

Welcome ... From Director Mitchell B. Cohen, MD and Associate Director Jorge A. Bezerra, MD

Introducing the Cincinnati DDRDC

Earlier this year, Cincinnati Children's received a grant from the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK) to support digestive health research in Cincinnati. As the centerpiece of this initiative, the Cincinnati Digestive Disease Research Development Center (DDRDC) was established.

This center seeks to promote interaction among scientists with diverse backgrounds in order to gain insight into the fundamental processes of growth and development in the digestive tract and liver. Out of this interaction, novel or improved therapies might be developed to correct intestinal, nutritional and liver diseases, improving the quality of millions of lives.

Already, the DDRDC includes 27 investigators and seven associate members from six departments at Cincinnati Children's and the University of Cincinnati: Pediatrics (ten divisions), Surgery, Molecular Genetics, Biochemistry and

Microbiology, Physiology, Internal Medicine and Pathology and Laboratory Medicine. Together, these scientists have more than \$12 million in annual digestive disease-related funding.

This inaugural issue of **DDRDC Digest** introduces the three biomedical research cores that make up the DDRDC: the Bioinformatics Core, Microarray Core and Integrative Morphology Core. For each core, we provide a summary of resources and services, as well as contact information for interested investigators. We also offer a brief introduction to the Biological Sample Tracking System (BSTS), Cincinnati Children's web-based application for submitting samples to labs and retrieving results.

In future issues, we will guide current and potential investigators on using the cores, highlighting new developments and spotlighting new resources. We will also highlight investigator research programs. 

Bioinformatics Core

Director: Bruce Aronow, PhD

Primary Contact: Sarah Williams (636-2055)

The Bioinformatics Core assists investigators in discovering, validating and assessing the functional significance of genes, gene groups and biological pathways responsible for health, adaptation and disease of the digestive system. To achieve these objectives, the core develops gene and gene expression databases and conducts training courses on mining these databases.

In addition, the core supports commercial sequence analysis packages including SeqLab and SeqWeb, as well as GeneSpring, the industry-standard software for gene expression analysis. The core also is developing several custom applications including:

GenomeTrafac (<http://genometrafac.cchmc.org>): a web-based tool for comparative genomics analysis; designed to identify ortholog-conserved cis-element regions, reducing not only the sequence search space but also the number of likely false positive binding sites.

Peak Analyzer - CisMols Server (<http://cismols.cchmc.org>): an extension of GenomeTrafac that identifies compositionally similar cis-regulatory element clusters that occur in groups of co-regulated genes; these computationally predicted cis-regulatory modules, also known as cis-mols, could serve as valuable probes for genome-wide identification of regulatory regions.

PathMaker: a component of the Genomics Knowledge Platform that enables investigators to visualize biochemical pathways, including the impact of variations such as genetic mutations.

Microarray Core

Director: Steven Potter, PhD

Primary Contact: Shawn Smith (636-0290)

The Microarray Core assists investigators in preparing RNA samples for gene expression analysis.

Investigators begin by using the Biological Sample Tracking System (BSTS) to submit detailed information about their samples. The core then analyzes the quality of the samples by running a RNA 6000 Nano assay with the Agilent 2100 bioanalyzer.

Next, the core performs the required reverse transcription and in vitro transcription reactions, fragments and then spikes the projects with controls, hybridizes, washes, stains and scans the gene chips.

Using the BSTS, investigators can monitor the core's progress at every step and obtain results as they become available. They also can set up experiment sets and projects to facilitate analysis with gene expression software such as GeneSpring.

The Microarray Core continues to look for ways to serve the needs of as many investigators as possible. For example, the core recently started offering a protocol for target preparation that enables the use of as little as 0.5 micrograms of total starting RNA. The reproducibility of this new micro-amplification protocol is similar to that of the standard Affymetrix target preparation procedure.

DDRDC members are eligible for exclusive discounts on services. Currently, for instance, the price for up to eight commercial probe arrays per year will be reduced by \$200 per array for members who use the BSTS and consult with the Bioinformatics Core for approval of their experimental design.

Integrative Morphology Core

Director: David Witte, MD

Primary Contact: Pam Groen (636-8445)

The Integrative Morphology Core provides DDRDC investigators with routine, critical and specialized histological and electron microscopic support.

The histology laboratory is a state-of-the-art facility that offers standard services (fixation, embedding, sectioning and staining of tissue specimens) and specialized services (decalcification, histochemical staining). Both human and animal tissues can be processed.

Similarly, the electron microscopy laboratory provides a range of services including tissue processing and embedding, routine electron microscopy, photomicroscopy and photographic processing.

Radioactive in situ hybridization also is available through the molecular pathology lab.

As with the Microarray Core, DDRDC investigators using the Biological Sample Tracking System (BSTS) receive priority handling of specimens and discounts on core services, including research consultation and interpretation by core pathologists.



Interested in becoming a member?

By becoming a DDRDC member, you will receive discounts on many core resources and services. Your orders also will receive priority.

Full membership is open to all Cincinnati Children's and University of Cincinnati principal investigators involved in digestive research, as well as their trainees. **Junior membership** is open to junior faculty members who do not yet have independent funding.

If you are interested in joining, email the director at mtchell.cohen@cchmc.org. Further instructions will follow.

For a comprehensive list of current members and the latest information about the DDRDC, visit our website:

<http://www.cincinnatichildrens.org/ddrdc>

The Biological Sample Tracking System: Online Sample Submission and Annotation

Primary Contact: Lisa McMillin (636-8159)

Recently developed by the Division of Pediatric Informatics, the Biological Sample Tracking System (BSTS) is a web-based system for tracking and annotating biological specimens. Already it is being used by several groups at Cincinnati Children's, including the members of the DDRDC.

With the BSTS, investigators create a permanently accessible record of each specimen they submit to a core lab, complete with demographic details and experimental descriptions. The lab then uses the system to retrieve this information, generate a bar code for each specimen and store data associated with the specimen such as text descriptions of microscopic interpretations, digital images of stain requests, even costs and charges.

At any time, investigators can access their data via the World Wide Web and check the status of their requests. Security is

insured by a network firewall, password protection and user roles.

In addition to automating the flow of information between investigators and labs, the BSTS promotes sharing of data, reagents, valuable tissue samples and experimental animal systems among investigators. Moreover, by using the BSTS, DDRDC investigators receive substantial discounts on services, and their requests receive priority status.

One-on-one training and support is available from Lisa McMillin (636-8159). In addition, a comprehensive user guide is available with links to animated demos of key procedures such as requesting an account and placing an order. Links to these demos and additional instructions for using the BSTS are available from the DDRDC's website: <http://www.cincinnatichildrens.org/ddrdc>.



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DDRDC First Retreat

The Digestive Disease Research Development Center (DDRDC) held its first retreat on April 20, 2004.

Dr. Mitchell Cohen, director of the DDRDC, began with a description of the DDRDC's history, current organizational structure, and future goals including the development of a CCHMC/UC Digestive Health Center and expansion of the DDRDC into a larger Digestive Disease Research Center (DDRC). This larger center would include more cores and a pilot and feasibility program. Dr. Cohen encouraged the DDRDC members to demonstrate collaboration in their research work and also effective use of core resources. Dr. Jorge Bezerra, associate director of the DDRDC, presented an introduction of the DDRDC Seminar Series held monthly to acquaint members with ongoing digestive disease research. Dr. Bezerra also discussed long-term goals of weekly enrichment seminars, annual lectureship, and special topic symposia.

The next segment featured the three DDRDC Cores: **Microarray**, **Bioinformatics**, and **Integrative Morphology**.

Dr. Steve Potter, director of the **Microarray Core**, introduced the work done by his team. There are three areas of specialty: Gene Expression Arrays, SNP Mapping Arrays, and Re-Sequencing Arrays. Dr. Potter discussed validating new RNA labeling procedures to permit microarray analysis on <50 ng of starting RNA, e.g. samples from developing embryos or laser capture microdissection experiments. His presentation also covered development of resequencing chips for disease based mutation analysis.

Dr. Bruce Aronow, director of the **Bioinformatics Core**, presented the development of a web based program, PolyDoms, (<http://polydoms.cchmc.org>) for mapping of human coding SNPs onto protein domains. Dr. Aronow also discussed intercomparison of three microarray platforms to provide accurate estimates of differential expression as function of graded changes in RNA concentration, labeling methods, and standardization among different microarray cores.

Dr. David Witte, director of the **Integrative Morphology Core**, presented the services provided by his labs. These services include: routine histology, radioactive in situ hybridization, immunohistochemical staining, and barcode tracking system of submitted work. Another service provided by this core is electron microscopy. In particular, Dr. Witte discussed the planned purchase of a new electron microscope with transmission and scanning electron microscopy capabilities and

a digital camera read out. The new equipment will replace an existing aged microscope and expand core capability.

The DDRDC has formed Working Groups to focus on areas of research programs impacting growth and development in the digestive tract. The rest of the evening was filled with presentations on the exciting work done by these Working Groups. Members of each Working Group participated.

Members of the **Differentiation Working Group** gave the following two presentations.

- 1) Dr. Aaron Zorn: Xenopus Embryos as a Model for Studying Endoderm and Liver Development
- 2) Dr. James Lessard: Mice of a Different Color-Transgenic Approaches to Study Smooth Muscle Development and Function

Members of the **Absorption and Secretion Working Group** gave the following two presentations.

- 1) Dr. Gary Shull: Compensatory Mechanisms in Small Intestine and Colon of Mice Lacking the NHE3 Na/H Exchanger
- 2) Dr. Patrick Tso: Lymph Fistula Mouse Model

Members of the **Inflammation Working Group** gave the following two presentations.

- 1) Dr. Alison Weiss: How Do You Relate a Clinical Syndrome To a Specific Molecular Alteration?
- 2) Dr. Ted Denson: Growth Hormone Regulation of STAT3 Activation in Colitis

Members of the **Regeneration and Repair Working Group** gave the following two presentations.

- 1) Dr. Jorge Bezerra: Gene Expression Survey to Understand Liver Biology and Disease
- 2) Dr. Karl Matlin: Repair and Regeneration of a Simple Epithelium

The retreat clearly demonstrated the collaborative spirit of the DDRDC members. The evening concluded with a short discussion of the next retreat, providing a preview of what may be expected. The program for the next retreat planned for early 2005 will include external reviewers: Drs. Alan Walker, Gregory Gores, and Philip Sherman.

Upcoming Seminars

Conference Date	Presenter	Title
Tues. June 1, 2004	Dr. Stephen Zucker	“Physiological Functions of Bilirubin: is it O.K. to be Mellow When Your Patient Turns Yellow?”
Tues. July 6, 2004	No seminar	
Tues. Aug 3, 2004	Dr. Marc Rothenberg	“Eosinophils and Gastrointestinal Inflammation”

DDRDC Research Seminars are held on the first Tuesday of every month throughout the year. Seminars are held in room 3490 Location R (CCHRF) from 8-9am. If you are interested in presenting a research topic at one of these future meetings, please email Dr. Jorge Bezerra (jorge.bezerra@cchmc.org).



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<http://www.cincinnatichildrens.org/ddrdc>

Welcome to Five New Investigators

The DDRDC is pleased to welcome five new investigators since July, 2003.

Dr. Lee (Ted) Denson is an Assistant Professor, in the Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition. Dr. Denson's research is focused on JAK-STAT signaling in colitis and the relationship of the inflammatory process to growth, chronic inflammation, and mucosal healing in Inflammatory Bowel Diseases.

Dr. Alex Lentsch is an Associate Professor, in the Department of Surgery. Dr. Lentsch's research is focused on regulation of inflammatory responses by cytokines, chemokines and adhesion molecules in response to ischemia reperfusion injury in the liver.

Dr. Marshall (Chip) Montrose is Professor and Chair, in the Department of Molecular and Cellular Physiology. Dr. Montrose's research is focused on biophysical analysis of events

at the gastric surface in vivo that help to protect against gastric mucosal damage.

Dr. Manoocher Soleimani is a Professor, in the Department of Medicine, and director of the Division of Nephrology. Dr. Soleimani's research is focused on epithelial transport and biology with an emphasis on cloning of acid-base transporters, identification of bicarbonate transporters in the gastrointestinal tract and kidney, and determining the pathways that are important to ischemic reperfusion injury.

Dr. Alison Weiss is a Professor, in the Department of Molecular Genetics, Biochemistry & Microbiology. Dr. Weiss' research is focused on Shiga Toxin encoding phage and the modulation of Shiga Toxin production in a mouse model of intestinal disease.

We welcome these five investigators to the DDRDC as we continue to promote opportunities for collaborative digestive disease research in Cincinnati.

New Grants Awarded to DDRDC Members Since October 2003

Investigator	Project #	A. D. C.	Source	Project Title
Dr. Ted Denson	IBD-0112R-2RT	\$90,217	Broad Medical Research Program	Mechanisms of growth hormone resistance in experimental colitis.
Dr. Hong Du*	None	\$328,457	Large Scale Biology Corp.	Studies of lysosomal acid lipase produced in GENEWARE system.
Dr. Kathleen Goss	Career Development Award	\$100,000	Susan G. Komen Foundation	Beta-catenin and Breast Cancer: A Transcription-independent Pathway to Tumorigenesis.
Dr. Joanna Groden	CA-84291-06	\$609,582	NIH/NCI	Mouse Models of Gastrointestinal Cancer.
Dr. Xi(Jason) Jiang	PR033018	\$640,637	USAMRMC/DoD	Development of strategies to treat and prevent Norovirus infection
Dr. Christopher Karp	R41 DK067744-01	\$90,910	NIH/NIDDK	Novel lipid-based therapies for cystic fibrosis
Dr. Jeffrey Matthews	R01 DK048010-12	\$235,000	NIH/NIDDK	Salt Transport in Surgical Diarrheal Disease
Dr. Marc Rothenberg	R01 AI45898-05	\$175,467	NIH/NIAID	Regulation of gastrointestinal eosinophils
	R01 AI057803-01	\$125,000	NIH/NIAID	Eosinophil, chemokine and IL-13 cooperativity in asthma
Dr. Jeffrey Rudolph	K08 DK066297-01	\$115,751	NIH/NIDDK	Cyclic-AMP Induced Crypt Cell Survival in the Intestine
Dr. Susan Waltz	None	\$2000	NIH/NCI Cancer phenotyping Core	Ron over-expression in the mouse mammary gland
	R01 CA100002	\$184,500	NIH/NCI	The Ron Receptor in Mammary Gland Biology
Dr. James Wells	2-2003-530	\$100,000	Juvenile Diabetes Research Foundation	Promoting endodermal & pancreatic differentiation of ES cells
	None	\$125,000	American Diabetes Association	Promoting endodermal & pancreatic differentiation of ES Cells
Dr. Christopher Wylie	R01 HD044764-01	\$225,000	NIH/NICHD	Maternal Control of Actin Assembly in Xenopus Embryos
	R01 HD45737-01	\$356,049	NIH/NICHD	Ectoderm Formation in the Early Xenopus Embryo

* (P.I.: Grabowski, G.A.)

Featured Investigator

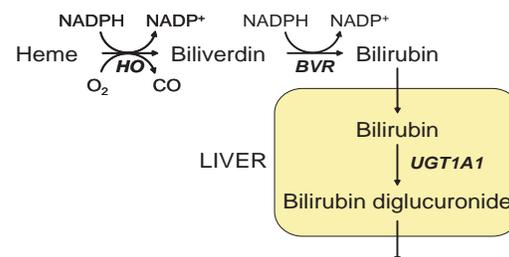


Dr. Stephen D. Zucker, M. D.,
Associate Professor of Medicine
Division of Digestive Diseases,
University of Cincinnati

The DDRDC hosts monthly research seminars on the first Tuesday of each month. In the recent June seminar, Dr. Stephen Zucker, a DDRDC investigator from the University of Cincinnati presented his research on bilirubin. Highlights from his seminar follow.

Bilirubin, the primary end-product of heme catabolism, is a key marker of liver and hematological disorders. As some nonmammalian vertebrates (e.g., birds, reptiles, amphibians) do not synthesize bilirubin, the evolutionary development of the energetically costly process of bilirubin production and elimination suggests that this bile pigment may serve an important physiological function. The inducible isoform of heme oxygenase (HO), the rate-limiting enzyme in bilirubin synthesis, has been shown to play an important role in attenuating tissue injury. In work spearheaded by Dr. Weizheng Wang, now an Assistant Professor at the New Jersey College of Medicine & Dentistry, we examined the hypothesis that bilirubin is a key mediator of HO cytoprotection, employing a rat model of endotoxemia. Bilirubin treatment resulted in improved survival and attenuated liver injury in response to lipopolysaccharide infusion. Serum levels of nitric oxide and TNF- α , key mediators of endotoxemia, and hepatic iNOS expression were significantly lower in bilirubin-treated rodents versus control animals. Both intraperitoneal and local administration of bilirubin was also found to ameliorate hindpaw inflammation induced by the injection of λ -carrageenan. Consistent with in vivo results, bilirubin significantly inhibited iNOS expression and suppressed NO production in LPS-stimulated RAW 264.7 murine macrophages, in the absence of an effect on LPS-mediated activation of NF- κ B or p38 MAPK, consistent with an NF- κ B-independent mechanism of action.

Metabolic Pathway for Bilirubin Synthesis and Degradation



During lymphocyte migration, engagement of Vascular Cell Adhesion Molecule-1 (VCAM-1) triggers the generation of reactive oxygen species (ROS) and activation of matrix metalloproteinases (MMPs), which facilitates endothelial cell retraction. Based on evidence that bilirubin is a potent antioxidant, studies conducted by Pavitra Keshavan, a graduate student in the Pathobiology and Molecular Medicine Program, examined whether this bile pigment inhibits VCAM-1-dependent inflammatory processes. The migration of splenic lymphocytes across confluent monolayers of murine endothelial cells constitutively expressing VCAM-1 (mHEV) was significantly inhibited by incubation with 20 μ M bilirubin (1.2 mg/dL) in a transwell assay, in the absence of any effect on lymphocyte or endothelial cell viability or adhesion. Concomitantly, bilirubin treatment inhibited VCAM-1-stimulated endothelial cell ROS generation, as assessed by confocal microscopy and substantially reduced cellular MMP-2 and MMP-9 activity, as determined by gel zymography. In an ovalbumin-induced mouse asthma model, characterized by VCAM-1-mediated pulmonary eosinophilia, the administration of i.p. bilirubin (30 mg/kg) decreased the total leukocyte count in bronchoalveolar lavage fluid by 60%, through specific inhibition of eosinophil and lymphocyte infiltration. Blood eosinophil counts were increased in bilirubin-treated animals, while serum cytokine levels and VCAM-1 expression in the capillary endothelium were unchanged, suggesting that the observed effects were the result of impaired cellular migration across the pulmonary capillary endothelium. Taken together, these findings support a potential role for bilirubin as an endogenous immunomodulatory agent.

A Multi-Center Grant for the Cincinnati Children's Hospital Rare Liver Diseases Network

Investigators at Cincinnati Children's Hospital has recently been notified by NIH that an award will be made to the Rare Liver Disease Network (RLDN). The RLDN will focus on investigations of genetic causes of intrahepatic cholestasis. This RLDN will be composed of five Clinical Sites within the United States, each with investigators who have extensive clinical care experience, patient populations and investigative programs for these disorders. The program is directed by Ronald Sokol, M.D., Denver Children's Hospital. Participating sites will include the Children's Hospital of Pittsburgh directed by David Perlmutter, M.D., Denver Children's Hospital directed by Ronald Sokol, M.D., Children's Hospital of Philadelphia directed by David Piccoli, M.D., Mt. Sinai Medical Center directed by Benjamin Shneider, M.D., and the Cincinnati Children's Hospital directed by James Heubi, M.D. Each Clinical Site is nationally recognized as a referral center for children with genetic causes of cholestasis, with investigators who maintain active clinical and basic science research programs in liver diseases. A unique aspect of this Network is that each individual clinical site includes investigators whose nationally recognized, NIH-funded research is focused on one of the five cholestatic disorders of the RLDN. Thus, scientific experts for each of these disorders will collaborate in the RLDN across clinical sites to bring the most cutting-edge, novel research strategies for each disorder to this Network. The investigators propose to focus this Network on five of the most important rare genetic liver diseases in childhood which share the common clinical presentation and the pathophysiology of chronic intrahepatic cholestasis of childhood. These five genetic causes of intrahepatic cholestasis are alpha-1-antitrypsin deficiency (α -1AT), Alagille syndrome, progressive familial intrahepatic cholestasis (PFIC), bile acid synthesis and metabolism defects and mitochondrial hepatopathies. The overall goal of the RLDN is to ensure that these important liver diseases, which have received little concentrated clinical and investigative support in the past, are subject to an intensive effort at better categorization and clinical investigation through the development of an integrated longitudinal database and related research and training programs.

The RLDN will develop a longitudinal hypothesis-driven database study of these diseases. The RLDN will also include three Biologic Core Facilities to ensure the highest quality analysis of genetic information directed by Nancy Spinner, Ph.D. at Children's Hospital of Philadelphia, liver histopathology directed by Kevin Bove, M.D. at Cincinnati Children's Hospital, a bile acid biochemistry core directed by Kenneth Setchell, Ph.D. at Cincinnati Children's Hospital for subjects enrolled in this study; an Administrative Core; a Pilot/Demonstration Project program to encourage innovative scientific investigation; a Training Program for new investigators in rare liver diseases; and development of electronic internet-based clinical, educational, histologic and research informational resources for these diseases. The RLDN will utilize a number of resources to ensure recruitment of subjects to the studies proposed, including the Pediatric GI Bulletin Board (internet list serve), websites and newsletters of the support/advocacy foundations, and mailings to pediatric gastroenterologists and hepatologists.

Upcoming Seminars

Conference Date	Presenter	Title	Location
Tues. July 6, 2004	No seminar		
Tues. Aug 3, 2004	Dr. Marc Rothenberg	“Eosinophils and Gastrointestinal Inflammation”	CCHRF: R-3490
Tues. Sept. 7, 2004	Dr. John Cuppoletti	“Translational Physiology: SPI-0211 activates intestinal chloride currents and recombinant CIC-2 chloride channels.”	UC Mont Reid Library, MSB 2461
Tues. Oct. 5, 2004	Dr. James Lessard	"Using the Muscle Actins to Study Function, Disease and Development"	CCHRF: R-3490
Tues. Nov 2, 2004	Dr. Gary E. Shull	"Role of Basolateral Cl/HCO ₃ and Na/H Exchangers in Gastric Acid Secretion"	UC Mont Reid Library, MSB 2461

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Promoting Immunohistochemistry in the DDRDC Integrative Morphology Core

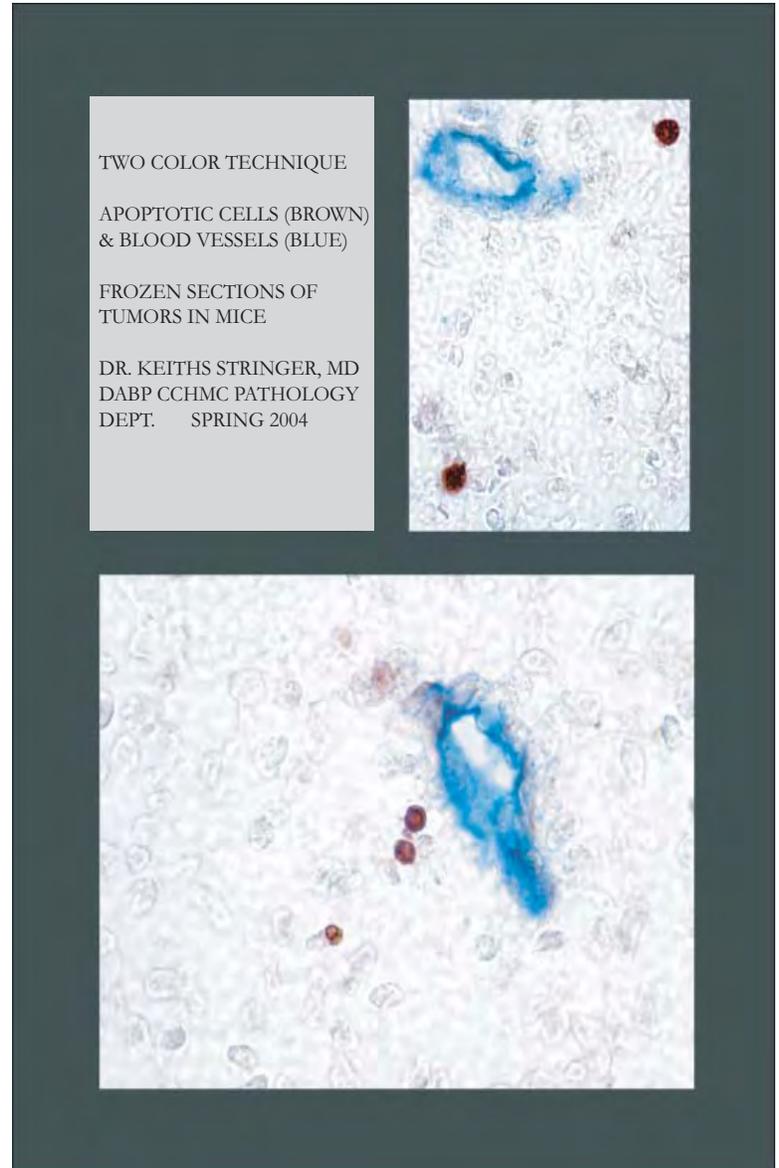
Dr. David Witte and the Integrative Morphology Core are enthusiastic about helping any investigator who is interested in immunohistochemistry.

Immunohistochemistry is the localization of antigens in tissue sections by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme or radioactive element.

Since immunohistochemistry involves specific antigen-antibody reaction, it has apparent advantage over traditionally used special and enzyme staining techniques that identify only a limited number of proteins, enzymes and tissue structures. Therefore, immunohistochemistry has become a crucial technique and widely used in many medical research laboratories

There are numerous immunohistochemistry methods that may be used to localize antigens. The selection of a suitable method should be based on parameters such as the type of specimen under investigation and the degree of sensitivity required.

The Department of Pathology houses an inventory of over 200 antibodies. These antibodies have human and some animal validation. Dr. Keith Stringer and Lisa McMillin are able to assist in immunohistochemical experiment needs. The goal of the Integrative Morphology Core is to expand opportunities for immunohistochemical studies throughout the research community.



Picture of Dr. Witte's team in the Integrative Morphology Core
Front row from the left: Pam Groen, Elain Raptis
Back row from the left: Guang Zhu,
Donna Diorio, Meredith Farmer, Lisa McMillin

Featured Investigator

Dr. Lee (Ted) A. Denson, MD

Assistant Professor - Department of Pediatrics

Division of Gastroenterology, Hepatology & Nutrition, CCHMC

The primary focus of Dr. Lee (Ted) Denson's laboratory is to determine the mechanisms by which the chronic inflammation associated with Inflammatory Bowel Disease (IBD) inhibits normal childhood growth and mucosal healing.

Normal growth and tissue healing are dependent upon an intact growth hormone/Insulin-Like Growth Factor 1 (GH/IGF-1) axis. Inflammation and reduced caloric intake combine to create a state of GH resistance in children with Crohn's disease (CD). Tumor Necrosis Factor α (TNF α) has been implicated in both growth failure and mucosal injury in CD (Figure 1). We have previously determined that TNF α suppresses GH receptor (GHR) gene expression by down regulating Sp1/Sp3 transcription factors and there by inhibits cellular GH signaling. Recent studies have aimed to 1) determine the molecular basis for GH resistance in CD and experimental colitis and 2) investigate potential immunomodulatory effects of GH in this setting.

We have determined that liver and colon GHR expression are reduced in Interleukin 10 (IL-10) null mice with colitis. This is coincident with the onset of growth failure in this murine model of CD. Down regulation of GHR expression has been associated with reduced nuclear abundance and DNA binding of the Sp3 transcription factor. Prior gene deletion studies have demonstrated that the STAT5b transcription factor transduces anabolic effects of GH via up regulation of IGF-1 gene expression. Consistent with an acquired GH resistance, we have determined that GH induced tyrosine phosphorylation of STAT5b and associated binding to the IGF-1 gene promoter cis elements are significantly reduced in mice with colitis at the onset of growth failure. Preliminary patient-based studies have confirmed that serum GH binding protein (a marker for tissue GHR abundance) and IGF-1 are reduced in children with CD and growth failure at diagnosis and that GH activation of STAT5b is reduced in colon biopsies from affected segments. TNF α blockade has up-regulated GHR expression and restored GH activation of STAT5b in experimental colitis; this has then led to improvements in colon histology and weight gain. The DDRDC Integrative Morphology Core has assisted with processing the samples for histological analysis.

Prior experimental and patient-based studies have indicated that GH administration may exert beneficial effects in IBD, despite the relative GH resistance.

See **Denson**, Page 2.

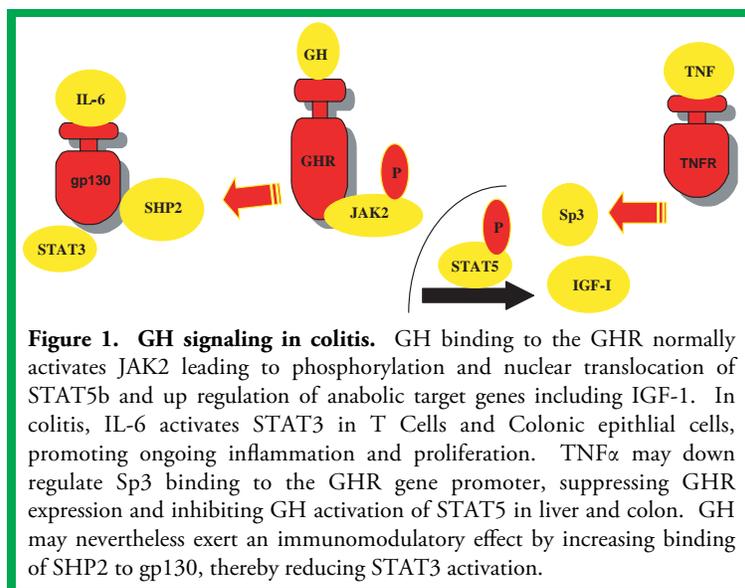


Figure 1. GH signaling in colitis. GH binding to the GHR normally activates JAK2 leading to phosphorylation and nuclear translocation of STAT5b and up regulation of anabolic target genes including IGF-1. In colitis, IL-6 activates STAT3 in T Cells and Colonic epithelial cells, promoting ongoing inflammation and proliferation. TNF α may down regulate Sp3 binding to the GHR gene promoter, suppressing GHR expression and inhibiting GH activation of STAT5 in liver and colon. GH may nevertheless exert an immunomodulatory effect by increasing binding of SHP2 to gp130, thereby reducing STAT3 activation.

Welcome to Two New Investigators

The DDRDC is pleased to welcome two new investigators since Sept. 1, 2004.

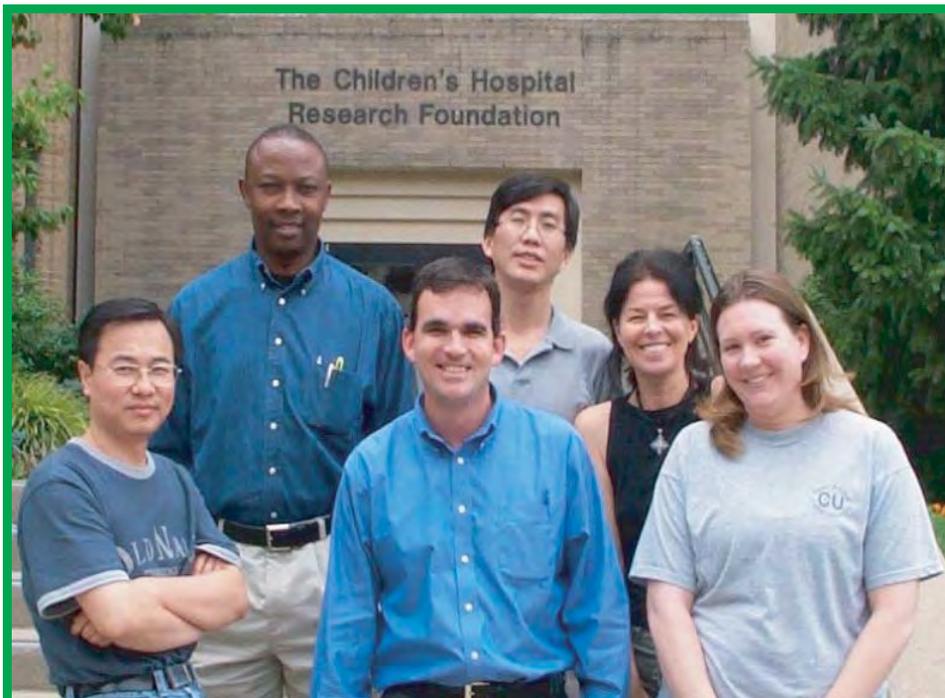
Dr. Jay L. Degen is a Professor of Pediatrics, in the Division of Developmental Biology. The long-term goal of Dr. Degen's laboratory is to define the role of hemostatic system components in innate immunity and inflammatory disease processes.

Dr. Gregory A. Grabowski is a Professor of Pediatrics, and Director of the Division and Program in Human Genetics. His research focus is on the evolution of the pathogenesis in selected lysosomal storage diseases. His studies include gene transfer, purification and characterization of recombinantly produced selectively mutated enzymes, knock-in and knock-out mouse generation, and genome wide studies of transcriptomes and proteomes.

We welcome these two investigators to the DDRDC as we continue to promote opportunities for collaborative digestive disease research in Cincinnati.

Denson: (cont'd)

We have tested the effect of GH upon disease activity and growth in IL-10 null mice with colitis, as well as the direct effect upon CD colon biopsies maintained in short term tissue culture. STAT3 is a transcription factor which has been linked to both chronic inflammation and epithelial proliferation in colitis. We have recently determined that GH administration will reduce STAT3 activation in both colon epithelial cells and lamina propria T cells in murine colitis, leading to an overall reduction in inflammation and epithelial hyperplasia. This was subsequently associated with improvements in colon histology and weight gain. Studies utilizing CD colon biopsies have confirmed that STAT3 is up regulated in affected segments, and that GH treatment significantly reduces this. Taken together, these studies have given new insights into regulation of the GH/IGF-1 axis in IBD. **They have pointed to two potential approaches for optimizing growth and mucosal healing in CD: specific cytokine blockade thereby restoring endogenous GH action, or administration of exogenous GH yielding a direct anti-inflammatory and anabolic effect.**



Picture of Dr. Denson's team

Front row from the left: Xiaonan Han, Dr. Ted Denson, Erin Bonkowski
Back row from the left: Bankole Osuntokun, Jiman He, Danka Sosnowska

New Microarray Discount Policy for DDRDC members

The DDRDC is pleased to announce that it will be able to double the number of discounted microarrays available to members using the Microarray Core in the current fiscal year. It will now be possible to purchase 16 microarrays (per member) with a discount of \$200 per microarray. This makes the cost of the mouse MOE430 version 2 microarray, for example, \$250 instead of the normal \$450. This microarray has over one million features, or hybridization squares, and measures the expression levels of about 34,000 genes.

The Affymetrix Microarray Core is also pleased to announce that we are now offering a new procedure that allow the analysis of gene expression profiles of very small samples. This target amplification system is offered by Nugen, and generates high quality microarray data with as little as five nanograms of total starting RNA. The cost will be the same as for the standard Affymetrix amplification procedure we have offered in the past, \$232 per sample.

Do not mix amplification procedures in a single study. But if you are starting a new study, and at least one sample will provide less than a few micrograms of total RNA, then we strongly suggest that you select the Nugen Ovation amplification system. If, however, there will be at least a few micrograms of total RNA for all samples then we recommend that the standard Amplification protocol is used, as the data quality is slightly better.

The Affymetrix Core has run a series of seven test amplifications using the Nugen Ovation system, all starting with the same high quality adult kidney RNA. When starting with 5-100 nanograms of total RNA we in each case generated sufficient target for microarray hybridization. The resulting microarray hybridization patterns, using the MOE430_2 microarray, were extremely reproducible, with correlation coefficients of 0.99. Background, noise, and Percent genes called expressed were comparable to the standard Affymetrix protocol. The 3'/5' ratios were, however, somewhat higher with the Nugen system.

If you have any questions regarding the Affymetrix System, or would like to use the Microarray core. Please contact **Shawn Smith** at 636-0290.

DDRDC members can also go to the link: <http://www.cincinnatichildrens.org/ddrdc> and use the Microarray Core link on that page to learn more about the facility, or call to learn more about the use of microarray technology and how it may assist in answering the research questions.

Upcoming Seminars

Conference Date	Presenter	Title	Location
Tues. Oct. 5, 2004	Dr. James Lessard	"Using the Muscle Actins to Study Function, Disease and Development."	CCHRF: R-3490
Tues. Nov 2, 2004	Dr. Gary E. Shull	"Role of Basolateral Cl/HCO ₃ and Na/H Exchangers in Gastric Acid Secretion."	UC Mont Reid Library, MSB 2461
Tues. Dec. 7, 2004	Dr. Cong Liu	"Creating a reversible immortalized human hepatocyte line."	CCHRF: R-3490

DDRDC Research Seminars are held on the first Tuesday of every month throughout the year. Seminars will run from 8-9am. If you are interested in presenting a research topic at one of these future meetings, please email **Dr. Jorge Bezerra** (jorge.bezerra@cchmc.org).



Interested in becoming a member?

By becoming a DDRDC member, you will receive discounts on many core resources and services. Your orders also will receive priority.

Full membership is open to all Cincinnati Children's and University of Cincinnati principal investigators involved in digestive disease research. Associate membership is open to junior faculty members who do not yet have independent funding.

If you are interested in joining, email the director at mitchell.cohen@cchmc.org. Further instructions will follow.

For a comprehensive list of current members and the latest information about the DDRDC, visit our website:

<http://www.cincinnatichildrens.org/ddrdc>



POSTER INVITATION

As you may already know, we are planning the Digestive Disease Research Development Center (DDRDC) research forum and external advisory board (EAB) meeting for January 22, 2005. The EAB includes Drs. Allan Walker (Harvard Medical School), Gregory Gores (Mayo Clinic) and Philip Sherman (Hospital for Sick Children), all of whom have agreed to attend.

The goals of this retreat and EAB meeting are to:

- Promote scientific interchange among DDRDC members
- Review the services of the current cores and plan how to better utilize these services
- Plan for our larger center grant (DDRDC) application to be submitted in July 2006.

The retreat program will include a limited number of oral research presentations, presentations by each of the three DDRDC cores, a keynote address, and a poster session. The entire program will be open to DDRDC members and all others at UC and CCHMC who are interested in digestive diseases.

Please plan to present a poster at the retreat. We welcome both new posters and posters that have been presented at local, regional or national meetings anytime in 2004. The poster format will be up to 4' x 8'. In order to present a poster, you must submit an abstract by January 4, 2005. Abstracts should be submitted electronically to: diane.liu@cchmc.org Please see the attached form.

Posters should be related to digestive diseases. Presenters may be DDRDC investigators or members of their laboratories. Undergraduate students, graduate students, postdoctoral fellows, investigators and others working in digestive diseases are welcome to attend the retreat and to submit an abstract for poster presentation even if they are not in the laboratory of a DDRDC member.

Prizes will be given for the best posters presented at the meeting.

- \$400 First Prize
- \$200 Second Prize
- \$100 Third Prize

I look forward to seeing you on January 22, 2005.

Mitchell B. Cohen, MD
Director, Cincinnati DDRDC

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Abstract Submission Form

Cincinnati DDRDC Research Forum

January 22, 2005

(Submit this form electronically by January 4, 2005 to: diane.liu@cchmc.org)

Title:

Authors:

Institution:

Background and Aims:

Methods:

Results:

Conclusions: