Mentored Professional Enrichment Experience

Applicant:

Name of Project/Experience:
Immunology of preeclampsia: Effect of type-1 pro-inflammatory cytokines on expression of the transcription factor GCM1 and placenta growth factor in human trophoblast.
Location Where Project/Experience Will Take Place:
Department of Medical Microbiology, Immunology and Cell Biology and Department of Obstetrics and Gynecology, SIU School of Medicine, Springfield, IL, USA.
Mentor Name and Contact Information:
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RATIONALE

During pregnancy, the human uterus undergoes many vascular and tissue changes. One of the most important changes involves the conversion of relatively low capacity, high resistance uterine spiral arteries into high capacity, low resistance uteroplacental vessels. Preeclampsia, a serious complication of pregnancy, is a disease that involves inadequate angiogenesis of the placenta (Rusterholz et al., 2007). Trophoblasts form the outer layer of the blastocyst and are important for implantation and placentation of the embryo. In order for proper vascular development of the placenta to occur, trophoblasts must express proangiogenic factors at the maternal-fetal interface, specifically placenta growth factor (PlGF) protein (Chang et al., 2007).

P1GF, a member of the vascular endothelial growth factor family, is expressed primarily in the placenta and is highly expressed by trophoblast cells. Functions of P1GF include inducing angiogenesis, trophoblast survival, and promotion of extravillous trophoblast proliferation. Additionally, P1GF is associated with the relaxation of human placental vessels, which plays a role in the reduction of blood flow resistance within the fetoplacental circulation (Chang et al., 2007).

Due to inadequate angiogenesis of the placenta, preeclampsia results in reduced placental perfusion, which in turn causes hypoxic conditions at the fetoplacental interface (Rusterholz et al., 2007). In non-trophoblast cells, hypoxia causes P1GF gene expression to increase. However, when exposed to hypoxic conditions, trophoblast P1GF gene expression decreases. Maternal serum levels of P1GF in women with preeclampsia support this finding, as they are significantly reduced when compared to levels in women with normal pregnancies (Tidwell et al., 2001).

The differential regulation of P1GF gene expression in trophoblast versus non-trophoblast cell types suggests differing mechanisms of regulation (Gobble et al., 2007). The expression of P1GF by trophoblasts has recently been shown to be regulated by the transcription factor glial cell missing 1 (GCM1). GCM1 functions to increase placenta growth factor promoter activity, which affects gene expression and thus levels of P1GF produced by trophoblast. Placentae taken from women with preeclampsia express less GCM1 protein and mRNA and also have fewer trophoblast cells expressing GCM1 when compared to women with normal pregnancies. Additionally, trophoblasts placed in low oxygen tensions to imitate the hypoxic conditions of preeclampsia also express less

GCM1. Less GCM1 expression leads to significantly decreased amounts of P1GF production (Chang et al., 2007).

Recent evidence has shown that maternal immune responses to the placenta play an important role in the development of preeclampsia (Dong et al., 2005). Immune cells are good candidates for control of invasion of trophoblasts because they are able to differentiate between foreign fetal cells and maternal cells. Preeclampsia is primarily a disease of women in their first pregnancies with subsequent pregnancies at low risk for developing preeclampsia. Additionally, studies have shown that the risk of developing preeclampsia rises to 30% in women pregnant with a fetus that was created via egg donation (Moffett & Hiby, 2007).

In women with preeclampsia, studies have shown that levels of pro-inflammatory cytokines are imbalanced. Specifically, women with preeclampsia have an imbalance between type I and type II cytokines. Type I cytokines primarily are proinflammatory in nature and include interferon-γ (IFN-γ), interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α). Type II cytokines down regulate proinflammatory immune responses and include IL-4, IL-5, and IL-10. During normal pregnancy, there is a movement of cytokine production towards type II cytokines. However, women with preeclampsia demonstrate just the opposite shift. Their levels of type I cytokines increase while levels of type II cytokines decrease (Dong et al., 2005). Collectively, this evidence indicates that a maternal immune response develops in preeclampsia relating to the fetus.

The proposed study will examine effects of specific type I proinflammatory cytokines on trophoblast production of P1GF. Because the imbalance between type I and II cytokines may decrease production of P1GF by trophoblast via effects on the GCM1 transcription factor, the effects of type I cytokines on the expression of GCM1 will be examined. Our working hypothesis is that type I cytokines will decrease expression of GCM1 leading to decreased P1GF mRNA expression in human trophoblast cells.

GOALS

Goal 1: Explore the effects of TNF-α, IFN-γ, and IL-1 (type I pro-inflammatory cytokines) on expression of PlGF in human trophoblast cells.
Goal 2: Determine the effects of type I pro-inflammatory cytokines on expression of the glial cell homolog 1 (GCM1) transcription factor in human trophoblast cells.
Goal 3: Become familiar with the pathogenesis and pathophysiology associated with immune mechanisms of preeclampsia in human pregnancy.
Goal 4: Learn methods of tissue culturing, RNA isolation, real time RT-PCR, and other molecular and cellular biology techniques related to project.
Goal 5: Collaborate with other students in the lab to assist and/or supplement their projects with information I gather.

METHODS

Cell culture and Cytokine Treatments.

Human trophoblast choriocarcinoma cell lines, JEG-3 and JAR will be grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin G, 50U/ml and streptomycin, 50 μg/ml). Cultures will be treated separately with 10 ng/ml of type I proinflammatory cytokines TNF-α, IFN-γ, and IL-1. Control cells will consist of cultures treated with IL-4 (a type II noninflammatory cytokine) and no cytokine treatment. Treatment time will be 16 hours.

Gene expression studies.

Effects of the cytokines on GCM1 and PlGF mRNA expression will be monitored by real time RT-PCR. Following cytokine treatment, cells will be lysed with RNA lysis buffer and extracted RNA (200ng) will be converted to cDNA with iScript cDNA Synthesis kit. The cDNA ($1/10^{th}$) will be subjected to quantitative RT-PCR as previously described (Chang et al., 2007). PCR reactions will be performed in iQ5 Real Time PCR Detection System with iQ SYBR Green Supermix. The cycling conditions for *PGF, GCM1* and *RPL32* will be: 95ºC for 4.5 min, 40 cycles of 95ºC for 30 sec, 62ºC (*PGF and GCM1)* or 53ºC (*RPL32)* for 30 sec, and 72ºC for 30 sec. The following quantitative PCR primers will be used: *PGF*: (F): 5'-AGAAGATGCCGGTC ATGAG-3', and (R): 5'-ACACTTCCTGGAAGGGTAC-3', *GCM1*: (F): 5'-AGAAAGTGAACACAGCACCTT CCT-3', and (R): 5'-TGAGTTCTGCTGAGGAGTGTTAGC-3' (Chang et al., 2007). Ribosomal protein (*RPL32)* will be used as a normalization control gene. RPL32 will be amplified via *RPL32* (F): 5'-CCCAAGATCGTCAAAAAGA-3' and *RPL32*(R): 5'-TCAATGCCTCTGGGTTT. *GCM1* and *PGF* expression from each culture will be normalized to *RPL32*, and the change in expression between the cytokine treatments and control cultures calculated using the $2^{-\Delta\Delta CT}$ formula (Chang et al., 2007).

ANALYSIS

To determine if *GCM1* and/or *PGF* mRNA expression is affected by Th1 proinflammatory cytokines, *GCM1* and *PGF* mRNA expression levels will be quantified using real time RT-PCR. *GCM1* and *PGF* mRNA levels will also be quantified after exposure to IL-4, a Th2 noninflammatory cytokine, in order to compare the effects of Th1 and Th2 cytokines. Relative changes in *GCM1* and *PGF* mRNA expression after cytokine treatments will be determined by comparing values between Th1 and Th2 cytokine treated cultures and control cultures (no treatments).

SUPPORT

1. Do you request support funds? Yes.
2. Would you be able to participate if a scholarship is not available? Yes.

References

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