammatory molecule during pregnancy maintenance is established in future, then SP-A administration may be useful in delaying pre-term labor and inhibition of SP-A induced ant-inflammatory signaling could be useful for induction of labor. We have no information on role of SP-D in parturition. This is important as SP-A and SP-D interact with each other, share some of the physiological actions and oppose each other in some physiological conditions to create homeostasis in human lung. Interestingly, SP-A gene deficient mice show manifold increase in levels of SP-D in bronchoalveolar lavage.

7.4. SP-A, SP-D and matrix-metalloproteinases

The parturition process involves increased expression of metalloproteases (40, 41). SP-A has been implicated in the regulation of protease and anti-protease activity (42). It has been shown that SP-A gene deficient mice showed increased secretory leukoprotease inhibitor (SLPI), an inhibitor of expression of serine proteases and reduced anti-neutrophil elastase activity in bronchoalveolar fluid. *In vitro* experiments revealed reduced matrix metalloproteinase MMP-12 mediated SLPI cleavage in the presence of SP-A. Targeted ablation of the surfactant protein D (SP-D) gene caused progressive pulmonary emphysema associated with pulmonary infiltration by foamy alveolar macrophages (AMs), increased hydrogen peroxide production, and matrix metalloproteinase (MMP)-2, -9, and -12 expression (43). Immunohistochemical staining of AMs from SP-D(-/-) mice demonstrated that NF-kappaB was highly expressed and translocated to the

nucleus. Increased NF-kappaB binding was detected by EMSA in nuclear extracts of AMs isolated from SP-D(-/-) mice (44). Antioxidants N-acetylcysteine and pyrrolidine dithiocarbamate inhibited MMP production by AMs from SP-D(-/-) mice (44). Trask *et al*, 2001, reported that recombinant rat SP-D dodecamers selectively induce the biosynthesis of collagenase-1 (MMP-1), stromelysin (MMP-3), and macrophage elastase (MMP-12) without significantly increasing the production of tumor necrosis factor alpha and interleukin-1beta (45). SP-D did not alter the production of these MMPs by fibroblasts. Phosphatidylinositol, a surfactant-associated ligand that interacts with the carboxyl-terminal neck and carbohydrate recognition domains of SP-D, inhibited the SP-D-dependent increase in MMP biosynthesis. So, in future it will be interesting to investigate the role of SP-A, SP-D and its effect on the proteases and anti-proteases during pregnancy and the parturition process.

7.5. Modulation of the adaptive immune response SP-A and SP-D

Repeated first trimester losses of pregnancy or recurrent miscarriages have been often attributed to unfavorable immune milieu contributed by infiltrating lymphocytes. In a recent study Mjosberg *et al*, 2010, reported that regulatory T cells (Treg) cells were significantly enriched in first trimester decidua and displayed a more homogenous suppressive phenotype with more frequent expression of FOXP3, HLA-DR, and CTLA4 than in blood (46). Th17 cells were nearly absent in decidua, whereas Th2-cell frequencies were similar in blood and decidua. CCR6(+) Th1 cells, reported to secrete high levels of interferon gamma (IFNG), were fewer, whereas the moderately IFNG-secreting CCR6(-) Th1 cells were more frequent in decidua compared with blood. The study concluded that local, moderate Th1 activity seems to be a part of normal early pregnancy, consistent with a mild inflammatory environment controlled by Treg cells.

Another recent study reported higher ratios of Th1/Th2 chemokine receptors in women with recurrent miscarriage compared to controls. The ratio of Th1/Th2 chemokine receptors was normalised in recurrent miscarriage women after immunotherapy, suggesting that, lymphocyte immunotherapy might influence pregnancy outcome via a shift in the balance of the Th1/Th2 chemokine receptors (47).

Administration of SP-A and SP-D to murine models of allergy with predominant Th2 type response led to the restoration of a balanced Th1-Th2 type of cytokines and alleviation of elevated IgE antibodies and hypereosinophilia (48). Furthermore, SP-A and SP-D gene deficient mice showed a skewed Th2 type of response when compared with wild type, and Th1-Th2 homeostasis could be restored on administration of recombinant SP-D in SP-D gene deficient mice (49). In view of this effect of SP-A and SP-D, we hypothesize they could be playing an important role in the physiology of first trimester pregnancy. It would be interesting to examine their function using gene deficient mice, other animal models and women with recurrent miscarriages.

7.6. Alteration of SP-A and SP-D function by post-translational modification

Effect of SP-A and SP-D on pregnancy maintenance and parturition may be conditioned by the the presence of post-translationally modified forms of SP-A and SP-D. S-nitrosylation is becoming increasingly recognized as an important post-translational modification with signaling consequences. The formation of S-nitrosothiol (SNO)-SP-D both *in vivo* and *in vitro* results in a disruption of SP-D multimers such that trimers become evident. SNO-SP-D but not SP-D, either dodecameric or trimeric, is chemoattractive for macrophages and induces p38 MAPK phosphorylation (50).

8. INFERENCE

Presence of SP-A and SP-D in the epithelial lining of various female reproductive tract tissues and amniotic fluid suggests that these proteins may be involved in immuno-regulation during pregnancy maintenance and may have a direct or indirect role in the onset of parturition. It is still a nascent field worthy for exploration in view of possible applications in miscarriage, pre-term labor and delayed labor. Recombinant SP-D has shown efficacy *in vivo* in murine models of allergy and infection and thus, the field looks promising.

9. ACKNOWLEDGEMENT

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