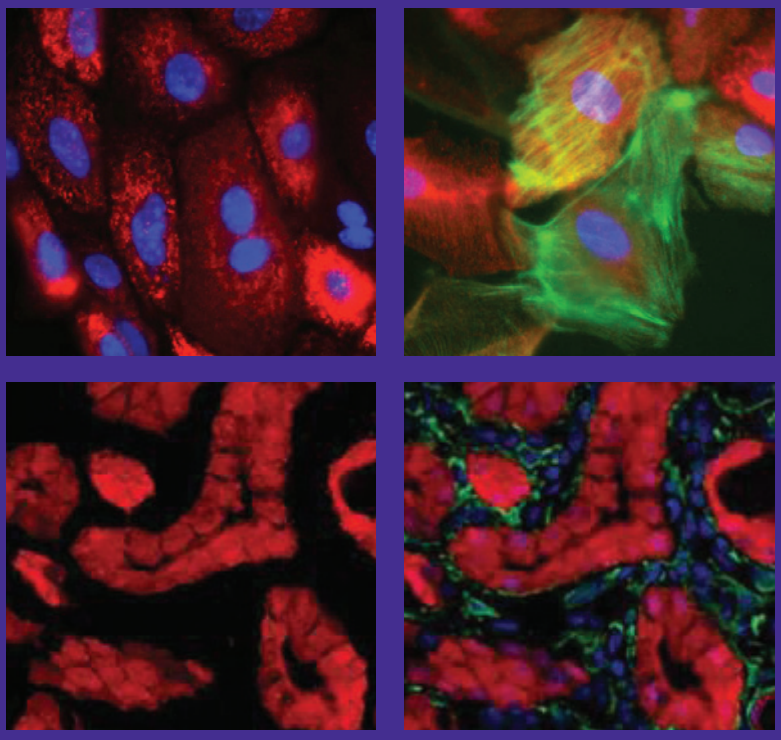

Mapping where cells move and how they act after injury can provide important information about the mechanisms of kidney injury and repair. New work has identified methods to unambiguously mark cells throughout their life. In culture, mouse kidney tubule cells (red, top left) can be transformed into primitive mesenchymal cells (green and yellow, top right). But in the intact mouse, this transformation does not occur, and injury does not cause tubule cells (red) to move out of the tubule and transform into primitive mesenchymal cells (green, bottom left). This research identified novel targets for treatment of kidney injury. For more information on this topic, please see the corresponding research advance in this chapter.

Images provided by Dr. Jeremy S. Duffield and reproduced with permission of the American Society for Investigative Pathology, from Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis, Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, and Duffield, JS, American Journal of Pathology, volume 176, edition number 1, 2010; permission conveyed through Copyright Clearance Center, Inc.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the U.S. They afflict millions of Americans, including children and young adults. The NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The goal is to increase understanding of kidney, urologic, and hematologic diseases to enhance prevention and treatment strategies.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, even for a short period of time or due to gradual deterioration, can result in life-threatening complications. Whether kidney function is lost suddenly or slowly represents an important health challenge.

Chronic kidney disease has two main causes: high blood pressure and diabetes. Recent estimates put the number of Americans with chronic kidney disease at more than 23 million.[1] If unchecked, the recent increases in obesity and type 2 diabetes in the U.S.—especially among children and adolescents—have grave implications, as individuals are likely to face any secondary health consequences at an earlier age than people who develop these conditions as middle-aged adults.

Chronic kidney disease, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. At the close of 2008, nearly 550,000 patients were receiving treatment for ESRD: over 380,000 were undergoing dialysis and over 165,000 were living with a kidney transplant. Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease and ESRD. African Americans are nearly four times more likely to develop kidney failure as non-Hispanic whites. American Indians and Hispanics have twice the risk for kidney failure as do non-Hispanic whites.[2]

The NIDDK supports a significant body of research aimed at understanding the biology underlying chronic kidney disease. The Institute's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease; the underlying mechanisms leading to progression of kidney disease to ESRD; and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. Also of interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome. The Institute's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat chronic kidney disease and prevent kidney failure. It represents a major educational outreach effort to patients, physicians, and the public. In October 2010, NKDEP hosted a meeting titled "Translating Chronic Kidney Disease Research into Improved Clinical Outcomes." It focused on research to identify factors that lead to adoption, maintenance, and sustainability of science-based interventions in real-world clinical settings.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's Urology Program supports basic and clinical research on the normal and abnormal development, structure, and function of the genitourinary tract. Areas of particular interest include the causes of and treatments for major adult urological diseases and disorders, such

[1] *Levey AS, et al: Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the U.S., National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2010.*

as benign prostatic hyperplasia, urinary incontinence and urinary tract infections. Other disorders of the genitourinary tract, such as interstitial cystitis/chronic pelvic pain syndrome in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of NIDDK's urology program. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating, chronic, and painful urologic disorder. IC/PBS affects both men and women, but it is nine times more common in women. NIDDK-supported basic and clinical research is focused on elucidating the causes of IC/PBS, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. The NIDDK's Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network supports studies designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. The goals and approaches of the MAPP Research Network reflect the most current thinking on IC/PBS pathology and involve significant new advancements in how IC/PBS is studied. All efforts are designed to provide insights that can be translated to improve the clinical care of patients with IC/PBS. A prospective epidemiological study in a racially and ethnically diverse sample of men and women, the Boston Area Community Health Survey (BACH), seeks to identify patterns and risk factors for those bothersome symptoms. A similar study, the Olmsted County (Minnesota) Study, is studying lower urinary tract symptoms in men.

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them women.[3,4] Many suffer in silence due to embarrassment and lack of knowledge about options available. The introduction of new surgical procedures has advanced the treatment of urinary incontinence dramatically in the last decade. The NIDDK's Urinary Incontinence Treatment Network recently completed the Trial of Mid-Urethral Slings showing that the two most common mid-urethral sling procedures are similar in their chance of cure for stress urinary incontinence, though each surgery has different risks. Additional information regarding the study can be found later in this chapter.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. The Institute is also keenly interested in the basic biology of stem cells, including adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

The year 2010 marked the 100th anniversary of the first detailed case report of sickle cell disease. In the U.S., between 70,000 and 100,000 people are affected with this disease, mainly individuals of African ancestry. In November 2010, NIH held the "James B. Herrick Symposium—Sickle Cell Disease Care and Research: Past, Present and Future." At the symposium, which was named after the physician who first described the disease, national and international experts in sickle cell care and research discussed the history and societal impact of the disease along with current and future basic, translational and clinical research. The NIDDK Director Dr. Griffin Rodgers, a renowned sickle cell disease researcher, was a featured speaker.

GENETICS OF KIDNEY DISEASE

New Genetic Regions Found To Be Associated with Kidney Function and Chronic Kidney Disease: Researchers have identified 20 new genetic regions in which variants seem to be associated with

[3] Nygaard I, et al: Urinary Incontinence in Women in Urological Diseases in America (pp. 157-191). NIDDK, NIH Publication Number 07-5512, 2007.

[4] Stothers L, et al: Urinary Incontinence in Men in Urological Diseases in America (pp. 193-221). NIDDK, NIH Publication Number 07-5512, 2007.

increased susceptibility to chronic kidney disease. Members of the CKDGen consortium analyzed data from genome-wide association studies of over 67,000 people to try to identify alternate genetic sequences that were correlated with either diagnosed chronic kidney disease or evidence of reduced kidney function. The researchers assessed reduced kidney function based on levels of the molecules creatinine and cystatin c circulating in the blood. These molecules are usually filtered out of the blood by the kidneys; thus, the circulating level of these molecules can be used to estimate kidney function, and their levels tend to rise as kidney function declines.

Once candidate genetic regions were identified in the initial analysis, they were tested again using a second set of samples from nearly 23,000 additional people. This two-step approach identified 13 genetic regions that appear to be related to kidney function and chronic kidney disease, and an additional 7 that are thought to be involved in creatinine production and secretion. The regions identified by these analyses are believed to include genes related to kidney development, filtration, transport of small molecules and salts, and other metabolic functions of the kidneys. The identification of multiple common genetic variants that seem to be associated with various aspects of kidney function and kidney disease furthers scientists' understanding of the basic biology underlying kidney development and function. These findings also may help explain some of the marked variability in the likelihood of developing kidney disease among people with diabetes and high blood pressure, the two most common causes of kidney disease and kidney failure.

Köttgen A, Pattaro C, Böger CA, et al. New loci associated with kidney function and chronic kidney disease. Nat Genet 42: 376-384, 2010.

Gene Variants that May Protect Against Parasitic Disease also Lead to Increased Risk of Kidney Disease: Researchers have found that variants in the *APOL1* gene that are more common in African Americans come with both health benefits and risks. On one hand, they provide protection from African sleeping sickness, but on the other, they confer an increased likelihood of developing kidney disease. In 2008, researchers reported that genetic variations on chromosome 22 were linked to greater incidence of non-diabetic kidney disease among African Americans. Although these variations were at first thought to be related to the *MYH9* gene, researchers have now found that much of the increased risk of kidney disease is due to two specific variations in the adjacent *APOL1* gene. This gene encodes a circulating protein that, in its mutant form, has been shown in experiments to destroy trypanosomes, which are parasites that carry African sleeping sickness, a degenerative and potentially fatal disease affecting tens of thousands of people in sub-Saharan Africa. These two *APOL1* variants appear to have evolved relatively recently—in the past 10,000 years or so. Their relatively recent appearance and frequency in chromosomes in individuals of African descent suggests that the protection these variants provide against parasitic infection is significant.

It is currently unclear what the precise biological function of the protein encoded by this gene is, nor is it clear how these mutations might contribute to kidney disease. Given the high frequency of these *APOL1* variants in people of African descent and their strong effect on kidney disease risk, unraveling the molecular mechanisms by which they contribute to kidney injury could provide important insights into the causes of and possible treatments for kidney disease in African Americans.

Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science 329: 841-845, 2010.

New, Improved Genetic Screening Method Identifies a Novel Genetic Cause of a Rare Form of Kidney Disease: Researchers have recently used a novel, faster genetic screening approach to identify a gene related to the development of a rare but devastating form of kidney disease. Nephronophthisis-related ciliopathies (NPHP-RC) are genetic cystic disorders that manifest in the kidney, eye, brain, and liver and lead to tissue degeneration. Collectively, nephronophthisis is the most common genetic cause of end-stage renal failure in people under age 30. Mutations in nine genes are known to result in NPHP-RC, but these appear to account for less than 1 percent of all cases. The identification of additional genetic contributors to disease has been

hampered by the fact that these diseases are very rare, often appearing in single families, and because standard techniques for identifying candidate genes return too many potential genetic variants to be able to narrow down the possibilities with only a small number of samples.

The researchers combined two different genetic screening approaches to advance their search for disease genes. One of these, called "exome capture," examines just that portion of the genome that codes for proteins. The other approach is based on the knowledge that these rare kidney diseases tend to occur when a person inherits the same gene variant from each parent, such that both of the person's copies of the gene are mutated. With this combined strategy and advanced technology for determining DNA sequences across genomes, researchers identified 12 new mutations in the *SDCCAG8* gene that were associated with NPHP-RC in 10 families. Characterization of the protein encoded by this gene showed that it is associated with the centrioles, barrel-shaped structures within the cell that are involved in cell division as well as in the formation of cilia, which are tiny, hair-like projections on the surface of many cells. Cilia collect information about the cells' environment, and defects in cilia and their signaling properties have been shown to play a role in several diseases. The SDCCAG8 protein was also found to interact with another protein that previously had been shown to be associated with the development of NPHP-RC. Studies in zebrafish—an important vertebrate model organism in scientific research—showed that depletion of the SDCCAG8 protein led to defects in body axis development and the formation of cysts in the kidneys. Moreover, cultured kidney cells in which SDCCAG8 protein had been depleted were unable to form higher-order structures, indicating a defect in cell polarity and tubule formation.

These results strongly suggest that loss of SDCCAG8 function can cause NPHP-RC, possibly by disrupting the ability of the cell to sense its orientation in three-dimensional space. They also validate exome capture in combination with other targeted genetic screening approaches as an experimental strategy for identifying candidate mutations in genetic disease. This approach may help speed the search for the causes of many other single-gene disorders. It may also facilitate the search for agents to treat them, as it would allow the screening of large numbers of compounds that may halt disease initiation or progression.

Otto EA, Hurd TW, Airik R, et al. Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. Nat Genet 42: 840-850, 2010.

SLOWING THE PROGRESSION OF CHRONIC KIDNEY DISEASE

Lower Blood Pressure Goal Benefits African Americans with Chronic Kidney Disease and Protein in the Urine: The latest results from a long-term study show that, on average, a lower blood pressure goal was no better than the standard blood pressure goal at slowing progression of kidney disease among African Americans who had chronic kidney disease (CKD) resulting from high blood pressure. However, the blood pressure goal did benefit patients who had protein in the urine, a sign of kidney damage. This same trial also found that among people with protein in their urine, keeping blood pressure at the lower level reduced the likelihood of kidney disease progression, kidney failure, or death by 27 percent compared to the standard blood pressure level—a statistically significant difference. These results come from the African American Study of Kidney Disease and Hypertension (AASK), the largest and longest study of CKD in African Americans.

In the U.S., high blood pressure causes about one third of new cases of kidney failure. The AASK trial has followed participants for approximately 12 years to measure the long-term effects of blood pressure control in African Americans with kidney disease attributed to high blood pressure. In its initial phase, the AASK study found that a drug that targeted the renin-angiotensin system, specifically an ACE inhibitor, was more effective at slowing the progression of kidney disease in African Americans than other classes of drugs. A subsequent follow-up study found that kidney disease worsened in about one-quarter of study participants in spite of the best available treatment, while another one-third of participants experienced only a slow decline in kidney function, about what is generally observed with aging.

This most recent finding that, in some patients, more intensive control of blood pressure may slow progression of chronic kidney disease adds important new information about which patients with kidney disease may benefit from lowering of blood pressure beyond the standard goal. It may help doctors practice evidence-based, personalized medicine, tailoring the treatment regimen to each patient's unique characteristics. This study also highlights the importance of conducting long-term clinical studies, because without the follow-up study, the benefits of the lower blood pressure goal in a subset of patients with protein in their urine might have been missed.

Appel LJ, Wright JT, Greene T, et al. Intensive blood-pressure control in hypertensive chronic kidney disease. N Engl J Med 363: 918-929, 2010.

KIDNEY FIBROSIS RESEARCH

Identification of the Cellular Source of Scar-Producing Collagen in a Model of Kidney Fibrosis: Scientists have recently pinpointed a type of cell in the kidney that appears to be a source of much of the scar tissue that is seen in some forms of kidney disease. "Fibrosis" is the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to scarring within an organ. It is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis can impair the removal of toxins and excess fluid from the blood, cause irreversible kidney damage and, in extreme cases, lead to kidney failure.

The origin of the collagen that comprises the fibrotic scar in kidney disease has remained a mystery. Many scientists have hypothesized that kidney fibrosis may be mediated, at least in part, by cells that migrate out of the kidney tubule into surrounding tissue and begin secreting collagen. This speculation stems from studies using cells grown in culture dishes in which kidney cells can become collagen-producing cells called myofibroblasts, a phenomenon referred to as "epithelial-to-mesenchymal transformation." To investigate whether this was occurring in whole kidneys, researchers genetically manipulated mice so that specific subtypes of cells in their kidneys contained an easily detectable molecule, or "label." They surgically induced fibrotic kidney disease in these animals and, after about 2 weeks, examined the kidneys for scar formation. Contrary to prevailing theories, the labeled cells in the kidney tubule did not migrate into the surrounding tissue and participate in scar formation. Instead, myofibroblasts that were already present in the kidney seemed to be the source of collagen. Myofibroblasts are derived from pericytes, a type of stem cell that is usually associated with blood vessels.

This study provides strong evidence that kidney tubule cells do not migrate or undergo epithelial-to-mesenchymal transformation in kidney fibrosis. Rather, kidney fibrosis appears to arise through a novel pathway involving pericyte-derived myofibroblasts. A more complete and accurate understanding of collagen deposition and scar formation is a key first step in devising novel therapies aimed at preventing kidney fibrosis. These studies illustrate the value of animal models in providing important insights into biological processes. They also suggest that therapeutic approaches targeting pericytes may prove beneficial in patients with fibrotic kidney disease.

Humphreys BD, Lin S-L, Kobayashi A, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol 176: 85-97, 2010.

Scientists Identify a Potent Inhibitor of Kidney Fibrosis: Researchers have identified the circulating protein serum amyloid P (SAP) as a natural inhibitor of fibrosis during inflammatory injury in the kidney. Using two different models of kidney injury and fibrosis in mice, researchers found that human SAP can potently inhibit fibrosis in this organ. SAP accumulated at sites of injury within the kidney, where it appeared to be associated with injured or dead cells. In the kidney, SAP acts on monocytes and macrophages, specialized white blood cells that are involved in the inflammatory response. SAP suppresses the activity of these inflammatory cells by binding to Fc-gamma receptors on their surfaces. This inhibition of cell activity is dependent on the increased production of the anti-inflammatory protein interleukin-10. SAP has previously been shown to suppress fibrosis in the lung, but through a different mechanism than that seen in the kidney. Taken together, these observations suggest that SAP may have the potential to act as a broad-based anti-fibrotic agent.

The repair of organ and tissue damage is a complex and multistep process, with an initial inflammatory response that attempts to resolve the insult coupled with wound healing and tissue remodeling. Under certain conditions, unrestrained tissue "repair" leads to the excessive deposits of fibrous scar tissue. This process, termed fibrosis, can impair organ function and, left unchecked, lead to organ failure.

This research identifies previously unknown mechanisms of action of SAP in regulating anti-inflammatory activity, and raises the possibility of using SAP or similar agents as a therapy for fibrotic kidney diseases. Subsequent testing in patients who have ongoing fibrotic diseases may determine whether the therapeutic potential seen in these mouse studies can be translated into a novel clinical intervention in patients.

Castaño AP, Lin S-L, Surowy T, et al. Serum amyloid P inhibits fibrosis through Fc-gamma R-dependent monocyte-macrophage regulation in vivo. *Sci Transl Med 1: 5ra13, 2009.*

AUTOIMMUNE KIDNEY DISEASE

New Insights into the Immunology of Goodpasture's Syndrome: Scientists have recently reported new findings on the immunological targets relevant to a debilitating autoimmune disease known as Goodpasture's syndrome. This disease is marked by kidney damage, often leading to kidney failure, and bleeding in the lungs. Previous studies of Goodpasture's syndrome have suggested that the body's immune system mounts a misguided antibody attack that targets the collagen networks found within the kidney. These networks are composed of bundles of rope-like collagen molecules that are linked together in a way that, among other functions, helps provide support for surrounding cells. In Goodpasture's syndrome, it has been postulated that a change in the shape of one of the collagen subunits exposed a normally hidden region of the molecule, triggering the immune response. Indeed, patients with Goodpasture's syndrome have circulating antibodies against a region of one of the collagen strands, but details regarding the actual target region(s) have remained elusive.

The new study identifies two specific anti-collagen antibodies in the kidneys and lungs of patients with Goodpasture's syndrome that react with two distinct sites on the collagen strand. The presence of the antibodies in these two tissues provides strong evidence that they play a role in disease progression. Using this knowledge, researchers may be able to design "decoy" targets for the self-reactive antibodies that could either prevent them from binding collagen molecules in the kidney or otherwise remove them from the circulation. An important issue to be clarified in future studies is determining what events lead to the initial shape change of the collagen molecule and whether this in fact provokes the immune response or whether some other, yet unknown, factor is responsible for both events.

Pedchenko V, Bondar O, Fogo AB, et al. Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. N Engl J Med 363: 343-354, 2010.

KIDNEY REPAIR

New Component of the Kidney Repair Machinery Characterized: Investigators studying a rat model of kidney injury recently identified a new component of the injury repair machinery. This finding has important implications because there is relatively little known about normal repair mechanisms, and new knowledge could provide targets for novel therapeutic strategies.

In normal mice, a protein called Gpnmb is highly expressed in lung, pancreas, and skin, but very little is found in the kidney. Using rodent models of kidney injury in which the blood supply to the kidney is temporarily restricted, scientists discovered that Gpnmb levels markedly increased during the repair phase after blood flow was restored. Gpnmb levels increased 15-fold in kidney tissue, more than 10-fold in an immune cell type called macrophages that were in the kidney, and 3-fold in surviving kidney epithelial cells, which help carry out many kidney functions. Gpnmb levels were higher in regions of the kidney that suffered more severe injury than in areas that were less seriously damaged.

Macrophages—the name means "big eater"—are a specialized type of immune cell that engulf and digest cellular debris and foreign bodies, and this process is an important part of recovery from injury. Recent studies have suggested that injured epithelial cells

might also play an important role in the clearance of debris and dying cells through a similar mechanism during tissue repair. To examine the possible connection between increased Gpnmb expression in both macrophages and kidney epithelial cells, these cell types were microscopically examined for the presence of "apoptotic bodies," the remnants of dead cells. Both macrophages and epithelial cells containing Gpnmb were found to have more apoptotic bodies than their counterparts deficient in Gpnmb.

Kidney tissue repair following experimentally induced ischemia (restriction of blood supply) was next evaluated in normal mice and in mice lacking Gpnmb. Gpnmb-deficient mice were found to have a five-fold increase in the number of dead cells in their kidney tissue compared to control animals, providing evidence that Gpnmb plays an important role in either cell death or in the removal of dead cells by macrophages and epithelial cells. Moreover, in an animal model in which macrophages had been depleted, recovery from injury was delayed compared to animals containing normal levels of macrophages.

Taken together, these results provide strong evidence that Gpnmb levels are increased in the kidney during injury repair, and that it promotes repair by facilitating the degradation of debris by both macrophages and epithelial cells. This finding is notable because these two cell types were not previously known to work collectively in the tissue repair process, information that may contribute to the development of new treatments for kidney injury.

Li B, Castano AP, Hudson TE, et al. The melanoma-associated transmembrane glycoprotein Gpnmb controls trafficking of cellular debris for degradation and is essential for tissue repair. FASEB J 24: 4767-4781, 2010.

UROLOGY RESEARCH

The Role of Bacterial "Capsules" in Urinary Tract Infections: The presence of a particular kind of capsule is required for infectious bacteria to grow and form large masses called intracellular bacterial communities (IBCs) within the urinary tract, according to a recent report. Infections of the urinary tract are common in women—about one-third of all women in the U.S. are diagnosed with a urinary tract infection (UTI) by the time they reach 24 years of age—and many women suffer repeated UTIs. While antibiotic treatments are available, better prevention and treatment strategies are needed. Most UTIs are caused by a common type of *Escherichia coli* (*E. coli*) bacterium. An acute UTI begins when bacteria attach to cells lining the inside of the bladder. This provokes a defense response in the infected individual, including activation of the immune system and sloughing off of bladder cells into the urine in an attempt to rid the body of bacteria. To escape the host defense response, UTI-causing *E. coli* are able to invade cells lining the bladder and form IBCs. After an IBC has grown extensively within an infected bladder cell, the bacterial community exits the infected cell and invades uninfected cells, establishing a cyclical pattern of infectivity.

How are IBCs so effective in evading the host defense response? To address this issue, scientists hypothesized that the K1 capsule that surrounds *E. coli* is involved in the bacteria's ability to form IBCs. Encapsulation is a well-established feature of bacteria that causes disease; for example, the K1 type of capsule has been shown to play a key role in the ability of bacteria to cause meningitis in the rat. Composed of molecules of sialic acid (a complex chain of sugar molecules), the K1 capsule has been shown to inhibit the activity of immune system cells called neutrophils.

In this study, scientists generated *E. coli* strains containing different mutations of the K1 capsule and compared these to normal UTI-causing bacteria to determine whether the capsule plays a role in various stages of UTI disease development in mice. Compared to non-mutated *E. coli*, K1 capsule mutants were found to be less efficient in different stages of disease development, including the formation of IBCs, cell growth, and prevention of neutrophil infiltration into infected bladders. The scientists also found that a specific K1 capsule mutation that results in accumulation of sialic acid within the bacterium has an even more significant negative effect on IBC formation, likely *via* sialic acid's putative role in controlling the levels of various proteins involved in the development of UTIs. By identifying the bacterial capsule as a factor that contributes to IBC formation, the scientists have found targets for potential novel therapeutic interventions to prevent or treat UTIs. As IBCs have

been observed in human bladder infections, these results likely have direct clinical implications.

Anderson GG, Goller CC, Justice S, Hultgren SJ, and Seed PC. Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. Infect Immun 78: 963-975, 2010.

Bladder Control in Women: A recent study reported that two common operations for stress urinary incontinence (SUI) help women achieve similar levels of dryness. SUI is the leakage of small amounts of urine during physical activity, such as coughing, sneezing, and exercising. This condition is commonly treated with surgery that is designed to provide additional support to the bladder neck and urethra during increases in abdominal pressure that occur with these activities. The two most common surgical procedures are called the retropubic sling and the transobturator sling. In both procedures a synthetic mesh material is implanted to act as a hammock, or sling, to support the urethra and prevent leakage. The retropubic sling places the mesh material under the urethra and behind the pelvic bone, while the transobturator sling places the mesh material under the urethra and out through the upper inner thigh or groin area. Although both mid-urethral sling surgeries have been approved by the Food and Drug Administration and have been performed in the U.S. for more than a decade, the overall comparative effectiveness of these clinical procedures was untested.

The NIDDK's Urinary Incontinence Treatment Network conducted the Trial of Mid-Urethral Slings by randomizing 597 women with SUI to receive either retropubic or transobturator sling surgeries, and the outcomes of the surgeries were compared. Twelve months after surgery, women who received the transobturator sling and women who received the retropubic sling had equivalent levels of treatment success: 78 to 81 percent of women achieved dryness as defined by no leakage during a stress test and a 24-hour pad test, and they had no additional treatment for the problem. Participants also completed validated questionnaires and a 3-day voiding (bladder emptying) diary, and reported additional treatment with surgery, behavioral therapy, or drug therapy. Results from the questionnaire showed that 62 percent in the retropubic group and 56 percent in the transobturator group reported they had been cured.

Each type of surgery had different risks and side effects. Serious adverse events were more common in the retropubic group (14 percent), compared to the transobturator group (6 percent). More bladder perforations during surgery and serious voiding problems requiring surgical correction occurred in the retropubic group, while more vaginal perforations during surgery and neurological problems like weakness of the upper leg occurred in the transobturator group. Blood loss during surgery, duration of surgery, and likelihood of post-surgery urinary tract infections were all modestly higher in the retropubic group, compared to the transobturator group.

This rigorous, large-scale trial represents a major milestone in treatment for stress urinary incontinence, an underdiagnosed public health problem affecting millions of American women. Investments in this kind of research enable women and their doctors to weigh more accurately the benefits and risks of available treatment options.

Richter HE, Albo ME, Zyczynski HM, et al. Retropubic versus transobturator midurethral slings for stress incontinence. N Engl J Med 362: 2066-2076, 2010.

Identification of Inhibitors of Crystal Growth in Kidney Stone Disease: Kidney stones are among the most painful—and, unfortunately, common—of urologic disorders. Now, scientists have uncovered new insights into how the stones form and grow, information that may lead to better treatments for a condition that accounts for approximately 3 million visits to health care providers each year.

Kidney stones are crystals, a type of structure in which the atoms or molecules that comprise it are arranged in an orderly, repeating pattern in three dimensions. The most common type of kidney stone is made up of calcium in combination with either oxalate or phosphate. Kidney stones composed of L-cystine—an amino acid that dissolves poorly in urine—while less common, tend to be larger, to recur more frequently, and to be more likely to lead to chronic kidney disease. There currently is no ideal treatment for L-cystine stones; current therapy consists of increased fluid intake to dilute the urine, modulation of salt intake to change the pH of the urine and make stone formation less likely, or sulfur-containing drugs that have unpleasant side effects.

Researchers used a powerful imaging technique called atomic force microscopy to observe, in real time, growth of L-cystine crystals in solution and to measure the rate of this growth. They found that addition of either of two chemically-modified derivatives of L-cystine—either L-cystine dimethylester (L-CDME) or L-cystine methylester (L-CME)—dramatically reduced the growth rate of the L-cystine crystals, with L-CDME appearing particularly effective. Further analysis revealed that this was because binding of the modified L-cystine molecules disrupted the ordered arrangement of the crystal and made it more difficult for additional molecules to join the structure.

Although this study did not address the therapeutic use of L-CDME for the prevention and treatment of kidney stones, the researchers suggest that the concentration of the molecule shown to inhibit crystal growth under experimental conditions is low enough that it might be achievable in people. Future research may show whether L-CDME, or other compounds designed to disrupt crystal formation, would be a viable prevention or treatment for L-cystine stones.

Rimer JD, An Z, Zhu Z, et al. Crystal growth inhibitors for the prevention of L-cystine kidney stones through molecular design. Science 330: 337-341, 2010.

HEMATOLOGY RESEARCH

Improving Cord Blood Stem Cell Transplantation for Patients with Various Blood Diseases: A team of researchers developed the first successful laboratory culture system for increasing or expanding the numbers of cord blood stem cells in order to shorten the time necessary for complete engraftment for bone marrow transplantation. Umbilical cord blood is a source of blood-forming cells used in transplants. However, its utility is restricted due to the relatively small number of stem cells in a unit of cord blood. Because of this limitation, compared with a conventional bone marrow transplant, cord blood transplants take longer to fully repopulate all the different types of blood cells in the body. The longer timeframe for engraftment places the patient at increased risk of acquiring life-threatening infections, owing to the inadequate number of white blood cells. For this reason, cord blood is used more often in patients with a small body size, for example children, as they require fewer cells. Patients with larger bodies may have to be transplanted with two or more units of cord blood and still may contend with engraftment times longer than conventional bone marrow transplant.

The investigators took advantage of their knowledge of the "Notch" signaling pathway which stimulates expansion (cell division) of stem cells. A protein was engineered in the laboratory that activates the Notch pathway. The protein was used to stimulate expansion of cord blood stem cells in culture. The presence of the protein resulted in a greater than 100-fold increase in cultured cord blood stem cells compared with cells grown in the absence of the protein.

The researchers then conducted a pilot study of 10 patients with leukemia to begin to assess the safety of infusing cord blood stem cells that had been expanded in the laboratory with this Notch-mediated procedure and to perform an initial evaluation of the engraftment properties of the expanded stem cells. Each patient received two units of cord blood—one unit of non-expanded blood and one containing expanded blood cells that had been expanded with this procedure or two units of non-expanded blood. In this small group of patients, the investigators reported that they did not encounter safety issues. The median time for engraftment using the expanded cells was 16 days versus 26 days when non-expanded units of cord blood were used.

The study's intriguing results suggest that engraftments derived from expanded cells may proceed more rapidly than those derived using conventional (non-expanded) cord blood. These results need to be followed up by a larger study in order to develop statistically significant results.

Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, and Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat Med 16: 232-236, 2010.

Potential New Strategy To Improve Bone Marrow Transplant Success Rate: Scientists have recently reported a translational research finding that may improve outcomes for patients undergoing bone marrow transplantation. Currently, granulocyte

colony stimulating factor (G-CSF) is used to mobilize hematopoietic stem cells (HSCs) from the bone marrow into the bloodstream where it is collected for use in bone marrow transplantation. Unfortunately, in up to 10 percent of donors, this procedure does not mobilize sufficient numbers of HSCs from the bone marrow, precluding self (autologous) transplantation in those donors, or at the very least delaying the recovery time from the procedure. Thus, additional approaches are needed to increase the number of HSCs in the circulating peripheral blood.

Using genetic and pharmacologic approaches in mice, researchers have discovered that the activation of cell-surface "epidermal growth factor receptor" (EGFR)—a protein that spans the cell membrane and transduces signals from outside the cell to inside it—inhibits the ability of G-CSF to mobilize HSCs. EGFR and the factors that bind to it are well-known cell signaling molecules involved in diverse cellular functions, including cell proliferation, differentiation, motility, and survival, and in tissue development. Mice carrying a mutation in their *EGFR* gene that results in diminished receptor activity showed greater HSC mobilization in response to G-CSF stimulation. Similarly, a pharmacologic inhibitor of EGFR activity, called erlotinib, increased G-CSF's ability to mobilize HSCs by three- to seven-fold in normal mice, depending on the dose of the EGFR inhibitor. Importantly, in mice lacking the *G-CSF* gene, erlotinib had no effect.

This pre-clinical study reveals a previously unknown role of EGFR in HSC mobilization and provides a new approach for improving mobilization through EGFR inhibition. Additional research will be necessary to evaluate this approach to improving bone marrow transplantation prior to its use in people, but it points toward a new pharmacologic means of improving transplantation outcomes.

Ryan MA, Nattamai KJ, Xing E, et al. Pharmacological inhibition of EGFR signaling enhances G-CSF–induced hematopoietic stem cell mobilization. Nat Med 16: 1141–1146, 2010.

Mesenchymal Stem Cells Get Good Neighbor Award: A team of researchers has discovered that mesenchymal stem cells (MSCs) are an essential component of the HSC niche, the microenvironment in bone marrow where the cells are found. HSCs, a type of adult stem cell, hold great promise for future biomedical applications because of their ability to self-renew and develop into any kind of blood cell. However, HSCs are estimated to be a very rare cell type in bone marrow—only 1 in every 20,000 cells. Current scientific inquiry seeks to further our understanding of how the niche environment maintains HSCs with the hope of one day being able to manipulate the HSC population for potential use in various therapeutic interventions.

Scientists identified a novel cell type in bone marrow—the MSC—through its production of a protein called "nestin." They observed that MSCs outnumber HSCs 10 to 1 in bone marrow and are in direct contact with or cluster around HSCs, suggesting that MSCs might contribute to maintenance of HSCs. In fact, MSCs were found to make an abundance of supportive factors for HSCs. They were also found to self-renew, a key characteristic of stem cells.

To obtain more evidence about whether MSCs maintain or support HSCs in the bone marrow of mice, MSCs were depleted from their normal levels in mice using genetic and pharmacologic means. The result of fewer MSCs was an approximate four-fold reduction in HSC metabolic activity—evidence that MSCs play an important supportive role for HSCs. To evaluate the possible role of MSCs in homing HSCs to the marrow, HSCs were transplanted into mice whose MSCs had been depleted. Homing of HSCs to the marrow was reduced by 90 percent, and those cells that did home to the marrow tended to be located near MSCs, indicating that MSCs participate in the migration process.

This study illustrates the importance of the neighboring MSC to the HSC and provides additional information regarding a unique niche in the bone marrow made up, in part, by these two adult stem cell types. Future research efforts may explore the pharmacological targeting of the niche to enhance HSC production for use in regenerative therapies.

Méndez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466: 829-834, 2010.

STORY OF DISCOVERY

Sickle Cell Disease

Sickle cell disease is an inherited, chronic, and painful blood disorder. In the U.S., approximately 70,000 to 100,000 people have sickle cell disease,[1] predominantly individuals of African descent. Worldwide, millions of people have this disease. People with sickle cell disease have episodic severe pain in their bones, joints, and other parts of the body, as well as leg ulcers, jaundice, organ damage, and other serious health problems that may lead to multi-organ failure. The episodic, severe pain and complications associated with this disease can have a profound impact on the quality of life, ability to work, and life span of affected individuals. However, there is considerable variation in the clinical manifestations and severity of sickle cell disease among patients that is not completely understood. It is estimated that one of every three people with sickle cell disease who are hospitalized for pain will return to a hospital or emergency department within 30 days because of recurrent pain.[2] In the U.S., the average life expectancy for individuals with sickle cell disease has improved in recent decades but is still only 42 years for men and 48 years for women.[1]

What is Sickle Cell Disease?

Understanding of sickle cell disease at the molecular and cellular level began 100 years ago. In 1910, Dr. James Herrick, a Chicago physician, observed that the red blood cells of a patient from the West Indies were "sickle" shaped. It was later discovered that this red blood cell abnormality characteristic of sickle cell disease is caused by a mutation in hemoglobin, a protein that gives red blood cells their color and carries oxygen as these blood cells circulate throughout the body. Normal hemoglobin, referred to as hemoglobin A or HbA, consists of four protein components—two alpha globin chains and two beta globin chains. In sickle cell disease, the beta globin chains have a slightly altered structure caused by a heritable, genetic mutation of the beta globin gene. This alteration causes hemoglobin to polymerize and assemble into rod-like structures when red blood cells release their oxygen to tissues. Polymerized sickle hemoglobin elongation distorts the red blood cell membrane such that the red blood cells develop a "sickle" shape. Because "sickle" red blood cells are stiff and may clump together, they can block blood flow in small blood vessels, causing muscle, bone, and joint pain and eventual organ damage. Individuals with sickle cell disease usually have severe anemia (low red blood cell numbers) because sickle red blood cells have a much shorter lifespan in the circulation than normal red blood cells, and the bone marrow cannot produce new red blood cells fast enough to compensate for the rapid destruction of sickle cells.

Pinpointing the Molecular Basis of Sickle Cell Disease

In an elegant series of experiments in the 1940s and 1950s, specific chemical differences between normal HbA and sickle cell hemoglobin (referred to as HbS) were discovered. First, Dr. Linus Pauling demonstrated that HbA had a more positive charge than HbS and could be readily distinguished from HbS by a technique called electrophoresis. Then, Dr. Vernon Ingram found that one of the 146 amino acids in the beta globin protein component of HbA was altered in HbS; specifically, he found that the glutamic acid at position 6 in HbA was replaced by a valine in HbS. Subsequently in the mid-1970s, NIH-supported scientists showed that this amino acid substitution was caused by a specific mutation of the beta globin gene in sickle cell disease.

This sickle cell disease mutation is inherited. Individuals with the disease have two copies of the "sickle" hemoglobin gene, inherited from each parent. However, when a child inherits a mutated gene from

one parent but a normal gene from the other parent, the child has what is referred to as "sickle cell trait" and typically lives an asymptomatic, normal life.

Past and Current Treatments of Sickle Cell Disease

Until the early 1990s, treatment approaches were limited primarily to pain management and prevention of infections. One variable found to influence the clinical severity of the disease was the relative amount of another form of hemoglobin, called fetal hemoglobin (HbF), in red blood cells. HbF consists of two alpha globin chains and two gamma globin (not adult beta globin) chains; this form of hemoglobin predominates during human fetal development. Because HbF contains gamma globin chains instead of beta chains, it is not affected by the genetic mutation that causes sickle cell disease. While levels of HbF decline to very low levels after birth, varying levels of HbF may persist in the red blood cells. In people with sickle cell disease, HbF, if present at sufficient levels, can reduce the tendency of HbS to form the rod-like structures within red blood cells and to cause "sickling."

In 1985, the drug hydroxyurea, which is used in cancer therapy, was shown to increase the levels of HbF in red blood cells. Subsequently, investigators in the Intramural Research Programs of the National Institutes of Health—including the current NIDDK Director, Dr. Griffin Rodgers—and at The Johns Hopkins University School of Medicine, conducted a clinical trial in adult patients with sickle cell disease to evaluate the ability of hydroxyurea to increase HbF in the red blood cells of these patients and to reduce the clinical manifestations of their disease. The results of their study, published in 1990, showed therapeutic benefit in 7 out of 10 patients and led to subsequent large multi-center studies that established hydroxyurea as an effective form of treatment for sickle cell disease. Although not all patients respond to hydroxyurea, those that do have an improved quality of life—they are often able to attend school or work normally and better enjoy the normal activities of daily life. While hydroxyurea was a major breakthrough in the treatment of sickle cell disease in adults—and remains the only Food and Drug Administration-approved treatment for this disease—it is not a cure and it must be taken continuously.

For children with sickle cell disease, transplantation of blood stem cells from a donor without this disease has been shown to be curative. However, this procedure is only performed when there is a bone marrow (or blood stem cell) donor whose immunologic tissue type matches that of the patient. Siblings without sickle cell disease can sometimes be immunologically matched and serve as transplant donors, as can, occasionally, unrelated individuals of the same racial background. However, most children with sickle cell disease do not have a sibling who is a potential donor for transplantation, and bone marrow registries do not currently have sufficient numbers of African American donors for matches to be identified for the many patients who might benefit from this procedure. Moreover, standard conditioning regimens used to prepare a patient for blood stem cell or bone marrow transplantation are very toxic, particularly for adult patients with sickle cell disease, and serious complications often occur following standard bone marrow transplantation.

New Hope for the Future

In 2009, a team of researchers reported results of blood stem cell transplantation in adult patients with sickle cell disease using a modified transplant regimen with greatly reduced toxicity that was developed to make this treatment approach safer and much less harmful to the patient. This study was conducted at the NIH Clinical Center by NIDDK researchers Dr. Rodgers and Dr. John Tisdale of the NIH Molecular and Clinical Hematology Branch, who led the study, as well as investigators from the National Heart, Lung, and Blood Institute and the National Institute of Allergy and Infectious Diseases. Instead of using standard high-dose chemotherapy to prepare patients to receive the donor blood stem cells, the researchers

used a relatively non-toxic, "non-myeloablative" preparative procedure that does not destroy the patient's own bone marrow. Rather, this new regimen, based on earlier research with mice, was designed to suppress immune responses of the patient only enough to allow donor blood stem cells to engraft. Moreover, after the blood stem cell transplants were delivered into the recipient, a modified immunosuppressive regimen was used to sustain the graft and to prevent post-transplant complications.

Around 2.5 years post-transplantation, the researchers reported in a December 2009 publication that all 10 of the adult participants who had sickle cell disease were alive and well, and 9 no longer suffered from clinical manifestations of sickle cell disease. The results of this transplantation study indicate that adult patients with sickle cell disease now have an additional—and potentially life-changing—treatment option if a matched blood stem cell donor is available.

This clinical trial represents a major milestone for developing a cure for sickle cell disease. According to Dr. Tisdale, if participants in this ongoing trial continue to do well, it may be possible to extend this treatment approach to the use of "haplo-transplantation" donors (that is, sibling or parent donors whose immunologic tissue type only half matches that of the patient). If this becomes possible, it would allow most people with sickle cell disease to be treated with a potentially curative blood stem cell transplant.

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[1] www.cdc.gov/ncbddd/sicklecell/about.html

[2] Brousseau DC, et al. Accute care utilization and rehospitalizations for sickle cell disease. JAMA 303: 1288-1294, 2010

Chronic Pelvic Pain: Opening the Black Box

Dr. Anthony Schaeffer

Dr. Anthony Schaeffer is the Herman L. Kretschmer Professor and Chairman of the Department of Urology at Northwestern University in Chicago, Illinois. Dr. Schaeffer has led pioneering work in basic and clinical studies of urinary tract infections and prostatitis, involving novel concepts regarding the cause and treatment of these conditions. Dr. Schaeffer has also made major contributions to the management of post-prostatectomy incontinence through the implementation of a mobile urethral sling procedure. Dr. Schaeffer earned his M.D. from the Feinberg School of Medicine at Northwestern University and interned at the Chicago Wesley Memorial Hospital, after which he pursued a surgery residency at McGaw Medical Center of Northwestern University and a urology residency at Stanford University Medical Center in California. Dr. Schaffer has been an NIH-supported researcher for the past 30 years, including research support from NIDDK for at least 20 of those years. At the September 2010 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Schaeffer presented on urologic chronic pelvic pain, sharing some new insights into what this condition might be.

Urologic chronic pelvic pain encompasses two major pain syndromes—interstitial cystitis/painful bladder syndrome, which primarily affects women, and chronic prostatitis/chronic pelvic pain syndrome, which only affects men. Both syndromes, however, share the characteristics of severe pain below the abdomen, often with urinary frequency and urgency, and in both cases their cause remains unknown. Research is revealing that millions of people worldwide may have symptoms of urologic chronic pelvic pain syndromes, with attendant suffering akin to patients with serious chronic illness. As fully effective treatments are elusive and there is no cure, people with these syndromes suffer and can also incur high medical costs for themselves and the health care system. Dr. Schaeffer described research suggesting that urologic chronic pelvic pain syndromes may be mediated by novel adaptations of well-known host-bacteria interactions, as well as evidence that the central nervous system can be permanently altered by these interactions—thus suggesting that these syndromes might actually be a disease of infectious origin.

Quest for a Possible Infectious Origin

Dr. Schaeffer related that, in many cases of urologic chronic pelvic pain, there appears to be an infectious beginning. In his experience, patients will recall contracting a urinary tract infection (UTI) prior to the onset of chronic pain symptoms. However, most of these people, when seen by a doctor later in life, have no detectable evidence of infection, and many of them do not have inflammation. So, how could a UTI, which is usually associated with acute, or short-term, pain, possibly trigger or transition into a chronic pain condition?

While one candidate might be the inflammation that results from infection, patient data suggest that there is a disassociation between infection, inflammation, and the presence of pain. For example, patients with urologic chronic pelvic pain syndrome may have pain but no current evidence of infection or inflammation. Also, whereas most patients with UTI experience pain that has long been assumed a natural consequence of infection-associated inflammation, some people have pain-free bacterial infections (*i.e.*, asymptomatic) who nonetheless also have inflammation. That is, there is evidence of inflammation, such as infection-fighting white blood cells in the urine, but no pain. So, Dr. Schaeffer and his colleagues performed experiments in animal models to determine if there

are differences between the bacteria that cause the acute, painful UTIs and those that are involved in asymptomatic infections. They did this by placing the different strains of bacteria—those from patients with either acute UTI or from patients with an asymptomatic infection—into mouse bladders, and then monitoring the pain the mice experienced over the course of an infection. They found that, indeed, only infection with the acute UTI strain caused pain. However, similar to observations in humans, other experiments showed that both strains were capable of causing inflammation—suggesting that the difference in their ability to incite pain lay elsewhere.

Searching for bacterial factors that could contribute to this difference, Dr. Schaeffer's team focused on lipopolysaccharide, or LPS. The LPS molecule, a large lipid-sugar molecule found on the surface of bacteria, is a so-called "virulence factor" that helps to optimize bacterial infection of a host. It is known to contribute to inflammation and shock, suggesting it might also somehow contribute to pain. Experiments with cells and in animal models revealed that LPS from either strain incited inflammation. When placed in mouse bladders, however, only LPS from the acute strain caused pain, and did so much more rapidly even than the acute infection itself. Dr. Schaeffer and his colleagues determined that the typical interaction between this molecule and a receptor on host cells, called Toll-like receptor 4, was indeed involved in mediating the pain response—a potential new function for this interaction, which typically mediates inflammation.

Molecular Studies and Potential Clinical Relevance

Through further analysis of the LPS molecule, Dr. Schaeffer and his team have uncovered some intriguing findings that suggest that a specific alteration in this molecule between different bacterial strains is somehow responsible for whether a bacteria induces an acute infection that can lead subsequently to chronic pain after the infection is cleared, or whether it only causes acute pain at the time of infection. Moreover, they now have evidence for how the LPS molecule may be used therapeutically. Shortly following infection with an acute UTI strain, mice were given either a mock treatment or LPS from asymptomatic bacteria. The LPS from the asymptomatic bacteria significantly reduced the pain associated with the UTI, implying a therapeutic response.

Dr. Schaeffer noted that they have found similar responses in mice in which they have caused interstitial cystitis-like symptoms using a herpesvirus. In other studies, bacteria isolated from a person with chronic prostatitis were transferred to the prostates of mice. These mice developed pain symptoms similar to human chronic prostatitis. Interestingly, the ability to cause pain symptoms also depended on the mouse model used in the experiments, suggesting that there are host-specific differences in susceptibility to pain. Moreover, Dr. Schaeffer and his team have observed in this spectrum of studies the same type of dissociation between inflammation and pain and infection as seen with the UTI and pain models.

Neuro-Mechanisms and the MAPP Network

In 2008, the NIDDK established the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network to enable multiple laboratories and investigators around the country to work collaboratively on novel ways of looking at these enigmatic syndromes. While the Network's focus is on two major forms of urologic chronic urologic pelvic pain syndromes, interstitial cystitis and chronic prostatitis, researchers are also exploring the possible relationship between these and other pain syndromes, including irritable bowel syndrome, fibromyalgia, and chronic fatigue syndrome. Through this Network, Dr. Schaeffer's group has begun to work with neurophysiologists and neuroimaging experts, using an imaging technology called functional magnetic resonance imaging (fMRI) to look at chronic pain states. For example, using a computerized system originally developed

by a back pain researcher to directly correlate a person's experience of pain with what is going on in the brain, their fMRI studies have shown some intriguing differences in brain activity between people experiencing acute pain from heat exposure, people with chronic back pain, and people with chronic prostatitis. These studies in people are made all the more intriguing by new studies in mouse models suggesting that, when instilled into the mouse bladder, UTI bacteria that cause chronic pain can also cause a persistent change in electrical signaling by part of the central nervous system.

In addition to apparent functional changes in the brain, Dr. Schaeffer and collaborators have examined changes in the brain structure of people with urologic chronic pelvic pain syndromes. These imaging studies reveal an apparent correlation between the intensity and duration of pain and the density in the brain's gray matter in different brain regions. Interestingly, some of the brain areas that appear to be affected by urologic chronic pelvic pain are important in human function and behavior, particularly in emotional decision making.

Summary and Future Directions

Dr. Schaeffer noted that the studies he presented provide evidence that there may be an infectious basis for the chronic pain experienced by people with urologic chronic pelvic pain syndromes, and that this pain persists well after the initial infection and inflammation has cleared. At a cellular level, host Toll-like receptors appear to be acting as novel "nociceptors" for pain in a way that is independent of inflammation. The bacteria appear able to modulate the pain response via differences in LPS, and there appears to be involvement of the central nervous system. Building on some of these new findings and other studies, Dr. Schaeffer's team is now exploring "designer bacteria" or bacterially based molecules that could be administered to alter the pain response in patients, providing hope that a better understanding of the genesis of pain in these conditions could lead to new treatments.

PATIENT PROFILE

Lindsey Duquette

For the Family of a Child with Nephrotic Syndrome It's Been a Real Roller Coaster Ride

Lindsey Duquette

August 27, 2004, was Pam and Jim Duquette's 10th wedding anniversary. "It also just so happened to be the date our roller coaster nightmare began," says Pam. It was on that day that the Duquette's up-to-then perfectly healthy two-and-a-half year-old daughter, Lindsey, was diagnosed with nephrotic syndrome, a condition in which damage to the kidneys causes large amounts of protein to leak from the blood into the urine. Nephrotic syndrome affects the tiny filtering units in the kidney, called glomeruli, and over time may lead to kidney failure and the need for dialysis or a kidney transplant. Currently, there is no known cure for nephrotic syndrome. Even removing the diseased kidneys and replacing them with transplanted organs does not guarantee that the disease will not return.

Lindsey is now a happy 9-year-old who loves gym, Silly Bandz®, and going to school—normal for most kids her age. But these are relatively new experiences for a little girl who for years has suffered the pain and trauma of nephrotic syndrome. In Baltimore, MD, Lindsey's mother spoke to approximately 50 researchers and others from the Nephrotic Syndrome Study Network (NEPTUNE), which seeks to find better diagnoses and treatments for nephrotic syndrome and to understand its primary causes. Pam related Lindsey's and her family's experience with the disease, while Lindsey distributed brochures and introduced herself individually to everyone present.

Nephrotic syndrome is sometimes the first sign of an underlying disease that damages the kidney's tiny blood filtering units, called glomeruli, where urine is made.

Lindsey's Story

It all started about 6 years ago, when Lindsey began waking up with puffy eyes. The Duquettes didn't think much of it. "As a child, I had allergies," says Pam, "so I assumed that that's what it was." Lindsey then started asking for ice, which is sometimes a sign that a person's body is low on iron, although Pam and Jim didn't know this at the time. Within days, Lindsey's feet began to get puffy. Pam contacted her pediatrician, but the doctor was not overly concerned about Lindsey's condition.

About 10 days later, the Duquettes were attending a New York Mets baseball game when they realized

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something might be seriously wrong with their daughter. Lindsey's face, arms, and legs were swollen, and she seemed to have difficulty walking. A friend's wife, who was sitting with the Duquettes during the game and happened to be a pediatrician, said to Pam, "I don't want to alarm you, but I think it might be nephrotic syndrome."

Nephrotic syndrome is sometimes the first sign of an underlying disease that damages the kidney's tiny blood filtering units, called glomeruli. The glomeruli filter waste products and excess water and salts into the urine while keeping proteins and other larger molecules in the blood. Damaged glomeruli may allow protein to leak from the blood into the urine. In fact, elevated protein levels in the urine are one sign of kidney damage. The leakage of protein into the urine causes a corresponding drop in blood protein levels, which can lead to fluid retention in body tissues and swelling.

After calling Lindsey's pediatrician from the stadium, the Duquettes rushed Lindsey to the nearest emergency room, where she was diagnosed with nephrotic syndrome. At the time, Lindsey's blood protein levels were critically low, putting her at risk of going into shock. Lindsey was rushed from the hospital in Queens to a hospital in the Bronx, where she spent the next 5 days receiving high doses of steroids to help modulate the immune response that triggers some forms of nephrotic syndrome. Three weeks later, she was once again hospitalized, this time for peritonitis, a potentially life-threatening inflammation of the membrane that lines the wall of the abdomen, and a complication sometimes associated with nephrotic syndrome. Lindsey spent 3 days in the pediatric intensive care unit (PICU) battling peritonitis. After being released from the PICU, she remained hospitalized for more than 2 months. "We got nothing but bad news the entire time Lindsey was in the hospital," says Pam. "Blood clots….high blood pressure….sky-high triglycerides….poor breathing to the point that Lindsey had to be placed on oxygen. It was petrifying." Jim, who is now on the board of the NephCure Foundation, divided his time between work and taking care of the couple's two older children, while Pam spent most of her time at the hospital with Lindsey.

The Roller Coaster Ride Continues

After 76 days of treatment, Lindsey's condition finally went into remission. However, for more than 2 years, the roller coaster ride continued, with Lindsey's nephrotic syndrome flaring up, then going back into remission. During all this time, Lindsey continued to take steroids and other immunosuppressants, which had devastating side effects: Lindsey stopped growing, her eyebrows became bushy, and the hair on her head became discolored, dry, and brittle.

In August 2006, the family moved to Baltimore. But things weren't getting much better for Lindsey. The long-term steroid therapy was causing her bones to weaken. She was in tremendous pain and "burning from the inside out," recalls Pam. "Her cheeks were all puffed out and she couldn't move as a result of the pain. I had to pick her up to take her to the bathroom." Lindsey was taking over 20 pills each day, including steroids and pain medications. Her small frame—all 40 inches and 60 pounds of her—was carrying 18 pounds of extra weight, also caused by the steroids. "Whenever we tried to wean her off the steroids, she'd relapse," says Jim. "It seemed like we were chasing our tails. Our daughter had no quality of life."

Currently there is no known cure for nephrotic syndrome. Even removing the diseased kidneys and replacing them with transplanted organs does not guarantee that the disease will not return.

"Mom, I just want to be a normal kid."

Physicians in Baltimore strongly recommended that the Duquettes approve having their daughter's two ailing kidneys removed, which meant Lindsey would either require a kidney transplant or be on dialysis for the rest of her life. In October 2008, the Duquettes

scheduled the procedure. Lindsey's reaction: "Mom, I just want to be a normal kid." "I just broke down and sobbed," says Pam. "It broke my heart."

On further reflection, the Duquettes reconsidered the surgery, both because nephrotic syndrome can reoccur in people with transplanted kidneys and especially because Lindsey's kidneys were otherwise functioning normally, which had always made her case an anomaly. They instead sought a second opinion. In the process they were referred, and were accepted, into a research study being conducted by physicians in NIDDK's Intramural Research Program on the NIH campus in Bethesda, MD. In many respects, it was a risky decision.

The research study that Lindsey is participating in, which is led by Dr. Jeffrey B. Kopp in the Kidney Disease Branch of the Intramural Research Program at NIDDK, is studying the effectiveness of novel anti-inflammatory therapies for treating patients with nephrotic syndrome and related diseases that do not respond to traditional steroid treatment. "The protocol called for Lindsey to be infused with rituximab," says Jim. Rituximab belongs to a class of drugs called monoclonal antibodies, and, like steroids, has serious side effects, including potentially life-threatening reactions. In addition, the long-term effects of the drug are unknown. However, after years of watching their daughter suffer, the Duquettes were desperate.

Fortunately for the Duquettes, Lindsey responded well to the rituximab. Nonetheless, it's been a rough ride. As of this writing, Lindsey has had nine total infusions of the powerful drug, which is designed to destroy the B cells in her body. Every 4 months, as the B cells repopulate, Lindsey's disease relapses. In addition, in March 2009, she started experiencing severe headaches resulting from inflammation in the back of the eye due to being taken off of steroids too quickly.

Things Take a Turn for the Better

But as fast as things seemed to be going badly, they seemed to turn around. By June 2009, Lindsey went into remission again. As of this writing, Lindsey has been steroid-free for 10 months. Her bones have started to regenerate and she is no longer going to physical therapy. She's down from her previous 20-plus pills each day to only two. Because she's no longer on steroids she's starting to grow again. She's lost the bushy eyebrows, and her hair is getting back to normal. She's also lost the 18 pounds of extra weight she gained. Best of all, after having been bed-ridden for the better part of 2 years, Lindsey has gone to school for a full year—and won attendance awards. "She's got tons of friends, gets invited to birthday parties, she can do back bends. She's a normal little girl," Pam says with great emotion.

The Duquettes know that they may not be off the roller coaster just yet. Rituximab is a powerful drug, and there's no telling what its long-term effects might be. But they feel they made the right decision to enroll Lindsey in the study. "Lindsey would not be in the position she is in today if it weren't for NIH," says Jim. "We've been treated with nothing but professionalism and kindness and have met some extraordinary human beings," Pam adds.

As for Lindsey, "she keeps asking me when she can go back to NIH," says Pam. "When I ask her why, she says 'I love their food.'"

Gaining a Better Understanding of Nephrotic Syndrome Through Research

Through research studies, such as the one that Lindsey is participating in, NIDDK is hoping to develop new and improved methods for treating people with nephrotic syndrome, which may ultimately lead to a cure. In addition to the rituximab treatment study, NIDDK also supports research efforts to better understand nephrotic syndrome and other glomerular diseases. The NEPTUNE study is collecting long-term

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observational data of patients with rare forms of underlying kidney disease that lead to nephrotic syndrome and combining this information with biological samples. The consortium's investigators hope to improve our understanding of the fundamental biology of the causes of these diseases and the factors that contribute to their progression. Concerted and innovative investigational strategies that combine basic, clinical, and translational science are expected to improve the diagnosis and treatment of these forms of kidney disease.

The Intramural Research Program of NIDDK conducts basic, translational, and clinical biomedical research related to: diabetes mellitus, endocrine, bone, and metabolic diseases; digestive diseases, including liver diseases, obesity, and nutritional disorders; kidney diseases; and hematologic diseases. More information on research conducted at NIDDK can be found at: www.niddk.nih.gov

The NIDDK, the NIH Office of Rare Diseases Research, and the NephCure and Halpin Foundations collaborate to support research on nephrotic syndrome and other glomerular diseases through the Nephrotic Syndrome Study Network (NEPTUNE). NEPTUNE is a multi-site, multidisciplinary collaborative research and education network designed to foster innovative approaches to the understanding of four glomerular disease areas: minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), and nephrotic syndrome due to other or unspecified cause. More information can be found at: http://rarediseasesnetwork.epi.usf.edu/NEPTUNE/index.htm