Helium ion microscopy (HIM) uses a beam of helium ions to produce striking, high-magnification images that are difficult or impossible to capture with other types of microscopy. Sample preparation for HIM is relatively simple and allows researchers to view never before seen details of intact biological structures. Shown above are HIM images of rat kidney epithelium illustrating a basic kidney filtering unit, called a glomerulus. Glomeruli filter the blood, which is the first step in producing urine. The top left image shows a whole glomerulus, including the capillary bundle in the center. The other images are taken at increasing magnifications and show the glomerulus with the capillaries removed. The top right and bottom left image show two different magnifications of protrusions, called podocyte processes (white arrows), on the glomerulus’ surface. The bottom right image was taken at yet higher magnification and shows the filtration slits on the podocyte processes (black arrows). As described in this chapter, researchers use imaging technologies such as HIM to study cell surface structures and membrane organization, which are the basic foundation for all organ function.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They afflict millions of Americans and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The overall goal of the NIDDK’s research programs in these areas is to increase our understanding of kidney, urologic, and hematologic diseases in order to enhance approaches to prevent and treat these serious conditions.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about two quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, represents a life-threatening condition.

It has been estimated that more than 20 million Americans have impaired kidney function—also called chronic kidney disease (CKD). CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the nation’s health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated.

CKD, 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. In 2011, nearly 616,000 patients received treatment for ESRD: over 427,000 received either hemodialysis or peritoneal dialysis and over 185,000 were living with a kidney transplant. Minority populations, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives, bear a disproportionate burden of CKD and ESRD. African Americans are nearly four times more likely to develop kidney failure than are non-Hispanic whites. American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic whites. In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in health disparities related to kidney disease susceptibility and progression in minority populations.

The NIDDK supports a significant body of research aimed at understanding the biology underlying CKD. The NIDDK’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 strategies to prevent development or halt progression of kidney disease. The Institute’s Chronic Renal Insufficiency Cohort (CRIC) Study, one of the largest and longest ongoing studies of CKD epidemiology in the United States, has recently been extended for an additional five years. A major goal of this extension is to recruit an additional 1,500 people to the existing group of nearly 4,000 volunteers. These new participants and the additional time will allow researchers to collect more data and to explore and build upon findings compiled over the past 10 years, and examine in much greater detail the broad range of illnesses experienced by people with CKD. The NIDDK also supports studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome.

The NIDDK’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 CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public.

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 research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urological diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, and urinary tract infections. As described in this chapter, NIDDK-supported research has identified a protein that functions to protect the urinary tract from bacterial infection. Other disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS)—also known as IC/bladder pain syndrome (BPS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the 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.

IC/PBS is a debilitating, chronic, and painful urologic disorder. Based on a recent large national interview survey, it is estimated that 3.3 million (2.7 percent) U.S. women 18 years old or older have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/PBS. Using a community-based epidemiological survey, researchers have estimated that 1.6 million (1.3 percent) U.S. men ages 30 to 79 years old have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with painful bladder syndrome.

NIDDK-supported basic and clinical research on IC/PBS is focused on elucidating the causes of these conditions, identifying “biomarkers” that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. These include the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) research network, which supports research designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. To continue the Network’s important efforts, the NIDDK recently issued multiple Requests for Applications (RFAs) to support the renewal and enhancement of this multi-center Network for a second five year funding cycle beginning in FY 2014. Other studies include the Boston Area Community Health Survey (BACH), which seeks to identify patterns and risk factors for a range of urological problems, and the Olmsted County (Minnesota) Study, which is studying lower urinary tract symptoms in men. As described later in this chapter, researchers recently used data from the BACH to look for possible relationships between consumption of certain beverages and lower urinary tract symptoms.

Based upon national public health surveys conducted over several years, it is estimated that 1 in 10 U.S. adults (18 years of age and older) suffer from daily urinary incontinence; most of those affected are women. Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence and other lower urinary tract symptoms in women and men and the effect of different diagnostic tools and interventions on patient outcomes. To address this challenge, the NIDDK launched and recently expanded the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The Institute is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression in women, thereby improving health. In FY 2014, the NIDDK will hold a major multidisciplinary scientific symposium and pursue additional efforts focused on prevention of urinary incontinence and other lower urinary tract symptoms in women.

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. NIDDK-supported research has recently identified a potential new approach to improve blood stem cell transplantation.

The NIDDK is also keenly interested in the basic biology of stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the NIDDK’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. As described later in this chapter, scientists report a potential new prevention or treatment approach for iron overload. Also later in this chapter, new research reveals how chemotherapy blunts blood stem cell renewal.

INSIGHTS INTO KIDNEY DISEASE

Deterioration in Heart Function Associated with Progression to Kidney Failure: The progression of chronic kidney disease (CKD) to kidney failure (end-stage renal disease or ESRD) is associated with less efficient pumping of blood by the heart. This decline in the “ejection fraction”—the amount of blood that leaves the heart with each contraction versus the amount that is left behind—may contribute to the increased risk of cardiovascular disease and death that is seen in patients who are undergoing dialysis. These findings come from the Chronic Renal Insufficiency Cohort (CRIC) Study, one of the largest and longest ongoing studies of CKD epidemiology in the United States.

In the current report, CRIC researchers focused on a subset of patients who had progressed from advanced CKD to ESRD over the course of the study, and who had undergone an echocardiogram—a test that produces a detailed moving image of a beating heart—both while they had advanced CKD and shortly after they had progressed to ESRD. These tests provided detailed “before” and “after” information about the patients’ heart structure and function as their kidney function deteriorated.

Nearly three-quarters of patients with advanced CKD or ESRD have a condition termed left ventricular hypertrophy, which means that the main pumping chamber of their hearts is larger than normal because it must work harder to pump blood throughout the body. The researchers noted that there was no difference in

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the degree of left ventricular hypertrophy as patients progressed from advanced CKD to ESRD. However, the average ejection fraction decreased slightly, but significantly, during the transition to ESRD. This was observed across all patients regardless of their age, race, diabetes status, or the type of dialysis (hemodialysis or peritoneal dialysis) they were receiving.

This is the first study to examine changes in heart structure and function in patients as they progress from CKD to ESRD. Future studies will explore the mechanisms responsible for the decline in ejection fraction that accompanies progression to ESRD.


**Tracking the Origins of Kidney Fibrosis:**
Researchers have used a series of genetically engineered mice to identify cellular sources of the fibrosis that follows kidney injury.

Fibrosis is a common final pathway for many diseases. Extensive kidney fibrosis, and the scar tissue that can sometimes arise from it, can impair the removal of toxins and excess fluid from the blood, cause irreversible organ damage and, in severe cases, lead to kidney failure.

It is widely accepted that the source of collagen that causes fibrosis in the kidney is a type of cell called a myofibroblast. Previous research suggested that these cells might be derived from pericytes, a type of cell associated with blood vessels. In the new study, the scientists developed a number of different strains of mice in which they could visualize, track, and selectively eliminate specific subtypes of cells in the kidney. The goal was to identify the contribution of each cell subtype to the process of fibrosis.

The researchers confirmed that myofibroblasts are a significant contributor to kidney fibrosis. They identified two primary sources for these cells: about half of them result from conversion of existing precursor cells in the kidney, which then proliferate, while about one third arise from precursors produced in the bone marrow that travel to the kidney and convert to myofibroblasts but do not proliferate. (The remaining myofibroblasts were found to be derived from other sources.) The scientists also found evidence that pericytes were not a significant source of myofibroblasts.

Understanding the cellular and molecular mediators of kidney fibrosis is a high priority for scientists studying kidney disease. This finding is significant because it suggests that treatments for fibrosis that target myofibroblast proliferation may only be partially effective, because they have an impact on only about one-half of the myofibroblast population in the kidney. A better understanding of fibrosis, in general, could yield insights into how this process unfolds in other tissues and organs, potentially opening new avenues to therapy for a range of diseases.


**KIDNEY DISEASE AND GENETICS**

**Mutations May Lead to Both Kidney and Blood Diseases:** A recently discovered set of gene mutations may be behind some cases of the serious blood disorder hemolytic uremic syndrome (HUS).

Hemolytic uremic syndrome is a condition that results from the premature destruction of red blood cells. These red blood cell fragments can clog the kidneys’ filtering system and cause kidney failure. While many cases of HUS result from a bacterial infection, so-called atypical HUS (aHUS) arises from genetic or autoimmune factors. Researchers have now identified a set of novel mutations in the gene for diacylglycerol kinase epsilon (DGKE) that is associated with aHUS in nine unrelated families. The protein encoded by the DGKE gene is found in several cell types, including the blood vessels and filtering cells of the human kidney; thus, its disruption has the potential to have wide-ranging effects throughout the body.
People with the mutated DGKE genes typically develop aHUS before one year of age, have persistent high blood pressure, blood and protein in their urine, and are likely to develop chronic kidney disease as they age. The DGKE mutations identified in all nine families seemed to produce a non-functional, sometimes truncated DGKE protein, suggesting that the loss of DGKE function within cells might be responsible for these cases of aHUS. These findings implicate loss of DGKE function as a cause of a distinct subset of aHUS cases that may lead to severe kidney complications not usually seen in other forms of aHUS.

DGKE is the first gene implicated in aHUS that is not part of the complement system, a blood-borne part of the immune system. Currently, patients with aHUS are usually treated with drugs that inhibit the complement system, but this approach may be ineffective in people with DGKE mutations. For these individuals, kidney transplantation may represent a more effective treatment. These findings underscore the importance of correctly diagnosing patients whose aHUS arises from DGKE mutations and tailoring their treatment accordingly.


**Regulation of Cyst Growth in Polycystic Kidney Disease:** Scientists have discovered a link between two proteins known to contribute to the most common form of polycystic kidney disease (PKD) and a cell-surface structure in a subset of kidney cells in mice.

PKD is a genetic disorder characterized by the growth of numerous fluid-filled cysts in the kidneys. For many people who have the more common form of the disease, autosomal dominant PKD (ADPKD), these cysts grow slowly over many years. Over time, they can profoundly enlarge the kidney and replace much of the organ’s normal structure. This results in reduced kidney function that can potentially lead to kidney failure.

It is thought that cyst growth arises from improper growth signals in the kidney. Previous studies have shown that the removal of cilia, tiny, hair-like structures on the surface of some kidney cells, could produce cysts. Mutations in the proteins polycystin-1 and polycystin-2 have also been found to cause kidney cysts and cause ADPKD.

It is thought that cilia act to sense fluid flow in the kidney. The polycystins are present in the cilia, where they form a complex that has been hypothesized to aid in this flow sensing. To explore this relationship, researchers used a series of genetically engineered mice in which they could selectively delete polycystin-1, polycystin-2, and/or the cilia in the kidney. When they deleted polycystin-1 and/or -2, the mice developed kidney cysts. When the scientists next deleted the cilia in these mice, they found that cyst growth slowed dramatically. Interestingly, the severity of the cysts was related to the time interval between the loss of polycystin function and the loss of cilia: the longer the interval, the worse the cysts became before their growth slowed. This suggests that, under normal conditions, the polycystins may act as a brake on cyst-promoting signals arising from cilia. Loss of polycystin functions allows the cilia signal to proceed unabated, while deletion of cilia removes this stimulation.

These findings in mice have important implications for researchers’ understanding of the molecular basis of kidney cysts. In people with ADPKD, most cysts are slow-growing, and people can live with the disease for decades before they develop symptoms. If similar mechanisms lead to kidney cyst development in people with ADPKD, then the design of strategies to inhibit the function of cilia that are associated with polycystins in these individuals before extensive kidney damage occurs could have profound implications for patient care.


**GUT MICROBES AND BLOOD PRESSURE**

**New Function Identified for Odor Receptors:** A family of complex cell-surface receptors, some of which were initially characterized as playing a role in detecting
odors in the nose, has since been found to be involved in the kidney’s integration of signals from gut microbial metabolism and the regulation of blood pressure.

These specific cell-surface receptors are members of a family of proteins known as seven-transmembrane receptors, so-called because the proteins snake back and forth across the outer cell membrane seven times, forming a complex structure that helps the cell sense and respond to molecules that are outside of the cell. At least six members of the larger receptor family have been identified in the kidney, where they appear to influence the release of the hormone renin, which helps to regulate blood pressure by influencing the amount of fluid excreted by the kidneys. Two members of this receptor family that are present in the kidney—Olfr78, which was initially identified in the nose, and Gpr41, another seven-transmembrane receptor found in other cell types—are activated in response to short chain fatty acids produced by bacteria in the gut during digestion of fats or fiber.

To study the relationship between gut microbial-derived signals and kidney regulation of blood pressure, researchers administered the short chain fatty acid propionate to normal mice; they observed a rapid, but quickly reversible, drop in blood pressure. In subsequent studies, mice lacking the Olfr78 gene were observed to have lower baseline blood pressure than normal mice, and propionate further lowered blood pressure in these mice. However, propionate administration raised the blood pressure of mice lacking the Gpr41 gene, suggesting that the Gpr41 protein, when present, may respond to propionate by lowering blood pressure. Treatment with antibiotics, which disrupt normal gut function by killing intestinal bacteria, resulted in a significant increase in blood pressure in mice lacking the Olfr78 gene, but had no effect on blood pressure in normal mice. Together, these results suggest that the presence of gut microbes producing propionate may play a role in the modulation of blood pressure in mice. This effect appears to be mediated, at least in part, by Olfr78 and Gpr41, which may work to balance one another to maintain normal blood pressure as propionate levels in the blood fluctuate in response to gut microbial metabolism.

This discovery identifies a heretofore unknown connection between the gut, kidney, and cardiovascular systems. This connection may contribute to further understanding of high blood pressure and the future development of novel treatments.


KIDNEY DISEASE AND DIABETES

Test Predicts Outcomes in Dialysis Patients with Diabetes: In dialysis patients with diabetes, measuring another set of modified blood proteins may better predict the risk of death and cardiovascular disease (CVD) than the current standard test to assess blood glucose control used in the general diabetes population.

Diabetes is the leading cause of kidney failure, also termed end-stage renal disease (ESRD). Patients with ESRD have extremely poor survival rates, with fewer than 50 percent surviving three years after initiating dialysis. The leading cause of death in these individuals is CVD. The standard test used to determine blood glucose levels over the previous three months—hemoglobin A1c (HbA1c)—detects the fraction of circulating hemoglobin that has a glucose molecule attached to it. However, in people receiving hemodialysis—a process in which the blood is removed from the body, filtered, and returned—red blood cells, which contain hemoglobin, survive much less than three months. Thus, HbA1c can be misleading as a measure of long-term blood glucose control in these people. Alternatives to the HbA1c test exist, but they have not been evaluated with respect to long-term outcomes in dialysis patients.

To test other approaches to measuring blood sugar levels, and to evaluate their association with outcomes in dialysis patients, scientists examined blood samples from over 500 participants in a clinical trial; samples were taken at the time that the participants initiated dialysis and again at an average of five months later.
The researchers measured total glycated proteins, which represent all blood proteins that have a glucose molecule attached to them, and glycated albumin, which detects the fraction of the relatively common blood protein albumin that has undergone the same modification. They asked whether there was a correlation between elevated levels of these two values and increased risk of cardiovascular events, hospitalization due to sepsis (a serious bacterial infection), or death over an average follow-up period of three and a half years.

The analysis of the blood samples showed that elevated levels of overall glycated protein and the fraction of glycated albumin were associated with an increased risk of death from any cause, death from CVD, or time to first CVD event (for example, a non-fatal heart attack or stroke). In participants on dialysis, measurement of these markers may provide a more accurate representation of a given participant’s blood glucose control, provide important information about risk of death and CVD, and could be useful for the management of diabetes in people who are on dialysis.


Shared Gene Networks Link Diabetic Kidney Disease in Humans, Mice: Scientists comparing networks of activated genes in the human and mouse kidney have identified several common patterns that may lead to the development of better mouse models of diabetic kidney disease.

While animal models have proven invaluable in biomedical research, their applicability to human disease can have limitations. Current mouse models of diabetic kidney disease most faithfully reproduce only the early stages of the disease; the disease does not progress in mice as it does in humans. To better understand these differences, researchers examined the patterns of genes that were turned on in three different mouse strains that have been used to model diabetic kidney disease and compared them with samples taken from people with diabetic kidney disease.

The researchers focused on genes in the glomerulus, the filtering unit within the kidney. Activation of certain signaling genes was observed in all comparison groups. Other genes were only activated in humans and one or two of the mouse models. This suggests that different mouse models may more closely replicate different aspects of human diabetic kidney disease.

Selection of the best mouse model to evaluate specific molecular pathways or potential treatments presents a significant research problem. The results of this study may help to unravel the complex network of gene activity that can lead to diabetic kidney disease. It may also facilitate future research endeavors by helping researchers select mouse models most applicable to a human disease process of interest, to focus on the biologic pathways most relevant to human disease, and to generate better mouse models of the disease for further study.


VISUALIZING THE KIDNEY

A Closer Look at Kidney Structure: Researchers have unveiled a much closer, more detailed picture of the microscopic details of cells in the kidney.

Helium ion scanning microscopy is a new imaging technique that has been used to produce highly detailed pictures of inorganic materials. Now, this approach has been applied to biological specimens. In a recent publication, scientists presented images of multiple cell types and structures within the rat kidney. Kidney elements that were examined included the glomerulus and the branching appendages of podocytes, where blood is filtered; the proximal convoluted tubule and its brush border; and the collecting duct, which resorbs water and regulates blood pH.

Images generated through helium ion scanning microscopy are far more detailed than those produced
by scanning electron microscopy; the images in this study were captured at a resolution of approximately 1.4 nanometers (a nanometer is one-one billionth of a meter; for reference, the width of a human hair is approximately 80,000 to 100,000 nanometers).

This technological breakthrough in fine-scale visualization of cellular structures promises to allow more detailed studies of the cellular structure within tissues and facilitate scientists’ understanding of cell architecture, organization, and the physical and spatial relationships that are involved in organ function.


FIBROSIS IN KIDNEY, BONE MARROW, AND UROLOGICAL DISEASES

The NIDDK is spearheading new efforts to learn more about fibrosis in kidney, bone marrow, and urologic diseases.

In October 2013, the NIDDK issued a solicitation, entitled “Novel Methods for Detection and Measurement of Organ Fibrosis in Kidney, Bone Marrow, and Urological Diseases,” to encourage research. A consortium will be tasked with the development of novel methods for detection and measurement of organ fibrosis after acute or chronic injury in the kidney, bone marrow, prostate, or urinary tract. Specifically, the consortium will conduct translational research that focuses on development and validation of targeting probes, imaging technologies, or biomarkers to detect and measure pathologic fibrosis for molecular classification, risk stratification, and morphologic prediction as a step toward therapeutic prevention or reversal of fibrosis progression. It is anticipated that the consortium will begin its work in early 2015.

In January 2014, the Institute sponsored a scientific meeting, entitled “Targeting Fibrosis in Kidney, Bone Marrow, and Urological Diseases,” to discuss detection and measurement of fibrosis in humans, in order to help inform future research efforts. The invited experts provided input regarding several issues including: the ability to differentiate between pathologic fibrosis and fibrosis in normal aging; whether fibrosis—as measured by imaging—correlates with organ dysfunction, recovery, and regression; and the novel biomarkers or technologies which should be pursued to detect and measure pathologic fibrosis.

“Fibrosis”—the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage—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 is a common final pathway for many diseases. It may arise as the result of a brief, severe injury to the kidney—causing acute kidney failure—or from a slowly-progressing, chronic condition. Extensive kidney fibrosis, and the scar tissue that can sometimes arise, can impair the removal of toxins and excess fluid from the blood, cause irreversible organ damage and, in severe cases, lead to kidney failure.
RESEARCH TOWARD PREVENTING URINARY TRACT INFECTIONS

Fending Off Infection in the Urinary Tract: Recent study has shown that ribonuclease 7 (RNase 7) contributes to defense of the human urinary tract against bacterial infection. The urinary tract is the body’s drainage system for removing wastes and extra water. The system includes two kidneys, two ureters, a bladder, and a urethra. Despite its proximity to the anus, the urinary tract is usually sterile—but how it maintains its sterility is not well understood.

Building on previous research that demonstrated that RNase 7 is produced in human tissues (free of microscopic signs of disease or inflammation) of the bladder, ureters, and a specific part of the kidney called the collecting tubule, and is present in uninfected urine in sufficient quantity to kill bacteria, the same group of investigators has now reported the initial characterization of the antimicrobial features of RNase 7 in the human urinary tract during infection. Significantly more RNase 7 was detected in acutely inflamed kidney tissue compared to either non-inflamed or chronically inflamed kidney tissue. Consistent with findings included in the scientists’ earlier study, non-inflamed collecting tubule produced RNase 7, as did acutely inflamed and chronically inflamed collecting tubule. However, in contrast to the non-inflamed condition, the kidney’s proximal tubules produced RNase 7 in the setting of acute and chronic inflammation. The urine from children with bacterial infections contained significantly more RNase 7 compared to urine from uninfected children. RNase 7 was shown to be a potent, broad-spectrum antimicrobial agent against different types of bacteria (scientifically categorized as Gram-positive and Gram-negative), which are commonly found to cause urinary tract infections. RNase 7 exerts its antimicrobial activity by disrupting the bacterial cell membrane—making perforations such that the bacterium is no longer self-contained.

This study adds considerable knowledge to understanding how the urinary tract maintains sterility. Future studies that reveal the regulatory machinery involved in RNase 7 production or detail how this protein exerts its antimicrobial activity at the molecular level may help develop new therapeutic approaches to maintaining the sterility of the human urinary tract.


“Survival of the Fitness” May Mean Multiple Reservoirs for Urinary Tract Infection-causing Bacteria: A new study suggests that the source of recurrent urinary tract infection (UTI) in women is more complex than previously thought, with potential implications for therapy. UTIs are common and occur more frequently in women, many of whom suffer repeated bouts of infection. UTIs are treatable with antibiotics, but the emergence of antibiotic-resistant microbes, combined with the personal and medical costs of care, makes finding better therapeutic strategies a priority. The primary culprit in UTIs is a bacterium called Escherichia coli (E. coli) that is found in the human gut. Most E. coli strains that live in the gut are harmless and actually play a number of beneficial roles, such as helping to prevent harmful bacteria from infecting the gut. Some E. coli, however, acquire the ability, through genetic changes, to infect the bladder, and these uropathogenic E. coli (UPEC) will cause a UTI if accidentally introduced to the urinary tract. Scientists have wondered whether genetic changes that enable E. coli to adapt to a new environment—in this case, the bladder—require a “trade-off” in which the bacteria are less fit to flourish in the gut. Answering this question could help in understanding UTIs and identifying the sites of bacterial reservoirs that could contribute to recurrent infection.

To test this idea, scientists studied 45 E. coli strains recovered from urine and fecal samples that were obtained from four women at each of three episodes of UTI. They sequenced the DNA and compared the genes, whole genome sequences, and growth and fitness profiles of the bacteria. Using this approach, the researchers uncovered two different sets of results. In two of the women, the dominant E. coli strains present
in the bladder and gut appeared to be genetically the same and did not change across the three UTI episodes. In the other two women, the dominant bacterial strains in the bladder and gut were also the same or very similar within each UTI episode, but changed between the first and third UTI episode. When the researchers compared the genetic profiles of strains from one woman whose bacteria changed between the first and third UTI, the results suggested that \textit{E. coli} from the third episode would be better at infecting the bladder than \textit{E. coli} from the first episode—and, thus, might be comparatively weaker in their ability to colonize the gut. However, when the scientists experimentally introduced the two strains into the bladder and gut of mice, they found that \textit{E. coli} from the third episode grew more robustly than \textit{E. coli} from the first episode not only in the mouse bladder, but also in the mouse gut—suggesting that greater infectivity in the bladder did not require a fitness trade off for growth in the gut. Previous research in rodent models has shown that some UPEC have the ability to “hide out” within bladder cells only to reemerge later—a possible source of recurrent infection. While this study focused on samples from a small number of women, the findings suggest that \textit{E. coli} well-suited to cause UTIs may exist and flourish simultaneously in both the gut and the bladder, an aspect of UPEC that can be explored further as researchers consider how to design effective preventive and therapeutic strategies to combat recurrent UTIs.


**BLADDER CONTROL AND OTHER LOWER URINARY TRACT SYMPTOMS**

**Feeling What You Drink:** A new report suggests that limiting intake of caffeinated beverages may help stave off troublesome bladder and urinary symptoms. Many women and men live with symptoms affecting the lower urinary tract, such as frequent or urgent urination, needing to get up multiple times at night to urinate, and problems with voiding, such as a weak urinary stream or failure to empty the bladder completely. Dietary advice for people with these symptoms often includes avoiding caffeinated, carbonated, and citrus beverages because these drinks can irritate the bladder and therefore might also contribute to lower urinary tract symptoms; however, direct evidence for this association is limited. The Boston Area Community Health Survey (BACH) is a population-based study in white, Hispanic, and non-Hispanic black adults designed to assess prevalence and determinants of urological symptoms. Researchers analyzed dietary and symptom data collected from over 4,000 BACH study participants at both study entry (baseline) and about five years later to see if they could uncover any relationships between types and amounts of beverages (coffee, juice, and carbonated soda, including diet sodas and decaffeinated/caffeine free coffee and soda) and several lower urinary tract symptoms. They found that men reporting higher average caffeinated coffee or total caffeine consumption in the year prior to baseline—\textit{e.g.}, more than two cups of coffee per day \textit{versus} none—had a greater likelihood of symptom progression five years later, particularly symptoms of frequency and urgency. Drinking citrus juice, however, was associated with lower risk of symptom progression in men.

When they looked at changes in beverage consumption, the researchers found that women and men who increased their total coffee intake by at least two servings per day between baseline and the five year follow-up were more likely to have progression of urgency and frequency symptoms compared to those who had smaller changes in consumption; also, women who increased their total consumption of soda by at least two servings per day were more likely to have worsening of urgency symptoms. The researchers also examined short-term relationships between beverage intake and symptoms, and found that women and men who drank more than two cups of coffee or soda per day within the week prior to symptom assessment were more likely to have symptoms than those who did not; caffeinated diet soda appeared to affect women’s symptoms at even lower consumption. While additional studies are needed to
verify these observations, the findings support current recommendations about limiting coffee and soda intake to help manage lower urinary tract symptoms and suggest that there are dietary components to be further explored for how they may cause urologic symptoms or, in the case of citrus juice consumption by men, possibly provide protection.


Mouse Model Provides New Insight into Bladder Control: Recent research has identified a protein, β1-integrin, as having an essential role in bladder control in mice. The inner surface of the urinary tract is lined with epithelial tissue, or urothelium, which functions as a barrier to bacteria, environmental carcinogens, toxins, and the numerous and variable waste products in urine. The bladder is a balloon-shaped organ that stores and releases urine. The bladder muscle relaxes and stretches when it fills with urine, and it squeezes when it is time to urinate. As the bladder fills with urine, nerves carry signals about its change in shape and stretching—mechanosensory signals—to let the brain know when the bladder is full. Nerves also carry signals from the brain to tell the bladder when it is time to urinate. Improperly operating signals can lead to one of several conditions, including urinary frequency, urinary urgency, and urinary incontinence. Urinary frequency is an excessive number of urinations. Urinary urgency is the sudden, strong need to urinate immediately. Urinary incontinence (UI) is the unintentional leakage of urine.

Scientists have studied the ability of mice to maintain bladder control. A group of mice was genetically modified to no longer produce β1-integrin in the urothelium; a second group served as a normal population. β1-integrin, a member of a family of proteins called integrins, helps to anchor cells to the surrounding tissue. Surprisingly, the bladders of mice lacking the integrin were found to be normal in appearance. However, in contrast to normal mice, mice lacking β1 integrin were found to have several abnormal bladder conditions that may reflect urinary incontinence, urinary frequency, and urinary urgency, and their bladders filled beyond normal limits before triggering urination. The study’s findings in mice strongly suggest that loss of β1-integrin signaling in urothelium results in abnormal mechanosensory activity; information about the bladder’s shape and level of fullness is not properly relayed or triggers improper responses. Future studies could explore whether people with urinary frequency, urinary urgency, and urinary incontinence also have abnormal integrin signaling in their urothelium. If the human bladder works similarly, then loss of normal urothelium mechanosensory signals due to disease or injury may lead to some forms of urinary frequency, urinary urgency, and urinary incontinence in people, and treatments based on this knowledge may follow.


Clinical Trial Follow‑Up Yields New Information on Surgery for Urinary Incontinence: A new report from a clinical trial comparing two surgeries to treat stress urinary incontinence (SUI) in women suggests that continued surveillance of outcomes is important in these patients. Women with SUI experience urine leakage under physical stress, such as coughing, laughing, sneezing, or lifting heavy objects. One treatment option for women with SUI is surgery to help prevent leakage; however, not much is known about how well different surgical approaches compare in terms of outcomes, both in the short and long term. The Trial Of Mid-Urethral Slings (TOMUS) study was conducted to compare the outcomes of two minimally invasive surgical sling procedures approved by the U.S. Food and Drug Administration to treat SUI in women. Both procedures use a synthetic mesh sling to support the urethra (the tube through which urine passes from the bladder to outside the body), thereby preventing urine leakage under stress; the procedures differ in how the mesh sling is inserted. In the trial, researchers randomly assigned nearly 600 women with SUI to either “retropubic” or “transobturator” midurethral
sling surgery, and then compared rates of treatment success at 12 and 24 months post-operatively. The trial used two measures of treatment success: surgery was considered an objective success if women had no leakage during a stress test and 24 hour pad test, and also had no retreatment for SUI; it was considered a subjective success if women did not self-report SUI symptoms, leakage, or a need for retreatment.

Previously, researchers reported the one-year follow-up results from TOMUS, which showed that both procedures help women achieve similar levels of dryness as measured by objective success measures; self-reported outcomes, although similar, did not meet the trial criteria for equivalence.

Now, the researchers have reported that, after 24 months and a modest drop in success rates for both procedures, the two procedures are no longer equivalent by either success measure; however, the researchers did not find clear enough differences between the success rates of the procedures to be able to recommend one procedure over another. Importantly, the TOMUS study also captured the risks and side effects of each type of surgery. For example, at 24 months, the group that underwent retropubic surgery had higher rates of voiding dysfunction requiring surgery, as well as more urinary tract infections; in contrast, women who had the transobturator surgery were more likely to experience neurological symptoms. Although the majority of adverse health events occurred in the first 12 months post-surgery, onset of one serious complication, mesh exposure through a surgical incision site in the vaginal wall, differed in its timing between the two surgeries: its occurrence was more likely within 12 months of retropubic surgery, but within the 13 to 24 month period after transobturator surgery. Still, participant satisfaction with both surgical procedures remained high, with accompanying improvement in symptom severity and quality of life measures.

Overall, these new findings from the TOMUS study highlight the evolution of outcomes and the continued occurrence of complications over time, and therefore suggest that continued follow up is important in women who have mid-urethral sling surgery for SUI.


UNDERSTANDING AND TREATING HEMATOLOGICAL DISORDERS

Potential New Prevention or Treatment Approach to Iron Overload: Mini-hepcidins may provide benefit to people at risk for iron overload or people with iron overload. Hepcidin, a hormone produced by the liver, is the master regulator of iron balance in humans and other mammals. Hepcidin inhibits iron transport from cells in the intestine by binding to the iron channel, ferroportin, thereby reducing dietary iron absorption into the body. Insufficient levels of hepcidin cause or contribute to iron overload anemias such as hereditary hemochromatosis. Hepcidin deficiency is the cause of most cases of iron overload in people with hemochromatosis, a disorder in which the body absorbs too much iron and the extra iron builds up in organs. Strategies that increase the effective level of hepcidin might help ameliorate or prevent damage to organs, including the liver, heart, or pancreas for individuals with hemochromatosis and potentially other conditions marked by iron overload.

Building on previous research that demonstrated that miniature forms of hepcidin (fragments from one end of this hormone) could mimic hepcidin activity in mice, the same group of investigators synthesized a number of different versions of miniature hepcidins, tested these to identify an optimized “mini-hepcidin” called “PR65,” and assessed its ability to prevent or treat iron overload in mice. Initially, PR65 was found to have superior potency and long-lasting action compared with natural hepcidin in normal laboratory mice.

Subsequent experiments utilized genetically engineered mice that no longer produced hepcidin. As hepcidin inhibits iron absorption, mice lacking hepcidin would be expected to absorb and store increased levels of iron. These mice were fed an iron-deficient diet for eight weeks and then placed on a diet high in iron for two weeks while simultaneously being given daily...
injections of either PR65 or control injection (without PR65). Under these conditions, PR65 significantly reduced blood iron levels for up to 24 hours. In addition, PR65 prevented iron accumulation in the heart, liver, and blood of mice initially placed on an iron-depletion diet and then challenged with an iron-loading diet. In mice “pre-loaded” with iron using a standard diet, injection of PR65 daily for two weeks significantly reduced liver iron content by 20 percent but did not reduce iron content in the heart or blood.

Past investments in basic science research provided the foundation for this exciting study. Ongoing research is evaluating this and other promising compounds to effectively combat iron-related blood disorders.


New Research Reveals How Chemotherapy Blunts Blood Cell Regeneration: A recent study conducted in mice has shown that chemotherapy damages nerves that regulate bone marrow niches responsible for making new blood cells (hematopoiesis). The hematopoietic niche of the bone marrow supports the survival and self-renewal of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs), yet prevents the ill-timed release of these cells into the circulation. When used to treat cancers such as non-Hodgkin’s lymphoma and multiple myeloma, high-dose chemotherapy also destroys normal cells such as the HSCs and HPCs in the bone marrow. The result of chemotherapy, therefore, is a reduced hematopoietic reserve and function. However, the underlying cause remains unresolved.

This study shows for the first time that chemotherapy is toxic to the nerves that interact with (innervate) the hematopoietic niche. The experimental model assessed the ability of mice whose HSCs and HPCs have been destroyed (in this case, by irradiation) to restore their hematopoietic reserve and function under various conditions. Mice without HSCs and HPCs are likely to die unless transplanted with bone marrow cells from another normal animal. In one set of experiments, mice were or were not subjected to chemotherapy prior to irradiation and then transplanted with bone marrow cells. Compared to mice not subjected to chemotherapy, chemotherapy-treated mice were significantly more likely to die and contained fewer nerves innervating the hematopoietic niche.

To confirm that chemotherapy causes nerve toxicity in the bone marrow, the investigators studied the effect of a drug called 6-hydroxydopamine, which is known to selectively destroy “noradrenergic” nerves. Mice treated with 6-hydroxydopamine, a level of radiation that destroyed their HSCs and HPCs, and bone marrow cell transplantation showed a significant decrease in survival and delayed hematopoietic recovery compared to a group of mice not treated with the drug. Thus, this experimental model provides evidence that noradrenergic nerves are required for normal hematopoietic function. The loss of nerves contributes to the inability of the transplanted bone marrow cells to home to the bone marrow and take up residence in the hematopoietic niche.

The scientists next tested whether a known nerve protective agent such as 4-methylcatechol (4-MC) would mitigate the harmful effects of chemotherapy on hematopoietic nerves. In mice treated with chemotherapy and 4-MC, and subjected to lethal irradiation and bone marrow transplant, the number of hematopoietic nerves was significantly more than that found in mice not treated with 4-MC. In addition, the mice treated with 4-MC had improved survival. Thus the addition of 4-MC acts to maintain hematopoietic function by specifically protecting nerves which innervate the bone marrow. Overall, these results in mice may lead to future research in humans to explore ways to reduce nerve damage so as to improve blood cell regeneration after chemotherapy.


Improving White Blood Cell Defense Against Bacteria: A recent study has shown that deletion of a protein in white blood cells improves their ability to eradicate infections with the bacteria Staphylococcus
*aureus* (*S. aureus*), although not the fungus *Aspergillus fumigatus* (*A. fumigatus*), in an animal model of chronic granulomatous disease (CGD).

CGD is a disorder that causes the immune system to malfunction, resulting in a form of immunodeficiency. Individuals with CGD have recurrent bacterial and fungal infections, and often have areas of inflammation (granulomas) in various tissues that can cause damage. The body’s white blood cells normally eliminate bacteria via two modes of action—a non-oxidative mode of action that involves the use of specialized enzymes to attack and cleave proteins that are necessary for bacterial survival, and an oxidative mode involving chemically reactive molecules containing oxygen. It is the oxidative mode of defense that is defective in patients with CGD.

Building on their previous research findings showing that a protein called “olfactomedin-4” (Olfm4) hinders white blood cells’ ability to eradicate bacteria, the researchers deleted the *Olfm4* gene in a mouse model of CGD and evaluated the impact of this deletion on host defense against *S. aureus* and *A. fumigatus*, both of which are common causes of infections in people with CGD. White blood cells obtained from mice lacking Olfm4 protein showed increased ability to kill *S. aureus* compared to white blood cells having Olfm4. Likewise, when mice lacking Olfm4 were exposed to *S. aureus* for six hours, they killed significantly more bacteria than mice with Olfm4.

Next, the investigators examined whether deletion of *Olfm4* in CGD mice enhanced host defense against infections of *S. aureus* and *A. fumigatus* as measured by survival over a two week testing period. When infected with *S. aureus*, all CGD mice with Olfm4 died within five days, while the majority (approximately 85 percent) of CGD mice without Olfm4 survived the two-week testing period. Notably, approximately 75 percent of normal mice with Olfm4 died within eight days. When infected with *A. fumigatus*, all CGD mice died within nine days, whether or not they had Olfm4. In contrast, all normal mice with or without Olfm4 survived the two week testing period following *A. fumigatus* infection. These results show that *Olfm4* deletion can enhance host defense against *S. aureus*, but not *A. fumigatus*, in CGD mice. Future studies are needed to determine the role of Olfm4 in human white blood cells and could lead to the development of a therapeutic inhibitor of Olfm4 activity to boost human defense against infection.


**Improving Blood Stem Cell Transplantation:**

A recent study conducted in mice, baboons, and human volunteers has shown that a non-steroidal anti-inflammatory drug approved by the U.S. Food and Drug Administration (FDA), called meloxicam, significantly increased the number of blood (hematopoietic) stem cells (HSCs) and their descendent hematopoietic progenitor cells (HPCs) entering the circulation from the bone marrow, where they typically reside until needed. This finding sheds light on how the body responds to injury and has implications for blood cell transplantation. The hematopoietic niche of the bone marrow supports the survival and self-renewal of HSCs and HPCs, yet prevents the ill-timed release of these cells into the circulation. When used to treat cancers such as non-Hodgkin’s lymphoma and multiple myeloma, high-dose chemotherapy also destroys normal cells such as the HSCs and HPCs in the bone marrow. To replenish the lost cells, HSCs and HPCs are routinely harvested from a donor’s or patient’s blood, and then transplanted back into the patient at the conclusion of the chemotherapy procedure to repopulate the bone marrow. The levels of HSCs and HPCs normally found in blood are very low, and strategies have been devised to mobilize HSCs and progenitor cells out of the bone marrow and into the circulation. The naturally occurring protein G-CSF often is used clinically to mobilize cells, but this strategy does not work for approximately 10 to 20 percent of individuals. Research continues in order to identify more effective strategies to mobilize cells out of the microenvironment of the marrow and into the circulation.
Building on their previous research findings, which showed that prostaglandin E2 enhanced HSC survival and homing to the bone marrow, researchers demonstrated that meloxicam treatment of mice greatly increased the numbers HSCs and HPCs in the circulation. Mice treated with a combination of G-CSF and meloxicam mobilized significantly more cells than those treated with either drug separately. Similar to findings in mice, meloxicam treatment of both baboons and healthy human volunteers increased HSCs and HPCs in the circulation. Additional experiments were conducted to determine how meloxicam exerts its biological effect. Previous research showed that prostaglandin E2 signals through one or more of the four E-prostanoid (EP1-4) receptors. Using genetically altered mouse strains each lacking one of the EP receptors, it was shown that mice lacking EP4 receptor had increased HSCs and HPCs in the circulation and meloxicam had no additional effect. These findings strongly suggest that meloxicam targets the prostaglandin E2/EP4 receptor signaling pathway.

Moreover, the researchers showed that mice deficient in osteopontin, a component of the HSC niche, mobilized HPCs but not HSCs when treated with meloxicam. This result demonstrates that meloxicam decreases osteopontin levels in the HSC niche, which then permits HSC, but not HPC, mobilization out of the marrow.

This study has several potentially important clinical implications. Meloxicam in combination with G-CSF may improve the success rates of blood stem cell transplantation by making it easier to obtain sufficient numbers of cells for transplant. Meloxicam is FDA-approved for use and therefore additional toxicology studies need not be conducted, saving both time and money. And, meloxicam has comparatively few side effects compared to other non-steroidal anti-inflammatory drugs.

Kidney Research National Dialogue

To inform Institute efforts, the NIDDK historically has solicited input from the research community, voluntary and professional organizations, and the public to identify research areas of particular opportunity or challenge. Recently, the NIDDK’s Division of Kidney, Urologic, and Hematologic Diseases initiated an effort to seek input from stakeholders that would help the Institute identify and prioritize goals in kidney disease research. To do so, the Institute developed an interactive website: “The Kidney Research National Dialogue,” or KRND.

The NIDDK used a Web-based interface to invite people who had an interest in kidney disease—scientists and clinicians, patients and their families, and others who wanted to contribute—to join in a national information exchange. Participants were asked to put forward their own ideas about the most exciting areas of kidney disease research, to read and comment on discussions from others about these ideas, and ultimately to prioritize these ideas by “voting” on the topics that they believed to be the most important. More than 1,600 participants posted approximately 300 ideas that had been broken out into 12 topic areas that cover the breadth of kidney physiology and disease, including: diabetic nephropathy, acute kidney injury (AKI), CKD, kidney biology, dialysis therapies, disease education, polycystic kidney disease, glomerular disease, pediatric kidney disease, and training.

The objective of the KRND was to engage a large number of people in a discussion of kidney disease, and to identify research strategies that would improve our understanding of normal kidney function and the mechanisms underlying kidney disease. The ideas emanating from these discussions are being distilled and published in a series of commentaries on different topics—making them available to the broader community interested in kidney disease research. The first commentaries were published in late 2013.

The KRND is a new, alternative approach in the evolution of the NIDDK’s strategic planning process. The extended, open, Web-based dialogue used in the KRND allowed a large number of people to contribute to multiple simultaneous discussions that cut across diverse topic areas, and to do so at a time that was convenient for them. This Web-based approach was successful in compiling numerous posts and comments from a large, heterogeneous group of stakeholders whose expertise and interests spanned the kidney disease spectrum. The Institute is grateful for their input.

As of December 2013, three commentaries had been published (see below), another three have either been submitted or are in press, and the remaining ones were under development.


Urothelium and Human Disease
Dr. Mark L. Zeidel

Dr. Mark L. Zeidel is the Herman Ludwig Blumgart Professor of Medicine at Harvard Medical School, and Physician-in-Chief and Chairman of the Department of Medicine at the Beth Israel Deaconess Medical Center. He is a widely recognized scientist, clinician and teacher, known for his research in the area of epithelial biology and water transport. Dr. Zeidel has been recognized by his peers for numerous accomplishments, including elected membership in the American Society of Clinical Investigation and the Association of American Physicians. A graduate of Yale College, he earned his M.D. from Columbia University College of Physicians and Surgeons. He trained in internal medicine and nephrology at Brigham and Women’s Hospital, and was chief of the renal section of the West Roxbury VA, and Assistant Professor of Medicine at Harvard. He then moved to the University of Pittsburgh School of Medicine in 1993, where he served as chief of the renal and electrolyte division of the Department of Medicine, and then as the Jack D. Myers Professor and Chair of the Department of Medicine. He has been an NIDDK-supported researcher for the past 24 years. At the September 2013 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Zeidel presented a lecture on the role of the bladder urothelium in urological diseases.

To maintain a constant internal environment, or homeostasis, all organisms must regulate the flow of water across their membranes. The ability of water to flow through membranes varies greatly—by up to 4 orders of magnitude—and this process enables such necessities as preservation of normal blood composition. One example of abnormal water flow is when kidneys fail to reabsorb water, causing diabetes insipidus, which is characterized by frequent urination and excessive thirst (but normal blood sugar levels, unlike more common forms of diabetes). Dr. Zeidel’s research group has led efforts to understand how specialized cells regulate ability of water and other molecules to flow through membranes; specifically how the urothelium—the lining of the urinary tract, including the bladder—maintains its homeostasis, and to gain insight into the diseases which affect the urothelium.

The Urothelium
The lower urinary tract is primarily made up of the ureter (the tube that connects the kidney to the bladder), bladder, and urethra (tube that carries urine from the bladder to the outside). Lining the lower urinary tract is tissue called the urothelium, which functions as a barrier to bacteria, environmental carcinogens, toxins, and the highly variable waste products in urine. The urothelium consists of three cell layers called the umbrella, intermediate, and basal cells. It is the outer (apical) surface of the umbrella cell which comes into direct contact with urine.

The urothelium’s umbrella cell apical membrane creates a barrier that prevents water, urea, ammonia, and carbon dioxide from leaving the urine and entering the bladder tissue. To accomplish this, the umbrella cell apical membrane employs two approaches. First, membranes contain fat molecules (lipids) that are tightly packed into the bilayers of the membrane, and can further increase barrier function when a certain type of fat, cholesterol, is present. Second, the apical
SCIENTIFIC PRESENTATION

The surface of these cells contains plaques that cover the majority of the membrane surface of the bladder. The plaques consist of an ordered arrangement of proteins called uroplakins, which also contribute to the barrier function of the umbrella cell apical membrane.

In studies using mice that no longer produce uroplakins in their bladders, Dr. Zeidel, working with Dr. Tung-Tien Sun, of New York University School of Medicine, and colleagues showed a large increase in apical membrane water transport (permeability). This finding confirms that uroplakins contribute to barrier function of the umbrella cell apical membrane. In addition, Dr. Zeidel and colleagues have shown the importance of the umbrella cell layer to regulate permeability using an animal model of urothelium injury. Using a chemical called protamine sulfate which strips the umbrella cell layer off the urothelium, it was shown that removal of the umbrella cell leads to increased water and urea transport. Of note, the barrier function is rapidly restored within 3-5 days in the animal model.

The bladder is a very flexible, balloon-shaped organ that stores and releases urine. The bladder muscle relaxes and stretches when it fills with urine, and it contracts when it’s time to urinate. As the bladder fills with urine, nerves carry signals about changes in bladder shape and stretching, i.e., mechanosensory signals, to let the brain know when the bladder is full. Nerves also carry signals from the brain to tell the bladder when it’s time to urinate. Thus, the apical membrane of the umbrella cell needs to be very flexible as the urothelium transitions from a “globular” structure when the bladder is empty to a “flagstone” shape when the bladder is full.

In studies conducted in collaboration with Dr. Jeffrey Fredberg at the Harvard School of Public Health, Dr. Zeidel has shown that the umbrella cell’s apical membrane is one of the most flexible membranes known. To measure flexibility, magnetic beads were attached to the apical surface of urothelium membrane and subjected to magnetic fields, and their movement was captured using video microscopy. Both urothelium and red blood cells were found to be very responsive to the magnetic field. Moreover, in studies using mice that no longer produce uroplakins in their bladders, the urothelium was determined to be more rigid—losing a significant portion of its flexibility.

Also contributing to the flexibility of the urothelium is the uneven distribution of actin. One of the most common proteins found in mammals, actin has many functions, including its role in maintaining the cell’s proper shape. Dr. Zeidel and colleagues found no detectable actin in the umbrella cell’s apical membrane in contrast to a different part of the umbrella cell membrane, called the “basolateral” membrane, which comes into contact with adjoining cells. This lack of actin in the apical portion of the membrane is thought to allow for more flexibility as the bladder expands and contracts.

Insights into the Role of the Urothelium in Disease

Dr. Zeidel then described several experiments conducted by Dr. Warren Hill and his collaborators in the Department of Medicine at the Beth Israel Deaconess Medical Center, which were designed to illuminate whether the urothelium “communicates” with the nervous system to regulate bladder control. The scientists compared the ability of two groups of mice to maintain bladder control. One group of mice was genetically modified to no longer produce β1-integrin in the urothelium and the other group of mice served as a normal population. β1-integrin, a member of the integrin family of proteins, helps to anchor cells to the surrounding tissue. Surprisingly, the bladders of mice lacking the integrin were found to be normal...
in appearance. However, in contrast to normal mice, mice lacking β1-integrin were found to have several abnormal bladder conditions that may reflect urinary incontinence, urinary frequency, and urinary urgency, and their bladders filled beyond normal limits before triggering urination. Urinary incontinence is the unintentional leakage of urine. Urinary frequency is an excessive number of urinations. Urinary urgency is the sudden, strong need to urinate immediately. Thus, the bladder urothelium senses bladder fullness and communicates this information through the β1-integrin to nerves that connect to the bladder.

An acute urinary tract infection begins when bacteria attach to the bladder urothelium. 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 bladder of bacteria. Dr. Zeidel showed images, published by Dr. Scott J. Hultgren and colleagues of Washington University in St. Louis, of bacteria attaching to mouse bladder urothelium. The bacteria use adhesive structures called “type 1 pili” which serve to tether the bacteria to the apical surface of the umbrella cell. Once attached, the umbrella cell “engulfs” the bacteria and this sets in motion a host immune response to eliminate the infected cell.

**Urothelium and Human Disease**

Human diseases and disorders of the urothelium include cystitis, lower urinary tract syndrome (LUTS), and urothelial cancer. Cystitis, LUTS, and urothelial cancer are debilitating, burdensome, and costly conditions and in the case of urothelial cancer can be life-threatening.

Cystitis is the inflammation of the bladder and can be caused by bacterial infection or chemical exposure. Interstitial cystitis/painful bladder syndrome (IC/PBS) or bladder pain syndrome is one of several conditions that causes bladder pain and a need to urinate frequently and urgently. Both men and women can acquire IC/PBS, though twice as many women are affected as men. It can occur at any age, but it is most common in middle age.

LUTS is a syndrome featuring symptoms which occur during urine storage, voiding, or post-voiding. The symptoms include urine leakage, frequent and/or painful urination, sudden and/or strong urges to urinate, problems starting a urine stream, and/or problems emptying the bladder completely. Dr. Zeidel presented data obtained from the Epidemiology of LUTS (EpiLUTS) study, which was a large population study of the prevalence of LUTS in women and men over 40 years old. Although men and women both report having symptoms such as leakage and weak stream, the study findings indicate that LUTS impacts both men and women in very different but nonetheless very bothersome ways.

Dr. Zeidel suggested that several different abnormal mechanisms could be responsible for LUTS, including those targeting the urothelium such as barrier failure, inadequate repair, and poor sensing. In addition, the bladder wall’s detrusor muscle may be deformed or in spasm. Due to the potential numerous causes of LUTS, therapies to address each of these have been developed and range from limiting fluid intake, to botulinum toxin injections to surgery. Dr. Zeidel commented that one need only go to a grocery store to find that the availability of diapers targeting adults now rivals that for infants. Drawing from his clinical expertise, Dr. Zeidel offered the following observations regarding LUTS. First, LUTS is extremely common and becomes more severe with aging. Second, the symptoms and their severity vary enormously between individual patients, and environmental elements play
a pivotal role. And third, the wide array of therapies, aimed at some but not all of the elements of normal voiding, indicate that we know little about the mechanisms of LUTS in individual patients.

**Current and Future Research Directions**
Dr. Zeidel’s current research efforts include the development of two new mouse lines: one line that has a defined genetic background and the other line having a more mixed, or heterozygous genetic background (e.g., similar to human genetics). These new laboratory resources will then be used to define the genetic contribution in mice displaying LUTS, particularly in older mice, which will increase our understanding of these symptoms, foster the design of new treatments, and ultimately pave the way to test prevention strategies.
IgA Nephropathy—Shedding Light on a Form of Kidney Disease

Immunoglobulin A nephropathy (IgAN) is a kidney disorder that occurs when a complex of two immune system proteins that helps the body fight infections settles in the kidneys, eventually disrupting kidney function. While the mechanisms underlying this condition have remained mysterious, recent NIDDK-supported discoveries have provided important insights into molecular mechanisms that play an important role in this disease.

What Is IgAN?

IgAN is caused by the abnormal deposition of immune system proteins in the small blood vessels within the kidneys. These proteins, which are types of antibodies called IgA and IgG, normally help the body fight infections, but when they form abnormal complexes that settle in the kidneys, serious problems can result. As these protein complexes accumulate over many years, they can lead to inflammation and scarring of the kidneys' filtering units, or glomeruli. This damage, along with the accompanying development of fibrotic scar tissue, can impair the kidneys' ability to effectively filter waste products and excess fluids and salts from the blood, and result in the leakage of blood and sometimes protein into the urine. Unchecked, this process can lead to a decline in kidney function or to kidney failure.

Because these proteins are part of the immune system, IgAN is considered to be an “autoimmune” disease. In conditions such as this, the body’s defense mechanism that is designed to protect against outside threats, such as bacteria and viruses, somehow turns inward, attacking the body’s own cells or molecules.

As is the case with many forms of chronic kidney disease, IgAN is often a “silent” condition. Many people with IgAN will not show symptoms for years, or perhaps decades. Unfortunately, about 25 percent of adults with IgAN will develop kidney failure, also called end-stage renal disease, requiring dialysis or a kidney transplant to live.¹

IgAN can occur at any age, though it is less common in the very young and the very old. It is more likely to occur in men than in women. It is more common among Caucasians and Asians, and less common among African Americans.

Currently, there is no treatment that specifically addresses the disease mechanism that underlies IgAN. In formulating treatment strategies, physicians aim to ameliorate the consequences of the disease, using approaches that reduce tissue scarring in the kidney and controlling blood pressure (which, when elevated, can also result in kidney damage). However, recent discoveries have opened a window through which researchers are able to glimpse hints of the disease’s underlying cause, providing hope for those seeking new treatments, whether they are patients or investigators.

History and Pathogenesis of IgAN
IgAN was first identified as a distinct clinical diagnosis in 1968, based on the accumulation in the glomeruli of the proteins IgA and IgG. IgAN remains a diagnosis that is characterized by the identification of deposits of these proteins in kidney biopsy tissue.

IgA, IgG, and Immune Complexes
Research results published in the early- to mid-1990s suggested that patients with IgAN had IgA antibodies that were deficient in a sugar called galactose. Specifically, researchers showed that IgA from the blood of patients with IgAN is deficient in galactose and that this deficiency occurs in a specific part of the antibody called the hinge region. The deficiency of galactose in the hinge region of IgA unmasks the presence of GalNAc (a sugar derivative of galactose), which is subsequently recognized by naturally occurring IgG and IgA antibodies in the circulation. Hence, an autoimmune condition is established when normal IgG and IgA antibodies now recognize the aberrant IgA as foreign and form immune complexes. In a study of European patients with IgAN, researchers found that increased blood levels of both normal IgA and IgG paralleled the severity of disease progression. In addition, patients with the highest levels of IgG against the aberrant IgA antibody at the time of diagnosis had the highest risk of kidney failure. In a similar study conducted in a Chinese population with IgAN, elevated blood levels of aberrant IgA were found to be associated with poor prognosis.

To understand and explore the mechanism(s) that lead to the formation of galactose-deficient IgA associated with IgAN, cell lines from circulating IgA1-producing B lymphocytes derived from patients with IgAN were established in the laboratory. The cell lines were shown to produce galactose-deficient IgA and allowed for the identification of the specific step in the biological pathway responsible for the addition of galactose to the IgA antibody. Thus, these cell lines may be a valuable resource in the development of new therapeutic strategies for IgAN. As IgAN disease onset and progression often coincides with an upper respiratory tract infection, the researchers evaluated immune-system regulatory molecules called cytokines for their ability to add sugar molecules such as galactose to IgA using the above mentioned cell lines. Cytokines are released from immune cells such as monocytes and macrophages. The study findings indicated that the cytokines interleukin-6 and (to a lesser extent) interleukin-4 significantly worsened galactose deficiency of IgA via the coordinated regulation of key enzymes.

Further research investigating the IgG antibody from IgAN patients using a cell culture approach revealed a change in the amino acid structure of the antibody; the IgG antibody has an “alanine” to “serine” amino acid substitution in the part of the antibody important for binding to its target—the aberrant IgA. In a proof of principle experiment, the investigators genetically engineered the serine-substituted IgG to contain the normal alanine amino acid and assessed its ability to bind aberrant IgA. The alanine-containing IgG was determined to have dramatically less binding capacity for aberrant IgA.

IgA Deposits
Researchers have shown that immune complexes containing aberrant IgA specifically associated with human mesangial cells more efficiently in laboratory experiments than did aberrant IgA alone. Mesangial cells are located within the central portion of the glomerulus and provide structural support. In addition, a greater amount of circulating immune complexes from a patient with IgAN bound to mesangial cells than did immune complexes from a healthy volunteer.
Because mesangial cells are critical for kidney glomerular function, investigators systematically evaluated kidney biopsy tissues from patients with IgAN to begin to discover the molecular mechanisms that take place when IgA deposits in the mesangium. Previous research showed that many tissues and cell types use the "MAPK/ERK" signaling pathway in processes such as survival, inflammatory responses, and cell growth control, and investigators sought to determine whether this pathway was involved when IgA deposits in the mesangium. The study showed that the MAPK/ERK signaling pathway was activated in the mesangium of patients with IgAN having significant loss of kidney function. In contrast, kidney biopsy tissues from IgAN patients having better kidney function did not show activation of the MAPK/ERK signaling pathway.

**Genetics of Disease**

Researchers measured aberrant IgA levels in patients with IgAN, their relatives, and other volunteers without the disease. High levels of aberrant IgA were detected in blood from patients with IgAN compared to controls. Somewhat surprisingly, approximately half of the family members of IgAN patients also had elevated levels of aberrant IgA but did not display disease symptoms. The study results suggest that the defect in sugar addition to IgA antibodies is an inherited trait, but that additional factors—either genetic or environmental—are required for kidney disease to develop. A genome-wide association study of people of Chinese and European ancestry with IgAN has identified several regions of the genome (loci) associated with this disease. Genome-wide association studies involve rapidly scanning markers across the genome to find genetic variations associated with a particular disease. Scientists conducting these types of studies analyze genetic differences between people with a particular disease and healthy people.

Of the five loci contributing to significant risk for IgAN, one localized to the major histocompatibility complex (MHC)—a cluster of genes that play an important role in the immune system. MHC determines compatibility of donors for organ transplant as well as one’s susceptibility to an autoimmune disease via crossreacting immunization.

The other loci contain genes associated with the immune response, leading to models of the disease as a multi-step process. Producing high levels of aberrant IgA can lead to the disease in individuals who are genetically predisposed to develop kidney injury due to an overactive or aberrant immune or inflammatory response.

**Looking Forward**

Despite recent progress toward discovering the molecular underpinnings of this multifaceted disease, an effective treatment for IgAN remains elusive. The NIDDK continues its robust support of basic research into normal kidney function and clinical research into the diseases that impair normal kidney function at the cellular and molecular levels. For example, the Institute established the “Cure Glomerulopathy Network” (CureGN) consortium in 2013 to support translational and clinical research that promotes therapeutic development for primary glomerular diseases, including IgAN (https://curegn.org). Research studies of families in which IgAN is prevalent continue in order to identify and understand the genes and genetic factors that influence disease onset and progression.

Through multiple avenues of research, the NIDDK aims to advance progress toward the development of new intervention strategies.