Doing Battle with Infectious Disease:
Why Less Might be More
A New Look at Immunity in Newborns
Turning traditional thinking upside-down

Taking Nature by Surprise
A simple amino acid could outwit troublesome bacteria

The Next Frontier in Herpes Simplex Research
‘Boldly going’ for new insights

Learning from Failure
Vaccine trial offers important lessons

Finding the Balance
Reducing antibiotic resistance

Outsmarting a Stealth Virus
Hearing loss offers insights into cytomegalovirus
AWARDS AND APPOINTMENTS

Amal Asa’ad, MD, Allergy and Immunology, received the American College of Allergy, Asthma and Immunology’s Woman in Allergy award for 2013, for her work in food allergy and science.

William Balistreri, MD, Gastroenterology, Hepatology and Nutrition, has received an award named in his honor: the William F. Balistreri Prize for Excellence in Pediatric Gastroenterology, Hepatology, and Nutrition, which will be presented annually at their national meeting.

Mitchell Cohen, MD, Director, Gastroenterology, Hepatology and Nutrition, received the Shwachman Award for life-long scientific contributions to his field.

The Society of Pediatric Psychology has named one of its top research awards in honor of Dennis Drotar, MD, for outstanding performance in teaching and research.

Louis J. Muglia, MD, PhD, Co-Director, Perinatal Institute and Director, Center for Prevention of Preterm Birth, has been elected to membership in the Institute of Medicine (IOM) of the National Academy of Sciences (see newsbrief this issue).

Marc Rothenberg, MD, Director, Allergy and Immunology, has been named a fellow by the American Association for the Advancement of Science (AAAS), for his distinguished contributions to the field of allergy/immunology, particularly in advancing the understanding of eosinophilic disorders and pioneering pediatric research.

Jeffrey A. Towbin, MD, of the Heart Institute, was awarded the 2015 American Heart Association Basic Research Prize for outstanding achievement in basic-cardiovascular science. Towbin has played a major role in advancing pediatric heart transplantation, including initiating the National Institutes of Health-funded Pediatric Cardiomyopathy Registry.

Mitch Cohen, MD, Gastroenterology, Hepatology and Nutrition, received the Association of Academic Physiatrists’ Young Academician Award for outstanding achievement in basic-cardiovascular science. Towbin has played a major role in advancing pediatric heart transplantation, including initiating the National Institutes of Health-funded Pediatric Cardiomyopathy Registry.

Heidi Kalkwarf, PhD, General and Community Pediatrics, was awarded $2.4 million over two years from the Eunice Kennedy Shriver National Institute of Child Health and Human Development over five years to study “Immune Pathways Controlling Cardiac Gene Expression.”

Steve Muething, MD, as recipient of a three-year, $3.3 million grant from the National Institute of Dental and Craniofacial Research (NIDCR) for “Molecular Genetic Analysis of Craniofacial Development.” Jiang also received a five-year, $3 million grant from the NIDCR to study “Molecular Patterning of Mammalian Dentition.”

For research updates by email, sign up at www.cincinnatichildrens.org/email-rh. If you no longer wish to receive this mailing, please send an email to marketing@cchmc.org.
The deadliest thing about sickle cell disease is not just the misshapen red blood cells it produces that cause vascular occlusions. Another threat appears to come from oxidative stress. Recent research shows that sickle cell disease changes the way reactive oxygen species (ROS) interact with the system that regulates blood pressure and fluids in the body, the renin angiotensin system. Over time, the activity of this harmful molecular pathway destroys kidney and heart function.

Five divisions at Cincinnati Children’s are now studying this process. They will use a five-year, $9.1 million grant from the National Heart, Lung and Blood Institute (NHLBI) to create the Cincinnati Center of Excellence in Hemoglobinopathy, one of nine cooperative projects nationwide exploring promising research in hemoglobin disorders.

“We found that the same renin angiotensin system that causes renal damage in diabetes and hypertension also is activated by sickle cell,” says Punam Malik, MD, a researcher in Experimental Hematology and Cancer Biology and principal investigator for the new grant. “More importantly, we found that blocking this signaling pathway in mouse models prevents organ damage when they are transplanted with sickle cell disease.”

Scientists from the Heart Institute, the Cancer and Blood Disease Institute, and the Divisions of Experimental Hematology and Cancer Biology, Hematology, Radiology and Developmental Biology will explore how this signaling pathway can be manipulated in mouse models, and whether a similar pathway exists and can be controlled in humans with sickle cell disease. They also will explore novel non-invasive imaging tools to detect early signs of cardiac damage in people with sickle cell disease.

Researchers here and at the Ulm University Medicine in Germany have found a molecular switch that could be a key to slowing the ravages of aging. Published online in October in Nature, the study builds on the team’s 2012 findings that they could rejuvenate aging hematopoietic stem cells (HSCs) in laboratory mice. HSCs originate in the bone marrow and generate all of the body’s red and white blood cells and platelets. Although it is well established that HSCs become less effective as we age, scientists do not understand how this happens. “This study puts us significantly closer to that goal through novel findings that show a distinct switch in a molecular pathway critical to the aging process,” says Hartmut Geiger, PhD, the study’s senior investigator.

The pathway Geiger refers to is the Wnt signaling pathway, which regulates communications and interactions between cells in animals and people. Geiger and his team observed in animal studies that the protein Wnt5a disrupted normal Wnt signaling patterns in aging cells. When the scientists increased Wnt5a in young HSCs, the increase activated the protein Cdc42 and the cells began to age. Cdc42 is the same protein the scientists targeted in their 2012 study, in which they showed that inhibiting Cdc42 reversed the aging process and rejuvenated HSCs.

In this current study, the researchers deleted Wnt5a from the HSCs of mice, thereby rejuvenating the HSCs and delaying the aging process in blood-forming stem cells. The authors emphasized the need for more research before the findings become therapeutically relevant to people. They hope their work will lead to strategies that will help the elderly boost their immune systems, fight illnesses and enhance overall vitality.

Dr. Hartmut Geiger has identified a process that appears to accelerate the aging process.

Flipping The Switch On Aging

Research From New Center Of Excellence Could Prevent Kidney, Heart Damage

Sickle Cell Grant Scientists

Principal Investigator
Punam Malik, MD.

Co-principal investigators
Jeffrey Towbin, MD, Cardiology
Jay Degen, PhD, Experimental Hematology and Cancer Biology
Charles Quinn, MD, Hematology

Co-investigators
Theodossia Kalfa, MD, PhD, Hematology
Michael Taylor, MD, Cardiology
Steven Potter PhD, Developmental Biology
Robert Fleck, MD, Radiology

Translational Scholars
Omar Niss, MD, Hematology
Paritha Arumugan, PhD, Experimental Hematology

$9.1M Grant Targets Sickle Cell-Related Organ Damage

Dr. Punam Malik will lead a five-year, cross-divisional project focused on organ damage caused by sickle cell disease.
Magnetic resonance elastography (MRE) accurately detects liver scarring in children with chronic liver disease, without the risks and expense of a needle biopsy, according to researchers at Cincinnati Children’s.

The technique has proven especially useful for children who have non-alcoholic fatty liver disease (NAFLD). The disease, which can lead to liver failure and is fueled in large part by obesity, affects an estimated 13 percent of adolescents. A study published online in September in the Journal of Pediatrics shows that MRE effectively and accurately detects the condition.

“Because many pediatric patients with NAFLD are severely obese, MRE is likely to be superior to ultrasound-based elastography in this population,” says gastroenterologist Stavra Xanthakos, MD, lead author of the study.

Ultrasound-based methods are less reliable in severely obese patients.

The researchers evaluated 35 patients aged 4 to 20 for chronic liver disease using both MRE and liver biopsy. They found MRE highly accurate in detecting advanced fibrosis, even in severely obese patients. The technique measures tissue stiffness and takes only a few minutes. If the findings are validated in larger studies, MRE could reduce dependence on needle biopsies, the standard practice for evaluating liver fibrosis.

“Having the ability to easily and non-invasively assess the degree of fibrosis in a child’s liver could help us identify the issue early and determine the right course of treatment in a timely manner,” says Daniel Podberesky, MD, chief of thoracoabdominal imaging at Cincinnati Children’s and a co-author of the study.

Detecting Liver Disease Without Needle Biopsy

Louis Muglia, MD, PhD, Co-Director of the Perinatal Institute and Director of the Center for Prevention of Preterm Birth at Cincinnati Children’s, has been elected to membership in the Institute of Medicine (IOM) of the National Academy of Sciences.

Muglia has pioneered in vivo analyses of regulation of the endocrine stress response and the molecular pathways leading to birth. His laboratory studies the biological process controlling the timing for birth in humans. Among his achievements are more than 175 publications and recognitions that include election to the American Society for Clinical Investigation and Association of American Physicians. In 2010, he was elected to Fellow in the American Association for the Advancement of Science. Muglia is chairman of the Board of Scientific Counselors for the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health.

In May, Muglia was appointed coordinating principal investigator for the $10 million March of Dimes Prematurity Research Center Ohio Collaborative, a research program aimed at finding the unknown causes of premature birth. Muglia joins six Cincinnati Children’s faculty previously elected to the IOM: Uma Kotagal, MD, (2009), Arnold Strauss, MD, (2007), Alan Jobe, MD, PhD, (2007), Jeffrey A. Whitsett, MD, (2003), Thomas Boat, MD, now dean of the University of Cincinnati College of Medicine and Vice President for Health Affairs, (2001), and Margaret Hostetter, MD, (2000).
A protein that regulates blood cell development could provide a way to treat acute myeloid leukemia (AML), according to a study led by researchers in our Division of Experimental Hematology and Cancer Biology.

The findings, posted online in August in the *Journal of Clinical Investigation*, found that the RUNX1 protein plays an unexpected role in supporting the growth of AML.

“RUNX1 is generally considered a tumor suppressor in myeloid neoplasms, but our study found that inhibiting its activity rather than enhancing it could be a promising therapeutic strategy for AMLs driven by fusion proteins,” says James Mulloy, PhD, the study’s lead investigator.

AML develops and progresses rapidly, requiring prompt treatment with chemotherapy, radiation or bone marrow transplant. Treatment can be risky or only partially effective. Mulloy and colleagues are searching for targeted molecular approaches that could be more effective and carry fewer side effects.

They developed a mouse model of AML driven by fusion proteins and the leukemic gene MLL-AF9. When researchers genetically inhibited RUNX1 and an associated protein in the mice, it stopped the development of leukemia cells.

Mulloy says the findings demonstrate that RUNX1 merits further research as a potential target for treatment of AML.

**Blocking Protein May Halt Aggressive Form Of Leukemia**

**Study Identifies Protein Crucial To Healthy Blood Cell Production**

A protein that controls the formation of mature blood cells could play an important role in new treatments for blood diseases.

A study led by researchers from Cincinnati Children’s, published online in October in the *Journal of Experimental Medicine*, clarifies the function of RhoA, a GTPase protein that serves as a molecular switch guiding blood cell formation.

Mutations in the RhoA pathway have been linked to certain immune disorders, including human combined immunodeficiency. Finding ways to control the pathway could lead to improved treatments for blood diseases, immune disorders and cancers.

“We show that RhoA deficiency causes hematopoietic failure in all lines of blood cells and results in defective hematopoietic progenitor cells,” says Yi Zheng, PhD, Director of Experimental Hematology and Cancer Biology and the study’s lead investigator. *“This is important to understanding diseases like pancytopenia, in which people don’t produce enough mature red and white blood cells and platelets.”*

Zheng and colleagues transplanted stem cells from mice bred to lack RhoA into another group of mice. The RhoA-deficient stem cells engrafted long-term, but did not produce new progenitor cells or differentiated blood cells. In another test, the researchers were able to reconstitute RhoA in the cells, which restored the normal function of hematopoietic stem and progenitor cells.

Zheng’s team is now testing prospective small-molecule inhibitors developed at Cincinnati Children’s that could treat disease by blocking abnormal RhoA pathway functions.

Cincinnati Children’s has launched one of the few pediatric lung transplant programs that will perform transplants for infants as small as 11 pounds.

Marc Schecter, MD, formerly at Texas Children’s Hospital, is Medical Director. David Morales, MD, is the Surgical Director.

Currently, only two US hospitals perform more than 10 pediatric lung transplants a year, a goal we plan to reach within three years. The program also will make it possible for the medical center to perform heart-lung and other multiple organ transplants.

Schecter has participated in more than 90 pediatric lung transplants. Morales has been involved in more than 50. Schecter plans to continue research that explores the risk factors affecting transplant outcomes and the impact of transplants on quality of life.

Cincinnati Children’s program for pulmonary disease is one of the nation’s largest and was recently ranked No. 2 in the nation by U.S. News & World Report. The medical center also has extensive experience in pediatric organ transplantation, including more than 520 liver transplants, 278 kidney transplants, 90 heart transplants and 30 intestinal transplants.

**Lung Transplant Program Launches**

**Repairing deficiencies in the RhoA protein could cure many blood disorders, says Dr. Yi Zheng.**

**Targeting the RUNX1 protein could stop the progression of AML, says Dr. James Mulloy.**

**Dr. Marc Schecter will serve as the lung transplant program’s Medical Director.**

**Dr. Yi Zheng**
NEW & NOTEWORTHY

Obesity A Major Factor In Earlier Onset Of Puberty In Girls

Obesity is the largest predictor of early onset puberty in girls, according to a study led by Cincinnati Children’s adolescent medicine specialist Frank Biro, MD. Results of the multi-institutional study were published online in Pediatrics in November. The findings add to a growing body of research documenting the earlier onset of puberty in girls of all races.

“The impact of earlier maturation in girls has important clinical implications involving psychosocial and biologic outcomes,” says Biro. Girls who mature earlier are at risk of lower self-esteem, higher rates of depression, norm-breaking behaviors and lower academic achievement. Early maturation also increases risk of obesity, hypertension and breast, ovarian and endometrial cancer.

Researchers followed 1,239 girls in San Francisco, Cincinnati and New York City from 2004 to 2011. The girls were between 6 to 8 years of age at enrollment and were evaluated at regular intervals using established criteria of pubertal maturation. They found that the age of onset of breast development varied by race, body mass index (obesity), and geographic location. But body mass index was a stronger predictor of earlier puberty than race or ethnicity.

Breast development began in white, non-Hispanic girls at a median age of 9.7 years, earlier than previously reported. Black girls in the study continued to start breast development at a median age of 8.8 years; Hispanic girls, 9.3 years; and Asian girls, 9.7 years. Although the research team is still working to confirm the exact environmental and physiologic factors behind the phenomenon, they conclude the earlier onset of puberty in white girls is likely caused by greater obesity.

Other institutions participating in the study were Kaiser Permanente Division of Research, Oakland, Calif.; Mount Sinai School of Medicine, New York; California Department of Public Health and the University of California at Berkeley and San Francisco; and the University of Cincinnati College of Medicine.

New Director Of Biomedical Informatics

Peter White, PhD, became Director of the Division of Biomedical Informatics at Cincinnati Children’s and the Rieveschl Chair of the newly created Department of Biomedical Informatics at the University of Cincinnati’s College of Medicine, effective February 1, 2014.

White comes to Cincinnati Children’s from the Children’s Hospital of Philadelphia, where he launched the Research Institute’s Center for Biomedical Informatics in 2006. He oversaw that Center’s expansion in genome analysis, translational informatics, application development, mobile health, data reporting and management, and informatics education.

“I’m most excited about the opportunity to further develop biomedical informatics capabilities at Cincinnati Children’s,” White says. “It is apparent that the hospital and the University are well positioned to make better use of research and clinical data for new discoveries and innovative science. Cincinnati Children’s high quality of translational and clinical research, along with exceptional clinical care, provides our new department with many opportunities to improve the lives of children through more thoughtful use of biomedical data.”

White’s research includes identifying the genomic contributors to risk for pediatric disorders that include neuropsychiatric disorders, cardiac defects, and solid tumors. White and his research team have developed methods for extracting, integrating and disseminating biomedical data using novel informatics approaches.

White has an undergraduate degree in biology and received a PhD in Molecular Genetics at Washington University in St. Louis in 1992. He plays a lead informatics role on a number of NIH consortia, including the Newborn Screening Translational Research Network, the NHLBI Bench to Bassinet Program, the Clinical Sequencing and Exploratory Research Consortium, and the Audiology and Genetics Database.

Dr. Frank Biro has found that girls’ early maturation due to obesity is fraught with physical and emotional risks.

Dr. Peter White will hold a dual appointment in biomedical informatics at Cincinnati Children’s and the University of Cincinnati.
Mutations Linked To Rare Deafness Could Damage Other Organs

A molecular process that causes an uncommon form of deafness may put affected individuals at higher risk of damage to the heart, thyroid and salivary glands, according to a multi-national research team led by scientists at Cincinnati Children’s.

The study, posted online in August in the Journal of Clinical Investigation, focused on finding possible treatments for DFNB49 nonsyndromic hearing loss, an inherited condition caused by mutations in the gene TRIC. But the mouse model developed for the research project demonstrated unexpected characteristics that suggest TRIC mutations also can damage cell structures in other organs.

“Our study in mice suggests we should clinically evaluate affected individuals more thoroughly, as they may have other, not very obvious clinical problems,” says Saima Riazuddin, PhD, senior investigator and a scientist in the Division of Otolaryngology-Head and Neck Surgery at Cincinnati Children’s.

The researchers developed a mouse model that cannot produce a critical protein, tricellulin. As expected, the loss of tricellulin disrupted the formation of cochlear hair cells, which resulted in hearing loss. But the researchers also saw damage to other organs.

Riazuddin states that earlier studies of DFNB49 families did not reveal conditions besides hearing loss, but the human families were not assessed to the same extent as were the tricellulin mutant mice.

“We are beginning to understand the broader function of tricellulin,” she says, “and this study will guide us for further follow-up clinical evaluations of affected families.”

Evidence Of Environmental-Genetic Connection To Preterm Birth

Environmental stress, combined with a genetic predisposition, has long been suspected of contributing to preterm birth.

Now, a research team at Cincinnati Children’s has evidence of this gene-environment interaction. In animal studies led by Sudhansu K. Dey, PhD, Director of Reproductive Sciences, the combination resulted in preterm births 100 percent of the time.

“The concept had not been experimentally interrogated,” says Dey. “Our studies in mice provide evidence that when a genetic predisposition is combined with mild inflammation, the rate of preterm birth is profoundly increased, provoking preterm birth in 100 percent of the females.”

Dey added that the molecular signatures observed in their mouse studies were consistent with those in tissue samples from women who had preterm deliveries.

The researchers also found a treatment strategy that appears to prevent preterm delivery. They published their findings in the Journal of Clinical Investigation in August.

Others participating in the study were first author Jeeyeon Cha, an MD/PhD candidate in Dey’s laboratory, and Yasushi Hirota, MD, PhD, of the Department of Obstetrics and Gynecology in the Graduate School of Medicine at the University of Tokyo.

The investigators developed a mouse model of preterm delivery in which they inactivated the Trp53 gene in the uterus. Trp53 encodes a protein that regulates cell growth and replication. The preterm birth rate in the mice went to 50 percent just from the genetic deletion. When they subjected the Trp53-deficient mice to mild inflammation with an endotoxin, the preterm birth rate jumped to 100 percent.

The scientists then designed a treatment combining rapamycin and progesterone, which was effective at preventing preterm birth in the mice, with no apparent adverse effects on maternal or fetal health. This suggests that a combined therapy with low doses of an mTORC1 inhibitor and progesterone may help reduce the incidence of preterm birth in high-risk women.

Mutations in the TRIC gene can cause deafness as well as heart and thyroid problems, says Dr. Saima Riazuddin.
Here in the US, most children born with sickle cell disease can expect to live at least into their 40s or 50s. But in Africa, the vast majority of children born with the disease die before they reach age 5. It does not have to be that way, says Russell Ware, MD, PhD, the new Director of Hematology at Cincinnati Children’s and Executive Co-Director of the Cancer and Blood Diseases Institute. Expanding the use of simple blood tests and inexpensive treatments could give hundreds of thousands of African children a chance to grow up.

Ware joined Cincinnati Children’s in July 2013, bringing with him years of experience working with sickle cell and other blood diseases. Two years ago, he helped launch a screening and treatment program in Angola that has shown promising results. He expects to accelerate that effort with three new projects in 2014.

**Uganda Sickle Surveillance Study (US3)**
Using funds from our Cancer and Blood Diseases Institute, Ware will launch an 18-month mapping study with the Ugandan Ministry of Health. The project will analyze blood sample cards collected from babies born to HIV-infected mothers. This will help identify the distribution of sickle cell throughout Uganda and help launch sickle cell screening programs.

**Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM)**
Ware is co-principal investigator for a two-year, placebo-controlled clinical trial in Uganda to evaluate whether hydroxyurea treatment for sickle cell disease makes children more susceptible to malaria. Up to 200 children will be involved. Ware is working with Chandy John, MD, MS, a malaria expert at the University of Minnesota, and a team in Uganda on this Doris Duke Charitable Foundation-funded project.

**Realizing Effectiveness Across Continents with Hydroxyurea (REACH)**
This Phase I/II clinical trial will involve up to 450 children in Angola, Kenya and the Democratic Republic of Congo to determine safe and effective dose levels of hydroxyurea in regions with high rates of malaria and malnourishment. Ware is working with colleagues at The Hospital for Sick Children in Toronto on this study.

The potential to make a difference against sickle cell in Africa is large because much of the medical care for the disease is not complicated or expensive, Ware says. “Hydroxyurea is taken once a day by mouth, and it costs about $1 a day. Once we get the right dosing and show that it’s safe, I think it will catch on and save many, many lives.”

Results from a recent “challenge” trial led by researchers at Cincinnati Children’s reveal that an investigational norovirus vaccine appears well tolerated and effective against the most common strain of the virus. The study, led by David Bernstein, MD, MA, involved 98 people who drank water dosed with norovirus, 50 of whom received the injected vaccine while 48 received placebo injections.

Among those who received the vaccine, 26 people were infected, the researchers said. In the other group, 29 people were infected. However, just 10 people who were vaccinated developed mild, moderate or severe vomiting and/or diarrhea, compared with 20 people in the placebo group. This means the vaccine achieved a 52 percent efficacy in preventing disease. It was even more effective in preventing severe disease.

Norovirus is a common and highly contagious cause of gastrointestinal illness that causes 200,000 deaths a year worldwide. The challenge trial involved volunteers who agreed to spend five days in a controlled hospital-like setting. The next step will be to test the vaccine in a larger clinical trial under real-world conditions. “Ideally, we would like a vaccine to do a little better than 50 percent symptom reduction, but a vaccine that reduces severe symptoms could save many lives and help keep many more people out of the hospital,” says Bernstein.

75 percent of the world’s children born with sickle cell disease are in Africa, says Dr. Russell Ware, and most die by age 5. He studies aim to get treatment to those children.

**Big Step Forward In Fighting Norovirus**

Results from a recent “challenge” trial led by researchers at Cincinnati Children’s reveal that an investigational norovirus vaccine appears well tolerated and effective against the most common strain of the virus.

The study, led by David Bernstein, MD, MA, involved 98 people who drank water dosed with norovirus, 50 of whom received the injected vaccine while 48 received placebo injections.

Among those who received the vaccine, 26 people were infected, the researchers said. In the other group, 29 people were infected. However, just 10 people who were vaccinated developed mild, moderate or severe vomiting and/or diarrhea, compared with 20 people in the placebo group. This means the vaccine achieved a 52 percent efficacy in preventing disease. It was even more effective in preventing severe disease.

Norovirus is a common and highly contagious cause of gastrointestinal illness that causes 200,000 deaths a year worldwide.

The challenge trial involved volunteers who agreed to spend five days in a controlled hospital-like setting. The next step will be to test the vaccine in a larger clinical trial under real-world conditions. “Ideally, we would like a vaccine to do a little better than 50 percent symptom reduction, but a vaccine that reduces severe symptoms could save many lives and help keep many more people out of the hospital,” says Bernstein.

75 percent of the world’s children born with sickle cell disease are in Africa, says Dr. Russell Ware, and most die by age 5. He studies aim to get treatment to those children.
A New Look at Immunity in Newborn Infants

A newfound role for active suppression

by Nick Miller

No wonder babies cry. These tiny, fragile people arrive abruptly – delivered from the dark and protective environment of their mother’s womb to a big, raw world of light and noise. If this is not intimidating enough, newborns also face a long receiving line of microbes eagerly awaiting a new home. Of course, all of this happens while their immature immune systems are exceptionally vulnerable to various onslaughts from the outside world.

Infants’ vulnerability to infections has been well known for a long time. Now, research from Cincinnati Children’s reported in the journal Nature offers a new explanation for why, and an idea about how we might make babies more resilient to harmful infections.

The study comes from a team of scientists led by Sing Sing Way, MD, PhD, a pediatrician in the Division of Infectious Diseases and Perinatal Institute. Way knows the territory well. He is often called in to help manage infectious complications among newborn babies at Cincinnati Children’s. He also spends considerable time in the laboratory looking for ways to improve immune function in this highly vulnerable population.

A NEW LOOK AT NEONATE IMMUNE RESPONSE

Prior to this study, the prevailing view was that newborns are susceptible to infection during the first few weeks of life because the cells that make up their immune system are immature or underdeveloped. But Way and his colleagues found that cells programmed to allow helpful bacteria to colonize the intestines of newborns also suppress the baby’s immune system – making babies more vulnerable to infection.

“The first few days after birth represent a critical developmental period when a baby’s immune system must adapt and discriminate between friendly microbes and those that may cause more serious infection,” he says.

These include environmental “commensal” microbes that are not present in the womb, but that immediately colonize tissues such as the intestine and skin. Way explains, “Our findings fundamentally change how we look at why neonates are susceptible to infection. They suggest that susceptibility is caused by active immune suppression during this developmental period, as opposed to immaturity of immune cells.”

BLUNTED IMMUNITY IS PART OF THE PLAN

Way says the finding could prompt a major shift in how pediatricians and scientists deal with the threat of neonatal infection – and in particular how researchers go about looking for ways to control or stop it. The study points to cells that suppress immune responsiveness in newborn infants. The suppressive cells in this case are CD71+ erythroid cells, precursors of mature red blood cells.

The researchers found that the cells are highly enriched in newborn mice and humans to prevent an over-reactive immune response as infants adapt to their new, microbe-filled world. Neonatal CD71+ cells express an enzyme called arginase-2, essential for its immune suppression properties. This plays a vital role in infants’ developing intestines by preventing an onslaught of inflammation in response to colonizing bacteria that help digestion and absorption of nutrients.

His research team used a series of laboratory tests in human neonatal cord blood cells and complementary infection studies in newborn mice to show that temporary immune suppression in newborns extends beyond the intestines into other parts of the body. Way and his colleagues started their study because earlier laboratory research showed the degree of compromised immunity in infant mice varies significantly depending on specific experimental conditions. This prompted the authors to hypothesize that there must be another reason for compromised immunity in neonates, other than just immature immune cells.

CELL SWAPPING PROVES THEORY

The scientists transferred immune system cells in bulk from adult mice into newborn mice. They wanted to see if this would boost neonatal immunity when exposed to infection. Instead of enhancing immunity, researchers say the production of protective immune system cytokines among adult cells remained suppressed in newborn mice. Similar results were observed when adult immune cells were mixed with neonatal cells in laboratory cultures.

In a complementary experiment, researchers transferred neonatal immune system cells into adult mice exposed to infection. The neonatal cells produced the protective cytokine TNF-alpha, which helps ramp up the immune system’s protective response against infection.

BLUNTED IMMUNITY RISKS

Way says the benefits of immune suppression by neonatal CD71+ cells are more uniformly advantageous by quenching undesired inflammation with commensal microbial colonization. On a population level, this probably outweighs the threat of infection among individual infants. He stresses the importance of follow-up studies to develop ways to protect newborns from systemic invasive infections, while still allowing CD71+ cells to do their job preventing inflammation in the developing intestine.

The goal is to find a way to help nurture and maintain the right balance of immune stimulation for optimal protection against infection and immune suppression to prevent immune cell-mediated damage. “Our goals are now to try and dissociate the harmful impacts of immune suppression that cause infection susceptibility,” Way says, “from their more universal beneficial roles to avert overwhelming inflammation during the transition from the womb into a more hostile external environment.”

Making babies more resistant to infection is a focus of Dr. Sing Sing Way’s research.
A SURPRISE ATTACK?

In the laboratory, Qualls models infection with mycobacteria, which cause tuberculosis, leprosy, and related illnesses. The deadly respiratory disease associated with active tuberculosis continues to plague us, especially in less developed parts of the world. He thinks boosting the activity of macrophages – white blood cells that act as sentinels for our immune system and our body’s first line of defense – might be the surprise he is looking for.

“The macrophage is a clever cell that is in every tissue of our bodies,” Qualls explains. “It’s continuously on patrol and able to recognize microbes in infected tissue, engulf them, and subsequently kill them. Macrophages exhibit intrinsic antimicrobial functions, yet those functions are enhanced by recruiting and interacting with other cells of the immune system.”

CALLING FOR REINFORCEMENTS

After “phagocytosing,” or enveloping, germs, macrophages depend on an arsenal of microbicidal molecules, including the free radical chemical nitric oxide (NO), to kill the microbes. The macrophages also rely on other immune cells to enhance their germ-killing activity. For instance, NO production does not become fully efficient unless the infected macrophages receive a second signal from interferon-gamma (IFN-γ) – a cytokine produced by T cells and natural killer cells.

Following this activation, macrophages produce a burst of NO to attack germs. After the initial burst, however, NO production declines to protect host tissue. Consequently, the macrophages lose a primary weapon.

GIVING THE BURST A BOOST

What if macrophages had a way to continue producing that burst of NO, at least enough to carry on a vigorous battle until the germs were zapped or additional reinforcements arrived? Qualls and his collaborators have research data suggesting this could boost immunity. Their data also suggest this might eventually help make vaccines and antibiotics – in particular those used to treat mycobacterial diseases – more effective.

The scientists studied cell culture and mouse models to find out what causes NO to deplete so rapidly in macrophages after the initial burst. They identified genes and their related proteins (in the form of amino acids and enzymes) that control this process.

A key player in the regulatory process is the amino acid L-arginine, which is fueled by another amino acid, L-citrulline. The macrophage’s initial burst of NO and other enzyme activity, L-arginine starts to deplete. This slows the production of NO, possibly in an effort to prevent excessive immune response.

Supplementing with amino acids might help fight infection. Qualls and his colleagues hope to prove that supplementing L-citrulline is an easy and inexpensive way to boost immunity in people who need it, especially as an additive therapy to existing vaccines and medicines. If it works for macrophages and mycobacterial disease, the next question they hope to answer is whether this or a separate yet still similar approach might work in other compos-
For two decades, Nancy Sawtell, PhD, has probed the mysteries of the herpes simplex virus (HSV), and the effects of its long-term residence in our bodies. Now, she is taking her research to the next frontier, exploring the effects of deep space radiation on the reactivation of HSV. Sawtell will begin a NASA-funded study this year, together with fellow Cincinnati Children’s researcher Michael Williams, PhD, and Richard Thompson, PhD, of the University of Cincinnati.

Estimates are that as much as 90 percent of the world population is infected with HSV. Once acquired, there is no getting rid of it – there is no treatment, and no cure. After initial infection through oral or mucosal tissue, the virus makes its home in our neurons, where it lives in a mostly latent state for our lifetime.

Sawtell has examined the toll that the lifelong cycle of latency and reactivation takes on our central nervous system.

Dr. Nancy Sawtell’s latest work with HSV will study the risks of space exploration on virus reactivation.
THE “SENSITIVE” VIRUS

The herpes simplex virus (HSV) not only has a feel for when the cell it inhabits is stressed, but also has the ability to restrain its reactivation, says Dr. Nancy Sawtell.

“It is a very aggressive virus. When allowed to replicate freely, it will kill a cell within 18 hours. But it has built-in regulatory mechanisms to make itself able to survive in the delicate environs of the nervous system.”

So reactivation often might affect only one or two neurons.

“Single neurons can reactivate with no symptoms at all,” says Sawtell. “People who get no obvious symptoms are most likely periodically reactivating in their peripheral and central nervous system.”

But even though we may be unaware of them, these episodic flareups take their toll, causing us to unintentionally transmit the virus to others while wreaking slow havoc on our own systems.

“Because this happens over and over again,” Sawtell says. “Not to the same cell, but to different cells. And depending on the immunological genotype-phenotype of the host, it can cause no problems or it can cause serious problems. Because the host responds in an overly abundant way to that insult. So the inflammatory response accrues over time, and you get these lesions.”

NOT YOUR AVERAGE ASSAULT

Astronauts are as likely as the rest of us to be carrying latent HSV infections in their central nervous systems. If a mere fever can trigger a reactivation of the virus here on earth, what effect might travel into space have?

“Astronauts, like most humans, have latent virus in the brain. And they are concerned about deep space radiation exposure to heavy ions and other molecules over prolonged time in space,” says Sawtell. “They know that after a year, every cell in the body will be hit by a heavy ion. They want to understand the effects of radiation in a brain that has the latent virus.”

WHAT CAUSES REACTIVATION

Sawtell and Thompson made a breakthrough discovery several years ago when they identified the culprit responsible for HSV’s exit from latency – the viral protein VP 16. Their discovery, published in PLoS Pathogens in 2009, paved the way for better understanding the workings of HSV.

Since then, Sawtell has examined the toll that the lifelong cycle of latency and reactivation takes on our central nervous system. She already has evidence from animal studies that the inflammation caused by the virus’s reactivation causes lesions in the brain, particularly in genetically predisposed individuals.

WORSENING WITH AGE

Working from an idea proposed by British researcher Ruth Itzhaki, PhD, that HSV’s chronic latency-infection cycle might cause Alzheimer’s disease in certain individuals, Sawtell pursued her own studies in which animals with the genetic variant APOE4 were infected with HSV. She found that more of the virus got into the brains of the animals with the APOE 4 allele. After reactivating the infection periodically, she watched lesions form in the brains of the animals as they aged.

It was the first evidence Sawtell had seen that reactivating the virus actually caused lesions in the brain. “We saw quite remarkable lesions related to repeated reactivation,” she says.

MICE IN SPACE

Now, she will continue her work into HSV’s effects on the central nervous system by exposing mice to simulated deep space radiation. Beginning in January, she will ship mice to the particle accelerator at the Brookhaven National Laboratory in New York.

“The animals will be exposed to heavy ions and protons, then we’ll bring them back here and do long-term studies looking at the combination of repeated stresses to reactivate the virus and behavioral outcomes. We’ll also be imaging the brain and in the end will look at lesions,” Sawtell says.

The mice in the simulation study will be compared to controls of animals exposed to the normal stresses experienced by most humans, to see whether and how deep space exposure accelerates the risk of neurological problems from HSV infection.

Sawtell believes that studying the effects of these simulated deep space assaults will provide new insights into a virus that continues to fascinate scientists and escape our grasp. And she wonders if our inability to fully eradicate it might not be such a bad thing.

“We talk about eliminating the virus, but what we don’t know is, what would that do to the viral biome?” she asks. “These viruses probably provide positive as well as negative effects. Some researchers report that if we didn’t have viral infections we might have more cancer. There are tradeoffs potentially that we don’t understand.”
As vaccine studies go, the Herpevac Trial for Women was a massive undertaking. Fifty medical centers in the U.S. and Canada screened about 31,000 women to find 8,323 who had not been infected with HSV-1 or HSV-2, the two common types of herpes simplex virus. The study ran for eight years.

When initial results came out in late 2010, the data showed that an investigational glycoprotein D adjuvanted vaccine was safe, well-tolerated – and ineffective at protecting women from genital herpes. Results were reported in January 2012 in the *New England Journal of Medicine*.

David Bernstein, MD, MA, Director of the Vaccine and Treatment Evaluation Unit (VTEU) at Cincinnati Children’s, was a co-author of the 2012 study and lead author of a related paper published in February 2013 in the journal *Clinical Infectious Diseases*.

“Although it did protect against HSV-1 infections, we found that this version isn’t worth pursuing,” Bernstein says. “The prevailing thought is that we need something that prevents genital herpes infection or disease whether it’s caused by HSV-1 or HSV-2.”

**MIXED RESULTS NOT GOOD ENOUGH**

In previous trials led at Cincinnati Children’s, the vaccine did not work in men at all and was not effective in women against HSV-2, long considered the more serious health threat. This result was unexpected because the vaccine was based on a protein found on HSV-2. Researchers are not yet sure why the vaccine did not work against HSV-2.

A pessimist might see the Herpevac trial as another failure in a long line of attempts since the 1940s to develop a vaccine against herpes simplex. But Bernstein does not see it that way. “For me, one of the lessons is that this can be done,” he says. “If we can find something that’s effective against HSV-1, then we must be able to find something for HSV-2.”

**MAJOR SHIFT IN EPIDEMIOLOGY**

For many years, herpes simplex disease had two classifications: HSV-1 caused oral “cold sores” and HSV-2 affected the genitals. The medical community worries more about HSV-2 because flare-ups tend to occur more often, making HSV-2 more likely to spread and to harm infants exposed to the virus during birth. Herpes simplex can be lethal to an infant without aggressive medical care.

But data gathered in the Herpevac trial challenged this understanding. As expected, the study found that HSV-1 caused most of the oral infections among study participants. But HSV-1 also was the more frequent cause of genital disease, with interesting differences by race and age, Bernstein and colleagues reported in 2013.

An editorial that appeared with Bernstein’s study linked this change in pattern of disease to changes in sexual behaviors.

“For so many of us, HSV-1 was thought to be only a trivial infection of the mouth or lips,” wrote Richard Whitley, MD, of the University of Alabama at Birmingham. “Now that sexual practices have changed with increased oral-genital sex, it is likely that we can account for the displacement of HSV-2 as the most common cause of initial infection. Because of this changing epidemiology of genital HSV infections, future vaccine trials will need to be rethought.”

**Learning From Failures**

*Herpes simplex vaccine trial provides valuable lessons despite missing the mark*

By Tim Bonfield
WHY THE VACCINE HUNT IS SO DIFFICULT

Herpes simplex infections are forever, which complicates vaccine development.

Some consider infecting people with live attenuated herpes virus, no matter how weak, too risky. Even killed-virus formulations pose a risk.

"Unless you know you have killed every single one, there’s still a chance of an infection that can last a lifetime, and we still don’t know what all the lifetime risks are," Bernstein says.

Now researchers are using various HSV proteins, presented to the immune system in different ways, to trigger an immune response without causing infection.

FUEL FOR FUTURE RESEARCH

Although the Herpevac trial did not produce an effective vaccine, it did produce a first, and an incentive for future clinical trials: researchers found a correlation between higher antibody levels in women and protection from HSV-1. Having a measurable correlate for immunity makes it easier to decide when to go forward with clinical trials, Bernstein says.

Now, researchers are looking for what they need to include in the vaccine to boost the response.

"To be effective against type 2, maybe we just need to trigger twice the amount of antibodies than was protective for type 1," Bernstein says. "Maybe we need other adjuvants that can get more T-cells or other elements of the immune system involved."

NEW VACCINE MAY REDUCE TRANSMISSION

While the search continues for a vaccine that prevents HSV infections from occurring altogether, there may be value in preventing infected people from shedding persistent virus that can infect others.

A formulation called GEN003 appears to do exactly that. After pre-clinical trials conducted by Bernstein and colleagues showed promise, a clinical trial involving 143 people already infected with HSV-2 was conducted. The vaccine significantly reduced the number of days that virus could be found in participants’ genital tracts. Initial findings from the trial were presented in October 2013 at the IntraScience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). The study was led by Anna Wald, MD, MPH, of the University of Washington. Bernstein was second author.

“It wasn’t a home run. It didn’t eliminate shedding, but it did make a big dent and that’s very important," Bernstein says. "Existing antiviral medications have to be taken every day, so adherence is a major issue. But a vaccine, either by itself or in combination with antivirals, could be effective for several years.”

A Common Disease

About one in every four US women has genital herpes, making it one of the nation’s most common infectious diseases.

The herpes simplex virus spreads through sexual or other skin-to-skin contact, and the virus can spread even when the infected person shows no symptoms. Once in the body, HSV migrates to nerve cells and remains there permanently where it can reactivate to cause painful outbreaks.

HSV can cause severe illness in infants born to HSV-infected women, and the virus has been identified as a risk factor for HIV transmission in adults.

Source: National Institute of Allergy and Infectious Diseases (NIAID).
Even as Alexander Fleming accepted the Nobel Prize in 1945 for his discovery of penicillin, he warned of the dangers of misusing antibiotics. In the decades since, we have increased our antibiotic arsenal by the hundreds, saving countless lives. But as Fleming predicted, the misuse of these drugs has given rise to “superbugs” – bacteria resistant to even the most potent treatments.

Mediating the relationship between man and microbe is what infectious disease specialist David Haslam, MD, will focus on in his new role at Cincinnati Children’s. Haslam joined the Division of Infectious Diseases in July to launch an Antimicrobial Stewardship Program at the medical center.

RESISTANT INFECTIONS ARE ON THE RISE

Haslam takes on this role as part of the medical center’s commitment to ensuring the safety and well-being of patients. Like hospitals everywhere, we grapple with infections that have grown more virulent and stubborn.

“The risk of hospital-acquired infection is actually decreasing, but the types of infections are worse,” Haslam says. “The bacteria we are seeing are often resistant to many antibiotics, and in some cases almost all antibiotics.”

It is a problem brought on largely by our overuse of antibiotics – usually, says Haslam, giving a drug that is not targeted well enough, or in a dose that is not appropriate for the infection.

“Our goal is to get patients on the right antibiotic at the right dose for the right amount of time. We believe that by being more focused and rational in our choices, we might decrease antibiotic use and decrease resistance rates.”

AUTOMATION TO THE RESCUE

With our daily inpatient census averaging more than 400 children, keeping tabs on antibiotic dosage in the medical center is no easy task. The Antimicrobial Stewardship Program has recruited a powerful assistant for the job – a software program called VigiLanz. Cincinnati Children’s is the first pediatric hospital in the country to deploy the program.

When fully operational, VigiLanz will gather information from all of the hospital’s electronic medical records, order entries, computerized laboratory and pathology reports, and provide doctors with real-time alerts and recommendations for antibiotic use.
and more. “It monitors in real time everything that’s happened to every patient in the hospital,” Haslam says. “We can look at every positive culture from every sample taken from every patient.”

Haslam scrolls through a computer screen where thousands of lines of entries reveal what each patient is infected with, what drug the child is being given and in what dose. “We have 3,000 positive cultures from patient samples in just the last few months,” he says. The list includes organisms the patients came into the hospital with as well as those they acquired while here, he explains, adding that a major focus of the Antimicrobial Stewardship Program will be on infections acquired while in hospital.

**A PERFECT STORM FOR INFECTION**

“A lot of what we do with and for patients suppresses the immune system and puts them at risk of infection,” Haslam says. “We have the country’s busiest bone marrow transplant unit; we treat many children who have cancer; we have an active operating room. Our patients do very well, but because of these interventions, children are at higher risk of infection.”

And here, as at all other hospitals, the infections children are at risk of acquiring tend to be what Haslam calls “the most nasty bugs,” the ones most resistant to treatment.

**KILLING BUGS SOFTLY**

Through the Antibiotic Stewardship Program, he hopes to decrease exposure to the broad-spectrum “big gun” super antibiotics and instead use more targeted treatments.

“We want to make sure we don’t put patients at risk, of course,” Haslam says. “But we believe we can decrease the potential downside by choosing antibiotics that are likely to be just as effective, but have less risk of increasing antibiotic resistance.”

If a patient is being treated with a drug or dose that is likely to be ineffective or overly broad, Haslam and his team of clinical pharmacists will step in – but gently.

“Some hospitals just say, ‘You can’t use that drug.’ Our approach will be, ‘You might want to consider,’” he says. “We hope physicians will view this as bringing a lot of potential benefits.”

**CLINICAL PHARMACISTS PLAY KEY ROLE**

The hospital’s clinical pharmacists will play a key role in the Stewardship Program. They are the first to know if a drug is not working well and the most knowledgeable about alternatives.

“The pharmacists offer a wider perspective on choice and dosing of drugs. We rely on them a lot,” Haslam says. “We’re fortunate to have clinical pharmacists involved in the care of essentially all admitted patients.”

Haslam is working closely with Joshua Courter, PharmD, to run the Antimicrobial Stewardship Program and implement the Vigi-Lanz system. Because clinical pharmacists are embedded with the hospital’s care teams, says Haslam, they are well-positioned to recommend alternative medications if needed.

Although in its early stages, Haslam is optimistic about the Antimicrobial Stewardship Program’s potential to bring about better outcomes for patients, short and long-term.

“We think there is no downside. We want to show that by narrowing our use of antibiotics, we are not putting our kids at risk and are in fact improving outcomes overall.”
A discovery by two scientists at Cincinnati Children’s could lead to the first vaccine that would protect against both rotavirus and norovirus. The viruses are two of the leading causes of severe diarrhea in children, and responsible for hundreds of thousands of deaths each year in developing nations.

This potential leap in vaccine technology is based on new understanding of the P particle, a microscopic vaccine platform developed at Cincinnati Children’s for use against norovirus by Xi Jason Jiang, PhD, and Ming Tan, PhD, researchers in the Division of Infectious Diseases.

“The P particle is very stable and very flexible. It can tolerate the insertion of larger fragments of other antigens, which makes it an excellent platform for vaccine development,” Jiang says.

Their findings were published in the Journal of Virology in 2011. The particle may also be useful against influenza, hepatitis E and other viral diseases. And it could serve as a drug delivery vector to carry therapy agents straight to targeted cells.

WHAT IS A P PARTICLE?

These man-made nanoparticles include 24 copies of a protruding (hence the ‘P’ in the name) domain found on the norovirus’ outer surface. The protrusions contain viral receptor binding sites, which make P particles especially effective as vaccine against noroviruses. Jiang and Tan have also developed a smaller P particle that includes just 12 copies of the protruding domain.

In a paper published in PLoS One in April 2013, the researchers demonstrated that P particles produced just as powerful an immune response as did a larger virus-like particle (VLP) now in development as a norovirus-only vaccine (see related story, page 28).
**THE ADVANTAGES OF P PARTICLES**

P particles have two major advantages over VLPs: they are easier and cheaper to produce, and they are not damaged when antigens from other viruses are attached to their surface.

“We have identified three major surface loops, plus some minor ones,” Tan says. “We can use these loops to insert foreign antigens of viral pathogens, such as rotavirus, for a dual vaccine. We also could insert a signal peptide that would allow the P particle to target specific tissues.”

The loops are found on each of the 24 P domains of the P particle. When a single antigen or epitope (the part of an antigen that is recognized by the immune system) is inserted into a P domain, it will be duplicated 24 times on the P particle, which increases the potential for inducing a strong immune response.

VLPs are much larger particles with many copies of the viral protein. Utilization of such complicated particles for foreign antigen insertion is technically difficult.

In addition, producing VLPs requires using certain eukaryotic, or membrane-bound, cell factories. This approach can be time-consuming and expensive. P particles can be made by genetically modified E. coli bacteria, a simpler and lower cost process.

“This is particularly important for a low-cost vaccine for developing countries, where they need the vaccine the most,” Tan says.

The technology for producing the first dual vaccine candidate against noroviruses and rotaviruses has been licensed to two pharmaceutical companies: Takeda Vaccine Montana (formerly known as LigoCyte) and PATH Vaccine Solutions, which will take on the next steps of preparing candidate vaccines for human clinical trials. PATH is a non-profit organization based in Seattle. Its efforts to develop a non-replicating rotavirus vaccine are funded primarily by the Bill & Melinda Gates Foundation, which strives to bring vaccines and other health technologies to developing nations.

Cincinnati Children’s is well-known for developing the first live attenuated polio vaccine and one of the first two rotavirus vaccines. “This legacy will be continued by us as well as by many others yet to come,” Jiang says.

**INFLUENZA AND OTHER APPLICATIONS**

Jiang and Tan are working with colleagues at Ohio State University to evaluate an influenza vaccine that uses the P particle platform. This vaccine is targeted for use in livestock, but may also have potential as a human vaccine.

Early stage tests also indicate that combining the P particle with a surface antigen of hepatitis E virus (HEV) significantly increases immune response. Researchers report that other vaccines under development include respiratory syncytial virus (RSV), a major cause of respiratory illness in young children, and polio.

“The P particle is a connector,” Jiang says. “It is a platform that can be used to do many things.”
There are eight herpesviruses that infect humans. Each has its own characteristics and method of transmission; each targets different cell types within the body. All share an astonishing ability to adapt and survive at our expense. Cytomegalovirus (CMV) is human herpesvirus number five. Like the rest of the herpes family, it is sneaky and persistent. The virus moves in quickly and sets up house for a lifetime in the body’s welcoming environment. It causes no obvious problems as long as we are healthy. But for the vulnerable — people with HIV, for instance, or who are undergoing organ or stem cell transplants, or newborns — it can wreak havoc.

Rhonda Cardin, PhD, is a researcher in the Division of Infectious Diseases. Along with her colleagues David Bernstein, MD, Fernando Bravo, MD, and Dan Choo, MD, she studies CMV and its effect on infants who are infected with the virus before or during birth. Human CMV can cross the placenta during pregnancy. “About 44,000 of all live births per year in the U.S. are CMV-positive at birth,” says Cardin. “If a woman has an active infection while pregnant, she can transmit the infection to the fetus.”

Outsmarting A Stealth Virus
Study of hearing loss could offer clues to cytomegalovirus
by Mary Silva

Nearly half of all babies born in the US are infected with CMV, which can infect the cochlea and cause hearing loss later in childhood. Dr. Rhonda Cardin examines how CMV infects the cochlea.
First-time CMV infection during pregnancy can cause problems for a developing fetus and lead to severe complications in newborn infants. “If a woman has previously been infected and her virus reactivates during pregnancy, because she has had an immune response, the infection may not be as severe,” Cardin says. “But a woman who already has built antibodies to one strain of the virus can be infected with a new strain while pregnant and transmit it to her baby.”

THE IMPACT OF CMV
Cardin says about 10 percent of babies born with CMV have symptoms that include neurological deficits, hydrocephaly and hearing loss. The other 90 percent of CMV-positive babies show no symptoms at all – at least not at first. “Around 18 months to 2 years of age, a number of these children can exhibit hearing loss or learning impairment,” says Cardin. “CMV is a leading cause of infection-related hearing loss and mental retardation in children.”

For now, Cardin and her colleagues are focusing their research on CMV’s impact on hearing loss. She says between seven and 25 percent of asymptomatic children born with CMV develop “progressive sensorineural hearing loss.”

CREATING A HUMAN-LIKE MODEL
Cardin’s research team uses guinea pigs, the only small animal model in which CMV crosses the placenta during pregnancy. They infect pregnant guinea pigs with CMV between the second and third trimester and have seen a 90 to 100 percent transmission of the virus to the pups.

“Within 7 days of birth, we can measure virus spread throughout the animals,” Cardin says. “Our latest results show that cochleas are infected in the newborn pups.”

The researchers have also tested the hearing of the pups infected with CMV both in utero and just after birth, and have found delayed onset of hearing loss in both by measuring auditory-evoked brainstem responses, similar to detecting hearing loss in children. “In 50 percent of pups, we see progressive hearing loss. This gives us a model to evaluate how CMV infection in the cochlea leads to hearing loss,” says Cardin. This finding is significant, she says, because researchers also have found CMV in the cochlea of newborn babies born with high levels of CMV infection and in the cochleas of some older children who require cochlear implants.

Cardin hopes to use the animal models to understand what structures and cell types within the cochlea are infected and what leads to the hearing loss.

MEDICINES THAT MODULATE
Cardin and Bernstein are also exploring treatments and vaccines that might limit the severity of CMV transmission and infection of the fetus. They have evaluated one drug, CMX001, which is currently in Phase III human clinical trials.

In animal studies by Choo, use of the antiviral medication ganciclovir also has stopped CMV-related hearing loss when caught early; a clinical study is in the planning stages (see story, next page).

Finding a vaccine that prevents CMV infection altogether is the ultimate goal, but one that has eluded researchers to date. “It’s very difficult to develop vaccines against the herpesviruses,” says Cardin. “These viruses have evolved with their hosts and know all the tricks to maintain themselves or evade the immune response.”
Clinical Trial Hopes To Catch, Halt CMV Hearing Loss Early

“Clinically, CMV-related hearing loss is one of the few hearing loss conditions in kids that you can treat and reverse,” says Daniel Choo, MD. “If you catch it early enough and treat it with antiviral drugs, you can potentially rescue a child’s hearing.”

The key is catching it early. Newborns are not currently tested for CMV. The state of Ohio, however, does require that all newborns have a hearing screen before they leave the hospital. If the screen indicates hearing loss, the babies are referred to an audiologist for a follow-up test.

Choo, Director of Otolaryngology/Head and Neck Surgery, is awaiting IRB approval of a clinical study that could benefit infants seen for this follow up test at Cincinnati Children’s. The audiologist conducting the test would perform simple, non-invasive cheek swabs to test infants for CMV. Those testing positive for CMV could receive antiviral therapy.

Choo is basing the study on his successful NIH-funded pre-clinical trial of the antiviral drug ganciclovir. Because antiviral drugs can have side effects when given systemically, Choo and his fellow researchers administered the drug directly into the ears of guinea pigs with CMV-induced hearing loss. Direct injection avoided the side effects and stopped or reversed hearing loss in the animals.

“Our clinical trial proposes that we will follow an infant who is CMV positive and put the child on an oral antiviral,” Choo says, adding that many children tolerate the medication well. “But if the infant starts to show side effects, we will put a tube in his ear and send him home with antiviral eardrops. The drops go right into the tubes and diffuse into the ear.”

Although early detection and treatment of CMV-related hearing loss offers the best chance of correction, the problem often goes undetected until later in childhood, when the damage has progressed. But even those children can be helped, says Choo.

“Regardless of what causes a child’s hearing loss, we can fit them with hearing aids to compensate. If their hearing is still poor, we can do a cochlear implant. Children do really well with cochlear implants. Somewhere we can always make them hear.”

In Ohio, 450 kids a year are born with nerve-related hearing loss. About half of those are genetic causes, another 30 percent have physical defects in the ear, and the remaining 20 percent are of unknown cause, although Choo suspects that CMV is a significant contributor.
In This Issue

Dialing back on antibacterial warfare
Newborn immunity: not what we thought
Build-a-vaccine with P particles