Children's Foundation Research Institute

Research Matters

Fall 2020

Untangling neural crest cell migration in Hirschsprung disease

Ankush Gosain, MD, PhD

Ankush Gosain, MD, PhD, has focused his research on determining the mechanisms underlying abnormal development of the enteric nervous system in Hirschsprung disease. Gosain recently published a new study in *The FASEB Journal* delineating interactions between migrating neural crest cells and the extracellular matrix in a model of Hirschsprung disease using a variety of in vitro and in vivo approaches.

Neurons in the wall of the gastrointestinal tract comprise the enteric nervous system, which controls gut motility, digestion, secretion and absorption. During development, neural crest cells, the precursors of neurons in the enteric nervous system, migrate throughout the digestive tract to provide innervation. However, in Hirschsprung disease, neural crest cells fail to migrate into the distal colon, resulting in a lack of innervation in this region. This lack of innervation is a common cause of neonatal bowel obstruction, which can progress to bowel distension, Hirschsprung-associated enterocolitis and death.

Gosain and colleagues specifically focused on abnormalities in laminin expression as laminin is a potential regulator of interactions between migrating neural crest cells and the extracellular matrix in the developing enteric nervous system. The investigators used a mouse model of Hirschsprung disease, the endothelin receptor B knockout mouse, to tease out specific changes in laminin expression.

In the knockout mice, the gene encoding laminin β1 was upregulated more than two fold. By contrast, the receptor for laminin β1, LAMR, showed decreased expression in samples from knockout mice and human patients with Hirschsprung disease. Application of exogenous laminin-111 suppressed NCC migration in an organ culture model, whereas YIGSR, a laminin β1 analog, promoted NCC migration. YIGSR also upregulated expression of LAMR and enhanced NCC migration in midgut slice culture. When LAMR expression was silenced, the beneficial effect of YIGSR was abolished. Furthermore, YIGSR application resulted in colonization of the distal colon in 80% of ex vivo organ cultures from endothelin receptor B knockout mice.

These experiments indicate alterations in LAMR contribute to neural crest cell migration failure in enteric nervous system development. The investigators think YIGSR may selectively enhance neural crest cell migration through LAMR with LAMR binding increasing LAMR expression and preferentially promoting migration. These results add to the current body of literature showing interactions between neural crest cells and the extracellular matrix are involved in enteric nervous system development with the extracellular matrix representing a potential target for intervention in Hirschsprung disease.

Fu M, Barlow-Anacker AJ, Kuruvilla KP, et al. 37/67-laminin receptor facilitates neural crest cell migration during enteric nervous system development [published online ahead of print, 2020 Jun 27]. FASEB J. 2020;10.1096/fj.202000699R. doi:10.1096/fj.202000699R

THE UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER.

New Faces in the CFRI

Joycelynn Renee Douglas, MS, CCRP, joined the CFRI as an IRB regulatory coordinator. Joycelynn has over 16 years of biomedical and clinical research experience. At St. Jude Children's Research Hospital, she worked as a clinical research associate on studies focused on long-term outcomes in various cancers and cancer treatments. She also gained extensive regulatory experience at George Clinical, Inc., and clinical coordinating experience at Baptist Cancer Center.

Stephen Espy, MSCS, is director of the Biomedical Informatics Core (BMIC) for the CFRI. His responsibilities include research data management and governance as well as informatics innovation. He has a bachelor's in Psychology from Rhodes College and a master's in Computer Science from the Georgia Institute of Technology. He previously served as CISO and Chief Data Officer for the City of Memphis and provided research technology architecture leadership at St. Jude Children's Research Hospital.

Rumana Siddique, MBBS, is the clinical data specialist for the CFRI. Rumana will provide assistance with data manipulation and statistical analysis for CFRI investigators. She has expertise in epidemiological study designs, epigenetics and SAS for data analysis.

Gosain, Towbin receive NIH R01 grants

Le Bonheur Pediatric Surgeon Ankush Gosain, MD, PhD, and Cardiology Chief and Heart Institute Executive Co-Director Jeffrey A. Towbin, MD, were recently awarded NIH R01 grants for upcoming research projects.

Ankush Gosain, MD, PhD

Gosain received $2 million to study Hirschsprung-associated enterocolitis (HAEC) causes. His project, "Dysbiosis in Hirschsprung-Associated Enterocolitis Pathogenesis," builds on more than a decade of work on HAEC. The objectives of the research project are to establish a causative relationship between dysbiosis and HAEC and identify which bacteria are the main drivers of HAEC. Gosain will also test therapeutic targets and examine how neurotransmitters made by bacteria influence the motility of the intestine.

Jeffrey A . Towbin, MD

Towbin is lead principal investigator on the project "Discovery of Modifier Genes in Cardiomyopathy." The objective of this study is to identify the modifier genes that alter the expression of myopalladin (MYPN), a gene that affects the type and severity of cardiomyopathy - an inherited disease of the heart muscle that may ultimately result in heart failure, transplant or sudden cardiac death in many patients. The project will examine how different genetic backgrounds effect the mutation of the MYPN gene which determines how cardiomyopathy is expressed in children.

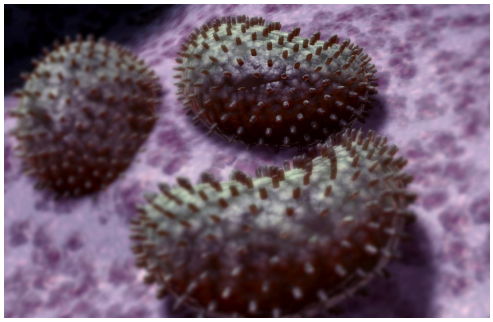
Fighting obesity family style with MEALS

Thomaseo Burton, PhD, and Webb Smith, PhD, have developed a novel intervention to address obesity in the kitchen. The intervention, Multidisciplinary Engagement and Learning/Mindful Eating and Active Living (MEALS), combines mindfulness, cooking techniques and education to teach children and their caregivers how to make healthier meals and engage in mindful eating. This program comprises education, cooking and discussion sessions that are tailored to fit the largely African-American and under-resourced communities served by the Healthy Lifestyle Clinic.

This intervention is unique compared to traditional clinical management, which usually comprises counseling without an experiential component. MEALs also takes barriers to healthy eating, such as cost, time, wariness of new foods and access to ingredients, into consideration by using low-cost ingredients and familiar recipes. Following the pilot program, the facilitators plan to assess the impact of the intervention by measuring participant characteristics (anthropometric measurements and vital signs), caregiver feedback (surveys) and youth feedback (surveys), with the hope of seeing meaningful improvement in participants' health and well-being.

Burton ET, Smith WA. Mindful Eating and Active Living: Development and Implementation of a Multidisciplinary Pediatric Weight Management Intervention. Nutrients. 2020;12(5):1425. Published 2020 May 14. doi:10.3390/nu12051425

Influenza A Virus and Eosinophils: A Charged Interaction



Eosinophils residing in the airways of mice respond to influenza A virus (IAV) infection through alterations in surface expression of various markers necessary for migration and cellular immunity responses, according to Le Bonheur research published in the *Journal of Leukocyte Biology*.

"Very little is known about how eosinophils respond to direct exposure to IAV or the microenvironment in which the viral burden is high," said Le Bonheur researcher Amali Samarasinghe, PhD. "We hypothesized that eosinophils would dynamically respond to the presence of IAV through phenotypic, transcriptomic and physiologic changes."

Researchers investigated eosinophil characteristics in different niches in a mouse model of fungal asthma and influenza, as well as responses to in vitro IAV exposure.

Results of the study showed:

1. Mice with fungal allergic asthma have a lower pro-inflammatory cytokine profile in their lungs during influenza than non-allergic mice.

2. Eosinophil surface antigens are differentially regulated in mice with fungal asthma and influenza. During influenza, eosinophils changed the surface expression of proteins involved in antigen presentation, activation and survival depending on both niche and allergic environment.

3. Following virus exposure, a discrete subset of eosinophils that decreased their surface expression of Siglec-F were also more active. Siglec-Flo expressors also increased expression of eosinophil survival receptor IL-5R and downregulated CD62L which is associated with activation. These data suggest that subpopulations of eosinophils may have differing functions during IAV infection.

4. IAV exposure alters the eosinophil transcriptome. IAV infected eosinophils reduced overall transcriptional activity but upregulated transcription of mRNAs encoding viral recognition proteins.

5. Eosinophils reduce mitochondrial respiration in response to IAV. Eosinophils had a lowered basal respiration rate and an overall reduction in mitochondrial respiration.

6. Flu-PB-1 pulsed eosinophils promote the generation of cytotoxic CD8+ T-cells by causing demethylation of the Tbx21 locus. IAV-exposed eosinophils can communicate with CD8+ T-cells, resulting in epigenetic changes that allow the differentiation of IAV-specific CD8+ T-cells into effector cells.

Overall, mice with fungal asthma that were protected from severe IAV morbidity had reduced levels of cytokines – which can contribute to pathology when present in excess. When exposed to IAV, eosinophils initiate self-preservation mechanisms to survive viral infection, such as conserving energy by reducing transcription activity and mitochondrial respiration. Concurrently, they increase their ability to recognize IAV and induce epigenetic changes in CD8+ T-cells that initiate their differentiation into cytotoxic cells known to be a critical component of the antiviral response.

LeMessurier KS, Rooney R, Ghoneim HE, et al. Influenza A virus directly modulates mouse eosinophil responses [published online ahead of print, 2020 May 9]. J Leukoc Biol. 2020;10.1002/JLB.4MA0320-343R. doi:10.1002/JLB.4MA0320-343R

IN BRIEF

Le Bonheur joins I-ACT

The Institute for Advanced Clinical Trials for Children (I-ACT) is a site network infrastructure of pediatric research sites created with funding from the U.S. Food and Drug Administration (FDA) with experts in conducting innovative and efficient pediatric trials. By implementing standard processes and practices, the I-ACT network can shorten study startup time when approached by a sponsor to conduct a clinical trial. The I-ACT network engages stakeholders to generate discussion and launch groundbreaking initiatives that raise awareness of the need to advance medical therapies, specifically for children, and promote a sense of urgency to achieve this goal. Top children's research organizations are in the network, including 62 sites in the U.S., Canada, Australia and Saudi Arabia. On May 12, 2020, Le Bonheur Children's Hospital was welcomed into the network, with the hope of expanding Le Bonheur's clinical research portfolio to offer even more innovative and ground-breaking research to patients.

FDA announces guidance regarding penalties for non-compliance with applicable clinical trial registration

The FDA has released new guidance to address non-compliance with registration of applicable clinical trials on clinicaltrials.gov. Under the PHS Act, investigators are required to register applicable clinical trials on clinicaltrials.gov and report any results or publications associated with these trials. The FDA's new guidance document indicates that investigators who do not register applicable clinical trials, fail to submit results of the trials or falsify trial records or results can be subject to monetary penalties. Upon discovery of non-compliance, the responsible party will receive a preliminary notice of non-compliance and will be given 30 calendar days after the notice is received to address the issue. After 30 days, the FDA will conduct further review of the submitted clinical trials information and associated documents. If the responsible party fails to comply, then the FDA may take further action, including monetary penalties, injunction or criminal prosecution. To avoid any penalties, please register all applicable clinical trials on clinicaltrials.gov and keep the trial information, such as recruitment, results and publications, up to date. If you have questions regarding this new guidance, please contact CFRI regulatory staff for more information.

Le Bonheur trial: Intravenous indomethacin more effective for hsPDAs

Intravenous indomethacin is more effective than intravenous acetaminophen in treating hemodynamically significant PDAs (hsPDAs) in very low birth weight (VLBW) infants, according to new Le Bonheur neonatology research published in the *Journal of Perinatology.*

Le Bonheur neonatologists, led by Jennifer M. Davidson, DO, conducted a randomized trial for the treatment of hsPDAs in very low birth weight (VLBW) infants. Echocardiogram criteria before and after treatment showed that IV indomethacin was more effective.

"We have several options for PDA closure in these very low birth weight infants with varying levels of effectiveness including intervention by medication therapies," said Davidson.

Commonly used medical therapies are indomethacin and ibuprofen, but these have variable success and notable side effects. Surgical PDA ligation and transcatheter PDA closure can be used if medical therapies fail to close the PDA, but each comes with risks and lack of access for some hospitals. Studies have shown that, for some neonates, acetaminophen may be equally effective for treating hsPDAs with minimal side effects. Le Bonheur neonatologists wanted to examine this option of IV acetaminophen for treating hsPDA in VLBW infants.

To be included in the trial, infants met specific criteria including gestational age at birth between 22 and 32 weeks, birth weight less than 1500 grams, 21 days of age or younger and no previous pharmacologic treatment for PDA. Infants also had to meet strict echocardiogram criteria including left to right ductal flow and two out of three of the following: ductal size greater than or equal to 1.5 mm at smallest diameter, reversal of flow in descending aorta or left atrial size to aortic root ratio greater than or equal to 1.5.

Seventeen infants received 15 mg/kg dose of IV acetaminophen every six hours for 12 doses. Twenty infants received three doses of IV indomethacin every 12 hours with the amount of medication based on age. Each infant had a follow-up echocardiogram within seven days of the initiation of treatment. Successful PDA treatment was defined as no longer meeting the echocardiogram inclusion criteria for hsPDA. Results of the study showed that IV indomethacin was more effective in successful treatment of hsPDA. The rate of successful PDA closure was 55% when using IV indomethacin and 6% when using IV acetaminophen.

"Through our study of very low birth weight infants, we were unable to show successful treatment of hsPDA with IV acetaminophen when compared to IV indomethacin in preterm infants born prior to 32 weeks," said Davidson. "In addition, many of our babies in the study treated with acetaminophen required interventional closure later on."

If acetaminophen continues to be used as a primary treatment of hsPDA, future studies should include a different dosing strategy or route of administration to learn more about its efficacy in PDA closure.

Davidson JM, Ferguson J, Ivey E, Philip R, Weems MF, Talati AJ. A randomized trial of intravenous acetaminophen versus indomethacin for treatment of hemodynamically significant PDAs in VLBW infants [published online ahead of print, 2020 May 21]. J Perinatol. 2020;10.1038/s41372-020-0694-1. doi:10.1038/s41372-020-0694-1