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Embryo-Placento-Maternal Interaction and Biomarkers: From Diagnosis to Therapy – A Workshop Report

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INTRODUCTION

The workshop focused on proteins of embryonic, placental or maternal origin involved in unique networks of interactions in establishing and maintaining healthy pregnancy. Special attention was given to various proteins throughout the full-time course of pregnancy, starting from the preimplantation period until labor. Structural and functional data on different polypeptides were shown, intending to evaluate their possible clinical role in healthy or complicated pregnancies and the associated negative consequences. Workshop presentations given by the participants are summarized.

Preimplantation factor (PIF): a novel immune-modulatory peptide: role and expression by gestational tissues

E.R. Barnea described studies on preimplantation factor (PIF), a novel peptide (9–15 aa) secreted only by viable embryos from the 2-cell stage and throughout a viable pregnancy. Pregnancy is an immune paradox, with no host-versus-graft response as an embryo avoids rejection while maintaining systemic immunity. In this study it was found that PIF, the first message of viability and acceptance from the embryo/implant to mother/host, promotes maternal tolerance without suppression. By a PIF antibody-based ELISA, PIF could be detected in viable human and mouse embryo media and in maternal serum 7 days after embryo transfer. Immunohistochemistry revealed high PIF expression in first and second trimesters but low

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expression at term or premature delivered placentas. Human fetal tissue microarrays confirmed high PIF expression in hematopoietic tissues.

PIF is inherently nontoxic (as it is embryo-derived), universally mammalian (has a cross-species effect) and acts at low doses (nM), and its synthetic counterpart mimics the native peptide's features. PIF induces immune tolerance by binding to apparently novel PBMC receptors blocking mitogen-activated but not basal immunity and modifies cytokine production. *In vivo* efficacy against acute mismatched (GvHD), JDM (NOD model, adoptive transfer), and multiple sclerosis (MS-EAE model) models, using the same low PIF dose and mode of delivery, led to disease prevention or lowered severity and increased survival. In conclusion, PIF was shown to be a pregnancy biomarker with diagnostic utility as well as a potential treatment for immune disorders without the toxicity associated with current therapies [1–3].

Decidual cell hemostasis: implications for adverse pregnancy outcome

M.J. Paidas reviewed the recent knowledge on endometrial stromal cells, unique cells capable of a major transformation following exposure to estrogen, progesterone and during pregnancy. Progesterone stimulates estrogen-primed endometrium to undergo decidualization, the process of growth and differentiation that transforms these precursor stromal cells into decidual cells. There is a risk of decidual hemorrhage as extravillous cytotrophoblasts penetrate spiral arteries, and it is the decidual cells that are keys to regulating hemostasis in this environment. Perivascular decidualized stromal cells are spatially and temporally positioned to paradoxically mediate hemostasis during menstrual bleeding during and trophoblast invasion in pregnancy. While the endometrium is the only tissue that undergoes cyclical angiogenesis, it is the decidualized stromal cells that regulate endometrial angiogenesis via ovarian steroid modulation. Decidual cells are not in direct contact with blood, yet these cells possess the cellular machinery to regulate thrombin generation.

Thrombin exerts a variety of effects beyond the coagulation cascade and includes myometrial activation and extracellular matrix degradation which are involved in the genesis of adverse pregnancy outcome. Recently, decidual cells have been shown to possess components of the innate immunity. It is possible that embryo derived proteins interact with decidual cells through mechanisms that involve this pathway, further implicating the importance of decidual cells in the establishment and maintenance of pregnancy and in the genesis of its complications [4–6].

Placental cell surface-aminopeptidases as a feto-maternal barrier

S. Mizutani demonstrated investigations on placental aminopeptidases in relation to bioactive peptides such as oxytocin, vasopressin and angiotensin, which are highly uterotonic and vasoactive and possibly derived from the fetus. Their studies were initially designed to clarify both the mechanism involved in the onset of labor and the etiology of preeclampsia (PE).

Primarily, oxytocinase and angiotensinase in the placenta were determined to be P-LAP and aminopeptidase A (APA), respectively. P-LAP hydrolyzes oxytocin, vasopressin and angiotensin III, and APA hydrolyzes angiotensin II, all quite actively [7]. It was also shown that changes in maternal serum P-LAP and APA are useful for predicting both the onset of labor and preterm labor as well as pre-eclampsia, respectively. Cloning P-LAP from a human placental cDNA library showed that the enzyme is a type II integral membrane protein, similar to APA. AP-2 and ikaros were found to cooperatively enhance P-LAP transcription in trophoblastic cells. In addition, forskolin (FSK) treatment was shown to upregulate P-LAP gene expression via AP-2, putatively AP-2 α , which is upregulated with trophoblast differentiation [8]. Animal studies with APA on not only APA deficient mouse but also

spontaneously hypertensive rat and Dahl salt-sensitive rat suggested a pivotal role of APA via degradation of A-II in hypertension [9].

The results of these basic and applied investigations suggested that the imbalance between the concentration of fetal bioactive peptides and placental aminopeptidase activities might result in the predominance of bioactive peptides in the feto-placental unit and their subsequent leakage into the maternal site occurs with the onset of labor, preterm labor, and pre-eclampsia.

Immune programming of preterm parturition

S. Sharma explained how a successful pregnancy is marked by an intricate regulation of the innate and adaptive immune responses at the maternal-fetal interface, resulting in tolerance of the semi-allogeneic fetus. In human placentation, fetus-derived trophoblast cells invade the decidua and replace the endothelium of the uterine spiral arteries to ensure the development of an adequate blood supply to the fetal-maternal unit. This also allows interactions between trophoblast cells and the maternal leukocytes richly populated by uterine NK cells. The placenta is known to produce an array of factors that may lead the maternal immune system toward anti-fetal responses. Thus, dysregulation of fetal immune responses may be causal for adverse pregnancy outcome such as preterm birth.

Preterm birth is the leading cause of perinatal mortality and morbidity, the latter contributing to a majority of long-term neurologic complications. Approximately 30–40% of preterm births are associated with mostly sub-clinical intrauterine infections, suggesting inflammation / infections as the disruptors of the balanced immunity at the maternal-fetal interface. Unscheduled activation of inflammatory responses, either by infection or other insults such as vascular pathology, stress, and uterine over-distention, may lead to the onset of preterm parturition. However, the precise mechanism(s) that invokes the immune system as the causative factor in adverse pregnancy outcomes is not yet understood [10–12].

It was hypothesized [13] that an intrinsic deficiency in an anti-inflammatory milieu coupled with gestational age-dependent inflammatory insult will result in an array of pregnancy-associated anomalies. The IL-10 knockout mouse model was used to address this hypothesis and recent results may have implications for the understanding of the mechanisms and therapy of infection/inflammation-induced adverse pregnancy outcomes, particularly based on control of NK cell activation and migration and elevated production of TNF- α [13].

Placental programming and intrauterine stress

J. Padbury described that fetal exposure to an adverse intrauterine environment leads to the long-term “programming” of physiological, behavioral and immunological systems in later life. Later deficits that have been associated with abnormal intrauterine life include ADHD, hypertension and other cardiovascular disorders, dyslipidemia and diabetes. The placenta is a novel site of expression of the norepinephrine transporter (NET) gene [14]. NET expression is critical to homeostasis *in utero*. Placental glucocorticoid metabolism by 11 β hydroxysteroid dehydrogenase (11 β -HSD 2) is regulated by norepinephrine. Regulation of NET and 11 β -HSD 2 in placentae from 32 women with adverse pregnancy histories and from low-risk controls was examined. Both 11 β -HSD2 and NET were highly correlated and were downregulated in response to maternal cocaine use, smoking, and depression ($p < 0.05$). Additionally, the DNA from patients exposed to cocaine/nicotine was hypermethylated. Direct sequencing of the NET and 11 β -HSD2 promoters is underway. This may represent a novel form of placental gene silencing seen in other tissues [15,16]. This gene-environment interaction suggests an important molecular mechanism for long-term programming.

Diagnostic value of Placenta Protein 13 (PP13) in the third trimester

N.G. Than presented their research conducted at Semmelweis University on Placental Protein 13 (PP13), a member of the so-called pregnancy-related proteins, which was purified and later cloned from human term placenta [17]. Detailed molecular biological analyses revealed its characteristic structural and functional features, identifying PP13 as galectin-13, a placental member of a beta-galactoside binding protein family. PP13 was shown to be localized at the apical membrane of the placenta, on the lining of the common fetomaternal blood-spaces [18]. In parallel, clinical tests identified PP13 as a promising first trimester biomarker for the prediction of PE [19].

Recent studies performed in the third trimester showed that the temporal and spatial expression pattern of PP13 in placentas was specifically altered in PE. Placental PP13 were significantly higher in early-onset PE compared to term controls while only a slight increase was detected in late-onset PE both at the protein and RNA levels. PP13 staining in the syncytiotrophoblasts, especially at the brush border membranes was considerably stronger in PE placentas. Moreover, the severity of the disease had major effect on maternal serum PP13 concentrations, as well. All of this data indicates that PP13 may be a promising tool for the differential assessment of early- vs. late-onset PE. Furthermore, these results also led them to conclude that PP13 may play a pathophysiologic role in the complex immunobiological processes at the fetomaternal interface, and its impaired expression may contribute to the development of severe pregnancy complications.

CONCLUSION AND FUTURE DIRECTIONS

Three themes have emerged from this workshop. These reflect better biomarkers for pregnancy, biomarkers from pregnancy to the newborn well-being, and better understanding of pregnancy as a unique phenomena and translating it for applications well beyond pregnancy. What that teaches us is that life is a continuum from conception until adult life and beyond. Consequently lessons and insights from one can be translated into other applications. Viewing life as an integrated picture instead of separating it to pre- and postnatal periods can be of great benefit for both periods. Therefore, emphasis should be placed on the earliest identification of disorders of pregnancy since the consequences as shown are for a lifetime. The development of specific biomarkers for identifying pregnancy complications and understanding pathophysiologic mechanisms that are involved in adverse pregnancy outcome as discussed in this workshop are important; however, developing the means of preventing and treating those disorders in a rational and specific manner is the next phase that we emphasize since addressing the one without the other is only part of the solution. The data presented in this workshop clearly addressed the second part as well. Overall, participants in the workshop felt that diverse cellular/molecular and translational studies target the treatment of pregnancy disorders, aspects which are being actively pursued.

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