

# DISCOVERY EXPLORATION

OPENING NEW DOORS  
FOR CHILDREN



# QUALITY COMMITMENT



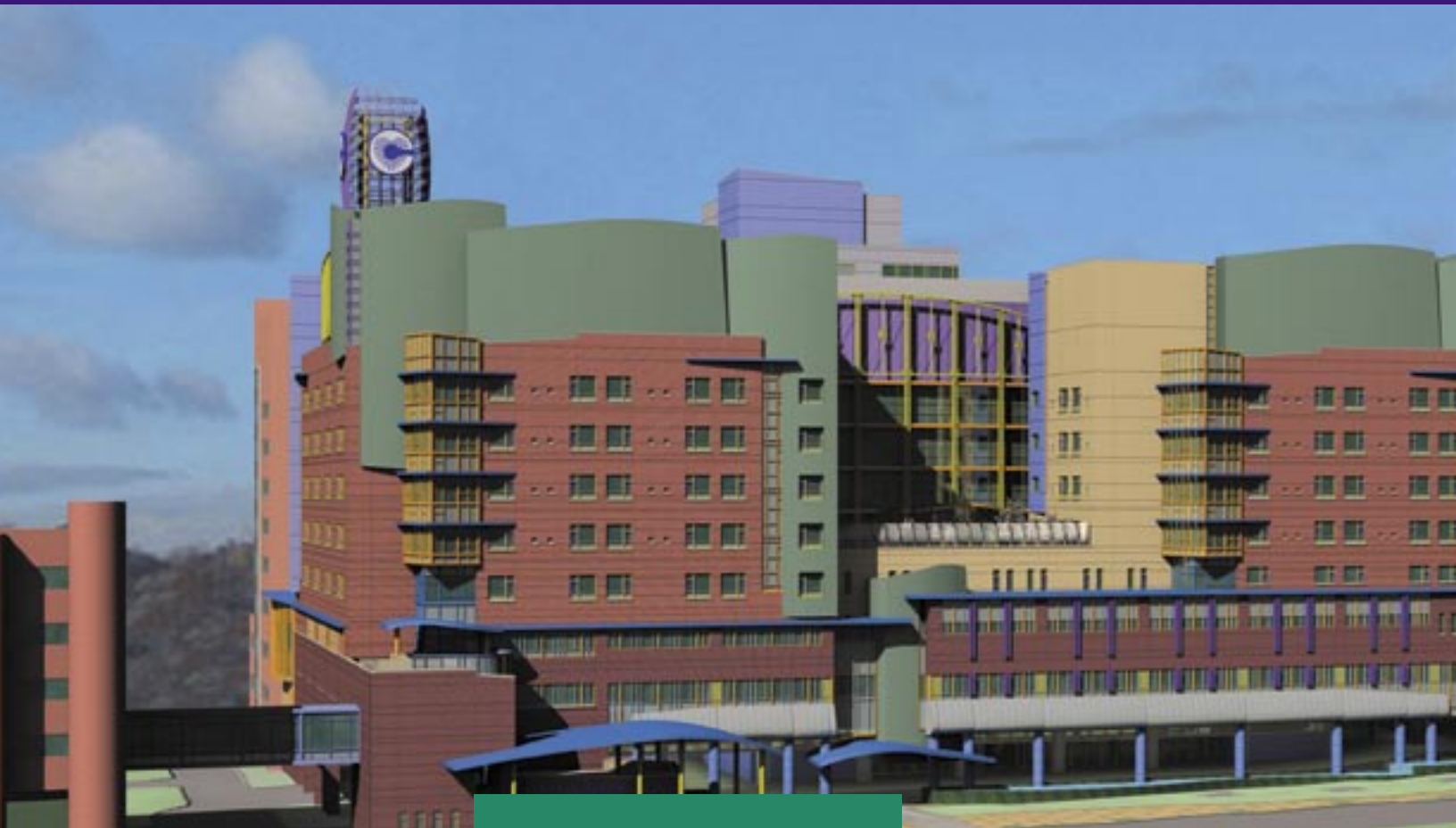


DISCOVERY

EXPLORATION

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*Above: Rendering of the new Children's Hospital of Pittsburgh in the Lawrenceville neighborhood of Pittsburgh, Pa., scheduled to open in 2007.*

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DISCOVERY



## DEAR COLLEAGUE:

At Children's Hospital of Pittsburgh, research and clinical care go hand in hand. We believe the most important discoveries in the laboratory are the result of exploring ideas born from our experience in treating the children who come to us for care.

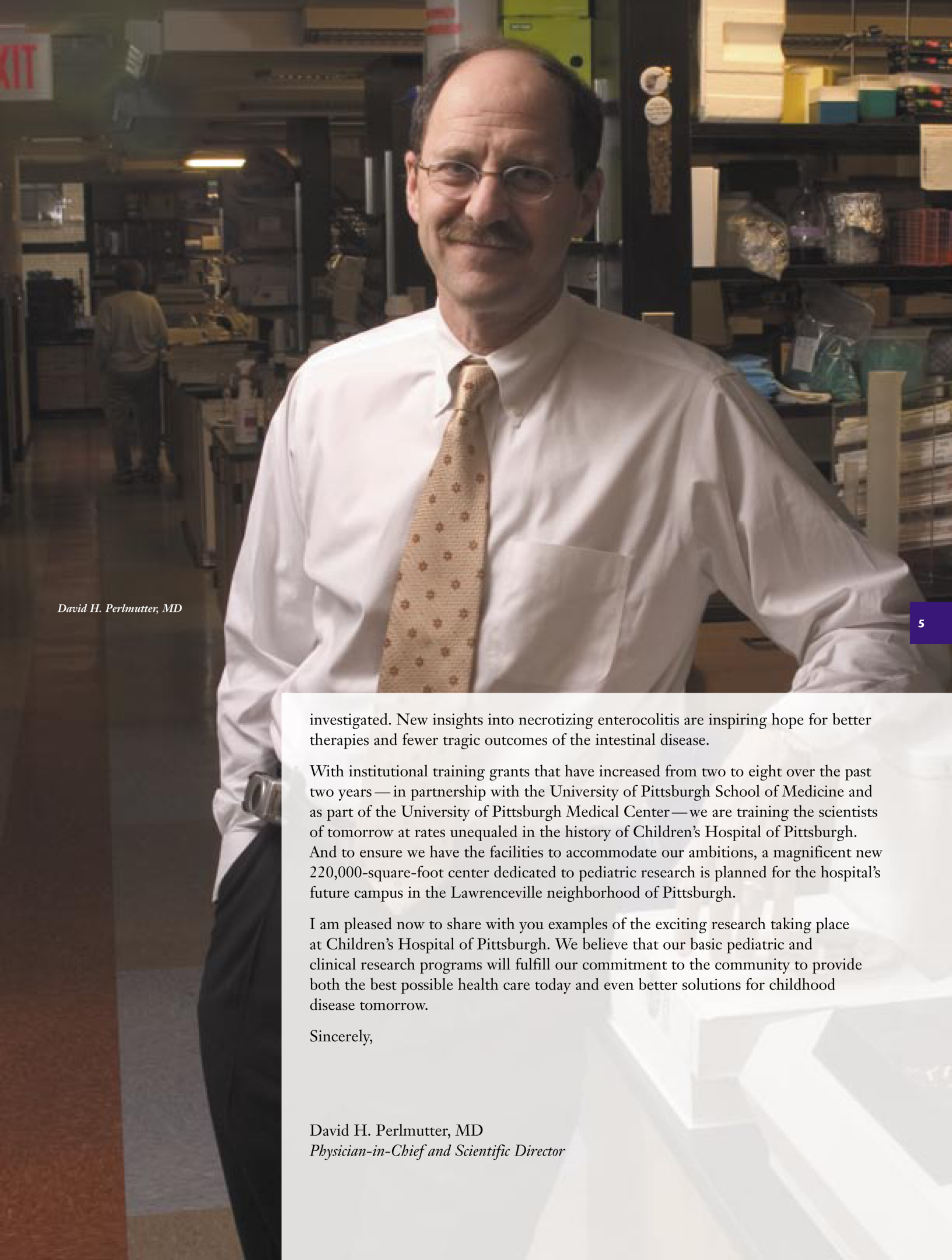
The hospital's contributions to medical science span half a century—from Jonas Salk's historic polio vaccine to advances in immunosuppressant management that allow surgeons to wean pediatric transplant patients from anti-rejection drugs and dramatically improve the quality of their lives. We are expanding our basic research programs to exploit new technologies that enable our scientists to investigate the molecular basis of disease and gain the knowledge necessary to develop new therapies for our patients.

Moreover, we are pursuing dynamic, new studies in world-class translational programs that bridge basic research and clinical care in units already well-established here, such as critical care, otolaryngology and neurosurgery, among others.

In the past two years, our National Institutes of Health funding increased from \$7 million to \$14 million, and our total funding for research reached record levels. Investigator-initiated grants doubled. And leading researchers in stem cell biology, pulmonology, gastroenterology, rheumatology and cardiology were recruited to expand the scope and depth of our work in the lab and to further build our critical mass of the best minds in medical science.

New research programs include a stem cell biology unit investigating the origins of stem cells in the body that might promote healing of damaged or diseased tissues. Gene therapies are being investigated to help fight antibiotic-resistant infections, such as pneumocystis, and for local delivery to joints for treatment of debilitating childhood arthritis. The role of lipases in digestion is being studied to advance therapies for feeding sick newborns and for controlling appetite. How the heart's chambers are formed in the embryo is being studied to develop novel interventions that prevent congenital heart defects.

These new programs complement established research that investigates the resistance of neuroblastoma to chemotherapy as a step toward developing an effective therapy for the childhood cancer. Transplantation of donor hepatocytes is being explored as a way to restore a child's diseased liver without surgery. Still more gene therapies are being developed to restore healthy insulin regulation and cure Type 1 diabetes. The physiological and social factors behind the rise of Type 2 diabetes in children are being



David H. Perlmutter, MD

investigated. New insights into necrotizing enterocolitis are inspiring hope for better therapies and fewer tragic outcomes of the intestinal disease.

With institutional training grants that have increased from two to eight over the past two years — in partnership with the University of Pittsburgh School of Medicine and as part of the University of Pittsburgh Medical Center — we are training the scientists of tomorrow at rates unequalled in the history of Children’s Hospital of Pittsburgh. And to ensure we have the facilities to accommodate our ambitions, a magnificent new 220,000-square-foot center dedicated to pediatric research is planned for the hospital’s future campus in the Lawrenceville neighborhood of Pittsburgh.

I am pleased now to share with you examples of the exciting research taking place at Children’s Hospital of Pittsburgh. We believe that our basic pediatric and clinical research programs will fulfill our commitment to the community to provide both the best possible health care today and even better solutions for childhood disease tomorrow.

Sincerely,

David H. Perlmutter, MD  
*Physician-in-Chief and Scientific Director*

## CARDIOLOGY

### *Learning the secrets of the heart's earliest days to ward off disease*

Cardiologist Bradley B. Keller, MD, and his research team are discovering details in the lab that explain how the heart is formed in the embryo — knowledge that improves the chances of doctors identifying fetuses at risk of heart disease for successful intervention to prevent congenital defects.

In some cases, Dr. Keller, chief of Pediatric Cardiology at Children's Hospital of Pittsburgh, turns to the embryo of a chick to shed light on how the heart functions and how it acquires its normal structure during its earliest days. In his lab, the avian embryo is a tool capable of being modified to allow for the study of specific heart conditions.

A model of hypoplastic left heart syndrome, for example, is created by simply tying a small suture around the developing atrium, which alters how blood flows into the heart and reduces the flow into the left side. "That side of the heart will not grow and that embryo will have hypoplastic left heart syndrome — exactly as we see it in patients," says Dr. Keller, author of *Development of Cardiovascular Systems: Molecules to Organism*. "We can measure the change in anatomy and structure of the heart wall responsible for that."

To get even more from such models, Children's is participating in the development of a new high-resolution imaging system that allows scientists to peer inside the embryo when it is as small as 0.2 millimeters, and measure blood flow.

Using mice as models, Dr. Keller's lab is studying how interactions between the pregnant mother and embryo influence how the heart forms and functions. To do so, an operating room environment was created — replete with anesthesia, surgical techniques and imaging capabilities — to study the mother and embryo simultaneously and observe interactions, such as the effect of low oxygen or how certain medications taken by the mother affect the developing heart.

Researchers in Dr. Keller's lab also are removing heart muscle cells from the embryo and putting them into cultures to understand how the mechanical environment is necessary for them to mature and divide. It is all part of learning more about the heart as a dynamic, moving element during development, when dividing cells are forming the heart while constantly exposed to stretching, twisting and other forces. "If we have a long-term goal of repairing the heart using individual cells — cell transplant, for example — we have to understand their environment," says Dr. Keller, professor, Department of Pediatrics at the University of Pittsburgh School of Medicine. "What triggers those cells to divide? To mature? To function with cells around them?"

Doing such work requires innovative tools. Multidisciplinary teams that include members of the Department of Biomedical Engineering at the University of Pittsburgh, the Robotics Institute at Carnegie Mellon University and several local Pittsburgh biotechnology start-up companies are working with Dr. Keller and his colleagues to develop novel imaging and instrumentation solutions for the repair of congenital cardiovascular defects prior to birth. "While we cannot care for every child with congenital heart disease in Pittsburgh," Dr. Keller says, "we can work to develop novel approaches for use everywhere."

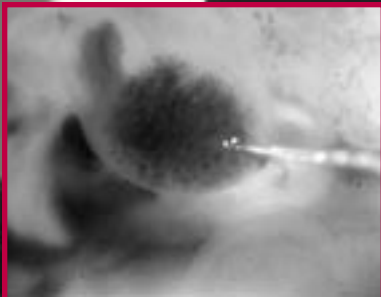
Children's is participating in the development of a new high-resolution imaging system that allows scientists to peer inside the embryo when it is as small as 0.2 millimeters, and measure blood flow.



**The Research Team**

*From left to right:* Kimimasa Tobita, MD; Jennifer Lucitti, PhD; Bradley B. Keller, MD; Joseph Tinney, BA; Li Jun Liu, MD





*Day four chick embryo ventricle instrumented for pressure measurement.*



## ENDOCRINOLOGY

### *Investigating the alarming rise of Type 2 diabetes among American children*



Silva Arslanian, MD

8

Five decades of diabetes research leaves Children's Hospital of Pittsburgh poised as a leading investigator of emerging issues in the field, including the alarming increase in children diagnosed with Type 2 diabetes, a disease once considered exclusive to adults.

The work of Silva Arslanian, MD, and her colleagues is defining characteristics of the disease in children to develop a better understanding of this trend and improve early diagnosis, treatment and prevention. For Dr. Arslanian, director of the General Clinical Research Center, investigating racial differences in Type 2 diabetes and obesity is of particular interest.

In the last decade, the incidence of Type 2 diabetes in U.S. children has increased tenfold. The increase in the childhood cases of the disease parallels a recent rise in childhood obesity and has been the greatest among minority adolescents. "Obesity is pulling the trigger for Type 2 diabetes," says Dr. Arslanian, professor, Department of Pediatrics at the University of Pittsburgh School of Medicine.

Dr. Arslanian and her colleagues are studying the metabolic and environmental factors that may account for the greater prevalence of the disease among African-American youths. Studies

report, for example, that young African-Americans generally have a higher rate of insulin resistance, a greater likelihood of being obese and are more likely than Caucasian youths to have a family history that includes diabetes.

They are exploring approaches to treatment and prevention of Type 2 diabetes and obesity. Children's is one of 12 U.S. centers participating in Treatment Options for Diabetes in Adolescents and Youth (TODAY). The National Institutes of Health (NIH) trial is investigating treatment options in adolescents with Type 2 diabetes, including medications and an intensive behavior-modification program designed to steer them toward a healthier lifestyle.

Children's has applied to NIH to participate in a school-based prevention program to reduce the rate of childhood obesity.

Researchers at Children's also are advancing the understanding of polycystic ovary syndrome, a disorder that affects 5 percent to 10 percent of women of reproductive age and is associated with clinical features that include irregular menstrual periods, hirsutism, acne and obesity. Studies suggest that insulin resistance and high insulin levels are driving the ovarian hormonal abnormality and that awareness of such

conditions is essential to proper diagnosis and treatment. "We have demonstrated that very early in the course of the disease these girls are severely insulin resistant," says Dr. Arslanian. "They are at very high risk of developing Type 2 diabetes."

Children's Hospital of Pittsburgh is a leader in training the next generation of pediatric diabetes researchers. Children's is one of seven U.S. medical centers supported by a major training grant from the National Institute of Diabetes and Digestive and Kidney Diseases to increase the ranks of medical students, postdoctoral fellows and junior faculty pursuing careers in childhood diabetes research.



**Children's is one of seven  
U.S. medical centers  
selected by NIH for  
a novel training grant  
in pediatric diabetes.**

## GASTROENTEROLOGY

*From lipases to liver disease, basic research opens door to new therapies*

At Children's Hospital of Pittsburgh, leading pediatric gastroenterologists are at work advancing potential therapies ranging from better ways to feed chronically ill infants to transplanting hepatocytes as a possible cure for childhood liver disease.



*Mark E. Lowe, MD, PhD*

In his laboratory, Mark E. Lowe, MD, PhD, is investigating how certain enzymes break down dietary fats so they can be absorbed. What he is learning may open the door to new therapies for acute pancreatitis, better ways to feed chronically ill infants, more effective appetite control and other developments.

Dr. Lowe, chief of Pediatric Gastroenterology, was recruited in 2003 from Washington University in St. Louis School of Medicine. He brings with him a National Institutes of Health-funded laboratory investigating the physiology of lipases, enzymes that digest fats, and, more specifically, the roles these proteins play in digestion and disease.

Digestive enzymes normally synthesized in the pancreas have long been suspected of being involved in acute pancreatitis, which, despite medical advances, remains a significant cause of illness and death in the United States. Work is under way in Dr. Lowe's laboratory to provide important details about how the disease develops and causes damage.

Using mice afflicted with the disease, Dr. Lowe, professor, Department of Pediatrics at the University of Pittsburgh School of Medicine, is investigating ways pancreatic lipases contribute to the damage the pancreas incurs during acute pancreatitis.

He also is defining the role of a membrane protein, Itmap 1, in determining the course of the disease. Early evidence suggests the protein is a protective mechanism. "Clearly, the mice missing this protein are more susceptible to pancreatitis," says Dr. Lowe.

Other projects seek a better understanding of the role certain lipases have in dietary fat digestion at all ages. Researchers, for example, are investigating the function of various lipases and procolipase, a pancreatic protein that aids lipases in newborns. Such basic knowledge is needed to improve nutritional therapies for infants with chronic illnesses (such as kidney failure and cystic fibrosis) who have high energy needs that are difficult to satisfy.

*Continued on Page 10*

### The Research Team

*From left to right:*  
Mark E. Lowe,  
MD, PhD;  
Rachel Dub, BS;  
Rita Miller, MS



Lipases also appear to have implications for regulating appetite and weight loss. Dr. Lowe is investigating the role of enterostatin, a peptide released by procolipase in the duodenum. When enterostatin is injected into animals, they tend to decrease their voluntary intake of fat, eat less and lose weight. “We’re very interested in understanding how it works,” says Dr. Lowe. “Our work, so far, has shown that it may play an important role in appetite regulation and in determining the body weight set point.”

Liver disease is under assault in the laboratory of David H. Perlmutter, MD, Children’s physician-in-chief and scientific director. Dr. Perlmutter is recognized internationally as an authority on alpha 1-antitrypsin (AT) deficiency, the most common genetic cause of liver disease in children.

“Part of the work in my lab has to do with understanding how that damage occurs and how the cells and tissues respond in a way to protect themselves,” says Dr. Perlmutter, chair, Department of Pediatrics at the University of Pittsburgh School of Medicine. “By understanding that, we will be able to develop more effective and specific therapies.”

A mutant protein retained in liver cells has been found to trigger the disease. Dr. Perlmutter has identified a class of compounds, called chemical chaperones, which partially correct alpha 1-AT deficiency. One of the chemicals has emerged as an excellent candidate for preventing liver and lung disease in children with alpha 1-AT deficiency and for preventing other diseases caused by mutant proteins, including Alzheimer’s disease.

Another novel strategy being explored at Children’s is the transplantation of donor hepatocytes to treat the disease. This would only require an injection of hepatocytes, eliminating the need for difficult transplant surgery.

Ultimately, this type of cell transplantation therapy could be combined with cells that prevent immune rejection. “We are envisioning developing cell-based therapies for children with liver disease that will not only cure the disease, but eliminate the need for anti-rejection medication,” Dr. Perlmutter says.



David H. Perlmutter, MD

Another novel strategy being explored at Children’s is the transplantation of donor hepatocytes to treat liver disease.



Massimo Trucco, MD, left, with Steve Ringquist, PhD

## IMMUNOGENETICS

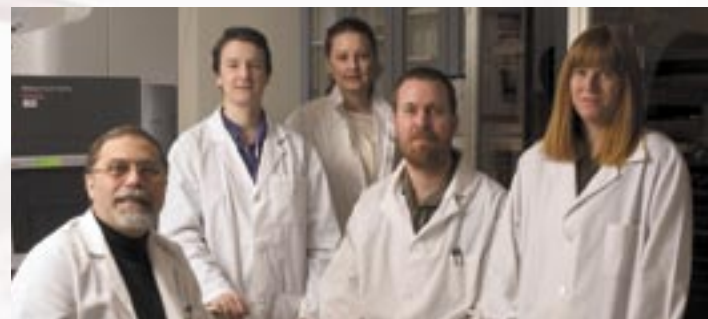
### *Altering immune response and using pig islets to conquer Type 1 diabetes*

To Massimo Trucco, MD, curing Type 1 diabetes is more than a theoretical possibility. It is a goal he and his colleagues draw closer to achieving with each discovery in the laboratory.

Dr. Trucco, head of Immunogenetics at the University of Pittsburgh School of Medicine, is director of the Juvenile Diabetes Foundation Center for Gene Therapy Approaches to Type 1 Diabetes at Children's Hospital of Pittsburgh. In October 2003, Children's and Dr. Trucco were awarded an NIH Center of Excellence grant for the study of autoimmune disorders, making Children's one of only nine centers in the nation selected by the National Institutes of Health to carry out the coordinated laboratory-based assault on these diseases. The five-year grant is intended to expand the study of new immune-based therapies for autoimmune diseases and help move new treatments into the clinic more quickly.

As part of their work at Children's, Dr. Trucco and colleagues are exploring fundamental questions, including scanning the human genome and mapping and comparing genes to see whether some children are genetically predisposed to certain complications of diabetes, such as blindness.

*Continued on Page 12*



### **The Research Team**

*From left to right: Massimo Trucco, MD; Therese Libert, MS; Angela M. Alexander, PhD; Steve Ringquist, PhD; Alex Styche, MS*

## IMMUNOGENETICS Continued from Page 11

At the same time, they are investigating emerging concepts in the field, such as using gene therapies to prevent the immune system from attacking and destroying healthy donor islets that are transplanted as a means of restoring normal insulin production in diabetes patients.

As long as powerful immunosuppressant drugs are necessary to prevent the destruction of transplanted donor islets, children are not likely to be candidates for the therapy. At Children's Hospital, scientists are hoping to eliminate the need for anti-rejection drugs by genetically altering the "gatekeepers" of the immune system — dendritic cells — to prevent them from signaling T-cells and B-cells to attack transplanted islets.

Another limitation of islet transplantation being addressed is the shortage of donor islets to transplant. As many as four donors may be needed to supply a single adult with enough islets to successfully restore insulin production. The supply of donor islets falls far short of helping the population of adults and children with diabetes.

To boost supply, University of Pittsburgh transplant pioneer Thomas E. Starzl, MD, PhD, suggested that Children's Hospital scientists explore using the islets of pigs, which are almost identical to human islets — except for an additional gene, 3-galactosyltransferase. "Scientifically," says Dr. Trucco, professor, Department of Pediatrics at the University of Pittsburgh School of Medicine, "it was challenging enough to say, 'why don't we try to do that?'"

Children's Hospital scientists modified the gene in vitro. And early in 2003, scientists from Children's Hospital and PPL Therapeutics in Scotland — the lab famous for cloning "Dolly" the sheep — produced a genetically engineered pig whose islets are compatible to those in humans. The pig islets have been transplanted successfully in mice; they are now being tested in monkeys. "Someday, this could be an infinite source of islets for human transplant," Dr. Trucco says.



Thomas E. Starzl, MD, PhD

At Children's, scientists are hoping to eliminate the need for anti-rejection drugs by genetically altering the "gatekeepers" of the immune system.



## NEUROLOGY

### *Scientists, new technologies kindle hope in the fight against neuroblastoma*

Advances in molecular technology are drawing scientists closer to being able to effectively treat diseases once considered hopelessly incurable. At Children’s Hospital of Pittsburgh, Nina Felice Schor, MD, PhD, is investigating the behavior of neuroblastoma cells in search of an effective therapy for one of the most common and deadliest childhood cancers.

Neuroblastoma occurs in about one in 100,000 children, about 60 percent of whom already have metastatic disease when the diagnosis is made. Only between 5 percent and 15 percent of those children survive long term.

Among her projects, Dr. Schor, chief of Pediatric Neurology, is working on ways to strip chemotherapy-resistant neuroblastoma cells of their protective shields. Her laboratory and colleagues at McGill University are investigating a group of compounds that block the activity of neurotrophins produced by the tumor cells and that erode the protective qualities of these proteins — leaving the tumor cells vulnerable to conventional chemotherapy.

Dr. Schor, assistant dean for Medical Student Research at the University of Pittsburgh School of Medicine, is also studying how to exploit Bcl-2, a protein that neuroblastoma cells produce in excess that prevents these cells from dying. Her lab has discovered a group of drugs that become more effective at killing cells when Bcl-2 is over-expressed. “You get increased kill of the tumor cell and decreased toxicity of the normal cells because the normal cells don’t make as much of the protein,” Dr. Schor says. “The protein that normally protects the cell becomes a death potentiator.”

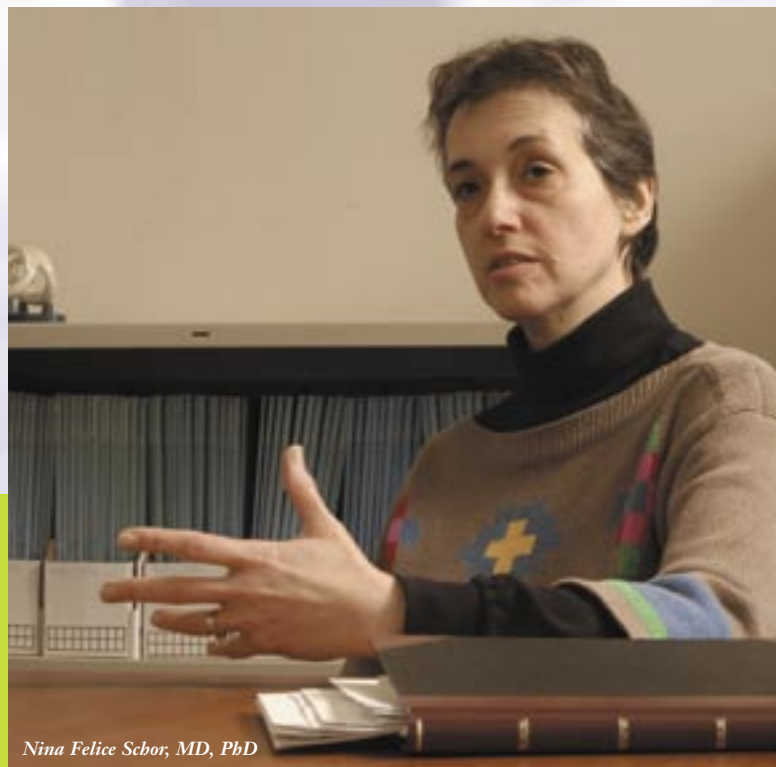
Although they are cancer cells, neuroblastoma cells are more like abnormal, primitive nerve cells. Like nerve cells, they often produce a neurotransmitter, dopamine. This is a characteristic that Dr. Schor is working to exploit, using a chemical analogue of dopamine that is made to be toxic to neuroblastoma cells that take it in. “The notion was to trick the neuroblastoma cells into taking up this dopamine analogue, thinking it was dopamine, but then blowing them apart,” Dr. Schor says.

Her lab also is working on protective compounds that gain entry into normal nerve cells — but not neuroblastoma cells — leaving tumor cells open to destruction while shielding the others.

These experiments unexpectedly led to the discovery of a tool for studying the peripheral neuropathy sometimes associated with Parkinson’s disease.

“It’s an example of how serendipity pulls you in a direction that you would not have anticipated,” says Dr. Schor.

**These experiments unexpectedly led to the discovery of a tool for studying the peripheral neuropathy sometimes associated with Parkinson’s disease.**



*Nina Felice Schor, MD, PhD*

## PEDIATRIC SURGERY

*In the lab, surgeons are looking for ways to treat a devastating intestinal disease*

Children's Hospital of Pittsburgh pediatric surgeons are working in the laboratory to better understand — and someday effectively treat — necrotizing enterocolitis (NEC), a devastating intestinal disease among premature infants that is not surgically treatable.

The team of pediatric surgeons, led by Henri R. Ford, MD, is taking several approaches to addressing NEC, which afflicts up to 20 percent of all premature babies born, and can cause bowel wall rupture, systemic infection and death. Dr. Ford began investigating the disease in 1993 after experiencing the tragic and frustrating outcomes of treating infants with NEC.

“We would perform great operations that should have worked, but the babies still died,” says Dr. Ford, chief of Pediatric Surgery and director of the Benedum Pediatric Trauma Center at Children's.

“We experimented with other surgical approaches, but no matter what we did, the infants seemed predestined to die, succumbing to overwhelming systemic infection. It was clear there was something we were missing, that the operation itself did not address all of the issues that were taking place,” says Dr. Ford, professor, Department of Surgery at the University of Pittsburgh School of Medicine.

NEC is the result of an imbalance between tissue injury and tissue repair mechanisms; that is, accelerated tissue injury occurs at the same time there is a defect in the intestine's ability to repair itself.

Jeffrey S. Upperman, MD, is leading the investigation of why cells die in the lining of the intestines of children with NEC. His work covers a number of possible causes

of enterocyte death, including the role of nitric oxide when it is over-expressed in the intestine.

*Continued on Page 15*



*Henri R. Ford, MD*



*Jeffrey S. Upperman, MD*





## PEDIATRIC SURGERY *Continued from Page 14*

In the laboratory, researchers have found that sustained release of nitric oxide in the intestine may lead to enterocyte death and the ultimate failure of the gut barrier in NEC patients. “Most of our work deals with understanding the steps involved in this destructive process,” says Dr. Upperman, pediatric surgeon at Children’s.

Five specific cytokines involved with inflammation are also being investigated as possible contributors to the death of enterocytes. In addition, Dr. Upperman’s lab is studying whether certain infants are genetically predisposed to NEC.

Enterocyte death during NEC is probably the result of many factors, says Dr. Upperman, assistant professor, Department of Surgery at the University of Pittsburgh School of Medicine. “But one of the factors that has not been thoroughly investigated is genetic predisposition. It is naïve of us to think as scientists that human diseases in general are caused by one gene, one protein, one problem.”

Meanwhile, David J. Hackam, MD, PhD, pediatric surgeon at Children’s Hospital, is focusing on how the intestine tries to heal itself when the enterocytes begin to die and how the healing process can be improved in babies at risk for developing NEC. One of the most important mechanisms involved is enterocyte migration, in which healthy cells near the sites of injury move in and replace the cells that have recently died. Scientists found that the gut barrier is kept healthy and intact by a constant movement of intestinal cells from healthy areas to sites of injury.

Dr. Hackam’s laboratory recently confirmed that the crucial cell migration is impaired in NEC, inviting damage. Endotoxin, a toxin released onto the surface of bacteria, is to blame. “When babies are sick, this toxin rises in the blood,” says Dr. Hackam, assistant professor, Departments of Surgery and Cell Biology and Physiology at the University of Pittsburgh School of Medicine. “We reasoned that the toxin may have something to do with gut barrier failure by altering the ability of the cells to move.”

Dr. Hackam reported that enterocytes sense and internalize the toxin, turning on a switch called Rho-GTPase that stops the intestinal cells in their tracks. As a result, there is no chance that healing will occur. Restoring these switches and preserving the ability of the intestinal cells to move is a possible therapy for protecting the gut barrier from toxins and reversing the development of NEC. Experiments are under way in animals to confirm these early findings. “Now we’re working backwards, finding ways to restore the switch and make cells move again,” Dr. Hackam says.

**Children’s Hospital of Pittsburgh  
pediatric surgeons are  
working in the laboratory on  
ways to prevent devastating  
intestinal damage that occurs  
in premature babies.**



*David J. Hackam, MD, PhD*

The investigation of cytokines is also leading to better understanding of their role in inflammation in the lungs of patients with cystic fibrosis.

## PULMONOLOGY

### *Developing gene therapies and new vaccines to fight lung infections*

Throughout most of the world, lung infections are a major cause of death and illness among children. Although death rates are low in the United States, lung infection is the leading reason children visit doctors. At Children's Hospital of Pittsburgh, the laboratory of Jay K. Kolls, MD, is investigating gene therapies and new vaccines to help doctors around the world win the battle against viral and bacterial infections of the lungs.

To broaden its ability to study lung infection, Children's is recruiting additional research-minded pediatric pulmonologists to join Dr. Kolls. "We are developing a program focused on lung immunology and host defense to address the fact that, worldwide, respiratory infections are the No. 1 killer of children," says Dr. Kolls, chief of Pediatric Pulmonology and the Laboratory of Lung Immunology and Host Defense and professor, Departments of Pediatrics and Molecular Genetics and Biochemistry at the University of Pittsburgh School of Medicine.

In one approach, he is investigating gene-based strategies to improve the immune system's ability to fight infection in the T-cell-depleted setting of HIV infection.

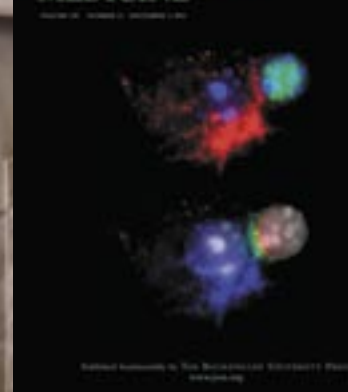
Even when T-cells are depleted, mice are able to protect themselves against infections when given an experimental therapy that takes certain proteins secreted by T-cells — the growth factor, interleukin-17, for example — and reconstitutes them into the system. Such an approach might someday treat infections that do not respond well to antibiotics, such as pneumocystis.

Researchers also are exploring how to overcome obstacles in the development of a vaccine to protect immune-deficient children from infection. Most vaccines require functional T-cells to generate antibodies that protect the child. Dr. Kolls' laboratory, however, developed a vaccine using a molecule that is normally expressed on the T-cell. The molecule, they discovered, stimulates antibody production even when the T-cells themselves are not present. The new therapy is being tested in mice.

The investigation of cytokines is also leading to better understanding of their role in inflammation in the lungs of patients with cystic fibrosis. "We can have an impact once we understand the inflammation process," says Dr. Kolls.

His lab is measuring mediators of inflammation in cystic fibrosis patients, focusing on interleukin-17. "If IL-17 is elevated — and we think it should be — it may be a good target for blocking the inflammatory response," he says.

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Jay K. Kolls, MD

## RHEUMATOLOGY

*Exploring the molecular pathways of arthritis, paving the way to a cure*

As a leading researcher in pediatric rheumatology, Raphael Hirsch, MD, labors in a field desperate for like-minded scientists, and answers to the most fundamental questions about juvenile rheumatoid arthritis (JRA) and other rheumatic diseases. At Children's Hospital of Pittsburgh, he is addressing both — searching for the cause of JRA in the laboratory, while recruiting some of the best researchers and clinicians to help pave the way to more effective therapies.

Dr. Hirsch came to Children's in 2002 as chief of Pediatric Rheumatology with a strong background in research and vitae that included positions at Children's Hospital of Cincinnati and the National Cancer Institute. His NIH-funded laboratory is one of the few dedicated to finding what causes pediatric rheumatic diseases and how to cure them.

To identify the genetic characteristics of the disease, researchers in Dr. Hirsch's lab are using DNA micro arrays, or "DNA chips" to examine the gene profiles of both mice with induced arthritis and JRA patients. Of particular importance is learning which genes are being over-expressed and which are being under-expressed in a particular patient at a specific point in time.

These gene expression profiles will help identify new targets for therapy and might someday help doctors more accurately diagnose JRA, predict outcomes and even determine to which therapies individual patients are most likely to respond. Gene expression analysis has already led to the discovery of several genes that appear to be involved in the destruction of the joint itself.

To improve the safety and effectiveness of new biologic drugs — such as etanercept — researchers are investigating how to deliver gene therapies that dramatically ease symptoms of diseased synovium of children with JRA.

"The concept is that the gene becomes the drug," says Dr. Hirsch, professor, Department of Pediatrics at the University of Pittsburgh School of Medicine. "Synovial cells then incorporate it into their genetic machinery and begin to make the protein locally."

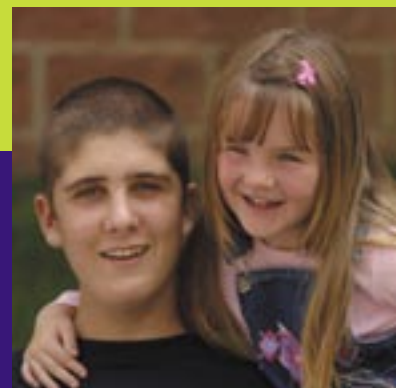
This, in turn, lessens the risk of possible side effects associated with tumor necrotizing factor-blocking drugs, whose long-term effects on the body's tumor surveillance capabilities is unclear.

"Everything we do is really focused on how to apply that understanding toward bettering the outcomes of our patients," says Dr. Hirsch.

Dr. Hirsch's NIH-funded laboratory is one of the few dedicated to finding what causes pediatric rheumatic diseases and how to cure them.



*Raphael Hirsch, MD*



Bruno Peault, PhD



Endothelium-adherent cell clusters within the five-week human embryonic aorta.

## STEM CELL BIOLOGY

*Finding new populations of stem cells and exploring their breathtaking potential*

At Children's Hospital of Pittsburgh, some of the world's leading stem cell researchers are studying questions as basic as where stem cells reside so that someday the promise of using the body's unassigned cells to repair damaged tissue and cure disease will become a reality.

The recent addition of Bruno Peault, PhD, brings the expertise of another internationally recognized stem cell researcher to the Rangos Research Center and provides an opportunity for Pittsburgh-based scientists to collaborate with researchers at Centre National de la Recherche Scientifique in Paris, where Dr. Peault splits his time as research director.

His work focuses on answering the fundamental questions surrounding stem cells. Many of these questions are basic, but necessary if the field is to advance. Among them are questions about where stem cells can be found in the human body, the answer to which might lead to a more readily available source of stem cells than the embryo now offers.

Dr. Peault's work in the laboratory includes developing assay systems to identify and locate populations of stem cells not assigned a specific role — ones that are capable of becoming anything from blood cells to brain cells. Locating these stem cells in adults, rather than embryos, is of particular interest — but a difficult task, given their scarcity.

The fact that unassigned stem cells live within adults is a relatively recent and revolutionary discovery. Developing a full understanding of these rare and difficult-to-observe cells is part of Dr. Peault's mission in the lab. "We try to identify stem cells in the embryo and fetus, and try to extrapolate to the adult patients," says Dr. Peault,

professor, Departments of Pediatrics and Cell Biology and Physiology at the University of Pittsburgh School of Medicine. "We try to understand where they are, what they are and how they function in the early stages of life."

Johnny Huard, PhD, came across muscle-derived stem cells while trying to develop an effective therapy for Duchenne muscular dystrophy (DMD). "I was not looking for stem cells," says Dr. Huard, director of the Growth and Development Laboratory at Children's Hospital, and associate professor, Departments of Orthopaedic Surgery, Molecular Genetics and Biochemistry, and Bioengineering at the University of Pittsburgh. "I was looking for that population of cells that do better in transplantation."

*Continued on Page 19*

### The Research Team

From left to right: Mihaela Crisan, MS; Bruno Peault, PhD; Solomon Yap, MD; Kristen Dering, BA

## STEM CELL BIOLOGY *Continued from Page 18*

The discovery offers promising approaches to developing treatments for the devastating disease, as well as the possibility of a new, more accessible population of stem cells that can become blood cells, bone, cartilage and even scar tissue.

Dr. Huard's work includes determining whether the transplantation of muscle-derived stem cells can be used to effectively treat DMD by delivering dystrophin and improving the structure and function of dystrophic muscle. Long-time proliferating cells with hematopoietic stem cell markers have been identified in the lab, and these cells have significantly improved the efficiency of muscle regeneration and dystrophin delivery to dystrophic muscle in mice.

To help DMD patients who develop progressive cardiomyopathy as a consequence of their disease, Dr. Huard and his colleagues are planning to explore the use of muscle-derived stem cells as a cell source for cardiac cell transplantation to help regenerate the heart muscle.

Another area of research explores ways to speed recovery from muscle injuries by blocking the formation of scar tissue, which typically slows the healing process. "Recently, we asked the question: When you form a scar in muscle, is the scar formed by the stem cells in your muscle or by the blood cells?" says Dr. Huard. "We found that scar tissue is formed by the stem cells if they are in a particular context, such as an injured muscle."

Researchers are developing biological approaches based on the anti-fibrosis agent, decorin, which block molecules involved in scarring and allow more efficient and effective healing of muscle.

"Stem cells offer the potential to restore, rebuild, reconstruct any tissue in the body that has been damaged because of trauma or disease," says Dr. Peault. "However, we are at the beginning of the path. There is a tremendous amount of basic research to be done."

"We try to understand where (stem cells) are, what they are and how they function in the early stages of life," says Dr. Peault.



*Jobmy Huard, PhD*

## OUTSTANDING RESEARCH AT CHILDREN'S HOSPITAL OF PITTSBURGH

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## NATIONAL INSTITUTES OF HEALTH (NIH) RECOGNITION

In fiscal year 2002, Children's Hospital of Pittsburgh ranked seven out of 30 children's hospitals that received NIH awards — with \$14.04 million in research funding.

The University of Pittsburgh School of Medicine ranked 10 out of 125 medical schools receiving NIH grants in fiscal year 2002 — with 600 awards totaling \$226.3 million.





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