

2010 Lewis A. Barness Lecture and Fellows' Forum

April 15, 2010

AGENDA

8:00 – 9:00 Lewis A. Barness Lecture
Tampa General Hospital MacInnes Auditorium
Cell-Free Nucleic Acids: Beyond Biomarkers, Towards Fetal and Neonatal Personalized Medicine
Diana Bianchi, MD
Zucker Professor of Pediatrics, Obstetrics and Gynecology
Tufts University School of Medicine

The Fellows' Forum Presentations will be held in room 5051 of the South Tampa Center

9:30 – 9:40 Welcome and Opening Remarks; Robert Nelson, MD, MS
9:40 – 10:00 Raquel Hernandez, MD, MPH
Parent's Healthy Weight Perceptions and Preferences for Obesity Counseling in Preschoolers: Pediatricians Matter
10:00 – 10:20 Ileana Arbona-Ramirez, MD
Fetal/Placental Immune Regulation: Analysis of FOXP3+ Regulatory T cells in Human Gestation
10:20 – 10:40 Alicia Diaz-Thomas, MD, MPH
Towards Functional Studies of a Novel CASR Mutation (c.2303G>T) Causing Neonatal Hypercalcemia
10:40 – 11:00 Suzanne Jackman, MD
Short Term Natural History of Pituitary Incidentalomas in Children
11:00 – 11:20 Anne Kotto-Kome, MD
Antibody PLAC 1 Autoimmune Response in Women with Reproductive Failure
11:20 – 11:40 Anne Lenz, MD
Cardiac Hormones Eliminate Some Human Squamous Lung Carcinomas in Athymic Mice
11:40 – 12:00 Luis Munoz, MD
Intravenous Ibuprofen Treatment for Patent Ductus Arteriosus In Preterm Infants Does Not Affect Cerebral Blood Flow Velocity
12:00 – 12:20 Yahdira Rodriguez, MD
PLAC1 (Placenta Specific-1) Expression in Trophoblasts of the Chorion Laeve
12:20 – 1:30 Luncheon
1:30 Adjourn

Fetal/Placental Immune Regulation: Analysis of FOXP3+ Regulatory T cells in Human Gestation

Authors: Ileana Arbona-Ramirez, Rene Ruiz, Mona Dorsey

Division/Year: Neonatology/Second Year

Background: FOXP3+ regulatory T cells (Tregs) play a central role in maintaining immune homeostasis, primarily by controlling the reactivity of self-aggressive T cells. During pregnancy, an increase in Tregs promotes fetal tolerance. Conversely, low maternal Treg counts can be associated with recurrent spontaneous abortion. Also, a decrease in fetal Tregs through the advancement of gestational age suggests that fetal Tregs may also play a role in fetal tolerance. These observations imply that Tregs may play an important role, not only in preventing autoimmunity, but also in inducing fetomaternal tolerance and, possibly, shaping fetal immunologic development. Consequently, it is important to determine the baseline proportions of circulating fetoplacental Tregs throughout gestation and determine if maternal factors influence these levels.

Hypothesis: Fetal Treg cells % decrease with advancing gestational age.

Objectives: The infant's cord blood Treg level will be quantified using Treg extracellular markers (CD3+, CD4⁺, CD25⁺) and intracellular transcription factor fork head box protein 3 (FOXP3⁺). Also, the Tregs phenotype will be further characterized with corresponding markers into naturally occurring cells (CD45RA), memory cells (CD45RO) and cells of thymic origin (CD31). After quantification of the Tregs we will observe the relationship between the gestational age and the level of the Tregs and correlate with demographic and clinical data.

Design/Methods: Umbilical cord blood is obtained from placentas from 24 to 42 weeks gestational age at the time of delivery. Infant's with history of maternal chronic steroid use or maternal HIV were excluded. Then, mononuclear cells are isolated by PBMC-Ficoll separation and Treg markers are identified by fluorescent staining and quantified by flow cytometry. Maternal data such as medical conditions, infections during pregnancy, medications during pregnancy, prenatal laboratories, and obstetrical history was obtained to correlate with results.

Previous results: Preliminary data from 17 infants between 35 and 41 weeks gestational age show that Treg cells comprise $5.8\% \pm 0.2$ (m \pm SD) of the umbilical cord blood T cell populations. A significant ($p=0.013$) negative correlation between Treg % and GA. There is a significant decline in Treg / Total T cells with advancing gestation.

Expected outcome: We expect to find a Treg is a discrete population in cord blood that will decrease with advance in gestational age.

Discussion: This research may prove useful in establishing a baseline of Tregs levels and phenotypic characterization in infants at different gestational age. This may provide further understanding on fetomaternal tolerance and how maternal/pregnancy factors may affect the fetal immunologic development. Ongoing studies can further correlate maternal/fetal Treg levels and phenotype and assess the suppressive function of Treg according to the Treg phenotype, as well as correlate materno/fetal Treg levels with autoimmune disorders during pregnancy and preterm clinical outcome.

Towards Functional Studies of a Novel CASR Mutation (c.2303G>T) Causing Neonatal Hypercalcemia

Authors: Alicia Diaz-Thomas, MD, MPH; Pallavi Iyer, MD; Allen Root, MD; John Cannon, PhD

Division/Year: Endocrinology/Second Year

Background: The calcium sensing receptor (CASR) is a transmembrane G-protein coupled receptor that transduces signaling via ERK1 phosphorylation and has a calcistat function. Hyper- and hypocalcemic states have been previously described in patients with mutations in the gene encoding CASR. We have identified two patients (siblings) with a novel mutation of the CASR who had neonatal severe hypercalcemia.

Aims: The aim was to functionally characterize this novel mutation *in vitro* using transfected HEK293 cells.

Results: HEK 293 cells were transfected with the CASR_{wt} gene. Functional studies comparing native HEK293 and CASR_{wt} transfected HEK 293 cells were carried out. Downstream ERK1 phosphorylation was compared after exposure to varying amounts of calcium; increased signaling was noted in the CASR_{wt} transfected HEK 293 cell. Site directed mutagenesis was carried out for the c.2303G>T (p.G768V) mutation.

Discussion: Similar functional studies will be performed for the target mutation. Characterization of the functional effect caused by the mutation could provide guidance in management of patients with the same mutation. Other mutations of the CASR can be examined in this fashion.

Parents' Healthy Weight Perceptions and Preferences for Obesity Counseling in Preschoolers: Pediatricians Matter

Authors: Raquel Hernandez, MD, MPH; Tina L. Cheng, MD, MPH, Janet R. Serwint, MD

Division/Year: General Pediatrics/Assistant Professor

Objective: To compare parental report of child body image to perceived healthy weight body image in preschoolers and describe weight-counseling preferences.

Methods: Parents of preschoolers receiving well-child care in an urban pediatric clinic were interviewed and asked to select body images that best resembled: 1) their own child's current weight, 2) a healthy weight preschooler and 3) friend and family report of a healthy weight preschooler. Those indicating that their overweight (age-gender specific BMI $\geq 85^{\text{th}} \leq 94^{\text{th}}$ percentile) or obese (BMI $\geq 95^{\text{th}}$ percentile) child resembled a healthy weight image were considered to misclassify their child's weight. Logistic regression was used to identify predictors of misclassification. Card-sorting exercises explored preferences for weight counseling.

Results: Of the 150 children in our sample, 32.7% (n=49) were overweight or obese. Misclassification occurred in 71.4% (n=35) of parents in this subgroup with some indicating a desire for a heavier child by sketch report. Absence of pediatrician comment on child weight strongly predicted misclassification (OR: 12.3, 95% CI 1.74-87.2). Pediatricians ranked as the most valued weight advisor.

Conclusion: Pediatricians' guidance is highly valued and strongly associated with parental accuracy in classifying child weight. Informing providers that their advice matters may promote more effective clinical discussions surrounding early childhood obesity.

Short Term Natural History of Pituitary Incidentalomas in Children

Authors: Suzanne M. Jackman, MD; Dorothy Shulman, MD; Verena Jorgensen, MD

Division/Year: Endocrinology/First Year

Introduction:

Pituitary incidentalomas are incidentally discovered asymptomatic pituitary lesions. There is a lack of studies that document the natural history of children with pituitary incidentalomas, thus clinical decisions for pediatric patients is based on adult data. This article presents data regarding our experiences with pediatric pituitary incidentalomas.

Patients and Methods:

The charts of 31 pediatric patients who presented with incidentally discovered pituitary lesions on MRI were retrospectively reviewed. All patients had some initial hormonal screening. The final repeat MRIs of the microincidentalomas (<10mm) and macroincidentalomas (> or equal to 10mm) were done on an average of 16.1 months and 15.8 months from the original MRI study, respectively.

Results:

The data demonstrate that over the given time period, 94% of the microincidentalomas and 87.5% of the macroincidentalomas had a favorable clinical course, including: remaining stable in size, decreasing in size, or resolving. Regarding the hormonal screening laboratories, only 1 (3.6%) out of the 28 patients who had prolactin levels drawn, had an elevated prolactin level (55 ng/ml) attributed to a prolactinoma.

Conclusions:

The percentage of pediatric patients with pituitary incidentalomas who had a favorable clinical course (MRI findings remaining stable in size, decreasing in size, or resolving) is similar to the percentage of adult patients who experienced a favorable clinical course. Thus it is reasonable to apply adult algorithms in management of pediatric patients with pituitary incidentalomas.

Antibody PLAC 1 Autoimmune Response in Women with Reproductive Failure

Authors: Anne Kotto-Kome, MD; Celso Silva, MD; Michael Fant, MD, PhD

Division/Year: Neonatology/Third Year

Background: Reproductive failure, including recurrent spontaneous abortion and infertility, result from multiple causes: chromosomal abnormalities, infection, uterine abnormalities, hormonal factors and systemic disease. After evaluation for these causes, about 10% of all cases remain unexplained. The proposed study will explore the possible association of an autoantibody to a placental-specific protein, PLAC1, in reproduction failure.

PLAC1 is an X-linked gene that encodes a 26-kilodalton protein primarily localized in membranous compartments of cells of trophoblast lineage. Although its function remains unknown, a role for PLAC1 in placental development is suggested by aberrant placentation, fetal growth retardation, and neonatal death in mice that have large deletions of the X-chromosome in the region where PLAC1 maps. While PLAC1 expression is restricted to the placenta under normal conditions, it is also frequently expressed in a variety of human cancer. In cancer, PLAC1 seems to be involved in proliferation, cell motility, migration, and invasion. Interestingly, a subset of patients with PLAC1-expressing tumors develops anti-PLAC1 auto-antibodies capable of modifying the tumor's behavior. A small percentage of women without cancer also appear to have circulating anti-PLAC1 antibodies, suggesting exposure to the PLAC1 protein during previous pregnancies. The prevalence and functional impact of anti-PLAC1 auto-antibodies on placental function and pregnancy maintenance remain to be determined.

Objective: The proposed study will determine if there is an association of circulating anti-PLAC1 antibodies pregnancy outcome. We hypothesize that the prevalence of anti-PLAC1 antibodies is higher in women with a documented history of reproductive failure compared to women without such history.

Methods: 1100 healthy, multiparous and primigravid women will be screened for anti-PLAC1 antibodies using a specific ELISA assay at their initial prenatal clinic visit at USF Health, STC Department of Obstetrics. These women will be considered "low risk" to develop pregnancy disorders. Additionally, 300 women referred to the Infertility clinic due to documented histories of infertility or recurrent pregnancy losses will also be screened. These women will be considered "high risk" for pregnancy disorders. This group will consist of women with at least two consecutive spontaneous abortions occurring before 20 weeks' gestation or women with unexplained infertility. Patients will be excluded if they have the pre-existing medical conditions of uterine malformations, cardiac disease, renal disease, infectious disease, thrombophilia, chromosomal abnormalities or cancer.

Expected Outcomes: Based on a small sample of normal women reported in the cancer literature, we can expect approximately 54 patients to be PLAC1-Ab positive. Assuming a type 1 error rate of 5% and type 2 rate of 20%, approximately 300 high risk patients will be required to detect a 2-fold difference in seropositivity compared to the low risk population.

This study will determine the prevalence of PLAC1 auto-antibodies in the general population and will also determine if its presence is associated with reproductive failure.

Cardiac Hormones Eliminate Some Human Squamous Lung Carcinomas in Athymic Mice

Authors: Anne Lenz, MD; Ying Sun, Ehrentraud Eichelbaum, William Skelton, Guillermo Pi, David Vesely

Division/Year: Endocrinology/Third Year

Introduction: Four cardiac hormones synthesized by the same gene, i.e., atrial natriuretic peptide, vessel dilator, long acting natriuretic peptide and kaliuretic peptide, have anticancer effects in vitro. The present investigation was designed to determine if they might have beneficial effects in vivo on human squamous cell lung carcinomas in athymic mice when treated for 28 days via subcutaneous pumps.

Methods: These cardiac hormones were infused subcutaneously for 28 days with weekly fresh hormones at 0.3 nM kg⁻¹ body weight in athymic mice bearing human squamous cell carcinomas. Tumor growth was followed using digital electronic Vernier calipers.

Results: Vessel dilator, atrial natriuretic peptide and kaliuretic peptide each eliminated 1 in 6 (17%) of the human squamous cell lung carcinomas. Long-acting natriuretic peptide, although it did not eliminate any of the human squamous cell lung carcinomas did decrease the volume of one carcinoma to only 2% ($p < 0.0001$) of the untreated carcinomas. The squamous cell lung carcinomas that were not eliminated, with the exception of the one LANP-treated tumor that decreased to only 2% of the volume of the untreated cancers, grew rapidly but their growth velocity compared to controls decreased by 76%, 40%, 38% and 25% in the vessel dilator, atrial natriuretic peptide, kaliuretic peptide and long-acting natriuretic peptide groups respectively ($p < 0.05$).

Conclusions: Three of four cardiac hormones synthesized by the atrial natriuretic peptide gene can eliminate human squamous cell lung carcinomas in athymic mice when treated subcutaneously for four weeks. The 4th cardiac hormones, i.e. long-acting natriuretic peptide, decreased the volume of one squamous cell lung carcinoma to 2% of that of untreated animals, suggesting that it, too, has beneficial effects on squamous cell lung cancers.

Intravenous Ibuprofen Treatment for Patent Ductus Arteriosus in Preterm Infants Does Not Affect Cerebral Blood Flow Velocity

Authors: Luis Munoz, MD; Jane Carver, PhD; Dawn Bruton, RN; Stacey Stone, MD; Roberto Sosa, MD; Rajan Wadhawan, MD

Division/Year: Neonatology/Third Year

Background: Patent ductus arteriosus (PDA) is a common complication in very low birth weight infants, with approximately 30% being diagnosed with a PDA. Pharmacological treatment of PDA includes the cyclo-oxygenase inhibitors indomethacin and ibuprofen. The medications have similar efficacy for PDA closure (60-80%). Infants may need repeated courses due to failure of the PDA to close, and some infants require more invasive procedures such as surgical ligation to close the PDA. The effects of indomethacin on systemic circulatory beds have been widely studied, and they demonstrate that indomethacin decreases intestinal, renal and cerebral blood flow. Indomethacin has also been used for prophylaxis of intra-ventricular hemorrhage (IVH), since it decreases cerebral blood flow. Ibuprofen is a newer medication that was approved for PDA treatment in 2006. Although ibuprofen is also a cyclo-oxygenase inhibitor, it has not been shown to be effective in IVH prophylaxis. We propose the reason that ibuprofen is ineffective in IVH prophylaxis is that the drug does not affect the cerebral vasculature.

Hypothesis: Intravenous ibuprofen for the treatment of PDA does not reduce middle cerebral artery (MCA) blood flow velocity in preterm infants.

Methods: This is an ongoing prospective study at All Children's Hospital. Low birth weight (<2500g) infants are enrolled after they are diagnosed by echocardiogram as having a hemo-dynamically significant PDA requiring medical therapy. Patients receive 3 doses of ibuprofen, 24 hours apart. The first dose is given as 10 mg/kg, followed by 2 subsequent doses at 5 mg/kg. Doppler ultrasound is used to measure MCA blood flow velocity and ductal size, thirty minutes before and thirty minutes after initial ibuprofen administration. Measurements are repeated with the third dose of ibuprofen.

Results: Preliminary data are available for 19 patients. Mean birth weight and gestational age are 899 ± 309 gm and 26 ± 2.1 weeks, respectively. The PDA closed in 68% of patients, with 21% requiring surgical ligation. Interim data analyses indicate that ibuprofen has no effect on MCA blood flow velocity. For dose 1, the mean pre- and post-dose MCA peak systolic velocity was 41 ± 12.3 cm/s and 39.6 ± 11.5 cm/s, respectively ($p=0.627$). For dose 3, the mean pre- and post-dose MCA peak systolic velocity was 41 ± 13.1 and 41 ± 12.8 cm/s, respectively ($p=0.988$). Similarly, there were no significant changes in MCA mean velocity before and 30 minutes after ibuprofen administration for the first and third doses.

Conclusion: Our preliminary results support the hypothesis that there is no reduction of MCA blood flow velocity after ibuprofen administration, which may explain the lack of efficacy of ibuprofen for IVH prophylaxis.

PLAC1 (Placenta Specific-1) Expression in Trophoblasts of the Chorion Laeve

Authors: Yahdira Rodriguez, MD; Michael Fant, MD, PhD

Division/Year: Neonatology/First Year

The placenta is a unique fetal organ that provides the necessary environment for fetal development. It forms a direct interface with the maternal compartment and facilitates gas exchange and nutrient transport to the fetus. It also has considerable endocrine activity involved in regulating both maternal and fetal metabolism. In early pregnancy, two distinct populations of trophoblasts are derived from the outer cell mass of the blastocyst: villous trophoblasts and chorion laeve cytotrophoblasts. Villous trophoblasts show marked proliferation in the peri-implantation period, resulting in formation of the villous syncytiotrophoblast and cytotrophoblasts that finally form the placenta. Trophoblasts of the chorion laeve, by contrast, are extra-villous and are an important component of the fetal membranes. They are in direct contact with both the amnionic membrane and the uterine environment and likely play critical roles in the maintenance of pregnancy. The mitochondria and endoplasmic reticula of chorion laeve cytotrophoblasts share morphologic features with the differentiated syncytiotrophoblast in chorionic villi suggesting functional overlap of these cell populations. Independent studies have suggested that genetic elements on the X chromosome are involved in early placental development. Analysis of the genomic region implicated by these findings led to the isolation of a novel gene, *PLAC1*. *PLAC1* expression is restricted primarily to the placenta and to cells of trophoblast lineage, specifically. *PLAC1* expression is coupled to trophoblast differentiation and is regulated by peptides known to be important for trophoblast differentiation. The functional role of *PLAC1* has not been determined. However, sequence analysis has revealed significant homology with the zona pellucida 3 protein, suggesting it may facilitate important protein-protein interactions at the maternal-fetal interface. In support of this, immunohistochemical studies have localized the *PLAC1* protein to the apical, microvillous membrane surface (MVM) of the syncytiotrophoblast as well as membranous locations within the differentiated syncytiotrophoblast. Preliminary, unpublished data indicate that *PLAC1* mRNA is also expressed in the fetal membranes suggesting trophoblasts of the chorion laeve may also express *PLAC1*. Demonstrating *PLAC1* expression by chorion laeve cytotrophoblasts will extend the role of *PLAC1* at the maternal-fetal interface and may point to important regulatory pathways important to membrane function. The proposed studies will confirm *PLAC1* expression in fetal membranes and identify the specific cell of origin. Additionally, its expression as a function of gestational age and specific gestational disorders will be studied. Finally, specific *PLAC1*-protein interactions will be identified in order to gain insight into its function at the cellular level.