

that distribution of HLA-G molecules on the cell-surface is uniform, indicating that HLA-G may not be confined to lipid rafts or other microdomains. Similar results were found when cells were incubated with W6/32, a pan-Class I specific mouse monoclonal antibody specific for all HLA-Class I antigens associated with beta-2-microglobulin. We have found that HLA-G molecules are present in close proximity of other existing HLA-Class I molecules and this spatial distribution allows them to interact with each other forming complexes with a possible functional relevance. The lifetime of HLA-G labelled with MEM-G/9 and FITC was 2.5 ± 0.1 ns. Upon co-expression with HLA-C, the lifetime was reduced to 2.1 ± 0.1 ns. This is indicative of FRET between HLA-G and HLA-C and strongly suggests physical interaction of these two proteins. Intensity based FRET studies for HLA-G and HLA-E interaction, using sensitized emission and acceptor photobleaching, also showed FRET occurrence; these results are currently under study using the more robust technique of FLIM-FRET.

Conclusions: Human HLA-Class I molecules such as HLA-Cw4, HLA-G and HLA-E are known to bind to NK cells inhibitory receptors (KIR) and thus prevent the NK cells mediated cell lysis at the fetomaternal interface. Our findings from *in vitro* studies suggest that HLA-G display physical interaction with other Class I cohorts on the surface of trophoblast model cell lines JEG-3 and ACH-3P. This interaction of HLA-G with its other class members might be functionally important in the modulation of uterine NK cell lysis, leading to a successful pregnancy. Our results are consistent in both cell lines under study. A corollary from this study is that ACH-3P can be used as a new model cell line for *in-vitro* trophoblast investigations, which can complement those studies performed so far with JEG-3 cells.

Keywords: HLA-G; MHC-Class I; Trophoblast; FLIM-FRET

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Fetal origin of chronic immune diseases: Role of prenatal stress challenge

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Introduction: Population-based studies show a dramatic rise in the incidence of chronic immune diseases such as allergies, inflammatory bowel disease, and diabetes over the last five decades. The prenatal period has been proposed to be highly vulnerable in inducing negative repercussions of postnatal offspring health. Recently, maternal stress during gestation has been identified as a

risk factor for the development of chronic immune disease in later life, such as allergies, but also schizophrenia or depression. To date, no studies have investigated the impact of maternal stress on the developing immune system, which commences during the fetal stage. In mice, the fetal liver represents a major source of hematopoietic stem cells (HSC) at mid-gestation, which seed into the thymus and differentiate during T cell maturation. Disturbance of this carefully orchestrated immune development may be a pivotal cause for immune impairment in later life. Additionally, prior studies have revealed the presence of a sex bias in prenatal response to maternal stress. Hence, the aim of the present study was to determine if stress challenge during gestation interferes with normal fetal immune development in mice.

Methods: Dams were stressed by exposure to sound stress (75 dB emitted for 1 second, 4 times/min by a device placed in the mouse cage) for 24 h on GDs (gestation days) 12.5 and 14.5. Fetal tissue from DBA/2J-mated $\times$ BALB/c females were collected on GD 16.5, absolute cell numbers in fetal thymus and liver were documented. Flow cytometric analysis was performed to investigate frequency and phenotype of fetal immune cell populations. Liver-derived HSC were identified as lineage(Lin)⁻, CD34⁺, c-kit(CD117)⁺, Sca-1⁺. T cell maturation stages were identified by the presence or absence of CD4, CD8, CD3, CD44 and CD25. Regulatory T cells were identified as CD3⁺CD4⁺CD25⁺Foxp3⁺. Fetal tails were collected on GD 16.5 for PCR analysis to determine fetal gender based on the presence or absence of the Y chromosome.

Results: We observed a significant decrease in overall hepatocytes in prenatally stressed fetuses compared to controls, whereby the total number of HSC among the hepatocytes was increased. Additionally, the total number of thymocytes was reduced in prenatally stressed fetuses compared to controls. Further, in the thymus we observed a significant stress-induced lag in T cell maturation, as cells were largely arrested in the pre-selection phases, whereas controls showed a timely progression to post-selection. Further, we revealed a significant decrease of regulatory T (Treg) cells within thymocytes in the prenatally stressed offspring compared to controls. Finally, we did not identify the presence of a gender bias in response to prenatal stress.

Conclusions: These results indicate that - as a consequence of prenatal stress challenge - the HSC seeding originating from the liver is extended, and expected HSC migration to bone marrow and spleen may be delayed. Stress challenge also mediates a decrease in overall organ cellularity. Further, we provide evidence for a significant delay in T cell maturation. Subsequently, this could result in altered selection or impaired central or peripheral tolerance and the fetus will be inadequately prepared for immune challenges faced after birth. Finally, the significant decrease in Tregs suggests that fetal ability to respond to immune challenges in later life is compromised. Given that T cells are long-lived, these impaired T cell functions could carry over into adulthood and may account for the impaired immune response seen in individuals with chronic immune disease.

Keywords: Immune ontogeny; Environmental challenge; Mouse model

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Aquaporin1, a water channel, and angiogenesis are novel targets of environmental toxicants that cause *in utero* complications

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Introduction: Toxicants such as polychlorinated biphenyls (PCBs) and bisphenol A (BPA) are ubiquitous in the environment and pose as serious risk factors to human health. BPA is under review by the United States Food and Drug Administration (FDA) for its disease-causing effects at different stages of life, beginning with *in utero* exposure. Our laboratory has recently identified a novel target of PCBs, aquaporin 1 (AQP1), in the mouse and human placenta. AQP1 regulates angiogenic activities, amniotic fluid levels and fetal development during pregnancy. Importantly, AQP1 protein content was significantly reduced in uteroplacental tissue, and this effect was mainly exerted by non dioxin-like (NDL) PCBs and was reversed by IL-10. We hypothesize that NDL PCBs act as anti-angiogenic agents, a surprising notion keeping in mind the xenobiotic and carcinogenic activities of PCBs. We further hypothesize that BPA induces adverse pregnancy outcomes by dysregulating the AQP1-angiogenesis-IL-10 axis.

Materials and methods: We are currently investigating BPA and dioxin-like (DL) and NDL PCBs for their temporal effects on pregnancy outcomes, hypertension and glucose tolerance. To this end, we are utilizing both *in vitro* cell culture and *in vivo* (murine) models of gestational exposure to these toxicants.

Results and conclusions: Upon completion, our investigation will further examine the pathways that lead to reduced AQP1 protein content and angiogenesis.

Keywords: Bisphenol A; Polychlorinated biphenyl; Aquaporins; Placenta; Angiogenesis

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Molecular regulation of trophoblast invasion

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Introduction: Invasion of extravillous trophoblasts (EVT) into maternal decidual stroma and spiral arteries is critical for placentation and successful pregnancy since remodelling of the uterine vessels ensures appropriate transfer of nutrients and oxygen to the developing fetus. Failures in endovascular trophoblast invasion have been observed in pregnancy diseases such as preeclampsia and severe form of intrauterine growth restriction. However, the underlying causes remain largely obscure warranting studies on the molecular regulation of trophoblast invasion under physiological and pathological conditions. Throughout the years an increasing number of growth factors, cytokines and angiogenic molecules controlling trophoblast motility have been identified. These factors are secreted from numerous cells of the placental bed such as trophoblast, maternal epithelial and stromal cells as well as uterine NK cells and macrophages. Hence, a complex network of cell types, mediators and signalling pathways is thought to control the invasive differentiation process of EVT. Despite these facts, the interplay between different cell types and growth factors, the cross-talk between signalling cascades, as well as transcription factors committing and differentiating invasive trophoblasts, remain largely elusive. Critical signalling cascades and growth factors studied so far will be summarised. Also, current work in the laboratory on the role Wnt-dependent transcription factors, Notch signalling and other regulatory factors in trophoblast invasion will be discussed.

Materials and methods: Gene silencing of critical transcription factors and signalling components was performed in trophoblast cell lines or primary EVT using siRNAs or retroviral transduction of shRNAmir-expressing plasmids. Functional analyses included invasion, proliferation and other assays.

Results: Functional analyses of untreated and gene-silenced cell cultures unravelled critical roles for Wnt-dependent TCF-4, Notch signalling and others in human trophoblast invasion.

Conclusions: An increasing number of growth factors, cytokines and chemokines signalling through different cascades are being identified. However, much remains to be learned whether changes in the activity of certain signalling pathways may contribute to the pathogenesis of pregnancy diseases with abnormal placentation or failed trophoblast differentiation.

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