

**NATIONAL INSTITUTE
OF
DIABETES AND DIGESTIVE AND KIDNEY DISEASES**

RESEARCH PROGRESS REVIEWS

FOR

FY 1999 PROGRAM PLAN

**Submitted to
The National Diabetes and Digestive
and Kidney Diseases Advisory Council
February 1999**



NOTE TO MEMBERS OF THE NATIONAL DIABETES AND DIGESTIVE AND KIDNEY
DISEASES ADVISORY COUNCIL

The document entitled "NIDDK FY 1999 Research Progress Reviews," is attached for your information. The reviews primarily describe examples of recent progress in biomedical research that has resulted from investigations supported by the NIDDK. This document is developed by our program staff and division directors, in consultation with their respective communities. Only a fraction of the many outstanding accomplishments of our grantees is presented.

All of us can take great pride in these accomplishments -- the fruits of our continued investment in this nation's biomedical research enterprise. We hope you will share this part of our Program Plan with your colleagues and friends in the scientific and professional community.

Over the years we have made a number of changes in the planning process and we are always interested in suggestions from you and others in the research and voluntary health community about ways to improve the process further.

A handwritten signature in black ink that reads "Phillip Gorden".

Phillip Gorden, M.D.
Director

Attachment

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE
AND KIDNEY DISEASES

FY 1999 PROGRAM PLAN

Research Progress Reviews

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DIVISION OF DIABETES, ENDOCRINOLOGY, AND METABOLIC DISEASES

FY 1999 Program Plan
RESEARCH PROGRESS REVIEWS
February 1999 Council

Richard C. Eastman, M.D., Director

DIVISION OF DIABETES, ENDOCRINOLOGY AND METABOLIC DISEASES

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DIABETES MELLITUS

I. TITLE: Intensive Glycemic Control Prevents, Delays or Reverses Complications of Diabetes

BACKGROUND: The Diabetes Control And Complications Trial (DCCT) established that intensive therapy of glycemic control, with a corresponding lowering of HbA1c, dramatically reduced the incidence in type 1 diabetes of microvascular complications, compared with conventional therapy. The DCCT findings showed that intensive therapy aimed at keeping blood glucose levels as close to normal as possible delayed the onset and slowed the progression of diabetic retinopathy, nephropathy, and neuropathy by 35 to 70 percent. The study also conclusively demonstrated that any sustained lowering of blood glucose reduces the risk of developing the microvascular complications of type 1 diabetes. At the close of the DCCT in 1993 all conventional treatment subjects were instructed in the use of intensive therapy and returned to their health care providers. The Epidemiology of Diabetes Interventions and Complications study (EDIC) continues to follow a large group of the original DCCT cohort of subjects to determine the long-term outcome of reduced glycemic exposure.

Although the DCCT was designed to study the effects of intensive therapy in individuals with type 1 diabetes, 90 percent to 95 percent of people with diabetes have type 2 diabetes and its prevalence is increasing. It is projected that worldwide there will be nearly 210 million individuals with this form of diabetes by the year 2010. The UKPDS was designed to determine whether intensive management of type 2 diabetes in controlling blood glucose levels resulted in a reduction in long term diabetes complications compared with standard care.

RECENT FINDINGS: The UKPDS is the largest study of individuals with type 2 diabetes ever performed, lasting 20 years and including 5,102 subjects. The study was designed to test whether intensive blood glucose control in type 2 patients reduced the risk for the macrovascular and microvascular complications of the disease compared to individuals randomized to diet therapy alone. It also tested whether a particular therapy was more effective and what the combined effects of lowering blood pressure and blood glucose were on diabetes and cardiovascular complications. The main clinical findings of this study have been reported in four papers, two in The Lancet and two in the British Medical Journal.

The UKPDS findings demonstrate that intensive control of blood glucose in type 2 diabetes significantly reduces the incidence of both retinopathy and nephropathy by 25 percent compared to standard treatment. Across all treatment groups combined it was also shown that for every 1 percent drop in

HbA1c (e.g. reducing HbA1c from 8.0 to 7.0) there was a 35 percent reduction in retinopathy, neuropathy, and nephropathy. Although there was evidence that intensive blood glucose control reduced the risk for myocardial infarction, the results were not significant for any of the interventions tested.

The results for the blood pressure lowering component of the UKPDS demonstrated the benefits of aggressive control of blood pressure in those randomized subjects that were hypertensive and diabetic compared to the group with less tight control of blood pressure. There was a 32 percent reduction in diabetes related deaths, a 44 percent reduction in strokes, and a 37 percent reduction in diabetes related microvascular end points.

Lachin et al,(1998) and Cleary et al, (1998) report convergence of HbA1c values for the original DCCT intensive and conventional treatment groups from 7.2 percent vs. 9.0 percent respectively during the DCCT to 8.1 percent and 8.3 percent respectively during the EDIC. In spite of this convergence, the former intensive treatment group had a 71-77 percent ($p<0.001$) reduced risk of worse retinopathy after four years of follow up in EDIC. There was also a reduced risk of further progression from the close of the DCCT of 72-87 percent ($p<0.001$), adjusting for the level of retinopathy at DCCT closeout. These measures of retinopathy also included the more advanced stages of severe non-proliferative and proliferative diabetic retinopathy, clinically significant macular edema, and the need for laser surgery for either retinopathy or macular edema. Risk of progression of albuminuria >40 mg/24 hours was reduced 53 percent ($p<0.001$) and progression of albuminuria >300 mg/24 hours was reduced 87 percent ($p<0.001$) in the intensive versus conventional treatment group. The relationship between HbA1c and the risk of further progression of retinopathy was assessed. In the former conventional treatment group, as mean HbA1c during the DCCT increased, there were dramatic increases in the risk of worsening retinopathy; however there was little further effect on the risk of worsening as the EDIC mean HbA1c increased. Among those in the former intensive treatment group, the risk of further worsening was weakly associated with mean HbA1c during the DCCT and not significantly with that during EDIC.

The DCCT research group examined whether there was an effect of intensive versus conventional therapy on residual B-cell function in type 1 patients who when randomized into the DCCT, were determined to have C peptide values that defined them as C peptide responders. At baseline, responders in intensive and conventional treatment groups had similar C-peptide levels. Responders receiving intensive therapy maintained a higher stimulated C-peptide level and had a lower likelihood of becoming nonresponders than responders on conventional therapy. Although C-peptide levels eventually were similar with later years of follow-up, the risk reduction in intensive versus conventionally

treated groups was 57 percent ($p < 0.001$) over the mean 6.5- years of follow-up. During the first four years of the DCCT among the intensive treatment group, responders had significantly lower HbA1c levels than nonresponders; this trend continued with longer follow-up but was not statistically significant. Among the intensive treatment group, there was a 50 percent reduction in the risk of retinopathy in responders compared with nonresponders (relative risk=0.50, 95 percent CI=0.28-0.88); after adjustment for HbA1c level, the reduction in risk was no longer significant. Micro-albuminuria also occurred less frequently in intensively treated responders than nonresponders but was not significant. Despite lower HbA1c levels in intensively treated responders, the risk for severe hypoglycemia with seizure or coma was 65 percent (95 percent CI=53-74 percent) less in this group versus intensively treated nonresponders. No differences in the development of complications were seen between the C-peptide responders and nonresponders in the conventional treatment group.

Fioretto et al, (1998) report that in type 1 patients who receive pancreas transplants but have not received kidney transplants, the lesions of diabetic nephropathy were not ameliorated over a 5-year exposure to normoglycemia. However, 10-years after pancreas transplantation, creatinine clearance rate, thickness of the basement membranes, and the mesangial fractional volume decreased significantly to normal levels. The authors conclude that pancreas transplantation can reverse the lesions of diabetic nephropathy, but that this process exceeds 5-years of exposure to normoglycemia and is evident when measured at 10-years.

SIGNIFICANCE: Since the completion of the DCCT, the major findings that a reduction in glycemic exposure reduced the long term complications of type 1 diabetes have been extended beyond the experimental design of the DCCT and to type 2 diabetes. The original cohort of subjects from the DCCT have been followed in the Epidemiology of Diabetes Interventions and Complications (EDIC) study. This surveillance study is being conducted to determine the long term benefit of intensive glycemic management. The findings suggest that intensive therapy aimed at maintaining normal glycemic levels has a beneficial impact on long-term complications that extend beyond the period of intensive therapy. Risk of microvascular complications does not appear to be acutely affected by the prevailing level of hyperglycemia. Rather these risks are associated with the long-term chronic effects of hyperglycemia that take considerable time to become manifest and are equally slow to dissipate with reductions in hyperglycemia. Intensive therapy should be implemented as early as is safely possible in subjects with type 1 diabetes, and maintained as long as possible.

Results from the DCCT cohort of C-peptide responders and non responders

support initiating intensive therapy as early in the course of type 1 diabetes as is practical and safe. Such treatment helps sustain residual endogenous insulin secretion which, in turn, allows better metabolic control for longer periods with fewer acute and chronic complications. The ability to maintain lower HbA1c levels with relatively fewer episodes of severe hypoglycemia makes earlier intervention especially appealing.

The importance of long-term glycemic control is also demonstrated by the reversal of the lesions of diabetic nephropathy following pancreas transplantation into individuals with type 1 diabetes. However, the reversal is not apparent upon the normalization of glycemia measured at 5-years, but is seen 10-years after transplantation.

The importance of glycemic control for type 2 diabetes has been conclusively demonstrated by the UKPDS that tested whether intensive control of blood glucose in type 2 diabetes significantly reduces the risk for developing the microvascular complications of the disease. This landmark study confirms the findings of the NIDDK supported DCCT that any reduction in blood glucose reduces the risk for developing the long-term microvascular complications of type 2 diabetes.

FUTURE DIRECTIONS: The UKPDS will be followed by a 5-year post-study to investigate longer-term responses to the intensive treatment protocol. This will allow the investigators to establish whether the indication of a reduction in risk for myocardial infarction reported in the UKPDS findings will achieve the level of statistical significance.

The Epidemiology of Diabetes Interventions and Complications (EDIC) study is a long-term epidemiologic surveillance study of the original cohort of subjects from the DCCT. At the close of the DCCT, all conventional treatment subjects were instructed in the use of intensive therapy and all subjects returned to their health care providers for further diabetes care. The EDIC study group is continuing to follow EDIC participants for the effects of former treatment group assignment during the DCCT, hyperglycemia, and other risk factors on the development and progression of microvascular and macrovascular disease.

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UK Prospective Diabetes Study Group, "Efficacy of Atenolol and Captopril in Reducing Risk of Macrovascular and Microvascular Complications in Type 2 Diabetes (UKPDS 39)," BMJ 1998;317:713-722.

II. TITLE: Type 1 Diabetes Mellitus: Etiology and Pathophysiology

BACKGROUND: Type 1 diabetes mellitus is an autoimmune disease in which an individual's immune system attacks and destroys its own insulin-producing β -cells in the pancreas. Normally, the immune system functions to protect us

against foreign agents, such as bacteria and viruses. However, in certain circumstances, the immune system identifies a normal part of the body as foreign and initiates a powerful attack to destroy and remove it from the body. Type 1 diabetes results when the pancreatic β -cells are attacked and destroyed.

Type 1 diabetes is a genetic disease with environmental influences. The function of the immune system is determined, in part, by the specific human leukocyte antigen (HLA) genes which each individual inherits. Certain HLA genes have been shown to be associated with a high risk of developing diabetes, while other HLA genes appear to exert a protective effect. The HLA genes influence the function of lymphocytes, which play a major role in the etiology and pathogenesis of type 1 diabetes. There are two predominant types of lymphocytes – β -cells, which produce antibodies, and T cells, which can directly kill cells. Both T and β -cells appear to be involved in the development of type 1 diabetes.

The antibody-producing β -cells gave researchers one of the first indications that type 1 diabetes was an autoimmune disease. Autoantibodies that react specifically against pancreatic β -cells are found in new onset patients with diabetes, as well as in numerous animal models of type 1 diabetes. One of the most well-studied animal models is the non-obese diabetic (NOD) mouse. These autoantibodies are directed against a variety of components of the β -cell. One of these β -cell components, or antigens, is glutamic acid decarboxylase (GAD).

During the activation of naive T cells, a protein on the T cell – the T cell receptor – interacts with a foreign protein or antigen. The set of T cell receptors an individual has is genetically determined. The combination of receptor and antigen appears to determine whether the resulting immune response will be autoimmune in nature. Naive T cells are activated by a multifaceted process requiring the interactions of different sub-populations of T lymphocytes (CD4+ and CD8+), cytokines, foreign antigens (e.g., viral proteins) and the genetic background of the individual. Cytokines are locally synthesized proteins that act on lymphocytes to induce an immune response. During the multiple step activation process, CD4+ lymphocytes can be further subdivided into T helper cell subsets, referred to as Th1 or Th2 cells, depending on which cytokines are secreted. Based on work in the NOD mouse, it has been proposed that the development of type 1 diabetes is controlled by the ratio of Th1 to Th2 cells, with Th1 cells promoting diabetes and Th2 cells protecting against disease.

Environmental factors, such as infections or dietary substances, have been postulated to act as triggers for the autoimmune response. Epidemiological

studies have indicated that Coxsackie B virus infection is very common in individuals who develop type 1 diabetes. Although it has not been definitively proven that Coxsackie B virus can trigger disease, researchers have focused a great deal of attention on possible mechanisms by which the virus could destroy pancreatic β -cells. One hypothesis is that the virus, which directly invades the pancreas, simply causes local inflammation and cell destruction. The inflamed cells, in turn, release autoantigens (e.g., GAD) which are normally sequestered, thus triggering an autoimmune response. This non-specific process has been termed “bystander damage.” Another hypothesis, termed “molecular mimicry,” is that the autoimmune response is triggered because of sequence similarity between a viral protein and GAD. In essence, the body mounts an attack against the invading virus, but ends up attacking its own β -cells because of sequence homology between GAD and virus.

RECENT FINDINGS: While both theories of how viruses might trigger diabetes are plausible, recent evidence suggests that, in the mouse model, Coxsackie virus appears to cause diabetes by bystander damage and not by molecular mimicry. Investigators infected different strains of mice with Coxsackie B virus, in order to determine if the development of diabetes was dependent on cross-reactivity of the virus with GAD. NOD mice, which develop spontaneous diabetes and show signs of autoimmunity against GAD, did not demonstrate accelerated development of disease when infected with Coxsackie, which would be expected if molecular mimicry was operative. However, a different strain of mice, BDC2.5 (which do not normally develop diabetes), rapidly developed disease after infection with virus. These mice carry T cells that are reactive to a pancreatic antigen distinct from GAD and not cross-reactive with Coxsackie virus. Thus, in these experiments, it appeared that autoimmunity resulted from bystander damage and release of antigens from damaged β -cells, rather than from an attack due to cross-reactive epitopes.

Whatever the trigger for β -cell destruction is, the end result is the appearance of autoantibodies, including those directed against GAD. One important question relates to whether GAD antibodies are merely markers for the autoimmune process, or whether GAD antigen itself is an inciting player in the autoimmune process. In two related studies in the NOD mouse, investigators have shown that CD4+ T cells specifically reactive against GAD can directly produce β -cell injury. Injection of GAD into NOD mice accelerated the development of diabetes. CD4+ T cells isolated from these mice were reactive against GAD and demonstrated an autoimmune Th1 cytokine secretion pattern. In addition, the GAD-specific CD4+ T cells from diabetic NOD mice could adoptively transfer diabetes into mice that would not normally develop the disease. This is the first evidence that GAD may actually have a primary role in the autoimmune process.

Much of the evidence for an environmental trigger of type 1 diabetes comes from epidemiological studies, including those which demonstrate that even identical twins do not have 100 percent concordance for developing disease. Studies of family members of patients with diabetes indicate that many individuals have evidence of autoimmunity (i.e., the presence of β -cell autoantibodies) yet do not develop disease. By studying sets of identical twins and triplets who are discordant for disease, researchers have found that there is an altered pattern of cytokine secretion in those who develop disease. Patients who develop type 1 diabetes secrete decreased levels of the cytokine Interleukin 4, which is known to promote a protective Th2 immune response. This extends previous findings from the NOD mouse to humans and is an important advance in understanding the nature of the autoimmune response.

SIGNIFICANCE: Understanding the mechanism by which the autoimmune process is triggered is essential for designing prevention/intervention strategies. For example, an approach focused on cross-reactive viral epitopes would be inappropriate if molecular mimicry is not operative.

The demonstration that the immune response against GAD appears to play a primary role in the development of diabetes, rather than merely reflecting pancreatic cell damage, offers a potential target for immunomodulation to prevent or ameliorate disease. Indeed, studies in the NOD mouse (see Type 1 Diabetes: Immunomodulation for Prevention and Therapy) suggest that GAD administration might be an effective therapeutic regimen.

Type 1 diabetes occurs because of a complex interplay between genetics, T cell activation and environment. Dissecting the role of different cytokines in the development of disease is critical to being able to manipulate the immune process away from a destructive, autoimmune pathway. In addition, differential cytokine secretion may provide a marker, which could predict which individuals with antibody evidence of ongoing autoimmunity are most likely to progress to actual disease. Such a marker would be useful for identifying those individuals who might best benefit from early intervention strategies to prevent the development of disease.

FUTURE DIRECTIONS: Our knowledge of the etiology and pathophysiology of type 1 diabetes has been expanded by these recent investigations. Much more work is needed to define the role of viruses and other environmental factors that may trigger the autoimmune process. Identification of such a trigger (e.g., a clear-cut viral etiology) could lead to an effective intervention (e.g., vaccination) to prevent the development of type 1 diabetes. Future research must also be aimed at refining our understanding of the role of cytokines and coactivators in controlling T cell subsets. The long-term goal would be to develop the ability to

control or alter the T cell phenotype, to intervene in the disease process. Only by understanding the pathophysiology of the autoimmune process in the development of type 1 diabetes, will we be able to develop modalities to prevent or treat the disease.

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III. TITLE: Type 1 Diabetes Mellitus: Immunomodulation for Prevention and Therapy

BACKGROUND: Type 1 diabetes mellitus is an autoimmune disease that

affects approximately 800,000 people in the United States. It is one of the most frequent chronic diseases of children with about 30,000 new cases diagnosed each year.

Prevention of type 1 diabetes is a major goal of research. In the past few years, investigators have been able to establish screening tests to identify individuals at high risk for the development of type 1 diabetes. They have also shown in animal models and in preliminary human trials, that low-dose insulin therapy may prevent or delay the onset of the clinical manifestations of type 1 diabetes. A controlled multi-center clinical trial is underway to assess the efficacy of parenteral or oral insulin to prevent or delay the onset of type 1 diabetes.

The search for therapies to prevent the autoimmune attack that eventually leads to type 1 diabetes has indicated the need for therapies that are highly specific for the disease process and for the individual. Current therapies would require an individual to be on systemic immunosuppression for their entire life. One of the screening tests presently utilized to identify individuals at high risk for the disease is genetic and defines the specific human leucocyte antigen (HLA) of the individual. Particular HLA genes [in animal studies these genes are referred to as the major histocompatibility complex, MHC] have been shown to have a high predictive value for identifying the development of an autoimmune attack on the β -cell. By combining specific varieties (alleles) of the HLA genes into an animal model [i.e., the non-obese diabetic (NOD) mouse], researchers can then study preventive therapies designed for the genetic background of the individual at high genetic risk for type 1 diabetes.

Lymphocytes play major roles in the cause and progression of type 1 diabetes. There are two predominant types of lymphocytes: β -cells, which produce antibodies, and T cells, which can directly kill a cell. Both cell types appear to be involved in type 1 diabetes. The antibody-producing β -cells of the immune system gave researchers one of the first indications that type 1 diabetes was an autoimmune disease. The presence of antibodies that react specifically against β -cells was found in new onset individuals with type 1 diabetes and in animal models of type 1 diabetes (i. e., the NOD mouse and the BB/Wor diabetic rat). The proteins or antigens with which these antibodies interact may signify important participants in the disease process. One of these antigens has been identified as glutamic acid decarboxylase (GAD). Naive T lymphocytes are activated by a multifaceted process requiring the interactions of different sub-populations of T lymphocytes ($CD8^+$ and $CD4^+$), cytokines, foreign antigens (viral proteins) and the genetic background of the individual. Cytokines are locally synthesized proteins that act on lymphocytes to induce an immune response. During the multiple step activation process, $CD4^+$ lymphocytes can be subdivided further into T helper cell subsets referred to as Th1 cells and Th2 cells.

It has been proposed that the development of type 1 diabetes is controlled by the ratio of Th1 to Th2 cells with the Th1 cells promoting diabetes and the Th2 cells protecting against the disease. Thus, researchers are interested in modulating the immune system enabling them to convert a Th1 destructive response to a benign Th2 response.

RECENT FINDINGS: The role of the major histocompatibility complex (MHC) in the NOD mouse model was examined by mutating the two amino acids in the MHC gene which are believed to cause this animal model to be susceptible to type 1 diabetes. The transgenic mice did not develop diabetes even after 8 months. Thus, this MHC gene plays a role of altering the immune regulatory networks.

Intravenous administration of GAD65 to 12-week old NOD mice suppresses the autoimmune attack on the β -cell. NOD mice at this age have an ongoing insulinitis, which progresses to overt type 1 diabetes between 13 and 25 weeks of age. The GAD65 treatment effectively prevents this disease progression. This prevention of diabetes is mediated by the induction of regulatory CD4⁺ T cells, which have a Th2 phenotype.

Type 1 diabetes is believed to be initiated by the development of autoreactive T cells reacting to a specific portion or determinant of an antigen, for example GAD65. With time the immune response spreads to additional determinants. This process is referred to as determinant spreading. Using an assay capable of characterizing T cells at the single cell level, the natural development of β -cell autoimmunity and the development of tolerization to a β -cell antigen in NOD mice was examined. These observations confirmed determinant spreading of a Th1 response during the spontaneous autoimmune process. They also demonstrated a new phenomenon, Th2 determinant spreading, which may be a mechanism underlying the efficacy of antigen-based immunotherapies.

Using a viral-induced murine model (lymphocytic choriomeningitis virus) of type 1 diabetes, researchers have shown disease prevention by developing transgenic mice with the immunoregulatory and cytokine inhibitory genes (E3) of the adenovirus genome. These results predict that the selective immune regulation at the level of the target cell is sufficient to prevent autoimmune diabetes without disrupting the function of the systemic immune response.

SIGNIFICANCE: Predisposition of an individual to type 1 diabetes is controlled by his HLA genes. Understanding how this control is manifested will empower researchers to intervene with specific prevention protocols.

Once the autoimmune attack on the β -cell has begun, intervention may be more

difficult. Methods to monitor the individual's response to a therapy are essential. These results indicate a method to induce tolerance in an animal model, thus preventing further autoimmune attack, and a procedure to monitor the effectiveness of the intervention by examining the autoimmune reactivity of single T cell responses in the periphery.

Selective immune regulation at the level of the β -cell is significant for several reasons. First, these results suggest if vectors are designed to transfect only the surviving β -cells, this strategy could give these cells the machinery necessary to inhibit the ongoing autoimmune attack. Second, β -cells could be transfected with the genes that offer immune protection *in vitro*. After the transfection, these cells could be utilized for transplantation into an individual with type 1 diabetes without fear that a continuing autoimmune process in the individual would destroy them.

FUTURE DIRECTIONS: Immunomodulation for the prevention and treatment of type 1 diabetes has made considerable advances in animal models. However, it is essential to move these findings forward into the clinical situation.

Development of protocols to intervene in the disease process in humans and development of assays to monitor responsiveness to therapies in humans are essential. Validation of a surrogate endpoint in humans would decrease the time required to move a given prevention/intervention protocol forward. Research in these directions must continue.

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IV. TITLE: Type 1 Diabetes Mellitus: Mechanical and Cellular Approaches to Therapy

BACKGROUND: Type 1 diabetes mellitus is an autoimmune disease that affects approximately 800,000 people in the United States. It is one of the most frequent chronic diseases of children with about 30,000 new cases diagnosed each year.

Currently available intensive insulin therapy is limited by the accompanying increased frequency of severe hypoglycemia and weight gain, resulting in an increasing prevalence of overweight patients with type 1 diabetes. Intensive therapy is also particularly problematic in children and adolescents due to difficulties in getting them to comply with current treatment regimens. This is especially troublesome since current clinical data support early intervention as being the most effective treatment strategy. Because, the results of the Diabetes Control and Complications Trial indicate that maintenance of near normal glycemic levels can reduce and delay the onset of the devastating complications of diabetes, establishing methods to achieve and maintain euglycemia will have enormous impact on the health and quality of life of individuals with diabetes.

Although the ultimate research goal is to prevent the onset of type 1 diabetes, investigators are currently developing approaches and technology to modulate blood glucose levels in patients with diabetes and to restore insulin-producing capacity through transplantation of the whole pancreas, or of islets from the pancreas. Today, the only method that offers normal blood glucose levels is pancreas transplantation. Several decades ago, islet transplantation was proposed in the hope of reducing the need for difficult surgical procedures by

allowing intravenous injection of islets. Initial animal studies on islet transplantation were encouraging. Unfortunately, the initial promise held out for islet cell transplantation has not been realized. Thus, of the 270 adult islet transplants performed by the end of 1995 in patients with type 1 diabetes, only ten percent of recipients did not require insulin injections for more than one week, and only five percent remained off exogenous insulin for more than one year. In light of these results, studies are needed to better understand what treatment regimens would allow successful islet transplantation using the least amount of immunosuppression, thereby minimizing the toxicity to the islet. For example, corticosteroids, a mainstay of immunosuppressive regimens for solid organ transplantation, interfere with the normal β -cell function to release insulin in response to elevated blood sugar levels. In addition, the recurrence of autoimmune-mediated destruction of transplanted β -cells is problematic, and many researchers are pursuing studies to abrogate the underlying autoimmunity that has led to type 1 diabetes.

While the "gold standard" for transplant success has been insulin independence, clinicians are observing a beneficial effect of islet transplantation even in recipients who have not achieved insulin independence. These patients require less exogenous insulin and show an improvement in metabolic control, which equates to fewer episodes of severe hypoglycemia. This effect may also lower the risk of long-term complications.

Other β -cell replacement strategies include bioengineered β -cells, β -cells grown in continuous or permanent culture to expand the number available for transplantation, and animal islets (xenotransplants). Bioengineered β -cells could be non-beta cells that would be transfected with specific genes to mimic β -cell function, could be "real" β -cells engineered to enhance engraftment or prevent rejection and autoimmune destruction, or could be a combination cell which functions to release insulin in response to glucose and prevents graft rejection. Animal islets, while offering an ample supply of islets, present additional risks, such as the transmission of animal diseases to humans and accelerated immune rejection.

Glucose sensors for the treatment of diabetes hold great promise for improving metabolic control and quality of life for persons with diabetes. The single greatest change in the management of both type 1 and type 2 diabetes in the past two decades has been the introduction and widespread implementation of reliable, accurate, and relatively "user-friendly" self-glucose monitoring devices. At present, state-of-the-art technology cleanly divides mechanical delivery devices and glucose sensing technology; however, the ultimate goal would be to develop a "closed-loop" delivery system by combining these two technologies.

Despite the enormous success of self-glucose monitoring, the technical challenges of developing methods for continuous monitoring of blood glucose and several highly publicized industry failures have overshadowed recent progress in the field of glucose sensing. Several approaches for continuous glucose measurement are close to clinical applicability. Designs that utilize an enzyme electrode (such as glucose oxidase) and either a hydrogen peroxide or an oxygen detection system appear to be the most successful to date. These electrodes can be placed subcutaneously or intravenously. Other glucose sensor designs being examined include acute microdialysis systems, transdermal extraction of tissue fluids for glucose assay, and non-invasive technologies. These designs require more basic research before demonstration of feasibility and subsequent development.

RECENT FINDINGS: Pancreas transplantation is usually considered for individuals with diabetes who require a kidney transplant. However, successful pancreas transplantation in individuals with diabetes without overt kidney disease can reverse the lesions of diabetic nephropathy. This reversal requires between five and ten years of normal glucose levels. This is in spite of the nephrotoxic effects of current immunosuppressive agents. Thus, the devastating effects of diabetes may be reversible if improved immunomodulation methods can be applied to pancreas or islet transplantation.

A long-term follow up (six years) of islet allografts in individuals with type 1 diabetes showed improvement in metabolic control even if an individual was not completely insulin independent. These immunosuppressed individuals had improved HbA_{1c} without the severe hypoglycemic episodes observed in the Diabetes Control and Complications Trial (DCCT). Consequently, even a partially successful islet transplant may offer a significant decrease in the progression of diabetic complications.

Human fetal islet-like clusters (ICC) transplanted into the kidney or the pancreas of a nude mouse (animal lacking T cells) were able to grow and mature producing sufficient insulin to restore euglycemia using 15,000 ICCs/kg. Transplantation into the lung, the liver or the spleen did not result in substantial growth or differentiation of the clusters. Thus, the site for transplantation of human fetal islet-like clusters is important, with advantages to transplantation either under the kidney capsule or in the pancreas.

Induction of immune tolerance has been achieved in the xenotransplantation of rat islets into mice treated with donor-specific spleen cell transfusion and anti-CD154 monoclonal antibody. Since the anti-CD154 antibody is directed against the CD40 ligand which is part of the co-stimulatory signal expressed by activated CD4⁺ T cells, these T cells must play a major role in the cellular immune

response to xenografts. Further studies suggest that a skin xenograft (considered more difficult to maintain without rejection) can survive on adult thymectomized mice treated with a donor-specific transfusion and a short-term

course of anti-CD154 monoclonal antibody. Thus, it appears that a durable allotolerance can be achieved without prolonged immunosuppression.

Technological advances have occurred in the area of glucose sensors. Glucose sensors coated with two cross-linked polyethylene glycol derivatives (hydrogel) were shown to have improved biocompatibility. Unlike prior coatings that can become encapsulated by tissue *in vivo*, the hydrogel coatings have very few adherent cells. Glucose sensors can be calibrated using as few as one calibration point. An algorithm for predicting blood glucose concentration from the subcutaneous glucose concentration, measured by subcutaneous-implanted glucose sensors, has been shown to improve blood glucose estimations.

A new chemical sensor for glucose is being developed. This sensor is a crystalline colloidal array of polymer spheres polymerized within a hydrogel that swells and shrinks reversibly in the presence of glucose. The material changes color in response to glucose. The hydrogel contains a glucose-recognition group. As glucose binds to this group the hydrogel expands causing an increase in the spacing between the crystalline colloidal array spheres. This causes a shift in the diffracted light to longer wavelengths. Future development of this technology will be necessary to apply it to the clinical situation.

SIGNIFICANCE: The DCCT demonstrated the importance of glucose control to prevent the complications of diabetes. Now we know from the pancreas transplant study that long-term maintenance of normal blood glucose control can reverse kidney damage caused by diabetes of long duration. Islet transplantation has been investigated as a means of normalizing blood glucose levels. However, the results to date, based on achievement of insulin independence have appeared disappointing. The present results indicate that even if an islet transplant recipient is not insulin independent, they may receive considerable benefit from the transplanted islets.

Eventually if islet transplantation is successful, sources, other than human cadaver, will have to be utilized to treat all individuals with diabetes. The ability to transplant human fetal islet-like clusters and have them grow and differentiate into islets *in situ* demonstrates one possible source for additional islet tissue. Several investigators have suggested the use of porcine islets as an additional source. For this to be successful, it is essential to develop immune therapies to circumvent both the autoimmune process and the xenograft rejection process. The present studies examine protocols that are effective for rat to mouse

transplants. Testing these protocols in larger animals will be crucial prior to their evaluation in humans.

The development of a glucose sensor has been a very challenging endeavor. This field is moving rapidly forward. A least one company has submitted a subcutaneous glucose sensor to the Food and Drug Administration for approval. Several other subcutaneous sensors are approaching this evaluation. All of the subcutaneous glucose sensors will require an algorithm to convert subcutaneous glucose levels to blood glucose levels. Such an algorithm is now available for testing. In addition, innovative ideas are being applied to develop noninvasive sensors.

FUTURE DIRECTIONS: The future of transplantation in the treatment of diabetes is extremely exciting. New methods to induce immune tolerance have been developed and are being tested in humans using islets isolated from cadaver pancreata. However, it is clear that additional sources of islets will be essential to treat all individuals with diabetes. Thus, it is essential to develop new sources of islets from human fetal tissue, other animals, or bioengineered cells in culture. Equally important will be the testing of new tolerance induction protocols to utilize these new islet sources without the need for long term immunosuppression.

The progress toward the development of a glucose sensor must be further advanced. We are only beginning to attract the expertise necessary to initiate novel devices that will be the next generation of glucose sensors. In addition, it will be necessary to evaluate the present glucose sensors to ascertain their usefulness to monitor blood glucose levels and to predict hypoglycemia. Once a sensor has passed these tests, it should be linked to glucose delivery system and further tested. Clearly substantial work is required to produce a closed-loop system. However, the recent progress in this area supports our enthusiasm for continuing research efforts designed to produce a closed-loop system.

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V. TITLE: Type 2 Diabetes Mellitus: Etiology and Pathophysiology

BACKGROUND: Type 2 diabetes mellitus affects more than 16 million Americans and is the predominant form of diabetes in the United States, with a disproportionate impact upon minority populations. It is a multifactorial disease with a significant genetic component, although the specific genes that underlie most cases of type 2 diabetes have remained elusive. In some families with a particularly rare form of type 2 diabetes, Maturity-Onset Diabetes of the Young (MODY), the gene for glucokinase, a key enzyme of glucose homeostasis found in the insulin-secreting β -cell of the pancreas and in the liver, has been implicated. Mutations in glucokinase, along with perhaps a half-dozen other mutations (e.g., in insulin, the insulin receptor, glycogen synthase, insulin-stimulated glucose transporters, other candidate genes, or in the mitochondrial genome), may account for a few percent of type 2 diabetes cases. Since the genetics of type 2 diabetes in humans follows a complex pattern of inheritance, it has been theorized that type 2 diabetes is not caused by a single mutation in one major gene. Thus, type 2 diabetes may develop as a result of different mutations at different genetic loci acting simultaneously and in a synergistic manner (epistasis). For the most part, however, information is still urgently needed about the interplay between genetic and environmental factors in producing full-blown type 2 diabetes, as well as its precursor, insulin resistance.

Type 2 diabetes is characterized by a complex and variable phenotype and often a long period of latency between the appearance of the first recognizable marker(s) and full expression of the disease. There is no doubt that type 2 diabetes has two basic components, peripheral insulin resistance and defective insulin secretion. The insulin resistance is postulated to result from alterations in the insulin signaling pathways, which allow the target cell to respond to insulin and increase glucose uptake. Defective insulin secretion results from a decrease in the signaling mechanisms of the β -cell that allows it to respond to elevated glucose levels and secrete insulin. Breakdown in both of these cellular response pathways occurs in type 2 diabetes and ideally correction of both abnormalities must occur to effectively treat this disease. The clinical course of this disease, as observed in the natural history studies on Pima Indians,

includes a preclinical stage in which the body increases its production of insulin in order to maintain blood glucose levels within the normal range while in an insulin-resistant state. As the disease progresses, insulin levels begin to fall and the patient becomes frankly diabetic. While significant

progress has been made in understanding the biochemical basis for, and genetic influences on, the development of type 2 diabetes, there also appear to be environmental and life-style factors that place individuals at higher risk for type 2 diabetes.

In mammals, insulin is the principal hormone controlling blood glucose and acts by stimulating glucose uptake and metabolism in muscle and fat tissue and inhibiting glucose production in the liver. Insulin action is mediated through the insulin receptor, a transmembrane glycoprotein with intrinsic protein tyrosine kinase activity. The mechanism causing insulin resistance is poorly understood. It is however a major feature of type 2 diabetes and results in a failure of target tissues to respond to normal levels of circulating insulin. Hence, to understand control of normal glucose metabolism, as well as the pathogenesis of type 2 diabetes, it is critical to understand the signaling pathways used by the insulin receptor and how abnormalities in this produce insulin resistance. It is now clear that addition or subtraction of a phosphate moiety to a protein or an enzyme plays a major role in the cellular response to insulin. Thus, enzymes that add phosphate moieties (kinases) and enzymes that remove these moieties (phosphatases) are integral constituents of insulin response pathways. The insulin receptor has kinase activity and can phosphorylate itself upon binding insulin. The insulin receptor also phosphorylates a series of proteins, including insulin receptor substrate-1 (IRS-1) and IRS-2. Once phosphorylated, IRS-1 has been shown to stimulate the phosphatidylinositol (PI) 3'-kinase pathway in liver and skeletal muscle, initiating a cascade of events leading to the cellular response. A primary effect of insulin signaling in muscle is the increase of glucose uptake. Insulin resistance leading to type 2 diabetes may be mediated by deficiencies in glucose transport. GLUT4 is the major insulin-responsive glucose transporter in skeletal muscle, heart and adipose tissue. In contrast, another glucose transporter, GLUT1, is found constitutively on the cell surface of the same tissues, and is not responsive to insulin regulation. Insulin regulates GLUT4 activity in two ways. Short-term regulation occurs via recruitment of GLUT4 from its storage site in intracellular vesicles to the plasma membrane. Long-term exposure to insulin results in downregulation of GLUT4 transcription. Defects anywhere along this signaling pathway from receptor phosphorylation to GLUT4 translocation may lead to increased insulin resistance.

Recent data have implicated tumor necrosis factor- α (TNF- α) in the insulin resistance of obesity and type 2 diabetes, and have suggested that TNF- α

participates in obesity-related systemic insulin resistance by inhibiting insulin receptor tyrosine kinase activity. In obese humans, adipose tissue TNF- α levels are increased compared to controls and are correlated with hyperinsulinemia. Both TNF- α and insulin levels decline with weight reduction, suggesting a role for the abnormal regulation of this cytokine in the pathogenesis of obesity-related insulin resistance. In mice where TNF- α and its receptor have been knocked out, obesity no longer induces insulin resistance, lending further credence to this hypothesis. An additional component involved in the linkage of obesity to insulin resistance has been identified. This component is the adipocyte fatty acid binding protein, aP2. This protein binds free fatty acids and may be involved in the trafficking of fatty acids to specific cellular compartments where the fatty acids elicit their effects on gene expression. Thus, aP2 is seen as a co-factor that enables elevated free fatty acids to increase the expression of TNF- α which in turn interferes with insulin action. These studies are beginning to link the metabolic abnormalities associated with obesity to the progression from insulin resistance to diabetes. In particular, it is now appreciated that triglycerides and fatty acids play a major role in this disease. These compounds, generally considered to be energy storage molecules, also control metabolic pathways utilized to metabolize glucose. The recently obtained knowledge of the role of mitochondrial uncoupling proteins in energy dissipation versus energy storage has added another dimension to our appreciation of the metabolic complexities involved in obesity, insulin resistance and diabetes.

Glucose-stimulated insulin secretion from the pancreatic β -cell is also diminished or lost in type 2 diabetes. However, unlike type 1 diabetes mellitus, the β -cells generally remain intact and, in fact, the β -cell mass appears to significantly increase in states of insulin resistance and type 2 diabetes. Significant progress has occurred in the past year in elucidating the role of triglycerides and fatty acids in the loss of β -cell function. In the presence of elevated intracellular fatty acid levels the β -cell loses its ability to respond to glucose. Leptin, an adipocyte hormone important for the regulation of body composition, has been recently shown to have direct effects on β -cells. Leptin increases the metabolism of intracellular fatty acids to energy dissipation rather than to energy storage in the form of triglycerides. Thus, leptin appears to have a beneficial effect on the β -cell in obese/insulin resistant states. Future studies will determine if this hormone may be an effective therapy for improving β -cell function in type 2 diabetes.

Recent findings shed light on the molecular mechanisms for both these phenomena.

RECENT FINDINGS: A number of investigators have utilized the power of

molecular ablation to study various components of the insulin signaling pathway that may play a role in type 2 diabetes. Last year, a group at the Joslin Diabetes Center in Boston, working with NIH intramural scientists, demonstrated in a landmark paper that diminished function at multiple steps in the insulin signaling pathway could lead to the development of diabetes. This provided a plausible multistep model in the development of diabetes in humans. Recently, they demonstrated that elimination of just one of the signal coupling molecules, IRS-2, can produce type 2 diabetes. Mice that have no IRS-2, but express normal levels of IRS-1 are characterized by insulin resistance and a progressive loss of pancreatic β -cell function leading to diabetes. In contrast, animals lacking IRS-1 are able to compensate for peripheral insulin resistance with β -cell hypertrophy and increased insulin secretion to maintain relatively normal glycemia.

Conversion from insulin resistance to frank diabetes occurs with the failure of the pancreatic β -cell to compensate with increased output of insulin. To understand the mechanism which results in the reduction of insulin secretion and eventual loss of β -cell mass, Roger Unger's group in Dallas examined the role of increased free fatty acids in obese Zucker rats. By studying islets from prediabetic and diabetic Zucker rats in vitro, they were able to demonstrate a progressive buildup of free fatty acids within the islets which eventually overwhelms the capacity to oxidize it. The high concentration of fat leads to increased nitric oxide levels and concomitant induction of β -cell apoptosis, or programmed cell death. Why don't all obese individuals who have marked insulin resistance go on to lose their β -cells and develop diabetes? It remains to be demonstrated that this mechanism acts in humans, and whether some individuals are resistant to fatty-acid induced apoptosis.

Because fatty acid metabolism is disturbed in type 2 diabetes, regulation of the oxidation pathway is an area of active research. One of the rate-limiting enzymes is carnitine palmitoyl-transferase I (CPT I), which carries long-chain fatty acids into the mitochondria so they can be used to produce energy. Kelly and colleagues recently showed that the amount of CPT I in normal heart is regulated by its substrate, long-chain fatty acids, but not by other, short-chain fatty acids. The putative fatty acid responsive element was shown to bind to a transcription factor called PPAR α . Mice missing PPAR α had only 50 percent the control levels of CPT I expression. It is possible that this pathway is impaired in diabetes.

Islet duodenum homeobox-1 (IDX-1) is a transcription factor whose elevated expression correlates with elevated insulin levels, while the CCAAT/enhancer binding protein β (C/EBP- β) factor inhibits insulin gene transcription. IDX-1 and insulin are reduced and C/EBP- β is increased in two hyperglycemic animal models of diabetes, Zucker diabetic fatty (fa/fa) rats, and after 90 percent

pancreatectomy. These findings are consistent with the notion that these transcription factors may mediate long-term down-regulation of the genes that encode for insulin in diabetes. Further support was provided by Dutta and others who also examined the relationship between IDX-1 (also known as PDX-1) and insulin levels. They reduced PDX-1 levels by making transgenic mice with only one copy of the PDX-1 gene. In these animals, insulin levels are inappropriately low for the level of glycemia. In addition, the islets in these animals contain a disproportionately high percentage of non- β cells. Conceivably, gene expression patterns could be reset through appropriate modulation of transcription factor activity. To that end, the pharmaceutical industry has recently embraced transcription factors as potential targets for drugs.

The low number of available animal models of type 2 diabetes has limited the ability to study insulin resistance. A potentially useful model is the heterozygous GLUT4 knockout mouse (GLUT4^{+/-}) which has a reduced number of the insulin-responsive glucose transporter protein, GLUT4. As it ages, this animal develops increased postprandial plasma glucose and insulin, reduced muscle glucose uptake, hypertension, diabetic hypertrophic cardiomyopathy and liver steatosis, without obesity. This indicates that a single impairment in insulin-stimulated glucose transport can initiate a process that leads to diabetes.

A focus on mutated GLUT4 as a locus for type 2 diabetes has yielded data that imply the existence of new factors that regulate GLUT4 transcription and insulin-stimulated movement in the cell. Lee and Jung investigated the role of the cytoplasmic domain of the GLUT4 protein in its movement between storage vesicles and its active location in the plasma membrane. Small proteins corresponding to the cytoplasmic domain were placed into fat cells at a very high concentration. Interestingly, these small fragments caused a dose-dependent increase in recruitment of native GLUT4 to the plasma membrane, similar to that caused by insulin. This implies that there is a regulatory interaction between an unknown molecule in the cell and the cytoplasmic domain of GLUT4 which tags it for cycling back to its intracellular storage site. When there is enough of this protein fragment present to completely bind the putative regulatory factor, GLUT4 remains in its active form at the cell surface. This factor remains to be isolated and characterized. Cooke and Lane found that the suppression of GLUT4 transcription by insulin is likely due to novel nuclear proteins. They transfected adipocytes with various lengths of the GLUT4 gene, all of which contained a special reporter gene. The region of the gene between 676 and 706 base pairs was found to be the important transcription regulatory site. Four different nuclear were found to bind this region as putative transcription regulators, and remain to be characterized.

SIGNIFICANCE: Major advances in our understanding of key transcription factors which control both pancreatic development and insulin secretion should provide keys to the production of insulin secreting cells which could be transplanted into patients with diabetes. The role of fatty acids in the development of diabetes is becoming increasingly clear. This suggests new therapeutic approaches that should be tested for their efficacy in ameliorating type 2 diabetes. Efforts to understand the inability of the pancreas to compensate for increased insulin resistance could lead to significant breakthroughs in treatment of type 2 diabetes.

FUTURE DIRECTIONS: Studies to explore the role of free fatty acid induced apoptosis in human islets should be a priority. If this mechanism is demonstrated in humans, then studies with inhibitors of nitric oxide production as a means of rescuing the pancreas should be considered. Studies aimed at elucidating the complete pathways in muscle, liver, and fat through which insulin regulates metabolism should be supported. Better methods to assess insulin sensitivity are needed both in humans and in rodent models. Studies aimed at characterizing the differential responsiveness of various tissues to insulin sensitizers as well as the mechanism of action of these drugs should be initiated. In vivo methods to assess β -cell mass would be a tremendous advance in both clinical and basic studies of the pathophysiology of diabetes. The insulin signaling pathway is one of the most heavily studied, but the key elements of this pathway that are involved in diabetes remain elusive. Increased emphasis should be placed on efforts to define the molecules that are primary effectors of insulin action in glucose homeostasis.

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VI. TITLE: Endocrine Regulation of Energy Balance

BACKGROUND: Homeostatic control of body weight and composition is an integrative function of a large number of complex variables resulting in a balance between food intake and energy expenditure. Caloric intake is a result of diet composition, nutrient absorption, cognitive cues, and satiety signals. The partitioning of calories into stored fat, protein, and carbohydrates or conversion to energy through exercise or thermoregulation is intimately controlled by neuronal and endocrine signals similarly to energy intake. Despite this apparent complexity the setpoint for an individual's body weight and lean to fat ratio is remarkably resistant to exogenous influences. When this equation is shifted toward energy storage the resultant obesity is exacerbated by the very same control mechanisms that resist efforts to lower weight. Over the last few years many of the components of this intricate regulatory pathway have been elucidated.

Using positional cloning techniques, NIDDK-supported investigators have made a number of major discoveries in the molecular endocrinology of obesity over the last three years. The identification of the mutations responsible for the obese/diabetic phenotypes of a number of rodent models has revealed a signaling pathway between the hypothalamus and brown and white adipocytes which appears to be a key pathway in the regulation of food intake and energy balance in humans as well as rodents. The discovery of a signaling hormone, leptin, secreted by adipocytes, began an explosion of discovery in the neuroscience and cell biology communities, which has uncovered new elements in the regulation of body composition and energy balance. The discovery of new uncoupling proteins, UCP-2 and UCP-3, in both rodents and humans suggests that thermoregulation may be an important component of the human energy balance equation. The cloning of the leptin receptor and its localization in the hypothalamus and the discovery that melanocortins play a key role in inhibition of food intake have demonstrated the importance of the hypothalamus in this regulatory axis. That a new class of drugs used to treat diabetes, the thiazolidinediones, can work through the nuclear hormone receptor, PPAR- γ , which is also involved in differentiation and function of adipocytes, demonstrates that intervention at any step in this axis can lead to alterations in the delicate balance between food intake, energy storage and energy utilization. Knock-

outs of melanocortin receptor (MC-4) and β 3-Adrenergic receptors have confirmed roles for their cognate ligands in regulation of energy balance. Unexpectedly the knock-out of one of the regulatory subunit isoforms of protein kinase A resulted in a superlean phenotype suggesting that this isoform, which has limited distribution in brain and adipocytes, is involved in intracellular signaling related to energy balance.

The discovery recently of a novel hormone, urocortin, and the localization of this hormone to discrete regions of the brain has led to a number of studies to define its role in control of behavior. Because this hormone is related to CRF (a hypothalamic hormone involved in response to stress) which has potent anorexigenic effects, and urocortin has a much more discrete localization in the brain, Dr. Vale's group investigated the role of urocortin in appetite control. Central administration of urocortin in the brain dramatically suppressed appetite without the concomitant anxiety producing effects of CRF. In addition, urocortin was able to suppress food intake stimulated by NPY treatment. This work suggests that urocortin works through the type 2 CRF receptor, a finding which has implications for the well known effects of CRF-like substances on the immune and cardiovascular systems.

While the necessity to deliver this compound to the brain directly probably limits its therapeutic potential in the treatment of obesity, further studies on the regulation of food intake by urocortin may provide the basis for future therapeutic strategies. In work that may well have immediate impact on drug discovery research, Dr. Roger Cone of the Vollum Institute, has extended his studies on the role of melanocortins in the development of obesity in the agouti mouse. Last year, Dr. Cone reported that the agouti protein is an antagonist at the MSH receptor. This family of receptors, called the melanocortin receptors (MCR), signals for a family of hormones involved in skin pigmentation (MSH) and response to stress (ACTH). Since a subtype of this family named the MC-4 receptor is expressed in hypothalamic regions known to be involved in control of food intake, Dr. Cone developed specific peptide agonists and antagonists to test whether the agouti phenotype could be recapitulated by blocking stimulation of the MC-4 receptor. Treatment of mice with the MCR agonist markedly inhibited feeding in 4 distinct models of hyperphagia whereas treatment with the antagonist at times when feeding is stimulated, such as following a fast or at night greatly enhanced the feeding behavior. Similarly, treatment with the antagonist concomitantly with the agonist blocked the actions of the agonist to inhibit feeding. This work demonstrates that the hypothalamic POMC neuronal system tonically inhibits feeding, and suggests a key role for this site in control of bodyweight.

NIDDK-supported researchers are actively investigating many aspects of adipocyte biology. Included among these efforts are attempts to understand the determinants of adipocyte differentiation, signal transduction in adipocytes, hormones produced by adipocytes, and insulin-induced responses including glucose transport. A number of transcription factors, gene regulatory proteins, have been identified that direct the differentiation of pre-adipocytes to adipocytes. One of these transcription factors, peroxisome proliferator associated receptor- γ (PPAR- γ), is a member of the steroid/thyroid receptor superfamily. Another, CCAAT/enhancer binding protein (C/EBP), is the founding member of the family of transcription factors called bZIP, named to convey the juxtaposition of a DNA-binding basic (b) region to a leucine zipper (ZIP) dimerization interface that characterizes the family of proteins. PPAR- γ and several C/EBP appear to cooperate to bring about a cessation of mitotic growth and the expression of adipocyte-specific genes that results in the cellular phenotype. Among the genes expressed in adipocytes is GLUT4, which encodes a glucose transporter that cycles between the plasma membrane and cytoplasmic vesicles in an insulin-dependent fashion. Ultimately, control over adipocyte differentiation and metabolism should afford an opportunity to control obesity, a pathological state closely associated with type 2 diabetes.

RECENT FINDINGS: Leptin deficiency appears to account for only a small proportion of human obesity. In the vast major of people with obesity leptin levels are elevated suggesting that a degree of leptin resistance may be involved in the development of obesity. The simplest mechanism of resistance, that is defects in the leptin receptor (as in the db/db mouse) have been reported in humans, but once again appear quite rare. Bjorbaek et al have discovered a novel mechanism in rodents that results in resistance to leptin. The leptin receptor is a member of the cytokine family of JAK-STAT receptors, many of which are involved in the immune system. Taking a cue from work in that field, Bjorbaek looked for an intracellular molecule which has been implicated in suppression of signaling through cytokine receptors. This molecule, SOCS-3 (suppressor of cytokine signaling), indeed appears to be involved in leptin signaling. When expressed in cells SOCS-3 blocks leptin signaling. In various animal models of obesity SOCS-3 is upregulated in hypothalamic cells which contain leptin receptors. Finally, this group showed that leptin treatment in normal animals results in upregulation of SOCS-3 in the hypothalamus. In aggregate, these results implicate SOCS-3 in the leptin resistance observed in most animal models of obesity and suggest that the same mechanism may be involved in human obesity. Left unresolved is whether the increase in SOCS-3 in obesity is secondary to elevation of leptin or is somehow involved in the pathogenesis of obesity.

Whether leptin acts to control food intake or simply informs the hypothalamus of

the amount of energy stored as fat is unclear. Two groups have published results which suggest that food intake may be at least partially independent of leptin. In the first report, Nonogaki and coworkers developed a mouse which lacks the serotonin 5HT_{2C} receptor. This receptor is the target for a number of drugs such as dexfenfluramine which have been used to treat obesity. In this animal model hyperphagia (overeating) precedes the development of obesity. The response to exogenous leptin in these animals appears to be normal despite the increased food intake. Leptin resistance develops over time as obesity develops in this model. Despite normal plasma free fatty acids and corticosterone, adipose TNF- α levels were significantly elevated. High levels of TNF- α have been implicated in insulin resistance as well as a number of autoimmune diseases. This model is likely to represent a primary defect in food intake. It is likely that subsequent development of obesity represents a failure of compensatory mechanisms to counterbalance this increase energy intake over a prolonged period. A group lead by Roger Cone at the Vollum Institute has come to a similar conclusion by studying a different brain control pathway. This group crossed mice that produce an endogenous antagonist of the melanocortin receptor in the hypothalamus, the agouti mouse, with mice lacking leptin, ob/ob mice. Whereas agouti mice are leptin resistant, the removal of the endogenous leptin gene by this cross restored leptin sensitivity. While leptin treatment has a marked effect in reducing weight in the ob/ob mouse, when the ob/ob mouse is crossed with the agouti mouse leptin no longer has this effect. Thus, the authors conclude that leptin does not work through the melanocortin system and that the leptin resistance in the agouti mouse is secondary to high leptin levels associated with obesity.

Balancing the inhibition of food intake by inputs through the serotonergic and melanocortinergic pathways are brain peptides that increase food intake. The most notably of these is NPY. This neuropeptide has potent orexigenic effects when injected into the hypothalamus. The lack of a major effect of removal of this peptide through gene knock-out approaches suggests that regulation of food intake involves multiple, redundant pathways. Workers at the Joslin Diabetes Center in Boston decided to test this hypothesis by developing a mouse lacking another hypothalamic peptide which increases food intake, melanin concentrating hormone, or MCH. In contrast to the NPY knock-out mouse, animals deficient in MCH exhibit a marked diminution in food intake along with a significant reduction in weight compared with aged matched controls. These animals lose weight despite reduced leptin levels which would be predicted to increase food intake. Even more strikingly, animals lacking MCH respond to exogenous leptin treatment with reductions in body weight and food intake that parallel that seen in normal animals indicating that the leptin response pathway is functional. Because the presence of this pathway appears obligatory in the stimulation of food intake, identification of the MCH receptor will be of particular

importance. Antagonists at this receptor may be able to control food intake and weight gain without compensation by other regulatory systems.

While much of the work in this field has been focused on regulation within the hypothalamus evidence is increasing that leptin may have a role in other tissues which are important in storage and utilization of energy. Rossetti and coworkers have demonstrated convincingly that leptin is produced in muscle as well as fat. Furthermore, they have demonstrated that both hyperglycemia and hyperlipidemia increase muscle synthesis of leptin. The functional significance of increase muscle leptin synthesis in light of the much greater contribution of fat to plasma leptin levels remains to be elucidated. However, the fact that the increased leptin appears in a physiologically relevant context suggests that it may contribute to energy regulation perhaps locally within muscle.

NIDDK-supported researcher and MERIT recipient, Bruce Spiegelman reported the discovery of a nuclear protein from brown adipose tissue that may be significant to adaptive thermogenesis. The protein, termed PGC-1, is a transcriptional coactivator of certain members of the steroid/thyroid receptor superfamily and its expression is cold-inducible in brown fat and skeletal muscle which are key thermogenic tissues. Previous work from Dr. Spiegelman's laboratory had suggested the presence of a factor in brown fat cells that was not present in fibroblasts which facilitated the transactivation of UCP-1, encoding an uncoupling protein present in mitochondria and linked to energy dissipation. In the recent report, Dr. Spiegelman and colleagues report the isolation of PGC-1 (PPAR- γ Coactivator -1) and its interaction with several members of the steroid/thyroid receptor superfamily including PPAR- γ . The researchers also show that PGC-1 serves as a coactivator of PPAR- γ in the transcription activation of UCP-1 in transient transfection assays. Finally, Dr. Spiegelman and co-workers also show that ectopic expression of PGC-1 in white adipose cells induces numerous mitochondrial genes and leads to an increase in mitochondria in the white adipose cells. The latter observation suggests that PGC-1 expression can coordinate a transformation from white adipose to brown adipose. While it remains to be seen whether the transformation includes energy dissipation instead of storage, the finding opens the possibility of combating obesity through manipulation of PGC-1 expression in white adipose.

SIGNIFICANCE: Obesity represents a major health risk in the U.S. inasmuch as it is associated with cardiovascular disease, diabetes and certain forms of cancer. Changes in lifestyle and diet have no doubt contributed to the epidemic of obesity seen in this country over the last few decades. The World Health Organization has recently recognized that obesity is a global menace to health, even in countries where starvation has been the primary concern in the past. As

more and more of the signaling pathways which control energy balance are uncovered, clues to the underlying pathology in obesity are emerging. Each of these regulatory sites provides a potential target for pharmaceutical development.

FUTURE DIRECTIONS: The characterization of regulatory proteins that distinguish energy storing adipocytes from energy dissipating adipocyte may afford the opportunity to develop novel therapeutic approaches to obesity. Research aimed at the isolation and characterization of regulatory proteins in adipose should continue to receive support. The development of a comprehensive database of adipose cDNAs should be considered. Studies aimed at the activation of the adaptive thermogenic pathway in white adipose should be supported. Key regulatory centers in the brain are likely to be good candidates for targeted pharmacotherapies in the treatment of obesity. While Leptin has only proven to be effective in a small number of patients suffering from leptin deficiency, drugs targeted at serotonergic pathways in the brain are widely used today. As the pathways and integration sites within the hypothalamus are uncovered more selective drugs are likely to emerge. A major initiative to define receptors in the hypothalamus should provide impetus for drug discovery efforts in academia as well as industry. The molecular/anatomical substrates for cognitive influences on food intake and energy metabolism should be a focus of investigation.

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VII. TITLE: Molecular Mechanisms Underlying Complications of Diabetes

BACKGROUND: Diabetes mellitus is one of the most prevalent chronic diseases in the United States. Based on the National Health Survey (NHIS), there were 7.8 million diagnosed cases of diabetes in the United States in 1993. It is estimated that about 625,000 new cases of diabetes are diagnosed each year, including 595,000 cases of type 2 diabetes and 30,000 cases of type 1 diabetes. The number of people with diagnosed diabetes increased five-fold between 1958 and 1993. In addition, it is estimated that there are probably 5.4 million undiagnosed cases of type 2 diabetes in the U.S. In the United States, diabetes is a major cause of amputations, blindness, cardiovascular disease and end-stage renal disease. Currently, diabetes is the seventh leading cause of death. In a recent study, it was estimated that the cost of medical care for diabetes in 1992 was \$91.8 billion.

The Diabetes Control and Complications Trial (DCCT) conclusively established the relationship between hyperglycemia and the complications of diabetes mellitus. Because of limitations in current therapies, it is often difficult to achieve normal glucose levels in patients with diabetes. Thus, an important therapeutic challenge of diabetes is the prevention and treatment of its chronic complications. However, the detailed sequence of events in the

pathophysiology of complications and the cellular, biochemical and molecular mechanisms that cause diabetes complications have not been elucidated. Several biochemical mechanisms by which hyperglycemia may cause cellular damage have been studied. One theory proposes that hyperglycemia causes an elevation in the activity of the aldose reductase enzyme, which, in turn, results in the abnormal accumulation of simple chemical compounds called polyols. These polyols cause a chain of events that lead to functional and structural dysfunction in the tissues where they accumulate. Another well-developed theory proposes that glucose reacts nonenzymatically with proteins to initiate a modification known as nonenzymatic glycosylation. During this process, the glucose molecules become attached to proteins in blood and cells. This attachment leads to the development of advanced glycation end products (AGEs), which have been implicated in the covalent modification of proteins. It is postulated that such modifications alter the structure of matrix proteins and the function of intracellular proteins, which may lead to diabetic vascular disease. In addition, interaction of an AGE with its specific receptor (RAGE) induces oxidative stress by altering free radical and cytokine production. In animal models, pharmacologic inhibition of AGE formation can prevent diabetic microvascular complications such as retinopathy and nephropathy. Clinical trials of an inhibitor of AGE formation, aminoguanidine, are underway in humans. A third theory attributes the adverse effect of hyperglycemia to the activation of protein kinase C (PKC), a member of the family of serine-threonine kinases that regulate many vascular functions.

Extensive epidemiologic and clinical evidence suggests that, in addition to hyperglycemia per se, genetic determinants are involved in the development of diabetic complications. However, very little is actually known about the identity or function of specific genes involved.

RECENT FINDINGS: Strong evidence exists in animals models that inhibiting the formation of AGEs can prevent or delay the development of microvascular diabetic complications. Now researchers have also shown that AGEs appear to be important in the development of macrovascular lesions in a diabetic mouse model. Investigators were able to prevent the development of accelerated atherosclerotic lesions in diabetic, hyperlipidemic mice by administering a soluble form of the receptor for AGEs.

Administering soluble RAGE prevented AGEs from activating cellular RAGE; in addition, blood and tissue AGE levels decreased, presumably because the AGE: soluble RAGE complex accelerated AGE clearance.

In animal models, microvascular diabetic complications can be prevented or delayed by aminoguanidine. Most studies of aminoguanidine have focused on

its ability to decrease AGE formation. Recently, investigators have demonstrated that aminoguanidine can also act directly as an antioxidant. Aminoguanidine was able to inhibit cell death (apoptosis) caused by hydrogen peroxide in cultured rat retinal cells. Aminoguanidine decreased reactive oxygen species and lipid peroxidation in the cells. In vivo, aminoguanidine decreased lipid peroxide levels in the vitreous of diabetic rabbits.

Clinical and epidemiologic observations suggest that hyperglycemia is not the only factor in the development of long-term complications of diabetes. Thus, some patients with good blood sugar control will develop complications, while, conversely, some patients with poor glycemic control appear to be spared. Previous epidemiologic studies have suggested a genetic influence for the development of diabetic nephropathy. Recently, a study of family members of patients who participated in the DCCT confirmed that familial factors (presumably, genetic) affect the development of nephropathy and demonstrated, for the first time, that familial factors appear to influence the severity of diabetic retinopathy. Further evidence for the role of familial factors in the development of retinopathy comes from data derived from the Third National Health and Nutrition Examination Survey (NHANES III). Analysis of this data revealed an increased risk for development of retinopathy in Mexican Americans with type 2 diabetes compared to non-Hispanic Whites.

SIGNIFICANCE: Much of the past work on the role of AGEs in diabetes complications has focused on microvascular complications. Also of great public health concern is macrovascular disease in diabetes. Patients with diabetes experience an excess risk of heart disease. Heart disease in diabetes occurs earlier in life, affects women almost as often as men, and is more often fatal. Although metabolic factors may influence this increased risk, it now appears that some of the same pathogenetic mechanisms (i.e., AGE formation) are responsible for macrovascular, as well as microvascular, disease. Clearly, this finding has important therapeutic implications.

Aminoguanidine, an inhibitor of AGE formation, has been useful in preventing complications in animals and is currently being studied in humans. The demonstration that aminoguanidine also inhibits oxidative stress has important implications in designing additional pharmacologic agents that might be useful in preventing or treating diabetic complications. In addition, the use of the soluble RAGE receptor to prevent accelerated atherosclerosis in mice provides another potential target for therapeutic intervention.

The long-term complications of diabetes remain a major public health problem. Since any drug carries some risk of side effects, it is imperative to be able to identify those patients with the highest likelihood of developing complications, to

allow targeted interventions. In addition, identifying those populations at highest risk may also lead to the discovery of additional factors and specific genes which determine the development of complications.

FUTURE DIRECTIONS: Further studies are needed to expand our understanding of the role of AGEs, as well as other potentially injurious pathways, in the development of diabetic complications. The molecular pathophysiology of altered protein function and gene expression leading to tissue injury is still unclear. In addition, the possible interrelationships between the various pathways have not been systematically explored. Further refinements in our understanding of the basis for complications will lead to new modalities for the prevention and treatment of these devastating long-term consequences of diabetes. Likewise, it is essential to continue to define population groups at highest risk for the development of specific complications and to identify specific genes and gene products involved in the development of diabetic complications.

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VIII. TITLE: Genetic Syndromes Provide Clues to the Etiology of Diabetes

BACKGROUND: Rare inherited forms of diabetes have provided clues to the genetic causes of the more common sporadic forms of the disease. The first gene identified to cause diabetes was the insulin receptor gene, which caused two phenotypic syndromes, leprechaunism and severe insulin resistance. The syndrome called Maturity Onset Diabetes of the Young (MODY) has also been studied to determine the genetic defects causing this dominantly inherited form of diabetes. MODY subjects exhibit a defect in glucose-stimulated insulin secretion from the β -cell of the pancreas resulting in diabetes before 25-years of age. A large kindred with MODY was studied and the genetic defect was shown to be linked to the adenosine deaminase (ADA) gene on chromosome 20. Surprisingly, other families with MODY did not show linkage with markers on chromosome 20. Studies on these other MODY kindreds identified a second MODY locus (MODY2) on chromosome 7 and a third (MODY3) on chromosome 12. The glucokinase gene was shown to be the genetic cause of MODY2 and mutations were identified in individuals with this form of MODY.

This gene is thought to function as a glucose sensor and regulate insulin release in the β -cell of the pancreas. Last year, the genes responsible for both MODY1 and MODY3 were identified. Using positional cloning and identifying all transcription units in the region, the gene responsible for MODY3 was identified as Hepatic Nuclear Factor - 1 α (HNF-1 α). Mutations in this gene were demonstrated in individuals from 7 different MODY3 pedigrees. The HNF-1 α gene codes for a transcription factor that regulates gene transcription in both the pancreas and the liver. This transcription factor functions as a dimer either with another HNF-1 α or the closely related HNF-1 β molecule. A dominant/negative effect may be seen if the abnormal subunit binds to a normal subunit inactivating

the entire complex. This dominant/negative effect could explain the dominant form of inheritance seen in MODY. With the identification of an HNF as the genetic defect in MODY3, the HNF-4 α gene was investigated as the cause of MODY1 since it was present within the genetic locus on chromosome 20. A single mutation was found to be inherited in the large MODY1 kindred, which would predict a protein of 267 amino acids containing only the DNA binding domain. The HNF-4 α , another transcription factor found in both the liver and the pancreas, is a member of the steroid/thyroid receptor supergene family. HNF-4 α has been shown to regulate the expression of HNF-1 α which may be the mechanism by which it causes MODY.

RECENT FINDINGS: This year, two additional genes have been shown to cause MODY, bringing the number of genes known to cause MODY to 5. Previously, NIDDK-supported researcher and MERIT Recipient Joel Habener had reported a patient with pancreatic agenesis who was homozygous for a mutation in the gene for the insulin promoter factor-1 (IPF-1), more commonly called PDX-1. The specific mutation involved the deletion of a single nucleotide pair in the IPF-1 encoding gene within codon 63 that resulted in premature translation termination. Subsequently, the research group showed that the entire pancreatic agenesis pedigree, who have a diagnosis consistent with MODY, were heterozygous for this mutation. The mutant mRNA encodes two proteins due to the use of a cryptic start site; one corresponding to the N-terminal region of the protein and a second that corresponds to the C-terminal region of the protein. Moreover, the C-terminal domain protein acts as a dominant-negative inhibitor of wild-type IPF-1. Thus, the manifestation of type 2 diabetes in the heterozygotes may not simply be a dosage effect but may be a result of the activity of the dominant-negative protein.

A second family was found to have mutations in the transcription factor HNF-1 β , a transcription factor that can dimerize with HNF-1 α . Four of the five genes known to cause MODY are pancreatic transcription factors. This finding confirms the importance of the HNF regulatory network in pancreatic β -cell function and in the development of diabetes. Clues for the interaction of these transcription factors were found by studying a patient with MODY3 who had a defect in the promoter region of HNF-1 α . This mutation disrupted the binding site for the transcription factor HNF-4 α , a factor that causes MODY1 when mutated. The mechanism for the development of MODY3 would appear to be that HNF-4 α binding is required to induce HNF-1 α .

Two groups have begun to address how defects in HNF transcription factors cause MODY. One paper studies the regulation of a variety of genes involved in glucose homeostasis and found that expression of HNF-4 α expression is required for expression of these genes including the glucose transporter 2,

aldolase B, glyceraldehyde-3-phosphate dehydrogenase and liver pyruvate kinase. Another strategy for studying the role of HNF-1 α is to analyze the HNF-1 α knockout mouse. Originally, the mouse was noted to have defects in liver gene expression. With the discovery that defects in HNF-1 α causes MODY, this mouse was examined for signs of diabetes. Studies showed that the heterozygous mouse has elevated glucose levels indicative of diabetes. Studies on insulin release demonstrated a defect in the ability of both glucose and arginine to stimulate glucose secretion. This appears to be the mechanism for the elevated glucose levels.

A new genetic syndrome has been described which demonstrates defects in insulin secretion. Mutations in the glutamate dehydrogenase gene have been identified in 8 children with a syndrome of hyperinsulinism-hyperammonemia. These mutations eliminate the ability of the enzyme to be inhibited by GTP and thereby increasing enzyme activity forming excessive amounts of α -keto-glutarate. This syndrome demonstrates the importance of these pathways in regulation of both insulin secretion and ureagenesis.

The gene for another rare syndrome of diabetes called Wolfram syndrome has been identified by positional cloning. The features of this syndrome are diabetes insipidus, diabetes mellitus, optic atrophy and deafness. In this syndrome, the diabetes results from the premature death of the β -cells. The gene located on chromosome 4 is unique. It is expressed in most of the relevant tissues including the pancreas and brain. It contains 10 regions that may be transmembrane segments. Further studies will be needed to determine the function of this gene in maintaining normal β -cell function.

SIGNIFICANCE: Positional cloning has identified new candidate genes for inherited forms of diabetes. These genes may provide insight into the mechanism for the development of the more common type 2 diabetes.

FUTURE DIRECTIONS: The role of the HNF transcription factors in the development of diabetes needs to be identified. In addition, whether these genes play a role in the more common form of type 2 diabetes needs to be investigated.

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GENETIC METABOLIC DISEASES

IX. TITLE: Cystic Fibrosis: Improved Understanding of the Function of CFTR has Lead to New Insights in Pathogenesis and Treatment of CF

BACKGROUND: Improved therapy has transformed CF from a disease characterized by death in early childhood to a chronic illness, with most patients living to adulthood. In recent years a number of new therapies have been shown to be effective in preventing or retarding the lung disease associated with CF. In 1993, the FDA approved the mucus-thinning drug DNase after research showed this drug reduced the frequency of severe episodes of lung infection and slightly improved lung function after 24-weeks of therapy. In 1995, a randomized controlled clinical trial showed that the anti-inflammatory drug ibuprofen reduced the rate of loss of lung function and improved body weight in patients with CF. In 1997, the FDA approved the use of an inhaled antibiotic which helps control lung infections and reduces the need for hospitalization in patients with CF. Clinical trials are underway to evaluate several other drugs that may prove useful in slowing the progression of lung disease in CF. Improved therapy for pancreatic insufficiency and attention to nutrition has also played a major role in the increased longevity and well being of CF patients.

The clinical presentation of CF can be extremely varied. Mutations, which result in no functional CFTR, cause a classical CF presentation with lung disease and pancreatic insufficiency. Other less severe mutations cause milder forms of the disease often with only some of the symptoms. Understanding how these

mutations cause the pathophysiology of CF requires an understanding of all of the cellular roles of CFTR and its functional domains. This year several new functions of CFTR have been discovered which might lead to a better understanding of the pathophysiology of the disease.

RECENT FINDINGS: The understanding of the pathophysiology of cystic fibrosis relies on the ability to understand the pleiotropic effects of the CFTR. Several studies have been published over the past year, which help define some of the disparate cellular functions of this protein. Several new functions of the CFTR molecule have been discovered. In addition, the roles of functional domains that comprise the CFTR protein are being elucidated.

The spectrum of clinical presentations that result from mutations in CFTR has been expanded and is being correlated to the functional properties of CFTR mutations.

One in 25 people are asymptomatic carriers of a mutation in CFTR. A new theory has been proposed to explain the high prevalence of CFTR mutations. A group from the University of Cambridge showed that *Salmonella typhi* uses CFTR to enter intestinal epithelial cells. Mice homozygous for CFTR with $\Delta F508$ mutation showed no uptake of *S. typhi* and heterozygous mice showed a significant reduction. This mutation, which predominates in CF, may reduce susceptibility to typhoid fever leading to a heterozygote advantage.

Studies on another member of the ABC transporter family, has shed new light on the function of many of the CFTR mutations including the most common $\Delta F508$ mutation. The crystal structure of the nucleotide binding domains (NBD) of histidine permease from *Salmonella typhimurium* has been determined. The NBD has been shown to adopt an L-shaped structure with one arm interacting with the adjacent NBD and the other interacting with the membrane-spanning domain. The NBD of most of the ATP transporters has been highly conserved. The most common defect causing CF, $\Delta F508$, occurs in the second arm of the NBD of CFTR, which interacts with the membrane-spanning domains. It may be that disruption of this critical interaction contributes to the mis-folding of this mutant protein. This structure would predict that mutations near the ATP-binding pocket are likely to disrupt ATP binding or hydrolysis where as mutations in arm 1 are likely to impair the ability of the NBD to dimerize.

Recent studies are developing a picture of CFTR functioning in the cell as part of a macromolecular complex. These complex interactions occur with other molecules within the cell including syntaxin, purine receptor, and other chloride-channels. On the cell surface, CFTR interacts with other membrane proteins and these interactions may play a role in fine-tuning CFTR and other channel

activity in response to physiological cues. Understanding how CFTR interacts with other membrane proteins has important implications for design of therapies targeted at activating CFTR or activating other proteins modulated by CFTR to overcome defects in cellular function induced by mutations in CFTR. Recent work identified a membrane protein (syntaxin 1A) that regulates CFTR. Syntaxin 1A was shown to physically interact with the N-terminal of the CFTR chloride channels and regulate movement of chloride both in model systems and in epithelial cells that normally express these proteins. Another protein-protein interacting domain has been discovered at the C-terminal of CFTR. This CFTR PDZ region has been shown to bind the Na⁺-H⁺ exchanger regulatory factor, a phosphoprotein that is known to regulate ion transport. Other proteins are likely to interact with this region. Understanding the physiologic regulation of CFTR and other chloride channels has important implications for strategies to activate alternate channels to compensate for defective CFTR function in CF.

Mutations in CFTR have been shown to cause a spectrum of manifestations of CF from a debilitating lung disease with pancreatic insufficiency to a mild form of the disease with congenital absence of the vas deferens (CVD) presenting as infertility due to the absence of sperm. A new clinical presentation has been recognized in this spectrum which consists of chronic pancreatitis without sinopulmonary disease. Two groups studied patients with pancreatic insufficiency of unknown causes. One of these groups supported by NIDDK found that 37 percent of these patients had one abnormal CFTR gene and a subset of these patients had mutations in both CFTR alleles. Because of the numerous CFTR mutations it is not clear at this time whether the patients with only a single identified mutation, in fact, have a second unrecognized CFTR mutation or whether heterozygosity for CFTR contributes to susceptibility to pancreatitis.

Multiple types of mutations have been shown to cause CF. Approximately 10 percent of patients have mutations that insert a stop codon into the CFTR gene resulting in premature termination of the protein. Agents such as gentamicin can cause the protein translation machinery to read through the stop codon resulting in the production of a full-length protein. Administration of gentamicin in a CF cell line has resulted in an increase in CFTR protein and restored cAMP-activated chloride transport. This potential therapy could help the CF patients who harbor this type of mutation.

SIGNIFICANCE: Recent studies have greatly expanded our understanding of the biologic importance of CFTR, the CF gene product. A greater understanding of the role CFTR plays within the cell and the function of its protein domains will lead to new methods to impact this disease.

FUTURE DIRECTIONS: With the discovery of a protein interaction domain within CFTR, studies are ongoing to identify other proteins that interact with CFTR. A complete understanding of the cellular role of CFTR is necessary to understand the contribution of different mutations to the pathophysiology of the disease.

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X. TITLE: Gene Therapy: Understanding the Role of the Immune System In Modulating Gene Expression

BACKGROUND: The ultimate goal for the treatment of genetic diseases is to replace the defective gene with a corrected gene that will remain active for the life of the patient. In theory, this is a simple concept but in reality there are many impediments to targeting a gene to the appropriate cell types and achieving long-term gene expression at physiologic levels.

Encouraging results have been obtained using viral vectors to introduce therapeutic genes into cells. Several different viral systems are being investigated, each with distinct advantages and disadvantages. Initial results with retroviral vectors have shown promise for long-term gene expression, however, the efficiency of transduction, the process by which vector DNA integrates into the cell, is low. In addition, cells must be actively dividing in order for retroviral transduction to occur limiting the application of this technology. Adenoviral vectors provide efficient gene expression in both dividing and non-dividing cells. However, expression has been short lived due to immunological destruction of cells expressing the therapeutic gene as well as viral proteins. Adeno-associated viral (AAV) vectors can transduce non-dividing cells and provide long-term expression but conditions, which are conducive to efficient transduction, need further elucidation. In addition to viral systems, investigators are developing non-viral delivery systems using lipoplexes and receptor-mediated conjugates. These systems have been characterized by transient gene expression but may provide a means of targeting expression to particular cell types. The ideal vector system may combine the advantageous features

from several of the current delivery systems. NIDDK-funded investigators are working on many different aspects of this problem.

One of the major impediments to gene therapy has been immunologic responses to both the viral proteins and to the transgenes that are novel to the host. Individuals can develop a cytotoxic T lymphocyte (CTL) response to these foreign proteins that results in the destruction of cells expressing these genes. This response has been documented with adenoviral vectors. In addition, many individuals have already developed humoral immunity to various viral agents that may interfere with initial attempts at gene therapy. Immune response to viral proteins or the transgenes at the initial administration can also inhibit readministration of the gene therapy vector. Immune response to novel transgenes has been shown to interfere with attempts at therapy. These can occur when a novel transgene such as β -galactosidase is used in the vector or when a therapeutic gene is novel to the host as is the case in null mutations. Significant advances in our understanding of the viral immune response have been reported this year.

RECENT FINDINGS: The observation that an adenoviral vector directly injected into muscle elicits a CTL response but an AAV vector does not, has led to studies on the mechanism of the immune response to adenoviral vectors. The induction of cellular immunity requires that the foreign protein be presented to the T cell on the surface of an antigen-presenting cell (APC). In addition to presenting the antigen, the APC has several costimulatory molecules such as B7 molecules and CD40 that bind to proteins on the surface of the T cell. NIDDK-supported investigator, James Wilson, has proposed that the mechanism for eliciting an immune response is based on the ability of the vector to transduce dendritic cells. The dendritic cell, also known as the antigen-presenting cell, is efficient at presenting antigen on MHC class I molecules thus activating the T cell response. Direct transduction of the dendritic cell allows efficient presentation of the vector expressed proteins as antigens. Adenoviral vectors efficiently transfect dendritic cells while AAV is a very poor transducer of dendritic cells. Cells transferred from adenoviral infected animals to an animal transfected with the same transgene in an AAV vector will mount a CTL response to the transgene. This process is called adoptive transfer and demonstrates that the transgene in the context of an AAV vector is susceptible to CTL recognition. The inability to transduce antigen-presenting cells appears to be the mechanism by which AAV vectors and their transgene cargo evade the immune system.

Humoral immunity has been shown to be a major component in inhibiting the readministration of both adenoviral and AAV vectors. The initial administration to a naïve animal results in the appearance of neutralizing antibodies that inhibit

future administrations. One strategy for interfering with the immune response is to block the costimulatory molecules required for activation of the immune response. Two molecules have demonstrated the ability to disrupt this process: CTLA4Ig, a soluble molecule which binds to B7 on the APC blocking the binding of CD28 on the T cell; and MRI, an anti-CD40 ligand antibody that blocks the interaction between the T cell CD40 ligand with both the APC and B cell CD40. Several groups have shown that transient immunosuppression with CTLA4Ig during IV adenoviral vector administration prevented antibody formation and permitted vector readministration. The situation in the lung is more complex since, in addition to systemic immunity, the lung is protected by mucosal immunity. Based on these observations, several immunosuppression regimens have been tested for both AAV and adenoviral vectors. IV administration of CTLA4Ig alone was not able to prevent antibody formation when the vector was administered to the lung. However, two papers from investigators at the University of Washington show that the combination of both CTLA4Ig and MR1, allowed for readministration to the lung. For AAV, a combination of both CTLA4Ig and MR1 administered intraperitoneally resulted in the ability to readminister the vector to the lung. For an adenoviral vector, intraperitoneal administration of both CTLA4Ig and MR1 as well as intratracheal coadministration of an adenoviral vector expressing CTLA4Ig was required to permit second administration. These immunosuppression regimens also increased the duration of transgene expression.

SIGNIFICANCE: Understanding the role of the immune system in modulating gene expression after gene transfer will allow for the development of more effective methods of gene delivery. Development of a protocol for readministration in the lung will aid in the development of gene therapy for cystic fibrosis.

FUTURE DIRECTIONS: Research to identify new serotypes of AAV for which the population has no prior immunity will be important. This, in combination with a method of immunomodulation like those described, would allow for multiple attempts at gene therapy.

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ENDOCRINE REGULATION OF BONE

XI. TITLE: **Clinical Trials of Parathyroid Hormone (PTH) Showing Efficacy as an Anabolic Factor in Bone to Treat Osteoporosis**

BACKGROUND: Bone is a dynamic organ, comprised of the extracellular matrix that provides the form and structure of the skeleton and the bone cells that sustain it. While the inert matrix represents both the major structural support for the body and a storage point for important minerals, such as calcium and phosphorus, bone cells must remain continuously active to maintain it. Once bone is formed it undergoes continuous cycles of formation and resorption (breakdown) in response to changing dietary, hormonal, and activity levels. Bone turnover determines bone mass and depends on numerous factors, including mechanical stress, physical activity, diet, and the need to maintain proper serum calcium levels. The balance between bone formation and resorption is regulated by numerous hormones, growth factors, and cytokines, which act upon the bone forming cells (osteoblasts), and bone resorbing cells (osteoblasts). Day-to-day, as well as chronic, fluctuations in serum calcium levels and body activity have important implications for bone mass. Early childhood, adolescence, and late adulthood represent key times during which proper regulation of bone turnover is especially crucial. Early growth and development of the skeleton is central to the development of peak bone mass

into adulthood, while the changes in hormonal balances that occur with aging are important for the maintenance of this peak bone mass through later life. Alterations during both of these periods can have profound effects on the development of osteoporotic fractures later in life.

Steroid hormones, such as vitamin D and estrogen, are particularly important through their actions on dietary supply of the key mineral calcium, as well as actions on bone cells to use these minerals to make bone. Hormones, such as Parathyroid Hormone (PTH) and the PTH-related Protein (PTHrP) are essential to maintaining the proper balance of minerals between bone and blood. Growth factors, such as insulin-like growth factor-I (IGF-I), are also key players in the regulation of bone turnover at key periods in the life cycle. In postmenopausal women, imbalances in hormones result in imbalances in bone turnover, such that there is a high rate of remodeling of bone, but with a net loss of bone mineral, weakening the bones. This high turnover bone remodeling as a result of loss of natural estrogen results in a rapid and sustained loss of bone mineral, weakening the microscopic structure of the bone to the point at which the risk of fracture is significantly increased. Hormone replacement therapy (HRT) has been used to stem and partly reverse this loss of bone mineral, but side effects, including potential increased risk of breast and uterine cancer limit the utilization of HRT. Considerable effort has focused on development of hormone-like substances that can exert the positive effects of the natural hormone on bone without unneeded or potentially deleterious side effects. Often the interplay in cell signaling responses between and among steroid hormones and growth factors, and other hormones, is an important feature in the regulation of bone turnover. Major progress in developing a complete understanding of how these factors work has been limited by an absence of knowledge of the precise molecular mechanism of action in bone of hormones such as vitamin D, estrogen, PTH and PTHrP.

RECENT FINDINGS: Two small-scale clinical trials testing the efficacy of PTH as an anabolic agent for the treatment of osteoporosis demonstrated that PTH can have a beneficial effect on bone mass. In the first trial, in young women treated with agents to induce a menopause as part of the treatment of endometriosis, PTH 1-34 administered once/day for 12 months increased bone mineral density in the spine, while eliminating further loss of bone mineral at other sites, including the hip and the arm. PTH was well tolerated, with good adherence to the administration regimen. In the second trial in women with secondary osteoporosis due to long-term administration of glucocorticoids in the treatment of chronic inflammatory diseases (e.g. rheumatoid arthritis), 12 months of intermittent PTH treatment results in increased bone mineral density in the lumbar spine, the hip, and the arm. When administered together with estrogen, an even greater effect was observed. Moreover, measurement of biochemical

markers of bone turnover indicated that bone formation had been uncoupled from bone resorption, in favor of continued bone formation. In another study post-menopausal women with osteoporosis and no other interventions received a 2-week trial of intermittent PTHrP 1-36. The drug was well tolerated, and while the duration was too short to demonstrate effects on bone mineral density, biochemical markers of bone turnover were also indicative of a shift to net bone formation.

SIGNIFICANCE: The hormonal regulation of bone turnover has been only incompletely understood. Proper hormonal regulation of bone formation early in development, coupled with other factors such as diet and exercise, combine to allow for the appropriate development of peak adult bone mass. Osteoporosis develops when the regulation of bone turnover is disrupted or in any way seriously impaired. Osteoporotic fractures are a major public health problem, causing significant amounts of pain and disability, primarily in women, but also including men. Hip fractures, in particular, often contribute to early death. With the results of these preliminary small trials hope has been raised that intermittent PTH or PTHrP administration may become a viable therapeutic intervention in osteoporotic women. These new developments in the understanding of hormonal mechanisms underlying the regulation of bone formation on the one hand, and bone resorption, on the other, it becomes possible to develop drugs or other PTH or PTH-rP hormone-like agents (analogues) which can be used at appropriate times to help maintain the proper regulatory balances and potentially rebuild bone. In women, the loss of normal hormonal balances that occur can sometimes be partly reversed through hormone replacement therapy, although for many women there are risks associated with hormone replacement therapy, including a slightly increased risk of the development of tumors in breast and uterus.

FUTURE DIRECTIONS: Further studies to investigate the combination of anabolic hormones with antiresorptive agents, such as Estrogen and the Selective Estrogen Receptor Modulators (SERMS), as well as bisphosphonates are needed to determine whether additive effects on bone mineral density may be obtained. Large studies will be needed to establish the safety and efficacy of these potential new treatments.

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XII. TITLE: Factors Which Mediate Cell Fate Determination of Bone Cell Precursors

BACKGROUND: Cell fate determination during development is a key event necessary for the ultimate development of a mature, functioning cell. Cell signaling at key times in development is essential for the proper expression of a sequence of genes that will determine the fate of that cell. For the bone forming cells or osteoblasts, that precursor, or undifferentiated stem cell, is found in the early mesoderm cells. For the bone resorbing cells or osteoclasts, pluripotent hematopoietic cells in the bone marrow represent the precursor cells. Cell signaling by local growth factors through receptors found on the surface of the undifferentiated cells is translated into a series of intracellular signals which reach the nucleus and either turn on or off important genes. When the full effects of these signals on gene expression are felt, the undifferentiated cell is stimulated to undergo differentiation down a pathway ultimately leading to the mature osteoblast or osteoclast. Often several different signals are required before the fully mature cell is obtained. In most instances the last step in the cascade of cell signaling is the action of a transcription factor in the nucleus, capable of altering the expression of a target gene(s).

RECENT FINDINGS: The osteoclast, or bone resorbing cell, is essential for the

process of bone remodeling. How it is regulated is still unknown, but one component of the process is the recruitment of osteoclast precursor cells to the site of bone resorption and the stimulation of the final stages of differentiation to produce a mature, working multi-nucleated osteoclast. One local cytokine, or growth factor, that has been implicated in osteoclast function is Colony-stimulating Factor-1 (CSF-1). CSF-1 exists in two forms, a soluble form, and a cell surface form. New findings now demonstrate that the cell surface form is capable of stimulating the last stages of differentiation of the osteoclast to form the large, multi-nucleated cells responsible for bone resorption. CSF-1 is, in turn, released by osteoblasts which also express the cell surface form, and do so in response to hormones such as Parathyroid Hormone (PTH), a key mineral regulating hormone, and Tumor Necrosis Factor (TNF), a local bone-active cytokine. It now appears that the cell surface form of CSF-1 when in close proximity to osteoclast precursor cells stimulates osteoclastogenesis, perhaps as part of the cascade of hormonal action stemming from PTH-induced bone resorption. When the cell-surface CSF-1 signal is received, a cascade of cell signaling events triggers the final differentiation of the pre-osteoclast to undergo terminal differentiation to form the mature, working osteoclast.

The osteoblast also arises from pluripotent cells, found in the mesenchyme. Several different local growth factors and bone-active cytokines have been implicated in differentiation of these pre-osteoblast cells. Now it appears that one factor, Bone Morphogenetic Protein-2 (BMP-2) signals mesenchymal precursor cell differentiation to begin the path of development toward becoming an osteoblast. Moreover, two transcription factors within these cells, Smad5 and DPC4 have now been found to mediate the BMP-2 signal in the pre-osteoblast, helping to mediate the final stages in osteoblastic differentiation. Smad5 and DPC4 are found in the cytoplasm of the precursor cells, and are phosphorylated in response to BMP-2 signaling. They then form a dimer complex, which is translocated to the nucleus where gene expression is regulated, with formation of a mature osteoblast the result.

SIGNIFICANCE: These studies have identified important steps in the process of cell fate determination of bone cells. The entire signaling cascade is not known for these cells, but it is now becoming clear that the expression of key signaling molecules at appropriate times in development is required for precursor cells to begin expressing the genes that allow for differentiation, ultimately giving rise to mature functioning bone cells. The regulation of the amounts and activities of these cells helps to determine whether bone will be formed or resorbed, and hence imbalances in cell function contribute to the development of osteoporosis.

FUTURE DIRECTIONS: Elucidation of the exact requirements of signals and signaling molecules may allow for the development of agents which can recruit

undifferentiated precursor cells to sites where bone remodeling is needed. It may then be possible to stimulate formation of bone forming cells to rebuild bone where it has been lost due to osteoporosis.

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BASIC RESEARCH RELEVANT TO DIABETES, ENDOCRINOLOGY METABOLISM

XIII. TITLE: Determination of Specificity in the Hormonal Regulation of Gene Expression

BACKGROUND: Two classes of circulating hormones exist: those that are peptide-based and cannot enter a cell, binding to receptors on the cell surface; and those that are lipid (e.g. steroid)-based and can cross into a cell, binding to receptors in the cell cytoplasm or the nucleus. Signal transduction for the steroid, or nuclear receptor, gene family involves receptors present either initially in the cytoplasm and/or the nucleus, but ultimately binding to target genes in the nucleus. These hormones act as regulators of gene transcription, the final manifestation of endocrine physiology. Often classified by their receptors, the nuclear receptor and steroid hormones include adrenal glucocorticoids and mineralocorticoids, sex hormones (androgens, estrogen, progesterone), vitamin D, thyroid hormone, derivatives of vitamin A (retinoids), and receptors for which hormones have not yet been found, the orphan receptors. Although there are differences in the structures of the different classes of these hormones, their receptors are structurally and functionally similar. In general each receptor has a domain which binds hormone, a dimerization domain which binds to itself or to other receptors to form a dimer pair, a DNA binding domain which binds to specific regions of DNA, and one or more transactivation domains which mediate the effects on the target gene(s). The region of DNA, to which the receptor dimer pair binds, the hormone response element (HRE), acts to either activate or suppress the transcription of the target gene. The enhancer region of the gene does not act alone, but rather requires the presence of a large protein/DNA complex at the actual transcription start site.

In the nucleus, a larger complex of proteins is required to regulate the expression of a target gene. Nuclear accessory proteins are those that either constitutes the transcriptional machinery, itself, or act to regulate the activity of the nuclear receptor. While basal transcription results in limited expression of a gene, higher, or regulated, levels of expression are obtained in response to hormonal signals. These nuclear accessory proteins bind to nuclear receptors or other hormonally regulated transcription factors, and act either as coactivators, or corepressors of receptor dependent gene expression. Specificity is often dependent on whether a particular coactivator complex or corepressor complex is present, and at what levels, in a given cell. While originally discovered and characterized for the thyroid hormone receptor, it is now becoming evident that many nuclear accessory proteins can be found that interact with numerous members of the steroid hormone superfamily. Recent

progress has stressed the role of co-activators, such as Steroid Receptor Co-Activator-1 (SRC-1) which acts to couple the thyroid hormone receptor/retinoic acid X receptor (TR/RXR) dimer pair bound to the promoter region of a gene to the general transcriptional protein complex. These proteins form complexes with other essential regulatory proteins, including CBP (CREB-binding protein), and other similar members of a family of nuclear proteins called the p160 family. All of these proteins are characterized by their ability to bind to hormone receptors, the transcriptional apparatus, and express enzyme activity toward DNA binding proteins. This activity allows for the acetylation or deacetylation of histones, the DNA binding proteins that keep the DNA tightly bound. When acetylated, DNA is "open" allowing for gene expression. When deacetylated, DNA is "closed" and gene expression is repressed. Often receptors from different hormonal pathways interact at the level of the nuclear accessory proteins and the gene in a process called cross talk, and several of these newly discovered nuclear accessory proteins have been found to mediate or act as the agents of this cross talk. The role of the ligand (hormone) is often to either recruit or dislodge a particular nuclear accessory protein, to either release repression or stimulate expression of a gene.

RECENT FINDINGS: Originally, it was felt that only receptors of the steroid hormone superfamily acted through nuclear accessory proteins to modulate gene transcription. Recently, it was shown that other hormonally dependent transcription factors also required nuclear accessory proteins. The POU-domain transcription factors have long been known to act in pituitary cells to regulate development of cell types and expression of genes for key hormones. One such POU-domain transcription factor is Pit-1. Pit-1 has many effects; one such is to regulate the expression of the prolactin gene in pituitary cells. It had also been observed that other hormones, including nuclear receptors, had effects on the regulation of this gene. To determine the mechanism of action of Pit-1 on hormonal gene expression, Rosenfeld and colleagues tested the ability of Pit-1 to interact with a number of nuclear accessory proteins, including CBP. Moreover, they found that phosphorylation of CBP by a number of different hormonal signaling pathways could affect CBP behavior and, consequently, its ability to partner with Pit-1. Rosenfeld and coworkers went on to show that Pit-1 appeared to exist in a balanced relationship with N-CoR and CBP, with the N-CoR complex leading to repression of gene expression and the CBP complex leading to activation of gene expression. The balance between the two was determined by which hormonal signaling pathway was dominant at a given time, with (e.g.) growth factors, such as EGF or insulin, or ligands acting through G-protein coupled receptors leading to activation, and other Growth Factors or hormones and/or nuclear receptors leading to repression. The ultimate effect was either acetylation or deacetylation of DNA-bound histones.

Since any cell is subjected to a myriad of sometimes competing hormonal signals at any time, understanding how ligands, or hormones, affect the balances between receptor-nuclear accessory complexes-DNA is an essential component in understanding the specificity of hormone action. Some of the nuclear receptor family members had all of the appearances of being classical receptors, able to bind DNA, and a ligand, but without a known ligand. One such "orphan" receptor is known as CAR. It was known to be constitutively active, always activating gene expression, but with no known ligand. Now, Moore and coworkers, have found that a naturally present metabolite of androgens (male sex steroids), androstane, acts to turn off CAR activity. It does so by altering the relationship of CAR to nuclear accessory proteins. As a constitutively active receptor, CAR is bound to one class of co-activator, SRC-1. When androstane is present CAR activity is shut down. Androstane apparently binds to CAR, changing its shape slightly and causing it to lose its association with SRC-1. Thus androstane acts as a reverse agonist of hormone action. Thus defining another aspect of selectivity and specificity in hormone action.

Finally, the application of the latest methods of transgenic mouse technology to the question of the role of nuclear accessory factors has shown that when SRC-1 is deleted resultant mice appear nearly normal. There are some rather important defects that become apparent as the animals age, and these relate to the maturation of the sex organs, which are reduced in SRC-1 deficient animals, suggesting a form of partial hormone resistance. Resistance, in this case, appears to be to estrogen, progesterone, and androgens. The implications of this study are that specificity of hormonal response for the nuclear receptor family may be depend on a given nuclear accessory protein(s).

SIGNIFICANCE: Insight into how hormone signaling results in change in gene expression will open major avenues in the understanding of the hormonal basis of diseases, such as breast and prostate cancer, osteoporosis, and diabetes. Delineating how hormones respond to ligands can help in the design of new therapeutic agents to modulate or control their actions.

FUTURE DIRECTIONS: Further research efforts are needed to fully define pathways of hormonal signaling, specificity of interaction with nuclear accessory factors, and ultimately with target genes in the nucleus.

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XIV. TITLE: Cell Surface Receptors and Regulation of Signal Transduction

BACKGROUND: Cell surface receptors bind hormones that cannot cross into the cell. These peptide-based hormones, growth factors, and cytokines require cell surface receptors to transduce their signals into cellular action. Thus, signal transduction requires the generation of second messengers at the surface of the cell that effect the hormonal signal within the cell. For G-protein coupled receptors (GPCR), a major class of cell surface receptor, the receptor is coupled to guanine nucleotide triphosphate (GTP) binding proteins as the first step in signal transduction. G-proteins then interact with other proteins in the cell to either manufacture cyclic adenosine monophosphate (cAMP), or derivatives of membrane phospholipids-in either case the result is further amplification of the hormonal signal. G-proteins are heterotrimeric complexes made up of 3 proteins, an α -subunit and a dimer of β and γ -subunits. Once G-proteins have been stimulated the α -subunit breaks off and can initiate a

signaling cascade in the cell through interaction with effector molecules, such as adenylyl cyclase (to form cAMP). The β - γ subunit may also initiate signaling cascades. These second messenger-initiated cascades of enzyme reactions amplify the hormonal signal throughout the cell. The ultimate effectors are ion channels, components of the cellular cytoskeletal machinery, or protein complexes, which enter the nucleus and regulate gene expression. The results for the cell can be membrane depolarization, secretion of hormones or other stored products, initiation of the synthesis of new protein(s), initiation of programmed cell death, cell movement, or change in the timing of the cell cycle. Unusual or deranged signaling through GPCRs can also lead to inappropriate cell behavior. If that occurs during embryogenesis it could lead to deformities; if occurring during adult state, it could lead to the development of disease or development of tumors.

RECENT FINDINGS: The GPCR is a large molecule, which loops through the cell surface 7 times. The segments outside of the cell bind the hormone, while the inner segments have roles in signal transduction within the cell. Recently, it was found that the 3rd intracellular segment of the M2- and M3-muscarinic receptors, a class of GPCRs active in muscle, has a specific docking site for one of the G- β / γ dimer complexes, thus providing a platform through which other parts of the signaling cascade can be recruited. This specific docking site may then serve to quickly amplify signal transduction through that class of GPCR.

While there are many known GPCRs, for which a hormone, or ligand, is well characterized, there are others for which a ligand is not known. These receptors are referred to as orphan receptors. Now, it has been shown that the orphan receptor EDG-1, a GPCR implicated in the formation of cell-cell junctions, does have a natural ligand. Sphingosine-1-phosphate is a circulating metabolite that appears to be the natural ligand for EDG-1. When overexpressed, EDG-1 causes very tight cell-to-cell coupling, and when not present, cells that ordinarily couple are unable to do so. When the ligand, sphingosine-1-phosphate, is present it stimulates cell adhesion through activation of a signaling cascade through the EDG-1 receptor. Defects in EDG-1 signaling could be involved in the loss of cell-cell contact that occurs during tumor formation.

One of the consequences of cell signaling through GPCRs is the activation of different classes of protein kinases. A class of kinase that initiates cellular proliferation is the mitogen activated protein kinase family or MAP kinases. One such MAP kinase, the extracellular signal-regulated kinase2 (ERK2) has been implicated in proliferation, programmed cell death, and other functions. New research now shows that when ERK2 is phosphorylated as part of a signaling cascade emanating from GPCRs it forms a dimer pair with another copy of itself and is translocated to the nucleus. There it is able to phosphorylate other

proteins, which are then able to regulate gene expression.

Another mechanism for the coupling of external signals through GPCRs is via the recruitment of small, intracellular G-proteins. This class of G-protein is distinct from the heterotrimeric α - β / γ class. For the rhodopsin receptor, the receptor for light in the rods and cones of the eye and one of the first member of the GPCR family to be identified, a class of small G-proteins has been found to signal in a new pathway that involves an enzyme that acts upon lipids in the cell. This enzyme, phospholipase D, in turn generates a message ultimately leading to changes in cellular calcium concentrations. The release of intracellular stores of calcium serves as a major signal for change in cell action. This new pathway thus explains how certain key cells are able to respond to hormones and other signals. Mutations in these small G-proteins have been implicated in developmental defects and cancer. Understanding how they function to couple signals in cells may help to find means for correcting mutations which lead to disease.

SIGNIFICANCE: Signaling through GPCRs is essential to every day function in most cells. The signaling cascades that flow from these receptors are responsible for cellular changes during development, in response to stress, disease, and signals for programmed cell death. Mutations in such receptors have been implicated in disease and many pharmaceutical agents are designed to selectively stimulate or block signal transduction through GPCRs.

FUTURE DIRECTIONS: Research to fully delineate the mechanisms of signal transduction through GPCRs will prove invaluable in discerning how and why cells become deranged in action and behavior, such as in cancer.

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XV. TITLE: Structure and Mechanism of Electron Transport Chain Proteins

BACKGROUND: The electron transport chain is an essential component of every living organism. It is composed of five protein complexes containing a variety of metal redox centers which are fixed in the inner mitochondrial membrane, plus the lipid soluble molecule ubiquinone and the soluble protein cytochrome c. These complexes facilitate transport of reducing equivalents from NADH and succinate, the products of the TCA cycle, to molecular oxygen, resulting finally in the production of water and the energy transducing molecule ATP. They contain iron-sulfur clusters, heme groups, and copper. The first protein complex I is NADH-ubiquinone oxidoreductase which transfers electrons directly from NADH to ubiquinone. Complex II, or succinate dehydrogenase, transfers electrons directly from succinate to ubiquinone. Complex III, cytochrome bc_1 , transfers electrons from ubiquinone to cytochrome c, and complex IV (cytochrome c oxidase) uses these electrons to reduce molecular oxygen to water. The product of the redox cascade is the generation of a proton gradient across the inner mitochondrial membrane, and this electrochemical energy is used by the membrane protein F_1F_0 -ATPase to drive ATP formation.

The molecular mechanism whereby the proton gradient is formed is still a matter of some speculation. It is clear from a variety of experiments conducted in intact tissues, isolated mitochondria, and submitochondrial particles that the mitochondrial proton gradient is generated by electron transport, and that use of substrates and oxygen is functionally and tightly coupled to proton gradient

formation and ATP production. Each of the protein complexes in the electron transport chain serve to pass protons across the membrane to build up this gradient, and it is very possible that each protein has its own unique mechanism. The complete structures of these complexes will certainly shed light on this problem, but they have been difficult to obtain because the proteins are hydrophobic and therefore require specialized conditions for crystallization. These complexes tend to contain multiple subunits and are too large to be easily studied with NMR.

RECENT FINDINGS: The complete crystal structure of the membrane protein cytochrome bc_1 from beef and chicken heart was solved to 2.8 Å. This structure was reported by three groups within the last year (one supported by NIDDK), and represents a large advance both in the technology of crystallization of membrane proteins and in our understanding of electron transport and proton gradient formation. In fact, the new structure yields a mechanism for the “Q-cycle” theory of proton gradient formation. Cytochrome bc_1 serves to pass electrons from ubiquinone, a redox molecule confined to the lipid interior of the membrane, to cytochrome c , a protein that moves freely on the exterior face of the inner mitochondrial membrane. The redox energy is used to move protons from the matrix side to the cytosolic side of the membrane, forming the proton gradient that is subsequently used to fuel ATP synthesis. The complete complex consists of a dimer of eleven subunits each, with a combined mass of 240 kD.

Cytochrome bc_1 contains three cytochromes (cyt) and one iron-sulphur protein (ISP) which serve as redox centers, in addition to two ubiquinone binding sites which were identified by co-crystallizing the protein with ubiquinone analogues antimycin, myxothiazol and stigmatellin. Cyt b_H and b_L are found within the membrane spanning α -helix region, one near each membrane face. A ubiquinol binding site is associated with each cyt b . The extramitochondrial domain of the protein complex includes cyt c_1 and the nearby cytochrome c binding site, as well as a moveable arm (ISP) with the Fe-S cluster on the free end. This arm crystalized in three different positions dependent on the site of the bound ubiquinone analogue, showing that it can swing between the cyt b_L and cyt c_1 of the opposite monomer, supposedly carrying an electron from one to the other.

These domains support the following “Q cycle” mechanism for coupling proton gradient generation to electron transport. Ubiquinol can accept one or two electrons, adding a proton with each. The fully reduced ubihydroquinone (electrons accepted from NADH or succinate via complexes I and II, and protons pulled from the mitochondrial matrix space) diffuses through the membrane lipid and binds in a hydrophobic pocket near cyt b_L (in the myxothiazol or stigmatellin site near the cytosolic face). Here it forms a hydrogen bond with the moveable Fe-S center. It donates one electron to cyt b_L and one to Fe-S. At the same

time, it gives up two protons to the cytoplasm. Cyt b_L passes its electron down to cyt b_H near the matrix side of the membrane, while the moving arm (no longer attracted to the oxidized ubiquinone) carries its electron up out of the membrane to cyt c_1 , which passes it directly to the freely moving cyt c . In the meantime, ubiquinone encounters and accepts an electron from reduced cyt b_H , picking up a proton from the matrix side of the membrane in the process. This mechanism thereby serves to move protons from the mitochondrial matrix to the cytosol using energy released from electron transport down the cytochrome chain.

Structural changes also occur in Complex I (NADH-ubiquinone oxidoreductase) associated with changing redox state. This enzyme contains 48 subunits, with 1 FMN and 5-8 Fe-S clusters. This complex has not yet been crystallized for x-ray diffraction studies. It contains three dissociable domains. The primary NAD(P)H dehydrogenase activity is found in a flavoprotein (FP) domain. The structure was interrogated by subunit cross-linking, or trypsin treatment following binding of the ligands NADPH, NADP or NAD(H). NAD(P) did not alter the pattern of subunit cross-linking or tryptic digestion, but NAD(P)H resulted in a new cross-linking patterns and digestion peptide product. This indicates that conformational changes occur upon reduction of the complex I redox centers, and that these changes expose trypsin sites and alter contact between subunits.

Cytochrome c is a globular protein with one cytochrome molecule that roams the intermembrane face of the inner mitochondrial membrane, passing electrons from cytochrome bc_1 (complex III) to cytochrome c oxidase (complex IV). It is important to understand the protein structural differences between the oxidized and the reduced state in order to interrogate function and the mode of interaction with its redox partners. The structure of oxidized horse heart cytochrome c in solution was found from NMR Nuclear Overhauser Effect and pseudocontact shift experiments. This new structure, obtained in a more physiological preparation, is similar to the crystal structure of the oxidized horse heart and yeast enzymes. The structural changes upon oxidation are subtle, confined to an increased proton lability in the residues around the heme axial ligands, a small rearrangement of one helix near the cytochrome binding site, and slight reorientation of one propionate residue.

SIGNIFICANCE: Despite great advances in understanding the structure-function relationships among molecules throughout the cell, these features of the proteins of the electron transport chain are only now being elucidated. These proteins are very large and contain many subunits, and because they reside in membranes, resist crystalization. Although an enormous amount of indirect evidence allows researchers to propose mechanisms linking proton gradient generation to electron transport, it is only through their structures, and the changes that take place due to reduction/oxidation that we will clearly pin down

these mechanisms.

FUTURE DIRECTIONS: The complete crystal or solution structures of cytochromes I, II and IV, and the F₀ subunit of ATPase remain to be solved. Work will continue toward this end.

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XVI. TITLE: Protein Trafficking in Animal Cells: Sorting Receptors and Membrane Fusion

BACKGROUND: Appropriate delivery of proteins to intracellular destinations and the control of protein maturation and overall abundance are crucial aspects of cellular metabolism. Typically, misfolded proteins fail to travel beyond the endoplasmic reticulum (ER). In fact, the most common mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene for a chloride channel, $\Delta F508$, causes misfolding and retention in the ER. Improper protein trafficking can result in hyperinsulinemia and insulin resistance. In addition, protein processing and trafficking malfunctions are at the root of certain neuroendocrine disorders as well as lysosomal storage diseases (e.g. Niemann-Pick C). Finally, the regulation of protein abundance is also significant to health, since overexpression of protooncogenes is a hallmark of cancer.

The secretory pathway compartments are subdivided into two central membrane populations, the endoplasmic reticulum and the trans-golgi network (TGN). The TGN is crucial to the sorting, export and recovery of soluble and membrane associated secretory proteins. In addition to being the site for an array of important biochemical reactions, the TGN plays a critical role in the routing of proteins to lysosomes, and to the regulated and constitutive exocytic pathways. The routing of membrane and protein traffic between different compartments of the secretory pathway involves the coordinated interaction of many components, including small molecules, lipids, soluble proteins, membrane proteins, and cytoskeletal elements. NIDDK-funded investigators are working to identify novel proteins that regulate membrane and protein traffic in the TGN.

Intracellular trafficking is mediated by vesicles which are comprised of lipid bi-layers that enclose an aqueous compartment. The vesicles emerge from and coalesce with larger subcellular structures that are similarly defined by the lipid bi-layers that enclose their compartments. The mechanism underlying vesicle fusion to target compartments is a focus of research in this area. Vesicles travel in the direction of secretion, anterograde, and in the return, retrograde, direction. Retrograde transport was hypothesized as necessary to preserve the biochemical identity of subcellular compartments, lest they be diluted as a consequence of proteins escaping in vesicles. Indeed, macromolecules have been identified that are subject to retrograde transport and these have allowed discrimination and comparison between vesicles trafficking in opposing directions. Proteins involved in directing traffic have been localized to the exterior of the vesicle in agreement with the expectation that recognition of target compartments by vesicles must occur at their interface in the cytoplasm. Several types of proteins with numerous family members have been implicated in the recognition process including Rabs (GTPases), SNAREs (membrane associated proteins that provide for recognition) and regulators of Rabs and SNAREs. NIDDK-funded investigators are engaged in the effort to determine if specific proteins within each family discriminate the discrete target compartment for each

type of vesicle.

Many proteins, especially secreted hormones, are synthesized in precursor forms and are modified after their initial translation. These changes, including additions, subtractions and three-dimensional folding, are often essential to the biological function of the final product. Investigators funded by NIDDK are active in identifying the enzymes that mediate these post-translational modifications. The molecular mechanisms that account for the specificity of these enzymes as well as accessory proteins that regulate the enzymes are areas of intense study. Cells also monitor the abundance of individual proteins and selectively regulate turnover rates via active destruction. The entities responsible for protein turnover are large structures called proteasomes. The molecular nature of proteasomes and the basis of their selectivity are also under investigation.

RECENT FINDINGS: In the past year, NIDDK-supported researcher Suzanne Pfeffer described the discovery of a cytosolic protein that directs a specific class of transport vesicles to their appropriate cellular destination. Using the recently-developed yeast two-hybrid system for isolating interacting proteins, Dr. Pfeffer identified TIP47, a novel protein that specifically binds to the cytoplasmic tail of mannose 6-phosphate receptors (MPRs). The MPRs are transmembrane proteins that have their tails exposed to the cytoplasm while the balance of the receptor is in the lumen of membrane-enclosed vesicles. The MPRs bind lysosomal hydrolases in the Golgi and, through mechanisms that are not clear, direct these hydrolases to vesicles that will fuse with the early lysosome. After delivering their cargo, MPR-enriched vesicles return to the Golgi for another round of transport. Dr. Pfeffer's work suggests that TIP47 plays a crucial role in this specific transportation pathway. In the report, the group demonstrates that TIP47 binding to vesicles is dependent on the presence of MPRs. Furthermore, depletion of TIP47 from cytosols prevents transport in a cell-free system. Finally, the investigators showed that the expression of antisense RNA against TIP47-encoding mRNA reduced MPR transport in living cells. The discovery and characterization of TIP47 should significantly propel effort to unravel the mechanisms whereby vesicles are appropriately routed to their final destination within the cell. In a paper published in *Cell*, NIDDK investigator, Nancy Dahms, reports the crystal structure of the cation-dependent MPR. The extracytoplasmic domain of the MPR crystallizes as a dimer, and its structure provides a rationale for the observed differences in binding affinity exhibited by the MPR toward various lysosomal enzymes.

Dr. Gary Thomas, an NIDDK-supported investigator, has identified PACS-1, a member of a novel gene family that encodes PACS (phosphofurin acidic cluster sorting proteins), as essential for the sorting of proteins to the *trans*-Golgi network (TGN). In this study, the intracellular sorting of the endoprotease furin

was utilized a model system with which to identify the factors that direct protein localization within the TGN. Localization of furin to clathrin-coated regions of the TGN is dependent upon a motif found within furin's cytosolic domain consisting of an acidic cluster (AC) of amino acids. Within the AC are a pair of serines that are subject to casein kinase II phosphorylation. Phosphorylation of these serines within the AC is essential for the TGN localization of furin. PACS-1 is a cytosolic connector protein that binds directly to the TGN localization signal on the furin AC, connecting furin to the clathrin-sorting machinery, and resulting in the localization of furin to the TGN. Cell-free assays demonstrate that the furin AC is not required for TGN budding or retention but functions in a PACS-1-mediated retrieval step. Furthermore, PACS-1 is required for the correct localization of the cation independent mannose-6-phosphate receptor, a TGN/endosomal membrane protein that, like furin, is sorted via its AC. PACS-binding motifs are found within many membrane protein cytoplasmic domains, suggesting a broad role for PACS family members in protein sorting within the mammalian secretory pathway.

The delivery of vesicle contents to their appropriate destination requires fusion of the vesicle membrane bilayer with the target organelle's membrane bilayer. Thus, understanding the determinants of membrane fusion is crucial to understanding protein trafficking in a cell. NIDDK-supported researcher and Advisory Council member James Rothman and his colleagues described the minimal machinery for required for membrane fusion in a manuscript published this year in the journal *Cell*. Rothman and colleagues reconstituted purified, recombinant v- and t-SNARE proteins into separate vesicles. It has been known for some time that the v- and t- SNAREs provide for recognition during membrane fusion. However, these proteins are always found in a large multi-protein complex and their individual roles are not entirely clear. In their recent work, these researchers showed that the v- and t- SNARE proteins alone are sufficient to direct docking and fusion of two membrane-enclosed compartments. The investigators propose a model wherein complementary recognition of v- and t- SNAREs generates a structure similar to viral proteins that direct the fusion of viral membranes with cellular membranes. By analogy to the hairpin structure formed by the viral proteins, Rothman and co-workers have christened the v- and t- complex a SNAREpin. In the model, the viral hairpin and cellular SNAREpin assemble into a configuration that places strain on the proteins. The researchers believe that the energy contained in these strained complexes is harvested to drive membrane fusion. The energy is required to overcome the unfavorable disruption of the membranes' integrity that accompanies fusion of the two membranes. Although the fusion rates of membranes with this minimal machinery is slow, the experimental design should allow the dissection of precisely how other proteins in the multi-protein complex increase fusion rates to those observed in intact cells.

Protein trafficking in a cell occurs not only from the center out to the plasma membrane but in the reverse direction as well. One example is the internalization of plasma membrane receptors. The half-life of these cell surface receptors is determined by controlled internalization and subsequent degradation. NIDDK-supported researcher and Presidential Early Career Award recipient Linda Hicke reported her discovery of the mechanism underlying internalization of well-studied plasma membrane receptor in yeast. Dr. Hicke had previously reported the revolutionary discovery that certain cell surface receptors are targeted for destruction by the same machinery used to target cytoplasmic proteins, the ubiquitin system. In the recent work, Dr. Hicke showed that phosphorylation of the cytoplasmic tail of the receptor is a prerequisite for internalization and subsequent degradation. Many cell surface receptors are phosphorylated on their cytoplasmic domains in response to their activation through ligand binding to extracellular domains. This phosphorylation event is known to be associated with down-regulation of the receptors. Dr. Hicke's recent findings establish the requirement for a specific sequence of amino acids in the cytoplasmic domain to elicit ubiquitination and subsequent internalization in response to receptor phosphorylation. The findings not only describe a specific mechanism for receptor turnover but also establish a connection between cellular signaling events and the ubiquitin-dependent turnover of a cell surface receptor.

SIGNIFICANCE: Ensuring that cells exhibit proper responses after hormonal signaling, environmental stimuli, and during steady-state activity requires that proteins are modified, sent to their proper subcellular compartment, and subjected to appropriate turnover rates. The delivery of proteins to intracellular destinations and the control of protein maturation and overall abundance are fundamental processes of all cells. Knowledge of the proteins involved in the targeting and turnover machinery provides the basis for our understanding of how changes in these processes can lead to metabolic diseases, neuroendocrine disorders, and lysosomal storage diseases.

FUTURE DIRECTIONS: The identification and characterization of proteins involved in directing intracellular trafficking is fundamental to all fields of biomedical research. The number of known traffic destinations suggests that many more recognition and regulatory molecules are waiting to be discovered. The development of in vitro assays such as those that mimic fusion with distinct destination compartments will aid in characterizing proteins involved in intracellular sorting. Further characterization of the ubiquitin-dependent proteasome should improve our ability to predict the relative stability of a given protein. The provision of high resolution structures of individual proteins involved in intracellular trafficking, as well as understanding these proteins in the context of a supramolecular structure is an exciting future direction in this field.

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R01DK42667	Dahms, N.M.	Med College of Wisc
R01DK37274	Thomas, G.	Vollum Institute
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XVII. TITLE: Hypothalamic-Pituitary Axis Regulation

BACKGROUND: Pioneering work by investigators supported by NIDDK led to the explosion of research in the nascent field of neuroendocrinology in the early 1970s. The discovery of factors released from the brain which in turn regulated

the pituitary gland was revolutionary in the development of our understanding of the coordinated regulation of bodily functions. The discovery of a substance, somatostatin, which inhibits release of GH from the pituitary gland has led to development of current therapies for control of acromegaly as well as diagnostic tools for imaging many cancers. The discovery of the releasing factor that controls reproduction, GnRH, not only led to the Nobel prize for NIDDK investigator Roger Guillemin, but has led to therapies for ovarian and prostatic cancers as well as a potential contraceptive. As, one by one, the hypothalamic factors that control the release of pituitary hormones have been discovered new fields of investigation have been born. NIDDK investigators have led and continue to lead the way in the discovery of the controlling factors involved in almost every important function of the body.

In the early 1980's Guillemin, Wylie Vale, and Michael Thorner discovered the hypothalamic factor that stimulates release of GH from the pituitary. While gargantuan efforts of the scale of the initial studies to purify releasing factors—studies which required literally millions of sheep hypothalami had failed to yield the releasing factors for GH and ACTH, serendipity did not fail. The discovery by Thorner and others that acromegaly could be caused by tumors of the pancreas led to the purification from tumor tissue of The GRF. Of the major regulatory axes which involved the pituitary only the hypothalamic pituitary adrenal (HPA) axis eluded investigators. Corticotropin-Releasing-Factor (CRF) was finally discovered by Vale and coworkers in 1981 opening yet another field to investigators all over the world. This peptide hormone appeared to be widely distributed both in the brain and in peripheral tissues of the body and was shown to have an extremely diverse repertoire of functions from regulation of the adrenal to effects on blood pressure, inflammation, and behavior. Following the discovery of the releasing factors secreted by the brain to regulate growth, reproduction, metabolism, and response to stress through the pituitary gland, literally thousands of investigators have focused on the coordinate regulation of these axes. In each case the subsequent discovery of the receptors for these factors has led to development of both therapeutic and investigational tools that have led to important advances in the clinical management of disease. In 1993, the receptor for CRF was finally identified concurrently by several labs including those of Vale and Michael Rosenfeld. A number of investigators have since worked to elucidate the pathways in the brain which impinge on the pituitary through CRF. One puzzling finding has been that several brain regions which are putatively involved in behavior express CRF but not its receptor as well as the converse. The discovery of Urocortin, a novel brain secretagogue that also works through CRF receptors, as well as the discovery of multiple subtypes of CRF receptors, suggests that there are multiple signaling pathways for this family of proteins which are likely to subserve different functions.

RECENT FINDINGS: To address the possibility that distinct functions of the CRF family of proteins are mediated by each member of the CRF receptor family, Vale and coworkers generated mice lacking the CRF type 1 receptor. Mice lacking this receptor appear relatively normal. It has been proposed that the hypothalamic CRF is necessary for development of the pituitary corticotropes, however, this does not appear to be the case. Basal levels of pituitary ACTH are relatively normal in mice lacking CRF-1 receptors and the adrenal, while quite small, does maintain the ability to secrete glucocorticoids if challenged with ACTH although at very diminished levels. When the animals are exposed to stress they do not show any of the signs of anxiety seen in normal animals. In fact, the mice lacking CRF-1 receptors will exhibit many behaviors that mice normally avoid because they are so stressful. The question remains as to which functions other CRF receptors or CRF-like molecules mediate. This work suggests that organogenesis and development of particular cell types can proceed to a certain point without communication from other parts of the regulatory axis. The programmed development of an organ up to that point appears to be mediated by a family of gene regulatory factors.

One factor, called Prophet of Pit-1 (Prop-1) is the site of the mutation leading to the mouse Ames dwarf phenotype. This factor can activate Pit-1, but only in the absence of another factor called RPX. RPX is expressed in the neural epithelium adjacent to the primordial pituitary gland and disappears at about the time that Pit-1 begins to be expressed. The inactivation of Prop-1 leads to a failure of the presumptive PRL/GH/TSH cells to migrate from the central proliferative zone to their sites of terminal differentiation. A collaboration between basic and clinical NIDDK-supported investigators has now discovered that mutations in Prop-1 can also result in abnormal pituitary development in humans leading to multiple hormone deficiencies. Wu et al demonstrated that familial combined pituitary hormone deficiency (that is, inherited CPHD) can be caused by mutations in Prop-1. An additional finding that was unexpected from extrapolation of the mouse data, was that these patients suffer from lack of gonadotropins in addition to deficits in PRL, GH and TSH. Cogan, Phillips and coworkers, in an international collaboration, have now demonstrated that mutations in Prop-1 are a cause of sporadic CPHD, as well. This group went further to study mutations in Prop-1 in families from many sites around the world. Their data suggest that mutations in Prop-1 occurred independently in these families. Further, their work suggests that DNA symmetry in the Prop-1 gene contributes to the frequency with which this gene is mutated. The finding that mutations in this gene in humans can result in both hypothalamic defects and defects in gonadotropin secretion suggests that this protein has as yet unidentified functions in development.

Taking a cue from work in the fruit fly, investigators recently identified a family of

genes (termed period genes, Per) which are expressed in a circadian rhythm in cells of the suprachiasmatic nucleus in the brain, the so-called circadian clock. Reppert and others have now identified a new member of this gene family, Per3, which raised important questions about how biological rhythms are maintained in mammals. Per1 and Per2 gene are exquisitely sensitive to light exposure to the eye. While the endogenous rhythm of expression of these genes in the brain is different in individual cells, the circadian regulation of multiple functions (for example, sleep) appears to be an integrated response that reflects the mean rhythms of all of the cells. Light, sensed through the retina, can then shift this endogenous rhythm somewhat to conform to the rhythm imposed by the earth's rotation. The mechanism by which individual cells communicate to synchronize their individual circadian rhythms is still unknown. Per3 exhibits an even more intriguing pattern of expression. First, it does not appear to be sensitive to light. Second and even more important, Per3 is widely expressed throughout the body. Per3 maintains a strong pattern of circadian oscillation in these tissues which is not regulated by light. The function of this endogenous clock in peripheral tissues is unknown.

SIGNIFICANCE: Work on neuroendocrine control of body function has broad implications for understanding and treatment of disease. The work by Rosenfeld and others confirms the importance of animal models of development in elucidation of the pathophysiology of human disease. This work also demonstrates that disruption of patterns of gene expression through dysfunction of transcription factors can have a cascade of effects on normal development. The perturbation of normal development of the hypothalamic-pituitary-adrenal axis through elimination of the CRF receptor confirms the role of signaling loops in the directed development of target organs. This latter work also suggests that the CRF type 1 receptor is responsible for mediating the behavioral stress response. By specifying the subtype of receptors involved in this subset of CRF actions, this work may lead to better pharmacotherapies for anxiety. Finally, work by the Reppert lab demonstrating that there are intrinsic circadian rhythms in cells throughout the body not just in the clock cells within the brain, reinforces the concept that application of therapeutic drugs should be based on the knowledge of the intrinsic rhythms of the cells or tissue that are targeted. For example, agents which are designed to kill cells which are actively dividing would be most effective and selective when delivered at a time when those cells are dividing and others are quiescent.

FUTURE DIRECTIONS: The discovery of leptin several years ago spawned an explosion of research into the hypothalamic control of energy balance and refocused attention on this important site of integration for cognitive, behavioral, and metabolic signals. Technological advances now make it possible to identify other novel regulatory pathways through sequencing of gene libraries and high

throughput screening for endogenous ligands. Investigations are also needed to define the essential elements that define a tissue both in its terminal differentiation and in commitment of the primordial tissues prior to evidence of differentiation. Both the mechanism of synchronization between cells with endogenous circadian rhythms and the role of these clocks in peripheral tissues remain to be elucidated. In particular, the function of Period genes in peripheral organs should be a priority since it has implications for both normal function and optimal therapy.

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XVIII. TITLE: Apolipoprotein and Isoprostanes in Lipid Oxidation

BACKGROUND: Lipid metabolism is intimately related to atherosclerosis, which is a major health problem in diabetes and obesity. Atherosclerosis is associated with hypercholesterolemia. Atherosclerotic plaque is currently thought to be initiated when the unsaturated fatty acids in circulating low density lipoproteins (LDL) are peroxidized by reactive oxygen species such as hydrogen peroxide and oxygen radicals. These damaged lipid molecules tend to aggregate, and are recognized by the scavenger receptor of macrophages. Macrophages take up these damaged LDL, and become filled with cholesterol, leading to the formation of "foam cells." These lodge in the smooth muscle wall of arteries and initiate plaque formation. In animal models of atherosclerosis, the plasma contains autoantibodies against epitopes of oxidized LDL found in atherosclerotic lesions, which likely participate in the associated inflammatory response in the vascular wall.

Low total cholesterol, high HDL cholesterol, and use of exogenous antioxidants are all associated with reduced risk for atherosclerosis. Great progress has been made in identifying the mechanisms responsible for this protection. An important animal model for these studies is the apolipoprotein E-deficient mouse. ApoE binds to the low density lipoprotein receptor (LDLR) with high affinity, which facilitates the LDLR-mediated hepatic lipoprotein uptake and disposal of excess cholesterol in bile. ApoE also binds to cell surface proteoglycans, which sequesters the apoE-containing lipoproteins to the surface of cells such as hepatocytes and vascular endothelial cells. These apoE-deficient animals become severely hypercholesterolemic, and quickly develop atherosclerotic lesions.

Overexpression of HDL apolipoproteins can impact on the atherosclerosis found in apoE^{-/-} mice. Even at elevated total cholesterol concentrations, ApoA1 reduces atherosclerotic lesions, while ApoAII increases them. At least some of the protection afforded by apoA1 can be attributed to its properties as an antioxidant. After oxidation of isolated HDL particles spontaneous reduction occurs, so that both cholesterol esters and phosphatidylcholine hydroperoxides are reduced to their hydroxide. The methionine residues in the protein component appear to act as the reducer. In fact, canine apoA1 has two fewer methionines than the human protein, and is far inferior as a reducer of damaged cholesterol ester. Peptide

Met(O) reductase is present in all mammalian tissues, and may serve to regenerate reduced apoAI.

RECENT FINDINGS: Another apolipoprotein, apolipoprotein AIV (apoAIV), also protects against diet-induced or apoE-deficient atherosclerosis when overexpressed in mice, and may be the most important antioxidant apolipoprotein found in vivo. This protection occurs despite elevated total cholesterol and is independent of changes in HDL cholesterol. ApoAIV is a soluble plasma protein which can also associate with chylomicrons or HDL. It appears to have several biological activities. ApoAIV promotes cholesterol efflux from cells, serves as a ligand for HDL binding to hepatocytes, and may act to stabilize HDL structure or as a cofactor involved in enzymatic remodeling of HDL. It activates lecithin:cholesterol acyltransferase, and modulates the activation of lipoprotein lipase by apoCII. It was recently shown that purified apoAIV protein acts as an antioxidant in vitro, and this may be its major anti-atherogenic property in vivo. ApoAIV was added to isolated lymph or LDL in the presence of either the oxidizing agent copper or peritoneal macrophages, and served to decrease and delay lipid damage and production of thiobarbituric acid-reactive substances. The addition of 2.5 µg/ml apoAIV increased the time of copper-induced conjugated diene formation by 2.4-fold. In comparison, apoE at 20 µg/ml only increased $T_{1/2}$ by 1.75 fold. ApoAIV interferes with oxidation directly, since addition of the protein after 90 minutes of copper-induced oxidation inhibited further damage to LDL. ApoAIV may be a very important endogenous antioxidant, and since it is mainly produced in the intestine, its production is directly correlated with fat intake. ApoAIV is amphipathic, soluble in both aqueous and lipid environments, and may therefore be able to act as an antioxidant in compartments inaccessible to other protective apolipoproteins.

The predominant oxidized lipids found in human atherosclerotic plaque appear to derive from linoleic acid. On the other hand, these lesions contain highly elevated concentrations of F_2 -isoprostanes. Isoprostanes are highly bioactive prostaglandin isomers derived from free-radical peroxidation of arachidonic acid. One of the isoprostanes, $IPF_{2\alpha}$ -VI, accumulates in the urine and blood of apoE-deficient mice even before atherosclerotic plaques appear, and may turn out to be an excellent surrogate marker for oxidative stress. When ten-week old apoE^{-/-} mice were fed vitamin E, a potent anti-oxidant, urine and plasma $IPF_{2\alpha}$ -VI are reduced to control levels. Most striking was the finding that vitamin E reduced $IPF_{2\alpha}$ -VI in the aorta by 50 percent ($p=0.0001$ for correlation), and this was accompanied by a reduction of atherosclerotic lesion from 18.0 percent to 6.2 percent of vascular surface area. Total cholesterol remained well above the level in control mice. Therefore, atherosclerosis was prevented in the presence of elevated cholesterol by the antioxidant vitamin E, which also reduced isoprostane production. This is further evidence that lipid oxidation is a

necessary event in atherosclerotic plaque formation.

SIGNIFICANCE: Atherosclerosis is a major factor in the morbidity and mortality associated with diabetes and obesity. Knowledge of the mechanisms that cause plaque formation, and that protect against it in vivo, will allow the development of new pharmaceutical agents that will increase life expectancy and quality in these patients. Large scale trials of new therapies to prevent or treat atherosclerosis are expensive and time-consuming, but it may be possible to decrease the time and expense by the use of good surrogate markers that can substitute for clinical endpoints. F2-Isoprostanes may be very useful markers of the oxidation damage that initiates and accompanies atherosclerosis.

FUTURE DIRECTIONS: Relatively little is known about lipoprotein metabolism, and our appreciation of the chemical properties of different classes of these molecules is still limited. Further research is needed in order to understand the metabolism, function and compartmentation of apolipoproteins. For those that act as cellular antioxidants, it is important to investigate their mechanism. It will also be important to fully assess whether isoprostanes can be used in the setting of a clinical trial as a surrogate marker for oxidative stress.

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HIV/AIDS-RELATED RESEARCH

XIX. TITLE: Endocrine and Metabolic Disturbances in AIDS

BACKGROUND: Wasting is a frequent AIDS-defining condition, which contributes substantially to the morbidity and mortality of AIDS. In addition to the diminished quality of life and functional impairments associated with wasting, weight loss has been shown to correlate strongly with mortality in AIDS. The pathogenesis of AIDS wasting syndrome is multi-factorial, with secondary diseases, such as infections and gastrointestinal disorders, playing important roles in development of wasting. HIV-positive individuals are often able to maintain stable weight for prolonged periods, with weight loss often occurring episodically in association with secondary infections. Anorexia, a frequent concomitant of infection, impairs the ability to compensate for the increased resting energy expenditure characteristic of AIDS, and weight loss ensues. Unfortunately, neither oral or parenteral nutrition or appetite enhancement with dronabinol or megestrol acetate have been successful in restoring lean body mass in individuals with AIDS wasting syndrome. In an effort to understand the metabolic mechanisms by which lean body mass is lost in AIDS and why, once lost, it has proven so difficult to restore, investigators have examined how HIV affects the anabolic hormones that help accrue and maintain muscle.

RECENT FINDINGS: Wasting imposes a substantial burden of increased morbidity and mortality in individuals infected with HIV. It has been suggested that metabolic alterations are involved in the pathogenesis of AIDS wasting and may prevent lean body mass repletion even when energy intake is adequate. A substantial fraction of men with AIDS have reduced levels of testosterone, a hormone essential for maintenance of muscle mass in men. From thirty to fifty percent of men with AIDS have been reported to have testicular dysfunction (hypogonadism) and low testosterone levels. A recent NIDDK-supported study assessed whether replacement doses of testosterone, administered by means of Androderm, a nonscrotal transdermal patch system, augmented lean body mass, body weight, muscle strength, and health-related quality of life in HIV-infected men with low testosterone. Investigators concluded that Androderm treatment of HIV-infected men with low testosterone levels is safe and is associated with a 1.35 kg gain in lean body mass, a significant reduction in fat mass, an increased red cell count and improvement in emotional measures.

Many of the clinically-important features of HIV/AIDS can be attributed to the immune deficiency which develops in infected patients. The destruction of the immune system by the virus results in opportunistic infections as well as risk of

autoimmune disease and malignancy. Kaposi's Sarcoma (KS), a multifocal tumor of vascular endothelium which typically involves skin and mucosal surfaces, was among the first recognized manifestations of the AIDS epidemic. The KS-associated herpes virus (KSHV) has been implicated in the pathogenesis of KS, but until recently the mechanism by which KSHV triggered KS was unclear. NIDDK-supported researchers have presented three lines of evidence indicating that one protein encoded by the KSHV is a G protein coupled receptor which appears to participate in tumor formation. Investigators at Cornell University have found that this receptor can transform cells to a malignant phenotype. Further they found that expression of this receptor is, by itself, sufficient to cause a switch to an angiogenic (blood-forming) phenotype by stimulating secretion of growth factors and by causing release of enzymes responsible for inducing growth of blood vessels from tissue surrounding the tumor.

SIGNIFICANCE: Favorable changes in body composition, lower cost relative to other anabolic agents, and lower frequency of side effects provide strong rationale for further evaluation of Androderm in the treatment of wasting syndrome. This is the first demonstration that a KSHV encoded gene is capable of both inducing transformation in the infected cells, and stimulating the growth of blood vessels from surrounding tissue which would support tumor growth. These complementary findings strongly support the concept that KSHV infection plays a primary role in the development of Kaposi's Sarcoma. That this work was generated in a laboratory whose primary focus is elucidation of basic mechanisms of G-protein-coupled receptor action underscores the need to continue a strong program in basic research as an essential part of disease based initiatives.

FUTURE DIRECTIONS: Current therapy for the treatment of AIDS has resulted in marked reductions in viral titers in this patients. There have however, been numerous reports of metabolic disturbances including the development of metabolic syndrome in these patients. Studies designed to define the prevalence of this complication in AIDS patients and to characterize these abnormalities should be a high priority of the AIDS program in NIDDK. Ongoing studies on anabolic therapies for the treatment of wasting in AIDS have demonstrated that these therapies may actually have an ameliorative effect on lipodystrophies seen in AIDS patients. As the extent of metabolic disturbances in AIDS patients becomes better defined, potential therapeutic approaches to combat these complications should be explored.

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HIGHLIGHTS OF DDEMD MINORITY PROGRAM ACTIVITIES

XX. TITLE: Type 2 Diabetes Mellitus: Prevention and Treatment

BACKGROUND: Type 2 diabetes mellitus is increasing in prevalence in the United States, with about 7 percent of the adult population affected and 600,000 new cases per year. Type 2 diabetes is even more common in the elderly and in minority populations including African-Americans, Hispanic-Americans, Asian and Pacific Islanders, and Native Americans. In these populations, type 2 diabetes may be present in 10 percent too as much as 50 percent of the adult population. Type 2 diabetes is accompanied by long-term complications including blindness, renal failure, amputations, and a two to four-fold increased risk for cardiovascular disease and stroke. The total health care expenditure for diabetes reflects the high cost of treating the attendant complications and is estimated at approximately one hundred billion dollars or 12 percent of the United States health care expenditure.

In response to the epidemic proportions of type 2 diabetes in the United States, its accompanying long-term complications, and the difficulty of treating type 2 diabetes successfully once it develops, the NIDDK launched the Diabetes Prevention Program (DPP) in 1996 with recruitment to end in May of 1999. The DPP is a randomized multicenter clinical trial to prevent or delay the onset of type 2 diabetes in a high-risk population with impaired glucose tolerance, of which 50 percent will be minority individuals. A cohort of 3000 subjects recruited over a three-year period will be randomized to one of three groups: (1) intensified lifestyle, (2) metformin, and (3) placebo. A fourth troglitazone group has been stopped and the subjects recruited to this intervention arm will be followed off drug for the duration of the study. The study duration will be for 3 to 6-years, with a median duration of 4.5-years. The study is scheduled to end in 2002.

RECENT FINDINGS: Important new information has emerged on the prevalence of diabetes and impaired glucose tolerance in U.S. adults based on the analysis of data from the Third National Health and Nutrition Examination Survey, 1988-1994 (NHANES III) and previous NHANESs. The prevalence of diagnosed diabetes, undiagnosed diabetes, and impaired glucose tolerance in U.S. adults were 5.1 percent, 2.7 percent, and 6.9 percent, respectively. The prevalence of diabetes in adults 40-74-years of age increased from 8.9 percent in the period 1976-1980 to 12.3 percent in 1988-1994. Prevalence was similar in men and women in each racial or ethnic group, but non-Hispanic blacks and Mexican-Americans had 1.6 and 1.9 times greater rates of diabetes compared to non-Hispanic whites. A separate study of the prevalence of glucose intolerance among Native Hawaiians over 30-years of age found the prevalence of diabetes

to be 20.4 percent.

SIGNIFICANCE: The World Health Organization has concluded that an increasing prevalence of diabetes is strongly related to lifestyle. Identification of populations at particular risk for diabetes and testing of interventions to prevent the development of diabetes is essential to reduce the prevalence of diabetes. The DPP will indicate whether lifestyle or pharmacologic interventions can prevent or delay the onset of type 2 diabetes in a study group including 50 percent representation of minorities. Successful strategies to prevent diabetes will reduce the human and financial cost of this disease.

FUTURE DIRECTIONS: Studies are underway to elucidate the basis of increased susceptibility to diabetes in minority populations and to develop interventions tailored to specific populations.

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XXI. TITLE: Complications of Diabetes Mellitus

BACKGROUND: Diabetes mellitus is one of the most prevalent chronic diseases in the United States. Based on the National Health Survey (NHIS), there were 7.8 million diagnosed cases of diabetes in the United States in 1993. It is estimated that about 625,000 new cases of diabetes are diagnosed each

year, including 595,000 cases of type 2 diabetes mellitus and 30,000 cases type 1 diabetes mellitus. The number of people with diagnosed diabetes increased five-fold between 1958 and 1993. In addition, it is estimated that there are probably 5.4 million undiagnosed cases of type 2 diabetes in the United States, based on fasting plasma glucose levels in representative samples of people without diagnosed diabetes. Of recent concern has been a rapid increase in the number of children and adolescents with type 2 diabetes. This increase has been a particular problem in minority populations. Indeed, certain minority groups, especially African-Americans, Hispanic-Americans and Native Americans, appear to have an increased risk of developing type 2 diabetes.

Chronically elevated blood glucose levels damage tissues and lead to a variety of long-term, potentially disabling complications. In the United States, diabetes is a major cause of amputations, blindness, heart attacks and end stage renal disease. Currently, diabetes is the seventh leading cause of death. In a recent study, it is estimated that the cost of medical care for diabetes in 1992 was \$91.8 billion.

The Diabetes Control and Complications Trial (DCCT) conclusively established the relationship between hyperglycemia and the complications of diabetes. The DCCT showed that tightly controlling blood glucose levels is an effective measure to slow the onset of microvascular complications (i.e., retinopathy, nephropathy and neuropathy). However, because of limitations in current therapies, it is often difficult to achieve normal glucose levels in patients with diabetes. Thus, an important therapeutic challenge of diabetes is the prevention and treatment of its chronic complications.

The detailed sequence of events in the pathophysiology of complications and the cellular, biochemical and molecular mechanisms that cause diabetes complications have not been elucidated. Several biochemical mechanisms by which hyperglycemia may cause cellular damage have been studied. However, the exact mechanisms by which elevated glucose levels lead to tissue damage remain incompletely understood.

Extensive epidemiologic and clinical evidence suggests that, in addition to hyperglycemia per se, genetic determinants are involved in the development of diabetic complications. However, very little is actually known about the identity or function of specific genes involved.

RECENT FINDINGS: Clinical and epidemiologic observations suggest that hyperglycemia is not the only factor in the development of long-term complications of diabetes. Thus, some patients with good blood glucose control will develop complications, while, conversely, some patients with poor

glycemic control appear to be spared. Previous epidemiologic studies have suggested a genetic influence for the development of diabetic nephropathy.

Recently, a study of family members of patients who participated in the DCCT confirmed that familial factors (presumably genetic) affect the development of nephropathy and demonstrated, for the first time, that familial factors appear to influence the severity of diabetic retinopathy. Further evidence for the role of familial factors in the development of retinopathy comes from data derived from the Third National Health and Nutrition Examination Survey (NHANES III). Analysis of this data revealed an increased risk for development of retinopathy in Mexican Americans with type 2 diabetes compared to non-Hispanic whites.

SIGNIFICANCE: The long-term complications of diabetes remain a major public health problem. Investigations are currently underway in an attempt to find drugs that would inhibit or reverse complications. Since any drug carries some risk of side effects, it is imperative to be able to identify those patients with the highest likelihood of developing complications, to allow targeted interventions. In addition, identifying those populations at highest risk may also lead to the discovery of additional factors and specific genes which determine the development of complications.

FUTURE DIRECTIONS: Further studies are needed to expand our understanding of the molecular events leading to the development of diabetic complications. Further refinement in our understanding of the basis for complications will lead to new modalities for the prevention and treatment of these devastating long-term consequences of diabetes. Likewise, it is essential to continue to define population groups at highest risk for the development of specific complications and to identify specific genes and gene products involved in the development of diabetic complications.

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XXII. NIDDK Minority Training and Career Development-FY 1998

Name of Program and Description	Division	# of NIDDK Awards	NIDDK Funding Level	ORMH Collab. Funding
<p><u>Minority Access to Research Careers (MARC) T-34</u> NIDDK Co-funds with NIGMS. Funds predoctoral faculty fellowships, visiting scientists, conferences for minority investigators and minority health issues, and honors undergraduate training in biomedical research. Summer Internship Program in the NIDDK Division of Intramural Research (students-managed by NIDDK-EEO).</p>	DK-wide	6	\$23,236	
<p><u>Minority Biomedical Research Support Program (MBRS)</u> NIDDK co-funds with NIGMS. Provides expanded opportunities for minorities to participate in biomedical research careers. Supports research projects of interest to the NIDDK at Minority and Equal Opportunity Institutions.</p>	DK-wide	25	\$1,985,728	
<p><u>R-13 (Conference Grant) to the American Physiological Society. FASEB</u> Provides support for underrepresented minority students to attend meetings of the Society, and for 36 minority high school science teachers to have summer research training in laboratories of Society members.</p>	DK-wide	1	\$74,315	

<p><u>Initiatives for Underrepresented Minorities in Biomedical Research</u> NIH-wide program initiatives to support minority undergraduate, graduate students, high school students, and faculty members on NIDDK active research grants through administrative supplements.</p>	DK-wide	120	\$4,500,000	
<p><u>Research Training of Underrepresented Minorities on Institutional Training Grants (T32)</u> Highly qualified Minority Investigators are assigned T-32 slots held in reserve for this purpose. DDEMD=5 DDDND=3 DKUHD=6</p>	DK-wide	14	\$175,174 81,437 183,000	\$41,917
<p><u>Pre-doctoral Fellowships (F-31)</u> To provide support to minority students for research training leading to M.D.-Ph.D. in the biomedical sciences. DDEMD=6 DDDND=2 DKUHD=1</p>	DK-wide	9	\$132,269	
<p><u>Cell/Molecular Biology Student/Teacher Learning Center (R-25)</u> Laboratory Research experience for minorities in the District of Columbia (managed by NIDDK-EEO).</p>	DK-wide	1	\$334,767	

<u>Small Research Grants (R-03) for Minority Researchers</u> DDEMD=5 DDDN=n/a DKUHD=1 ORMH Collaboration provides additional support for minority researchers.	DK-wide ORMH	6	\$367,229 84,750	\$466,933
<u>Minority High School Student Summer Research Training Supplement</u> In conjunction with the National Minority Organ Tissue Transplant Program award to Howard University, NIDDK provides meaningful laboratory research experience to minority high school students to stimulate their interest in careers in biomedical science.	DK-wide	1	\$70,138	
Totals		183	\$8,012,043	\$508,850

DIVISION OF DIGESTIVE DISEASES AND NUTRITION

FY 1999 Program Plan
RESEARCH PROGRESS REVIEWS
February 1999 Council

Jay H. Hoofnagle, M.D., Director

DIVISION OF DIGESTIVE DISEASES AND NUTRITION

FY 1999 Program Plan

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LIVER DISEASES PROGRAM

I. **TITLE: Pediatric Liver Disease and Genetics: Further characterization of the major pediatric forms of liver disease-Progressive familial intrahepatic cholestasis (PFIC) and benign recurrent intrahepatic cholestasis (BRIC)**

BACKGROUND: Bile formation is an osmotic secretory process of the liver that is driven by the active concentration of bile salts and other biliary constituents in the bile canaliculi. The transport of solutes from the blood to the bile is driven by two transport systems, one in the plasma membrane of the basolateral (sinusoidal) surface and one on the apical (canalicular) surface of the hepatocyte. The sinusoidal systems for bile-salt uptake in hepatocytes include a sodium-taurocholate cotransporter (NTCP) and a sodium-independent organic-anion transporter (OATP). The canalicular membrane contains several ATP-dependent export pumps: the multidrug-resistance gene or P1-glycoprotein (MDR1); the phospholipid transporter multidrug-resistance –P3-glycoprotein (MDR3); the multi-organic-anion transporter (MRP2 or cMOAT); and the bile-salt-export pump (BSEP or SPGP). Recent studies have identified the genes coding for these transporter systems allowing for an analysis of the role of these transporter systems in inherited cholestatic disorders.

RECENT FINDINGS: Molecular changes have been identified in patients with cholestatic disorders related to the genes of the hepatocellular transport systems. Decreased or even absent expression of specific hepatocellular transport proteins have been found in several clinical forms of cholestasis. Progressive familial intrahepatic cholestasis (PFIC) is a severe type of cholestatic liver disease that is inherited as an autosomal recessive trait. The disease presents in infancy and results in liver failure. Three types of PFIC are now recognized with differing molecular defects: Type I (Byler's disease) has been mapped by positional cloning to chromosome 18q21-22 with the molecular defect a mutation in P-type ATPase (FIC1); Type 2 (found in Middle East populations and Greenland and Sweden) results from a mutation in SPGP gene on chromosome 2q24; Type 3 results from a mutation in MDR3 on chromosome 7q21. Benign recurrent intrahepatic cholestasis (BRIC) is characterized by recurrent episodes of intrahepatic cholestasis lasting days to months and that can resolve spontaneously without lasting liver damage. The mutation has been mapped to the 18q21-22 locus with a defect in the P-type ATPase (FIC1).

SIGNIFICANCE: The molecular abnormalities explain the impairment of transport functions, with a subsequent reduction in bile flow and the development of cholestasis.

FUTURE DIRECTIONS: Hepatocytes transporter defects have not been identified in the MDR1 transporter or in sinusoidal transporters and related to cholestasis. In addition, other pediatric liver diseases that develop neonatal cholestasis, such as biliary atresia, need to be reevaluated for the known gene mutations that cause cholestasis.

ACKNOWLEDGMENTS

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R01DK25636	Boyer , J. L.	Yale Univ School of Med
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Trauner M, Meirer PJ, Boyer JL. "Molecular Pathogenesis of Cholestasis." N. Engl J. Med 1998; 339:1217-27.

Bull LN, van Eijk MJT, Pawlikowska L et al. "A Gene Encoding a P-type ATP-ase Mutated in two forms of Hereditary Cholestasis." Nature Genet 1998;18: 219-24.

II. TITLE: Liver Gene Therapy: New Approaches to Altering Abnormal Genes

BACKGROUND: Human gene therapy has been directed at the genetic diseases of the liver. To date, limited clinical efficacy has been shown. Major problems exist with regard to the generation of the appropriate vector to be utilized, long-term expression of the transgene and regulation of transgene expression within a therapeutic concentration range. Thus, new and novel methods for genetic -based therapeutics need to be developed. One such novel approach is based on the premise that single-base (point) mutations within a particular gene are the cause of many of the known inherited liver diseases. This is true for α -1 antitrypsin deficiency or hemophilia where the mutation results in either the production of a nonfunctional mutant protein or the entire loss of the protein product. For the case of a mutation is a single gene, the most desirable form of gene therapy would be targeted homologous recombination between the mutated gene and a new normal version of the gene. The frequency of this form of therapy is very low and is subject to random insertion into the genome of the correct version of the gene. Thus, a novel form of

therapy is the formation of RNA/DNA hybrid (chimeric molecules) at the site of the mutation. These molecules are highly active in homologous pairing reactions resulting in correction of the mutation in vivo.

RECENT FINDINGS: A chimeric RNA/DNA oligonucleotide was constructed to induce sequence mutation in the rat factor IX gene. Oligonucleotides were targeted to hepatocytes in cell culture or in vivo by intravenous injection. Nucleotide conversion was both site-specific and dose-dependent. The mutated gene was associated in vivo with significantly reduced factor IX coagulation activity.

SIGNIFICANCE: The results of this study demonstrate that single base-pair alterations can be introduced in hepatocytes in situ by RNA/DNA oligonucleotides suggesting a potentially powerful strategy for hepatocyte gene repair without the use of viral vectors. The study also showed that both isolated hepatocytes and intact liver are amenable to targeted genomic nucleotide conversion. This methodology may have enormous implications for the future of gene therapy and allow for correction of many serious genetic diseases.

FUTURE DIRECTIONS: Future studies need to address the reproducibility and stability of the nucleotide conversions. Ultimately this method needs to be attempted in a genetic liver disease of humans such as α -1 antitrypsin deficiency or hemophilia.

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R01DK44649	Steer, C. J.	Univ Minnesota Med sch

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Kren BT, Bandyopadhyay P, Steer CJ. "In vivo site-directed Mutagenesis of the Factor IX gene by Chimeric RNA/DNA Oligonucleotides." Nature Medicine 1998; 4:285-90.

III. TITLE: Liver Transplantation and End Stage Liver Disease

BACKGROUND: Mortality from end-stage liver disease declined yearly for over 20-years, but in the last 2-years has begun to increase killing more than 25,000 Americans in 1997. Liver transplantation is the only effective treatment for end stage liver disease with more than 4,000 liver transplantations now performed annually in the United States. Because of a shortage of donor livers, it is currently not feasible to increase further the number of transplantations. Thus, there is acute interest in optimal allocation of this scarce resource. One approach, as shown below, is to determine optimal timing of transplantation with the goal that patients would receive transplantation when needed but before they become so sick that the risk of death and graft loss becomes unacceptably large. Large databases have also begun to evaluate predictive factors of survival for chronic hepatitis C, a major reason for liver transplantation.

RECENT FINDINGS: In 1989 the efficacy of liver transplantation in primary biliary cirrhosis (PBC) was demonstrated by showing that actual patient survival following transplantation was significantly better than without transplantation as predicted by a mathematical survival model ("Mayo natural history model"). Using this model and outcomes following transplantation, the optimal time to perform liver transplantation in PBC has been determined. A risk score is constructed using age, bilirubin, albumin, prothrombin time, and the presence or absence of edema. The risk of death following transplantation remains low until reaching a risk score of 7.8. In contrast, risk scores greater than 7.8 are associated with a progressively increased mortality. Resource utilization measured by the days in the intensive care unit and hospital and the requirement for intraoperative blood transfusions is significantly greater in recipients who have higher risk scores before transplantation.

This model has been adapted to a web site that allows easy calculation of expected natural history without transplantation in PBC and in primary sclerosing cholangitis, another major cause of end stage liver disease. This site also allows calculation of predicted blood usage, length of stay in ICU, and occurrence of significant complications in patients undergoing liver transplantation for these diseases.

Analysis of a large cohort of patients transplanted for chronic hepatitis C in the Liver Transplantation Database has shown that the pre-transplantation level of hepatitis C viral RNA in serum was the sole predictive factor for decreased survival in this cohort of patients. Indeed, in patients with low levels of HCV RNA in serum, survival after liver transplantation is excellent, and as good as survival after transplantation for cholestatic liver diseases, such as PBC. In contrast, among patients with high levels of HCV RNA in serum before transplant, survival is poor and a major cause of mortality is recurrence of hepatitis C.

SIGNIFICANCE: Clinicians can now easily use this natural history model to make management decisions for their patients with chronic cholestatic liver diseases. Furthermore, studies can now be undertaken to evaluate therapy of hepatitis C aimed at patients with the highest risk for complications, i.e. those with high initial levels of HCV RNA. Such work demonstrates the successful translation of NIH funded research into clinical practice.

FUTURE DIRECTIONS: This successful modeling approach is now being adapted to hepatitis C, which has become the most common reason for liver transplantation. Studies of pre-emptive therapy of hepatitis C and of therapy of recurrent disease are now being designed.

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U01DK55883	Detre, K.	Univ of Pittsburgh
R03DK54627	Charlton, M.	Mayo Clinic-Rochester

Publication Data:

Kim, WR, Wiesner, RH, Therneau, TM, Poterucha, JJ, Porayko, MK, Evans, RW, Klintmalm, GB, Crippin, JS, Krom, RA, Dickson, ER. "Optimal Timing of Liver Transplantation for Primary Biliary Cirrhosis," Hepatology 1998;28:33-38.
Charlton M, Seaberg E, Wiesner R, Everhart J, Zetterman R, Lake J, Detre K, Hoofnagle JH. "Predictors of Patient and Graft Survival Following Liver Transplantation for Hepatitis C." Hepatology 1998;28:823-830.

Mathematical Models in Cholestatic Liver Disease at:
<http://www.mayo.edu/int-med/gi/model/mayomodl.htm>

IV. TITLE: Chronic Hepatitis B and C: Further Advances in Therapy

BACKGROUND: Hepatitis B and C are the two most important causes of acute and chronic hepatitis in the United States and account for approximately one-third of liver transplantations done in adults in the United States. Therapy of hepatitis B has progressed slowly since the initial studies of alpha interferon conducted at the Clinical Center of the NIH in the early 1980's. More recently, oral nucleoside analogue drugs have been developed for therapy of hepatitis B and are now undergoing clinical evaluation. The hepatitis C virus was discovered in 1989, yet even before the discovery of the virus and development of diagnostic tests, several advances had been made in therapy of this disease. The first report of successful use of alpha interferon in chronic hepatitis C was from the Clinical Center of the NIH where NIDDK investigators showed that a prolonged course of treatment led to improvement in the liver disease and fall of liver enzymes into the normal range. Once the virus was discovered, it was shown that these patients had hepatitis C and that therapy led to disappearance of HCV RNA from the serum. Based upon these preliminary results, controlled trials were carried out using various doses and regimens of alpha interferon, which led to the approval of interferon for treatment of hepatitis C in 1991. Nevertheless, therapy was still problematic: less than half of patients responded to therapy and many relapsed when treatment was stopped.

RECENT FINDINGS: In the last year, alpha interferon was approved for use in children with hepatitis B, the result of a multi-national study in which members of the Liver Diseases Section, NIDDK took part both in the design and conduct of the trial. For hepatitis C, several large, multicenter trials have shown that the response to alpha interferon can be increased two to three fold by the addition of ribavirin, an oral nucleoside analogue. Thus, a twelve month course of the combination of alpha interferon and ribavirin led to sustained eradication of viremia in 40 percent of patients. This combination was first used in the United States by intramural scientists in NIDDK who subsequently helped in the design of the study, but did not actually participate in the final trial. The results of these studies led to the FDA approval of this combination therapy for hepatitis C in December 1998.

At issue, however, is whether the responses to alpha interferon are sustained and whether treatment truly results in permanent eradication of virus infection and resolution of the disease. In long term follow-up studies by members of the Liver Diseases Section, NIDDK, patients with hepatitis C who had a sustained response to therapy in 1984-1986 were evaluated carefully 10 to 12- years after treatment. All responders were found to be without evidence of disease and without detectable HCV RNA in either blood or liver. Thus, sustained responses to treatment most likely represent a eradication of virus infection and "cure" of the disease. Hepatitis C is the first chronic viral infection found to be curable by antiviral therapy.

SIGNIFICANCE: Hepatitis C affects 1-2 percent of the United States population, probably 4 million adults. This disease results in cirrhosis in approximately 20 percent of infected individuals and now ranks as perhaps the major cause of cirrhosis and end-stage liver disease in the United States. A safe and effective therapy of hepatitis C would decrease the mortality and morbidity of liver disease considerably. With current therapies, 30-40 percent of patients might be cured of this chronic viral infection and liver disease.

FUTURE DIRECTIONS: Improvements in therapy of hepatitis B and C are needed, as well as translational efforts to inform the public and the medical care profession about the importance of hepatitis C and means of prevention and treatment.

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None	None	None

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Lau DYT, Kleiner DE, Ghany MG, Park Y, Schmid P, Hoofnagle JH. "Ten-year Follow up After Interferon- α therapy for Chronic Hepatitis C." Hepatology 1998;28:1121-1127.

McHutchinson JM, Gordon S, Schiff E, Shiffman M, Lee W, Rustgi V, Albrecht J for the Hepatitis Interventional Therapy Group. "Interferon α 2b alone or in Combination with Ribavirin in Naïve Chronic HCV Patients: a US Multicenter Trial." N Engl J Med 1998;339:1485-92.

Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, Shiffman ML, Zeuzem S, Craxi A, Ling MH, Albrecht J for the International Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1493-99.

Sokal EM, Conjeevaram HS, Roberts EA, et al. "Interferon alfa therapy for Chronic Hepatitis B in Children: a Multinational Randomized Controlled Trial." Gastroenterology 1998;114:988-995.

V. TITLE: Liver Cell Development and Use in Treatment of Genetic Liver Diseases: Identification of a New

Animal Model of Liver Cell Transplantation and Repopulation and the Use of Liver Cell Transplantation To Treat Genetic Liver Disease

BACKGROUND: Repopulation of a chronically diseased liver via hepatocyte transplantation would represent a valuable alternative to whole organ transplantation. Major problems in hepatocyte transplantation studies have been the limited growth of transplanted cells in the recipient organ as well as the identification of pluripotent stem cells. The identification of such liver stem cells would represent a major advance in the field by providing a population of cells with a large growth potential coupled with a cellular differentiation capacity to perform the complex biological functions of the liver. In addition, such cells may have a selective growth advantage over endogenous hepatocytes a property needed to repopulate a dysfunctional organ.

RECENT FINDINGS: A new animal model has been developed which allows for hepatocyte repopulation through the selective proliferation of transplanted cells. The selective growth advantage is derived using a new strategy that interferes with the proliferative capacity of resident hepatocytes followed by transplantation of normal hepatocytes in conjunction with partial hepatectomy. This strategy allows for the tracking of genetically marked transplanted hepatocytes thereby elucidating the proliferation, expansion and integration of the transplanted hepatocytes into the hepatic parenchymal structure of the liver. The current studies have noted near total replacement 98-99 percent of hepatic mass by hepatocyte transplantation for up to nine months with normal liver function. In other studies, hepatocyte transplantation was reportedly used for the first time in a patient with Crigler-Najjar Syndrome Type I. Crigler-Najjar Syndrome Type I is a recessively inherited disorder characterized by severe unconjugated hyperbilirubinemia beginning at birth. Hepatocyte transplantation represents an alternative to whole organ transplantation because hepatic architecture and function (except for the hyperbilirubinemia) is normal in this syndrome. The present study reports that allogeneic hepatocytes were safely infused through the portal vein, survived for more than eleven months and partially corrected the metabolic disorder in the patient.

SIGNIFICANCE: The development of a new animal model for liver repopulation will allow for a more detailed analysis of liver cell transplantation and the characterization of liver stem cells for use in transplantation studies. The report of the initial use of liver cell transplantation for the therapy of genetic liver disease indicates the feasibility of the approach in a clinical setting.

FUTURE DIRECTIONS: Future studies need to identify liver stem cells and characterize their use in animal models of liver cell transplantation and

repopulation. In addition, numerous biological properties of the cells need to be identified in vivo, most importantly their function, growth and longevity.

ACKNOWLEDGMENTS

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R01DK50636	Shafritz, D.A.	A. Einstein Coll of Med
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Laconi E, Oren R, Mukhopadhyay DK et al. "Long Term, near Total Liver Replacement by Transplantation of Isolated Hepatocytes." Am J Pathol 1998;153:319-29.

Fox IR, Roy Chowdhury J, Kaufman et al. "Treatment of Crigler-Najjar Syndrome Type I with Hepatocyte Transplantation." N.Engl. J Med. 1998;338:1422-26.

PANCREAS PROGRAM

VI. TITLE: Acute and Chronic Pancreatitis: Identification of Tumor Necrosis Factor-alpha (Tnf- α) Production by Pancreatic Acinar Cells and the Presence of the Cystic Fibrosis Transmembrane Receptor (Cftr) in Pancreatic Acinar and Ductal Cells- Potential Roles in Pancreatitis

BACKGROUND: Acute and chronic pancreatitis are major health care problems in the United States. The pathological mechanisms that cause acute pancreatitis and perpetuate chronic pancreatitis remain to be elucidated but are related to pancreatic secretion. Alternatively, there appears to be a genetic predisposition or susceptibility to pancreatitis through the presence or mutation of specific genes. Most recently, mutations have been identified in the cationic trypsinogen gene which leads to hereditary pancreatitis. Thus, the genetic basis for the inherited form of pancreatitis has been identified.

RECENT FINDINGS: In a study using an animal model of pancreatitis,

pancreatic acinar cells were shown to produce, release and respond to TNF- α . TNF- α is a major mediator of the acute inflammatory response and stimulates cell death through receptor mediated events leading to either apoptosis or necrosis. In this study, TNF- α messenger RNA as well as the mediator (TNF- α) were present in the pancreas of animals with experimentally-induced pancreatitis. In addition, pancreatic acinar cells expressed the cellular receptors for TNF- α . On receptor activation, pancreatic acinar cells were shown to have activated transcription factors and subsequently undergo cell death via apoptosis.

In other studies, experiments using immunological and functional reagents showed, for the first time, the expression of the CFTR on pancreatic acinar and ductal cells. These studies indicate that potential mutations in the CFTR could lead to modified secretion of digestive enzymes in the pancreas.

SIGNIFICANCE: More than 800 mutations in the CFTR have been identified and attempts have been made to elucidate relationships between CFTR genotype and disease phenotype. Between 6-37 percent of individuals with idiopathic pancreatitis have a CFTR mutation on at least one chromosome. The present study provides a potential link between genotype and disease phenotype by providing evidence that a mutation in the CFTR could modify both acinar and ductal enzyme secretion thus leading to pancreatitis. Additionally, acinar cells have now been shown to have cellular receptors for TNF- α thereby defining a pathogenic mechanism for pancreatic cellular apoptosis seen in pancreatitis.

FUTURE DIRECTIONS: Further studies are needed to define the molecular and genetic mechanism of acute and chronic pancreatitis. Molecular regulation studies are needed to define gene expression requirements in the induction of pancreatitis and new animal models of pancreatitis using gene knock-out techniques would facilitate this understanding. Furthermore, future studies modulating expression of mediator(s) of inflammation could potentially identify targets to reduce the inflammatory response in the pancreas.

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Zeng W, Lee MG, Yan M et al. "Immuno and Functional Characterization of CFTR in Submandibular and Pancreatic Acinar and Ductal Cells." Am J. Physiol 1997;42:C442-55.

Freedman, SD. "New Concepts in Understanding the Pathophysiology of Chronic Pancreatitis." Int. J. Pancreatology1998;24: 1-8.

Gukovskaya AS, Gutovsky I, Zaninovic V et al. "Pancreatic Acinar Cells Produce, Release and Respond to Tumor Necrosis Factor- α . Role in Regulating Cell Death and Pancreatitis." J. Clin Invest. 1997;100:1853-62.

GASTROENTEROLOGY PROGRAMS

VII. TITLE: Inflammatory Bowel Disease

BACKGROUND: Our understanding of Inflammatory bowel disease over the last ten years has increased significantly. In the past diagnostic approaches were limited to contrast radiographic studies and endoscopic examination while medical therapy centered on sulfa-derived drugs and corticosteroid therapy. The year 1998 was a landmark year in the diagnosis and management of Crohn's disease, one of the inflammatory bowel diseases. Translational research has led to useful diagnostic and therapeutic options for patients with Crohn's disease.

RECENT FINDINGS: Inflammation of the intestinal mucosa in inflammatory bowel disease is characterized by increased production of inflammatory mediators. These inflammatory mediators known as cytokines orchestrate the inflammation with the gut in Crohn's disease and ulcerative colitis. There are two classes of cytokines proinflammatory and anti-inflammatory. The pro-inflammatory cytokines include tumor necrosis factor (TNF- α), interleukin -1 (IL-1B), interleukin-2, and interferon. The production of these pro-inflammatory cytokines is increased in Crohn's Disease.

Targan and coworkers have used anti-TNF- α therapy in a series of clinical trials in Crohn's Disease that appear promising. Administration of human-mouse chimeric monoclonal antibody (Infliximab) infused at doses of 5 mg/kg has provided excellent clinical results in patients with chronically active and refractory Crohn's disease and has prolonged improvement and remissions with repeated infusions.

In addition to the pharmacological advances in IBD, a new diagnostic test developed by Rummele and Targan et al, was recently published. This assay shows promise in the pediatric group who present with nonspecific gastrointestinal complaints. It also appears useful in differentiating Crohn's disease from other gastrointestinal diseases.

Several recent reports have shown that antibodies against *Saccharomyces cerevisiae*, (ASCA), baker's and brewer's yeast are found more commonly in the sera of adults with Crohn's disease compared to controls and patients with ulcerative colitis. Likewise, antibodies to peripheral neutrophil cytoplasmic antigen (pANCA) are typically found in patients with ulcerative colitis. The sera of patients with a broad spectrum of well-characterized gastrointestinal diseases were tested for ANCA and ASCA using enzyme immunoassays. The specificity

and predictive value of the assays in distinguishing IBD from non-IBD gastrointestinal diseases were 95 percent and 96 percent, respectively. Children with Crohn's disease typically had both IgA and IgG ASCA.

SIGNIFICANCE: Anti-TNF- α is the first drug approved by the FDA specifically for the treatment of Crohn's disease. This breakthrough was the direct result of years of clinical and basic investigation into inflammatory bowel disease that has defined the cascade of inflammatory events that occurs in Crohn's disease. From these studies, it was clear that TNF- α was a pivotal factor in the inflammatory process underlying inflammatory bowel disease. The use of anti-TNF- α , known commercially as infliximab, provides new hope for patients with severe Crohn's disease who have failed conventional therapy.

The expression of ASCA and p ANCA are highly specific for Crohn's disease and ulcerative colitis respectively. These new serological tests may help clinicians more accurately distinguish between Crohn's disease and ulcerative colitis; a distinction that is important in determining treatment options.

FUTURE DIRECTIONS: Other agents that act by inhibiting TNF- α or other pro-inflammatory cytokines such as IL-1, IL-6 or interferon are worthy of evaluation in animal models, and if promising, in human trials. In reference to the new serological assay for IBD, future studies are needed to determine the predictive value of using ASCA in combination with other laboratory markers in screening patients with nonspecific gastrointestinal complaints.

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Ruemmler FM, Targan SR, Levy G, Dubinsky M, Braun J, and Seidman EG: "Diagnostic Accuracy of Serological Assays in Pediatric Inflammatory Bowel Disease." Gastroenterology 1998;115:822-829.

VIII. TITLE: Helicobacter pylori and Ulcer Disease

BACKGROUND: Helicobacter pylori infection has been shown to persist for a lifetime if it is not treated and is responsible for a major morbidity and mortality worldwide. Preventative immunization may be a practical approach to the elimination of the bacterium in high-risk population groups. High rates of protection have been experimentally demonstrated in the *H. felis* mouse model, utilizing antigens from whole cells to purified recombinant proteins selected because of their role in pathogeneticity. In some situations, immunization is followed by resolution of the infection. At this time, urease remains the most commonly used target antigen in vaccine development studies.

Helicobacter pylori produces a urease that catalyzes the hydrolysis of urea to yield ammonia and carbonic acid. It is this urease which aids in the colonization of the host by neutralizing gastric acid and providing ammonia for protein synthesis. Host defenses are avoided by urease by continuing to neutralize acid locally and by shedding urease from the surface of the bacterium.

RECENT FINDING: Our understanding of the physiology of urease has been enhanced recently by studies conducted by the laboratory of Dr. George Sachs. In this study, the H. pylori urease system was found to be highly adaptive to an environment of varying acidity. Utilizing a microphysiometer, these investigators were able to measure bacterial metabolism and urease activity and acid or alkali resistance of Helicobacter pylori. These observations further validate the findings that urease activity is the major reason that H. pylori is an acid tolerant organism. A better understanding of how H. pylori affects the stomach as demonstrated in the Sachs study provides further support for prevention strategies such as vaccine development.

In another scientific area, several researchers demonstrate that oral immunization of *H. felis* infected mice with recombinant urease induces an immune response that eliminates the bacterium. Utilizing recombinant H. pylori urease antigen, Saldinger and Michetti showed both therapeutic as well as preventive effects of mucosal immunization in mice. The major responses induced by immunization were Th2 CD4 T cell responses. The elimination of H. pylori was shown to be attributable to this cellular immune response.

SIGNIFICANCE: H. pylori infection of humans has been linked to both duodenal and gastric ulcers as well as severe pre-cancerous conditions, including gastric metaplasia and atrophy. H. pylori decreases the concentration of ascorbic acid in the gastric lumen, a change that decreases the protective antioxidant mechanisms. Also ongoing observations show that H. pylori infection is

suspected to contribute to DNA damage and alteration in immune response. Saldinger and Pierre Michetti et al have shown that effective immunization with recombinant H. pylori urease generates a de novo T helper type response in infected and noninfected mice. These investigations indicate that mucosal immunization with recombinant H. pylori urease may be capable of eliminating the pathogen. These findings have major implications in the development of vaccines for humans both for the therapy and the prevention of H. pylori infections.

FUTURE DIRECTIONS: Powerful new tools such as the microphysiometer have become available and are providing new methods to understand the bacterial metabolism of H. pylori and will undoubtedly provide important strategies for new treatments and development of new vaccines. Newer techniques to understand the biology of this organism are needed to clarify mechanisms that are exhibited by different strains of H. Pylori.

In view of the success of oral immunization for prevention of H. pylori infection in mice, the next logical step is to develop and evaluate a vaccine for humans. Vaccine induced immunity may be an important means of prevention of peptic ulcer disease and gastric cancer especially in high-risk populations.

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R29DK53706	Michetti, Pierre	Beth Israel Deaconess
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Saldinger PF, Porta N, Laumois P, Louis JA, Waanders GA, Bouzourene H, Michetti P, et al. "Immunization of BALB/C Mice with Helicobacter Urease Induce a T Helper 2 Response Absent in Helicobacter Infection." Gastroenterology 1998;115:891-897.

Scott DR, Weeks D, Hong C, Postius S, Melchers K, and Sachs G. "The Role of Internal Urease in Acid Resistance of Helicobacter Pylori." Gastronterology1998;114: 58-70.

IX. TITLE: Diarrheal Illnesses and Food Safety

BACKGROUND: Escherichia coli comprises a group of bacteria many of which are found normally in the intestines of humans and animals. Some E. coli, however, are highly pathogenic. The 0157:H7 strain of E. coli is perhaps the best known pathogenic strain. This organism was first identified in 1982 and has been designated as an emerging infection by the Centers for Disease Control and Prevention because of its increasing incidence over the last two decades.

E. coli 0157: H7 infection afflicts the very young and the very old. It produces a spectrum of illness, from mild diarrhea lasting 6 to 8 days to severe hemorrhagic colitis characterized by grossly bloody diarrhea, severe abdominal cramps, low grade fever and vomiting. Ten percent of infected individuals develop hemolytic uremic syndrome a life threatening illness characterized by acute renal failure, hemolytic anemia, and various central nervous system abnormalities.

Generally, most individuals who have E. coli 0157:H7 associated diarrhea have a complete recovery. The treatment of this illness is usually supportive with rehydration and with blood products for severe anemia. Because of its gastrointestinal presentation, physicians may fail to consider the diagnosis, and misdiagnoses, including ischemic colitis, pseudomembranous colitis, and inflammatory bowel disease, sometimes leading to unnecessary invasive diagnostic and therapeutic procedures, or inappropriate antibiotics therapy.

RECENT FINDINGS: Strains of E. coli are characterized by their ability to produce two type of Shiga toxin. These toxins are important factors in the pathogenesis of Hemolytic Uremic Syndrome. However, the mechanisms of how these toxins cause disease are unknown. Now, a recent discovery by Bieber and Schoolnik give insight in how this strain of E. coli causes diarrheal states in humans and animals. A unique property of Enteropathogenic E. coli is that it possesses certain features known as bundle forming pili (bfp), seen by electron microscopy. These bundle-forming pili, allow the bacteria to adhere to the lining on the intestines and confirms virulence. The type IV bundle forming pili of the enteropathogenic E. coli have been found in in vitro studies to be responsible for promoting bacterial adherence and subsequently diarrheal states. Bundle forming pili expression is required for the development of bacterial microcolonies on tissue cultures known as localized adherence phenotype and formation of spherical bacterial aggregates in tissue culture, known as autoaggregation phenotype.

In a study conducted at Stanford University, NIDDK-supported researchers inoculated normal human volunteers with either a bfp negative or a bfp positive

(wild type) strain of E coli. Volunteers who received the wild type solution had a dose dependent diarrheal response, and those who had the bpf negative solution had less diarrhea. Thus, this study further confirms that bpf is a determinant of virulence in human diarrheal illnesses related to E. coli.

A recent study by NIDDK-supported investigators from the University of Washington provided insights into the importance of the proper diagnosis of E. coli hemorrhagic colitis and ischemic colitis. Ischemic colitis is probably the most common ischemic disorder of the gastrointestinal tract and usually presents with left lower abdominal pain, and bloody diarrhea. The specific cause for ischemic colitis is not usually identified. In a retrospective study by Tarr and coworkers, E. coli 0157: H7 bacteria were identified in a proportion of patients with presumed ischemic colitis. Utilizing immunochemical-staining techniques, the investigators identified E. coli 0157:H7 organisms in tissue sections from over one-third of cases diagnosed as having ischemic colitis. This study underscores the importance of identifying patients who present with acute bloody diarrhea and the importance of performing stool cultures for E. coli 0157:H7.

SIGNIFICIANCE: The understanding of the pathogenesis of E. coli 0157:H7 is not merely of theoretical interest but rather aids in the development of new approaches to the management of E. coli 0157 induced foodborne illnesses. The study by Bieber and Skoolnik provides insights into how E. coli colonizes and produces diarrheal illnesses in man. Their discovery that bundle-forming pili of enteropathogenic E. coli are necessary for the development of diarrheal illnesses provides a better understanding of the role of adherence in the virulence of this organism.

The second study by Tarr et al. provide important information for clinicians. The assessment of patients who present with bloody diarrhea should include a stool culture for E. coil 0157:H7 even though colonoscopic and radiographic studies may be suggestive of ischemic colitis. The finding of the organism in tissue of patients with presumed ischemic colitis raises the possibility that the organism may have a role in initiating the event.

FUTURE DIRECTIONS: Future studies on the adherence of E. coli 0157:H7 will help elucidate the mechanism on how this organism colonizes the gastrointestinal tract and how the toxin from this organism leads to systemic disease.

In addition, there is a need for large prospective epidemiological studies of patients with bloody diarrhea to define whether E. coli 0157:H7 is a triggering event in ischemic colitis.

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RO3DK52038	Schoolnik, Gary	Stanford University
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Bieber D, Ramer SW, Wu CY, Murray WJ, Tobe TT, Fernandez r. Schoolnik GK. "Type IV Pili, Transient Bacterial Aggregated, and Virulence of Enteropathogenetic Escherichia Coli." Science 1998; 280:2114-2118.

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GASTROINTESTINAL AIDS PROGRAM

X. TITLE: Mucosal Immunity and Aids of the Gastrointestinal Tract: Identification of the Gastrointestinal Tract as a Major Site of Viral Replication and Cd4 T Cell Depletion

BACKGROUND: The gastrointestinal tract is a major target organ in the pathogenesis of AIDS. Progressive weight loss, chronic diarrhea, intestinal malabsorption as well as the AIDS Wasting Syndrome are prominent clinical features of HIV infection. In addition, the gastrointestinal tract is the target organ for numerous opportunistic infections characteristic of late stage AIDS. Although it is well established that in acute HIV infection, the gastrointestinal tract is a portal of entry for the virus in a large percentage of cases, it is unknown what specific early events occur in the gastrointestinal mucosa that promote systemic viral infection and pathogenesis.

RECENT FINDINGS: The human and simian immunodeficiency virus (HIV and SIV, respectively) replicate optimally in activated memory CD4 T cells which are abundantly found in the gastrointestinal mucosal tissues. SIV infection of Rhesus monkeys resulted in profound and selective depletion of CD4 T cells in the intestine within days of viral infection. The depletion of T cells occurs prior to any changes in T cells in the peripheral lymphoid tissues. The loss of T cells

in the intestine is concomitant with the productive infection of mononuclear cells in the intestine.

SIGNIFICANCE: The mucosal tissues of the gastrointestinal tract comprise the gut-associated lymphoid tissue (GALT) which represents the largest reservoir of T cells in the body. The present study shows that in an animal model of human HIV infection, the GALT reservoir of T cells is the major target for virus replication. The infection of such a large number of T cells and replication of virus in these cells likely accounts for the "viral burst" or spike in viral load seen in acute human infection. In addition, this study showed a dramatic loss of infected T cells in the intestine within four days of infection indicating the initial pathogenic effect of infection with the immunodeficiency virus.

FUTURE DIRECTIONS: With the observation that the gastrointestinal tract is the major target of the immunodeficiency virus in acute infection with regard to both infection and pathogenesis, future research efforts toward a vaccine for HIV can now target infection via the gastrointestinal mucosal surfaces. A vaccine capable of preventing infection and or transmission of the virus at mucosal surfaces would prove effective at halting or decreasing the systemic infection by HIV.

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R01DK50550	Lackner, A. A.	Harvard Med School

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Veasey RS, De Maria MA, Chalifoux LV et al. "Gastrointestinal Tract as a Major Site of Cd4 T Cell Depletion and Viral Replication in Siv Infection." Science 1998; 280:427-31.

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NUTRITION PROGRAMS

XI. TITLE: Characterization and Expression of Intestinal Cotransporters

BACKGROUND: In recent years Dr. Ernest Wright and co-workers at the University of California cloned and sequenced the gene responsible for glucose/galactose malabsorption and accompanying water transport perturbations with severe diarrhea and dehydration. The syndrome results from a defect in the Na/glucose symporter (SGLTI), which couples glucose and galactose transport to Na gradients across the brush border membrane of cells lining the small intestine and renal proximal tubule. In addition to glucose/galactose malabsorption syndrome, other hereditary diseases believed to be due to defects in Na/glucose cotransport proteins include renal glycosuria, Hartnup disease and prolinuria. Furthermore, perturbations associated water transport result in severe health problems. For example, it is reported that worldwide, more than 10,000 children under 5-years of age die daily from dehydration. Another 3,000 suffering from dehydration are saved each day by oral rehydration therapy. This therapy is based on the fact that glucose stimulates salt and water transport across the small intestine, but basic mechanisms remain obscure. One explanation is that water transport is directly coupled to the movements of solutes by cotransporters.

RECENT FINDINGS: After cloning the gene responsible for glucose-galactose malabsorption, work has continued resulting in efficient methods for screening patients for mutations and further elucidation of mechanisms involved in defective SGLTI proteins. Studies have confirmed that each mutation causes malabsorption by reducing the number of transporters in the plasma membrane. The mutations affect the Na/sugar and water cotransport by blocking the transfer of SGLTI protein from the endoplasmic reticulum to the plasma membranes. The mutant genes possess heterozygous missense mutations and produce poorly glycosylated proteins that fail to reach surface membrane and thus do not promote glucose absorption.

Additional studies by Dr. Wright and co-workers have confirmed that water transport is directly linked to solute transport by cotransport proteins such as the brush border Na/glucose cotransporter. The Na/glucose cotransporter was expressed in *Xenopus* oocytes, and the changes in cell volume measured under sugar-transporting and non-transporting conditions. These investigators demonstrate that 210 water molecules are directly coupled to each sugar molecule transported and estimate that the SGLTI could account for approximately half the daily re-uptake of water from the small intestine.

SIGNIFICANCE: The current studies have led to improved methods for genetic screening of patients, with malabsorption syndromes and other related, inherited diseases. In addition, the elucidation of processes involved in cotransport of solutes and water may provide a better understanding of basic mechanisms underlying several diarrheal diseases and improved treatment of the diseases.

FUTURE DIRECTIONS: Future work should include the screening of additional patients with more direct studies of SGLTI protein processing and degradation in model expression systems to determine how missense mutations actually impair sugar and water transport. Additional studies will also be needed to establish the existence of other water/solute cotransporters and the mechanisms involved in transport perturbations in diarrheal diseases.

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<u>Grant or Contract #</u>	<u>Principal Investigator</u>	<u>Institution</u>
R01DK19567	Wright, Ernest	Univ of Calif-LA
R01DK44582	Wright, Ernest	Univ of Calif-LA

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Martin MG, Lostao MP, Turk E, Lam J, Kreman M, Wright EM, "Compound Missense Mutations in the Sodium-D-Glucose Cotransporter Result in Trafficking Defects," Gastroenterology 1997;112:1206-1212.

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XII. TITLE: Assessment of Zinc Status in Human Based on Metallothionein Gene Expression

BACKGROUND: Zinc status is difficult to evaluate in humans and all methods used to date have been met with significant skepticism. Although zinc is an essential mineral with numerous physiological roles, a specific, reliable biomarker or index for zinc status has not been developed. The assessment of nutritional status usually is based on either the level of the nutrient in a blood component or on a biomarker related to a function that responds to dietary intake and/or body stores of that nutrient. Zinc status measurements are problematic because measurement of zinc levels in plasma or blood cells, while

technically straightforward, is not a reliable and sensitive predictor of zinc status. Plasma zinc concentration is homeostatically regulated at 10-15 micromole/liter. There appears to be adaptation to low zinc intake that decreases zinc excretion to achieve conservation and maintenance of normal plasma concentration. This is achieved through several mechanisms using zinc transporters and binding proteins that control cellular influx and efflux.

RECENT FINDINGS: Recently, Dr. Robert Cousins and colleagues have demonstrated that erythrocyte metallothionein (MT) levels reflect changes in dietary zinc status in humans using an enzyme linked immunosorbant assay (ELISA). Since dietary zinc intake is directly related to cellular MT mRNA levels in rats, these investigators reasoned that an assay based on MT mRNA levels would provide a reliable measurement of zinc intake and body zinc status in humans; the MT gene is transcriptionally regulated by zinc. Using competitive reverse transcriptase-polymerase chain reaction (CRT-PCR) assays, mRNA levels for MT was found to be a reliable biomarker for zinc status during both zinc supplementation and zinc depletion. MT mRNA levels in monocytes do not show the same homeostatic responses found in plasma levels of zinc. Furthermore, actual MT protein levels in erythrocytes show similar responses to changes in zinc intake/depletion levels. Thus, both erythrocyte MT and monocyte MT mRNA should prove to be measures useful for assessment.

SIGNIFICANCE: With increasing evidence concerning the essentiality of zinc to human health, there is a need for specific, reliable indicators of zinc status and cellular responses to dietary zinc. However, several factors have made identification of such indicators difficult. Zinc metabolism is under tight homeostatic control, therefore, the most commonly used variable for measuring nutrient status such as zinc concentration in body fluids and zinc metalloenzyme activities have not been reliable for the assessment of status. Consequently, marginal zinc deficiency, which may be prevalent in several populations in the U.S. and the world, is especially difficult to detect.

FUTURE DIRECTIONS: Further studies are required to evaluate the use of the ELISA and CRT-PCR assays in dietary zinc status assessment, and the variables that may influence those measurements in various cells and tissues and under different disease states. This will be critical in further studies focusing on evaluation of dietary zinc supplements and on effects of zinc depletion in health and disease.

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R01DK52412	Cousins, Robert	Univ of Florida
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Sullivan VK, Burnett FR, Cousins RJ, "Metallothionein Expression is Increased in Monocytes and Erythrocytes of Young Men During Zinc Supplementation," J Nutr; 1998;128:707-713.

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XIII. TITLE: New Roles for Vitamin D: Effects on the Immune System

BACKGROUND: Until the 1980's, the function of vitamin D was thought to be primarily related to the maintenance of plasma calcium and phosphorus concentrations required for normal mineralization of the skeleton and for normal neuromuscular function. In more recent years the vitamin has been targeted to a number of tissues, and with the discovery of the nuclear receptor (VDR) its roles as a member of steroid-thyroid hormone super family became clearer. These earlier investigations have led to treatment of rickets, hypocalcemia of hypoparathyroidism, renal osteodystrophy, osteoporosis and psoriasis.

The VDR has been detected in cells not previously thought to be target organs, and investigators continue to discover new functions of vitamin D, most recently in control of cellular growth and differentiation. The VDR binds to response elements in the promoter region of target genes to stimulate or suppress transcription of those genes encoding for proteins that carry out a wide variety of functions, including those in the immune system.

RECENT FINDINGS: One of the most interesting sites of action of vitamin D recently described is in the immune system where the VDR has been demonstrated in activated lymphocytes. Dr. DeLuca and collaborators have found that T-cell-mediated immune responses such as delayed hypersensitivity could be inhibited by excess vitamin D or its analogs. Furthermore, a deficiency of vitamin D also interferes with the T-cell-mediated immunity. It is now clear

that vitamin D and its analogs can influence T-cell-mediated disease states in animals.

Dr. DeLuca and colleagues have used a mouse model of the human disease multiple sclerosis (MS); experimental autoimmune encephalomyelitis (EAE), a mouse disease, can be both prevented and reversed by injections or dietary ingestion of vitamin D. Furthermore, if vitamin D treatment is removed during regression, the disease process continues. Thus, the action of the vitamin D hormone can be used both to prevent the disease entirely or it can be used to arrest its development in this model of human MS. Similarly, preliminary data using vitamin D in animal models of rheumatoid arthritis and transplant rejection experiments show equally promising results. It appears that the vitamin D compounds target either the CD-8 cytotoxic lymphocytes or the Th2 helper lymphocytes that in turn suppress Th1 lymphocytes that are known to induce the inflammatory response.

SIGNIFICANCE: It appears likely that vitamin D and its analogs may be useful in modulating some immune-mediated diseases and rejection of transplants.

FUTURE DIRECTIONS: Clinical trials and studies are needed to assess effects of vitamin D and its analogs on autoimmune diseases in humans. It will be critical to ascertain effectiveness levels, concomitant effects on calcium homeostasis, and whether the compounds can be used without compromising the skeleton and without compromising the host to opportunistic infection. In addition, further basic studies are needed on mechanisms involving cytokine actions and vitamin D in the immune system.

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P01DK14881	DeLuca, Hector	Univ of Wisc
R01DK46820	Hayes, Colleen	Univ of Wisc

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DeLuca H, Zierold C, "Mechanisms and Functions of Vitamin D," Nutrition Reviews 1998;56:S4-S10.

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Positive Regulator for the Two Anti-Encephalotogenic Cytokines TGF- β 1 and IL-4," J Immunol 1998;160:5314-9.

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OBESITY AND SATIETY PROGRAM

XIV. TITLE : Etiology and Pathogenesis of Obesity

BACKGROUND: Obesity is the most common nutritional disorder in the United States. Though commonly trivialized as primarily a cosmetic problem resulting from "lack of will power," a growing body of evidence from epidemiological, basic and clinical research indicates that obesity has a biologic basis and represents a major - and costly - threat to health. Obesity contributes to diabetes, hypertension, elevated blood lipids and certain types of cancer. Over half of all US adults are overweight (BMI >25), and more than 20 percent are obese (BMI >30). The costs of obesity to the U.S. health system are enormous. It is estimated that direct and indirect health care costs attributable to obesity approach \$100 billion. Obesity is particularly common among African American and Hispanic women, with prevalence rates of more than 33 percent in these groups. Studies involving twins, adopted children and extended families suggest that as much as 80 percent of the susceptibility to obesity is due to genetic factors. The term susceptibility is used because such genes do not act in isolation, but have their effects through interactions with a host of developmental and environmental factors.

Treatments for obesity are notoriously ineffective when measured by the yardstick of success in the maintenance of reduced body weight. An understanding of the nature of the components for the set point system would provide physiologic and pharmacological tools for the successful treatment of obesity and diabetes. The advances described below result from efforts to understand the bases for body weight control at both the physiologic and the molecular levels.

RECENT FINDINGS: Interactions between Melanocortins and Leptin. Researchers at the University of Cincinnati and the University of Washington have recently identified a role of the central nervous system (CNS) melanocortin system in mediating the effects of leptin in the brain to reduce food intake and body weight. Leptin is the fat-cell derived hormone that is synthesized and secreted in direct proportion to amount of body fat. When administered either peripherally or directly into the central nervous system, leptin potently reduces

food intake and body weight in rodents. The current data indicate that CNS melanocortin system is an important target of leptin action.

The melanocortins have traditionally been implicated in the control of skin and hair color but they also have potent effects in the CNS to alter food intake. For example, several stimulators of CNS melanocortin receptors decrease food intake, while receptor blockers stimulate food intake. Importantly, leptin's ability to reduce food intake depends critically on activity at CNS melanocortin receptors. Doses of melanocortin receptor blockers that have no effect on their own can completely reverse the reduction in food intake and increased brain activity usually observed after leptin administration.

SIGNIFICANCE: Illuminating the CNS mechanisms responsible for the normal regulation of food intake and body weight provide insight into how the system may be altered in diseases characterized by energy balance dysregulation such as obesity and wasting in response to AIDS and some cancers. Understanding the signaling cascade for leptin actions in the CNS also provides other potential systems beyond the leptin receptor to target for therapeutic intervention in obesity. The current work points to the CNS melanocortin system as one important piece of that cascade and one potential point to intervene for therapeutic effect.

FUTURE DIRECTIONS: To further understand the CNS mediation of leptin actions, it will be necessary to determine how many of leptin's effects are mediated by the melanocortin system. Leptin clearly alters sympathetic nervous system activity and a variety of metabolic and endocrine factors. How many of these effects are influenced by the melanocortin system is unknown. Additionally, the functional relationship between the hypothalamic melanocortins and other CNS neurotransmitter systems important in the control of food intake is yet to be determined.

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R37DK17844	Woods, S.	Univ Cincinnati
P30DK35816	Chait, A.	Univ Wash
R01DK52989	Schwartz, M.	Univ Wash

Publications:

Seeley, RJ, Yagaloff, KA, Fisher, SL, Burn, P, Thiele, TE, van Dijk, G, Baskin, DG and Schwartz, MW (1997). "Role of Melanocortin Receptors in Leptin Effects." Nature. 390, 349.

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Woods, SC, Seeley, RJ, Porte, D and Schwartz, MW (1998). "Signals that Regulate Food Intake." Science, 280, 1378-1383.

RECENT FINDINGS: Development of Fat cell Hyperplasia with Obesity. Previous reports have suggested that reaching a critical fat cell size during the development of obesity may trigger the proliferation of new fat cells, however this hypothesis remained unproven. The investigators set out to test this hypothesis systematically by determining the association between fat cell size distribution, the presence of local growth factors in adipose tissue, and subsequent change in the number of fat cells in various fat depots of lean and obese Zucker rats. The obese rats had a greater percentage of large fat cells than did lean rats. The investigators found a strong correlation between the percentage of large cells in fat tissue and the release of substances from the tissue that had a greater ability to stimulate the proliferation of fat cell precursors grown in special media. They also found that this increase in proliferative activity was associated with subsequent increases in fat cell number. Therefore, this controlled study supports the hypothesis that large fat cells (which occur as obesity develops) secrete growth factors that cause more fat cell precursors to develop.

SIGNIFICANCE: This finding has implications for the prevention and treatment of obesity, because it suggests that once a certain level of obesity develops, fat cell number (as well as fat cell size) increases, making it more difficult for the very obese person to maintain weight loss over the long-term. This makes prevention of obesity more critical as a strategy for improving public health.

FUTURE DIRECTIONS: Future studies should determine the effects of dietary change on adipose tissue growth, and better characterization of local factors that stimulate fat cell growth and development. Understanding the factors that trigger the development of new fat cells may lead to the development of new strategies for the prevention and treatment of obesity.

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RO1DK47246	Martin, Roy	Univ of Georgia

Publications:

Marques, BG, Hausman, DB and Martin, RJ. "Association of Fat Cell Size and Paracrine Growth Factors in Development of Hyperplastic Obesity." *Regulatory Integrative. Physiol.* In press [Dec publication].

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Loh, MY, Flatt, WP, Martin, RJ and Hausman, DB. "Dietary Fat Type and Level Influence Adiposity Development in Obese but Not Lean Zucker Rats." *Proc. Soc. Exp. Biol. Med.* 218:38-44, 1998.

RECENT FINDINGS: Role of Non-Exercise Activity Thermogenesis (NEAT) in resistance to fat in humans.

Researchers at the Mayo Clinic in Rochester, MN and Minnesota Obesity/Nutrition Research Center have investigated the observation that humans show considerable inter-individual variation in susceptibility to weight gain in response to overeating. Continued energy intake in excess of energy expenditure is necessary for the development of obesity, and understanding the physiological basis of this variation in response to overeating could improve our understanding of the factors that predispose to obesity. Changes in energy storage and expenditure were measured in 16 non-obese subjects who were fed 1000 kcal/day in excess of weight-maintenance requirements for eight weeks. Two-thirds of the increases in total daily energy expenditure was due to increased non-exercise activity thermogenesis (NEAT), which is associated with fidgeting, maintenance of posture and other physical activities of daily life. Changes in NEAT accounted for the ten-fold differences in fat storage that occurred, and directly predicted resistance to fat gain with overfeeding. Changes in basal metabolic rate and the thermic effect of food were observed but did not predict resistance to fat gain. These results suggest that as humans overeat, activation of NEAT dissipates excess energy to preserve leanness and that failure to activate NEAT may result in ready fat gain.

SIGNIFICANCE: These studies document that some lean, sedentary individuals can consume excess food and yet gain very little weight. The source of the increased energy expenditure that accomplishes this feat is not passive (e.g. uncoupling) but active. Additional energy is expended, albeit not consciously, in every day activities. If failure to activate NEAT predisposes to obesity, developing means to more readily activate activity specifically in response to overeating could help prevent obesity.

FUTURE DIRECTIONS: Little is known about the components of NEAT and their relative contribution to the changes in energy expenditure we observed in response to overeating. Additional studies will be required to fully characterize this previously unappreciated component of daily energy expenditure and to determine to what degree it is inherited and modifiable.

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R01DK45343	Jensen, M.	Mayo Clinic
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Publications:

Levine JA, Eberhardt NL, Jensen MD. "Role of Non-exercise Activity Thermogenesis (Neat) in Resistance to Fat Gain in Humans." Science. In press.

XV. TITLE: Pathophysiology and Treatment of Eating Disorders

BACKGROUND: Eating disorders are a serious health problem, particularly among adolescent and young adult women. It is estimated that about 3 percent of this population suffers from a diagnosable eating disorder, while many more suffer symptoms of subthreshold eating disorders. Bulimia nervosa is an eating disorder characterized by the uncontrollable urge to binge-eat usually followed by self-induced vomiting. In the majority of cases, young women voluntarily initiate these behaviors under societal pressure to maintain a slim, "attractive" figure. At some point the voluntary control ceases and patients feel "driven" to engage in binge eating and vomiting. While some progress has been made in treating disorders such as bulimia nervosa and binge eating disorder with

psychotherapy and psychotropic drugs, less research has focused on treatments based on hypothesized physiologic mechanisms that might either initiate or perpetuate disordered eating.

RECENT FINDINGS: Treatment of Bulimia Nervosa with a Serotonin Receptor Antagonist. Researchers at the University of Minnesota have obtained physiological and clinical evidence that a biological mechanism driving (i.e. perpetuating) the bulimic symptoms involves overactivity in the afferent branches of the vagus nerve. The main functions of this bilateral nerve is to relay information from the internal organs, including the stomach, to the brain for initiation of multiple effector responses including: (1) the feeling of “fullness” and satiety in response to meal consumption; and (2) a decrease in the detection of somatic pain (pain detection threshold). Ondansetron (Zofran), a serotonin type-3 (5HT₃) receptor antagonist, was used to assess the effect of reducing vagal neurotransmission on binge eating and vomiting and on pain detection thresholds in severe (>7 bulimic episodes per week) and chronic (>2-years illness duration) bulimia nervosa patients studied under a randomized, double-blind, placebo controlled experimental design.

Double-blind treatment with ondansetron treatment (n=13) was associated with a significant reduction in binge/vomit (B/V) frequencies compared to double-blind placebo control values (n=12). Specifically, the patients randomly assigned to ondansetron displayed a 70 percent reduction in B/V frequencies from a mean of 17 episodes per week during baseline to 6 episodes per week after 4-weeks of ondansetron treatment. Two secondary indicators of disorder severity, namely the time spent engaging in bulimic behaviors and the number of normal meals consumed, provide additional characterization of the effect of ondansetron. The subjects also significantly reduced the duration of time spent binge eating/vomiting. In addition, the number of "normal" meals and snacks increased, indicating that patients were not achieving a reduction in binge/vomit frequency by restricting food intake altogether, but rather were more capable of terminating meals without having an eating episode turn into an uncontrollable binge. In addition, pain detection thresholds (PDT), previously found to be abnormal in bulimic patients also normalized in those treated with ondansetron. The re-establishment of normal PDT (and presumably normal vagal tone) by the pathological behaviors of binge eating and vomiting was also evident in a different group of bulimia nervosa patients studied in a highly controlled hospitalized setting and asked to voluntarily engage in the bulimic behaviors.

SIGNIFICANCE: Pharmacological blockade of neural transmission in the vagus nerves by ondansetron is associated with improvement in both clinical and physiological variables. Not only was ondansetron associated with a relatively rapid improvement in the primary disease symptoms of binge-eating and vomiting, but equally important, patients also displayed a return toward normal

satiety and meal patterning. Furthermore, the physiological data on PDT from untreated bulimia nervosa patients indicates the existence of a self-perpetuating, vicious cycle in which binge-eating/vomiting results in a normalization of vagal tone and conversely, not engaging in the pathological behaviors is associated with a worsening of the underlying physiology. Since binge-eating and vomiting both result in vagal nerve stimulation, it is likely that the initial voluntary acts of engaging in these behaviors result in a self-induced, oscillatory pattern of vagal hyperactivity which is no longer under cognitive control. Collectively, these findings provide one of the first indications that alterations in the function of peripheral vagal afferents can produce complex “psychiatric” symptoms and thereby suggest new classes of compounds for potential use in the treatment of this debilitating disorder.

FUTURE DIRECTIONS: Two inter-related clinical and physiological questions need to be addressed. The first involves clinical usage issues such as determining the effectiveness of ondansetron in less-severe bulimia nervosa patients; the generalizability of vagal involvement to other eating disorders involving either binge eating (binge-eating disorder) or vomiting (purging-type anorexia nervosa); and the effectiveness of using ondansetron in combination with conventional psychotherapy or anti-depressant treatments. The second experimental series involves examination of the detailed physiological mechanisms underlying the therapeutic action of ondansetron, such as the involvement of peripheral serotonin from gastric enterochromaffin in perpetuating both the binge/vomiting behaviors and the dynamic fluctuation in pain thresholds; the functional status of the peripheral 5HT 3 receptor in bulimia nervosa subjects prior to and following ondansetron treatment; and a comparison of the acute effects of ondansetron versus another 5 HT 3 antagonist with higher CNS penetrability (granisetron) on the abstinence-induced elevation in PDT and the “psychological urge” to engage in the bulimic behaviors.

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<u>Grant or Contract #</u>	<u>Principal Investigator</u>	<u>Institution</u>
R01DK42291	Faris, P.	Univ of Minnesota

Publications:

Hartman BK, Faris PL, Kim SW, Raymond NC, Goodale RL, Meller WH and Eckert ED. “Treatment of Bulimia Nervosa with Ondansetron” (letter). Archives Gen Psychiatry 54 (10):969-970, 1997.

Faris PL, Kim SW, Meller WH, Goodale RL, Hofbauer RD, Oakman SA, Howard LA, Stevens ER, Eckert ED and Hartman BK. "Effect of Ondansetron, a 5 HT3 Receptor Antagonist, on the Dynamic Association Between Bulimic Behaviors and Pain Thresholds." Pain. In press.

Raymond NC, Eckert ED, Hamalainen M, Evanson D, Thuras PD, Hartman BK, and Faris PL. "A Preliminary Report on Pain Thresholds in Bulimia Nervosa During a Bulimic Episode." Comprehensive Psychiat. In press.

NIDDK MINORITY TRAINING AND CAREER DEVELOPMENT PROGRAMS-FY 1998

Name of Program and Description	Division	# of NIDDK Awards	NIDDK Funding Level	ORMH Collab. Funding
<p><u>Minority Access to Research Careers (MARC) T-34</u> NIDDK Co-funds with NIGMS. Funds predoctoral faculty fellowships, visiting scientists, conferences for minority investigators and minority health issues, and honors undergraduate training in biomedical research. Summer Internship Program in the NIDDK Division of Intramural Research (students-managed by NIDDK-EEO).</p>	DK-wide	6	\$23,236	
<p><u>Minority Biomedical Research Support Program (MBRS)</u> NIDDK co-funds with NIGMS. Provides expanded opportunities for minorities to participate in biomedical research careers. Supports research projects of interest to the NIDDK at Minority and Equal Opportunity Institutions.</p>	DK-wide	25	\$1,985,728	
<p><u>R-13 (Conference Grant) to the American Physiological Society, FASEB</u> Provides support for underrepresented minority students to attend meetings of the Society, and for 36 minority high school science teachers to have summer research training in laboratories of Society members.</p>	DK-wide	1	\$74,315	

<p><u>Initiatives for Underrepresented Minorities in Biomedical Research</u> NIH-wide program initiatives to support minority undergraduate, graduate students, high school students, and faculty members on NIDDK active research grants through administrative supplements.</p>	DK-wide	120	\$4,500,000	
<p><u>Research Training of Underrepresented Minorities on Institutional Training Grants (T32)</u> Highly qualified Minority Investigators are assigned T-32 slots held in reserve for this purpose. DDEMD=5 DDDND=3 DKUHD=6</p>	DK-wide	14	\$175,174 81,437 183,000	\$41,917
<p><u>Pre-doctoral Fellowships (F-31)</u> To provide support to minority students for research training leading to M.D.-Ph.D. in the biomedical sciences. DDEMD=6 DDDND=2 DKUHD=1</p>	DK-wide	9	\$132,269	
<p><u>Cell/Molecular Biology Student/Teacher Learning Center (R-25)</u> Laboratory Research experience for minorities in the District of Columbia (managed by NIDDK-EEO).</p>	DK-wide	1	\$334,767	

DIVISION OF KIDNEY, UROLOGIC AND HEMATOLOGIC DISEASES

FY 1999 Program Plan
RESEARCH PROGRESS REVIEWS
February 1999 Council

Josephine P. Briggs, M.D., Director

DIVISION OF KIDNEY, UROLOGIC, AND HEMATOLOGIC DISEASES

FY 1999 PROGRAM PLAN

Research Progress Reviews

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HEMATOLOGY PROGRAM

I. TITLE: Zebrafish Models for Human Blood Diseases

BACKGROUND: Sideroblastic anemias may derive from a number of causes, resulting in the presence of ringed cells in the marrow called sideroblasts, ineffective erythropoiesis, increased levels of tissue iron, and pale erythrocytes in the blood. The congenital form in humans often is caused by a mutation in delta-aminolevulinate synthase, the enzyme required for the first step in heme biosynthesis. Other heme enzyme mutations in humans cause human porphyrias, a group of rare and clinically complex syndromes. The most prevalent porphyria is porphyria cutanea tarda (PCT), which manifests skin photosensitivity and excessive excretion of uroporphyrin, due to reduced uroporphyrinogen decarboxylase (UROD). Animal models have not been available for these human disorders.

RECENT FINDINGS: The popular, small, aquarium fish called *Danio rerio* (or more commonly the zebrafish) is emerging as a powerful new tool to understand human genes and human development. Two recent studies highlight the success of this organism as a model to study human blood diseases. A zebrafish mutant called *sauternes*, because of its pale blood, has a form of anemia in which the red blood cells have a reduced volume and hemoglobin content. The gene responsible for this condition was just discovered to be the same gene that is defective in human patients with sideroblastic anemia. This gene codes for delta-aminolevulinate synthase, the enzyme required for the first step in heme synthesis. This enzyme is very similar in humans and zebrafish.

A second zebrafish mutant, *yquem*, has photosensitive red blood cells that are destroyed by exposure to bright light. Further study of these fish has established that they have a form of porphyria, very much like the human disease. Patients with this disease are very light-sensitive and have disordered liver and hematologic function. Fish with this mutation die when exposed to bright light; the mutation is in the same gene that is defective in humans with hepato-erythropoietic porphyria.

SIGNIFICANCE: These studies contribute to the increased awareness that many of the key proteins that determine the function of cells are widely conserved across many life forms, and that simple life forms can provide experimental models with results directly applicable to understanding human disease. Further studies in mutant fish may help identify new therapies for patients with defects in these enzymes.

FUTURE DIRECTIONS: Using these animal models, it will be possible to design protocols for gene therapy, to rescue affected animals from mutant enzymes, and cure the disease. These models may be useful for evaluating a large number of DNA constructs for gene therapy. Similar approaches may allow molecular characterization of other heme enzyme mutants in zebrafish, and the mutants should be useful in elucidating the pathogenesis of human disorders of heme biosynthesis.

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Brownlie A, Donvan A, Pratt S, Paw B, Oates A, Brugnara C, Witowska H, Sassa S, Zon L, "Positional Cloning of the Zebrafish *Sauternes* Gene: A Model for Congenital Sideroblastic Anemia," Nature Genetics 1998;20:244-50.

Wang H, Long Q, Marty S, Sassa S, Lin S, "A Zebrafish Model for Hepatoerythropoietic Porphyria," Nature Genetics 1998;20:239-43.

II. TITLE: Targeted Expansion of Genetically Modified Stem Cells

BACKGROUND: Hematopoietic stem cells are considered to be ideal target cells for gene therapy. However, their use is limited without efficient methods of transferring genes into early hematopoietic progenitors and stem cells. To overcome this obstacle, researchers have developed techniques to select and expand the numbers of genetically modified cells. The standard technique involves the transfer of a drug-resistance gene followed by exposure to the corresponding cytotoxic drug. However, the toxicity of the drug limits the use of this technique *in vivo*, and selection based on this method is difficult to apply since stem cells are resistant to most cytotoxic agents.

RECENT FINDINGS: NIDDK-funded researchers have developed a new method to select cells that results in a genetically modified cell population with a restricted and reversible growth advantage. The method uses a system that

permits intracellular protein dimerization to be reversibly activated in response to a lipid-soluble dimeric form of the drug FK506, called FK1012. FK1012 is used to bring together two FK506-binding domains from a cellular protein called FKBP12. In the experiment, FKBP12 was linked to the signaling domain of the thrombopoietin receptor, allowing interleukin-3-dependent cells to become FK1012-dependent. Dimerization of the fusion protein through the addition of FK1012 resulted in a marked proliferation of marrow cells that was restricted to the genetically modified cells. A preference for differentiation along the megakaryocyte lineage was observed.

SIGNIFICANCE: This innovative approach allows the specific delivery of a signal to divide a population of genetically modified primary cells. The technique may provide insights into blood cell development. The *in vivo* application of this approach may allow for the specific expansion of a minor population of genetically modified stem cells and progenitors, making it useful for gene therapy.

FUTURE DIRECTIONS: It needs to be demonstrated that the technique can be applied to other cell lineages, such as the erythroid and myeloid lines, through activation of the erythropoietin and GCSF receptors, respectively. Testing so far has been done only in cell lines, and must be tried in animal models.

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Jin L, Siritanaratkul N, Emery DW, Richard RE, Kaushansky K, Papayannopoulou T, Blau CA. "Targeted Expansion of Genetically Modified Stem Cells," Proceedings of the National Academy of Sciences 1998;95(14):8093-7.

III. TITLE: A More Stable and Effective Erythropoietin Molecule *in vivo*

BACKGROUND: Erythropoietin is a hormone, produced by the kidney, which

regulates the production of red cells. Its use in treating the anemia of renal failure has markedly improved the quality of life for many of those patients and for people with certain other forms of chronic anemia. Nevertheless, the drug's high cost and the need to administer it parenterally have limited its use.

RECENT FINDINGS: NIDDK-funded investigators reported recently in the *Proceedings of the National Academy of Sciences* that they have created a modified form of erythropoietin, in which two copies of the molecule are linked. The plasma half-life of erythropoietin dimers in rabbits exceeded 24 hours, compared with 4 hours for the monomers. Importantly, erythropoietin dimers were biologically active *in vivo*, as shown by their ability to raise the hematocrits of mice when injected under the skin. Finally, the dimers exhibited more than 26-fold higher activity *in vivo* than did the monomers, and were effective after only one dose.

SIGNIFICANCE: This modified molecule is more stable in the circulation and, molecule for molecule, much more effective. This advance raises the possibility of improved and more cost-effective treatment of a variety of forms of anemia.

FUTURE DIRECTIONS: Studies are needed to test this modified erythropoietin in people. The approach may be generally applicable to other therapeutic proteins, including but not limited to hematopoietic growth factors and coagulation factors.

ACKNOWLEDGMENTS:

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R01DK38841	Sytkowski, A.J.	Harvard Medical School

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Sytkowski AJ, Lunn ED, Davis KL, Feldman L; Siekman S, "Human Erythropoietin Dimers with Markedly Enhanced *in vivo* Activity," Proceedings of the National Academy of Sciences 1998;Feb 3;95(3):1184-8.

IV. TITLE: Potential Safety Issue With Widely Studied Chelator, Deferi prone (L1)

BACKGROUND: Patients with beta-thalassemia (Cooley's Anemia) continue to

suffer from the sequelae of transfusion-induced iron overload due to the inadequacies of current iron-chelation therapy. Compliance with the use of subcutaneous desferioxamine continues to be a major problem, despite convincing evidence that it markedly reduces morbidity and prolongs life. The full potential of iron-chelation therapy will not be realized until an orally-effective drug is available.

One widely studied oral candidate, deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one, L1), until recently has been considered by many to represent the most likely to be selected for clinical use.

RECENT FINDINGS: A long-term study in thalassemia patients at the Toronto Hospital for Sick Children identified a potential problem with the iron-chelator deferiprone. Of 14 patients given the drug, 5 developed progressive hepatic fibrosis. This liver complication was found in none of the patients who received deferoxamine. The authors raised questions about the safety and efficacy of long-term deferiprone therapy. However, an accompanying editorial urged further analysis, since iron overload itself causes hepatic fibrosis. The editorial also raised the possibility of sampling errors in the study and pointed out differences between deferiprone-treated patients in whom the fibrosis worsened and those in whom it did not.

SIGNIFICANCE: These results indicate that deferiprone may be associated with worsening of hepatic fibrosis, even in patients whose hepatic iron concentrations have stabilized or declined.

FUTURE DIRECTIONS: Prospective clinical trials are needed to evaluate the possibility of irreversible hepatic damage from L1. Improvements in the administration of iron chelators may prove useful, as in a study now being supported by the NIDDK involving a combination of two oral iron chelators, deferiprone and HBED, in a small number of patients with complete metabolic studies. Based on preliminary patient studies, there appears to be synergistic iron excretion when these chelators are used in combination. The search for additional new chelators needs to continue.

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<u>Grant or Contract #</u>	<u>Principal Investigator</u>	<u>Institution</u>
R01DK49108	Bergeron, R.J.	University of Florida

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Olivieri N, Brittenham G, McLaren C, Templeton D, Cameron R, McClelland R, Burt A, Fleming K, "Long-Term Safety and Effectiveness of Iron-Chelation Therapy with Deferiprone for Thalassemia Major," New England Journal of Medicine 1998;339(7):417-23.

V. TITLE: Identification of a Novel Cofactor, Friend of GATA-1 (FOG), Regulating Erythroid Development

BACKGROUND: GATA transcription factors have emerged as central regulators of diverse developmental processes in both vertebrate and invertebrate species. A hematopoietic-restricted transcription factor, GATA-1, is expressed at high levels in hematopoietic cells. Fundamental to the understanding of how GATA-1 functions is the elucidation of the mechanisms by which it alters transcription of genetic information.

RECENT FINDINGS: NIDDK-funded researchers have identified a novel, multitype zinc-finger protein, FOG, which now has been demonstrated to be required *in vivo* as a GATA-1 cofactor in erythroid cells. FOG also has been established as a pivotal, GATA-1-independent factor in the earliest stages of megakaryocyte development.

SIGNIFICANCE: It is apparent that the activity of GATA transcription factors is modulated by interactions with FOG, and possibly with certain other related proteins. Loss of FOG leads to the specific ablation of the megakaryocytic lineage, pointing to an absolute requirement for FOG during early platelet development. These findings have important implications for the role of FOG and other FOG-like proteins in regulating transcription and development. FOG and GATA-1 have been established as components of an essential protein complex in erythroid cells.

FUTURE DIRECTIONS: The possibility exists that the FOG/GATA-1 complex may contain additional proteins, which need to be identified and characterized. FOG also may act as a cofactor for other hematopoietic GATA factors. Defining how FOG functions in early megakaryocytopoiesis is likely to reveal novel mechanisms by which FOG and related proteins regulate transcription and development.

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P50 DK49216 Orkin, S.A. Boston Children's Hospital

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Tsang P, Fujiwara Y, Hom D, Orkin S, "Failure of Megakaryopoiesis and Arrested Erythropoiesis in Mice Lacking the Gata-1 Transcriptional Cofactor FOG," Genes and Development 1998;12(8):1176-88.

UROLOGY PROGRAM

VI. TITLE: The First Oral Drug is Approved for Male Erectile Dysfunction

BACKGROUND: Erectile dysfunction, also called impotence, affects an estimated 30 million men, including up to 70 percent of men with diabetes. The development of non-surgical treatments for erectile dysfunction has accelerated rapidly since NIDDK's 1992 Consensus Development Conference on erectile dysfunction. The conference established a clinical definition of erectile dysfunction and increased public awareness about the extent of the problem. Treatments have become sequentially less invasive, moving from prostheses to penile injections to drugs inserted into the penis. However, there was no FDA-approved oral treatment. Earlier NIDDK-funded studies had shown that erection occurs when smooth muscles in the corpora cavernosa relax following a chemical reaction in which nitric oxide from cavernous nerves triggers guanylate cyclase to form cyclic guanosine monophosphate (cGMP). The recently approved oral drug sildenafil inhibits cGMP-specific phosphodiesterase type 5, the isozyme that metabolizes cGMP in the corpora cavernosa.

RECENT FINDINGS: Sildenafil's inhibition of cGMP-specific phosphodiesterase type 5 would be expected to restore the natural erectile response to sexual stimulation, but not without such stimulation. Recent results from two short-term, double-blind clinical efficacy studies in men with erectile impotence demonstrated that 69 percent of all attempted sexual intercourse was successful in men receiving sildenafil, compared to only 22 percent in men taking a placebo. Men in the study had erectile dysfunction of organic, psychogenic or mixed causes, including diabetes, hypertension, and ischemic heart disease, and following radical prostatectomy. Sildenafil increased erectile function but did not change sexual desire.

SIGNIFICANCE: This is the first reported double-blind, clinical study to demonstrate the effectiveness of an oral drug for erectile dysfunction. Sildenafil opens a new era for the effective treatment of male erectile dysfunction and for research.

FUTURE DIRECTIONS: Sildenafil is an important tool for treating impotence, but not all men respond to the drug. Both basic and clinical research can increase both the effectiveness and number of available treatments, and identify treatments that can be targeted at specific causes of erectile dysfunction. Understanding how cGMP relaxes the cavernosal smooth muscle and how diseases such as diabetes cause erectile dysfunction will help accomplish these

goals. In addition, knowing how sildenafil and other oral agents in development work in men with different causes of impotence will contribute to our ability to rationally choose treatments for individual patients.

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R29DK45087	Azadzo, K.M.	Boston University

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Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. "Oral Sildenafil in the Treatment of Erectile Dysfunction," New England Journal of Medicine 1998;338:1397-1404.

VII. TITLE: Molecular Evidence of Stem Cell Compartments in the Prostate

BACKGROUND: Understanding cell growth in the prostate is important to help explain the development of benign prostatic hyperplasia (BPH). In several types of rapidly renewing tissues, stem cells, transiently proliferating cells, and mature terminally differentiated cells occupy discrete locations and often form stratified layers. The prostatic epithelium has two major compartments, basal and secretory. Most epithelial or secretory cells in the adult prostate are androgen-dependent. Although the cell layers of the adult prostate renew more slowly than layers in other organs, a similar stem-cell-driven hierarchical arrangement has been postulated. However, there has been little molecular evidence of a transiently proliferating cell compartment in the prostate, and the location and nature of the stem cells still is unknown.

RECENT FINDINGS: NIDDK-funded researchers have identified and characterized cells in the human prostate capable of entering the proliferating phase of the cell cycle. The investigators used differential expression of the cyclin-dependent kinase inhibitor p27Kip1 to demonstrate distinct cell populations in the normal prostate. A cell layer that was consistently p27Kip1-negative was identified between the basal and secretory layers. Cells in this middle layer were accentuated in the periurethral (BPH forming) layer and in

tissue subjected to androgen blockade. These cells are likely to be the population that undergoes abnormal growth in BPH.

SIGNIFICANCE: The identification of this transiently proliferating cell compartment demonstrates for the first time a method to identify the existence, location and interrelationship between the three significant cell layers (stem cell; transiently proliferating; and mature, terminally differentiated) in the adult prostate.

FUTURE DIRECTIONS: Identification of specific layers involved in cell development will allow gene therapy to be targeted to those layers to influence the growth of adult prostate tissues.

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<u>Grant or Contract #</u>	<u>Principal Investigator</u>	<u>Institution</u>
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P01DK19300	Coffey, D.S.	Johns Hopkins Univ

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DeMarzo AM, Meeker AK, Epstein JI, Coffey DS, "Prostate Stem Cell Compartments. Expression of the Cell Cycle Inhibitor p27^{kip1} in Normal, Hyperplastic, and Neoplastic Cells," American Journal of Pathology 1998;153 (3):911-9.

VIII. TITLE: Insights into Genetic Factors Involved in Kidney Stones

BACKGROUND: Kidney stones affect many adults. The most prevalent type of stone is made of calcium oxalate. Although stone disease has been reported since the age of Hippocrates, and the chemistry of kidney stones has been extensively studied, little is known about the genetic mechanisms predisposing to stone formation. Oxalic acid (oxalate) is made in the liver, where it has no known function, and excreted by the kidneys. Excessive production can cause a number of problems, including kidney stones, nephrocalcinosis and systemic oxalosis. The latter condition occurs in people with primary hyperoxaluria, an inherited disorder in which hepatic enzyme deficiencies promote excessive oxalate production. Despite the clinical significance of calcium oxalate, many features of the biosynthetic pathway have not been determined, and there are no

therapies to inhibit oxalate production by the liver.

RECENT FINDINGS: Investigators funded by NIDDK have now defined the terminal steps of the oxalate synthetic pathway and determined that oxalate synthesis is modulated by the metabolic state of the liver and the resultant changes in NAD:NADH ratios, and lactate and alanine levels.

- Glycolate is the most important source of glyoxylate, which is then catalyzed to oxalate by the enzyme glycolate oxidase (GO). The major enzymes associated with the terminal steps of these conversions are in peroxisomes. NADH was shown to be a potent inhibitor of oxalate production. These findings have led to development of drugs that inhibit specific enzymes such as GO, which may be useful in decreasing oxalate synthesis and urinary oxalate excretion.
- A phase I clinical study to evaluate the safety and pharmacokinetics of one such enzyme inhibitor, (L)-2-oxothizolidine-4-carboxylate (OTZ), demonstrated decreased urinary oxalate excretion in healthy men.
- Another investigator has described a gene and gene product responsible for an inherited form of hypercalciuric nephrolithiasis (X-linked hypercalciuric nephrolithiasis). Using positional cloning, the gene, CLCN5, was identified as a member of the CLC family of voltage-gated chloride channels. Abnormalities in thick ascending limb function might explain defective renal tubular calcium reabsorption, and the clinical findings of nephrolithiasis and nephrocalcinosis evident in persons with this disorder.

SIGNIFICANCE: These findings provide significant insights into the genetic basis for a predisposition to develop calcium oxalate stones. They also present preliminary data on a novel enzymatic inhibitor that may reduce oxalate synthesis in the liver. From these findings, additional treatments might be developed based on mechanisms to alter cellular NADH levels. These strategies provide a therapeutic option not only for those with the rare and devastating primary hyperoxaluria, but also possibly for people with idiopathic calcium oxalate stones.

FUTURE DIRECTIONS: Basic animal studies designed to characterize the oxalate metabolic pathway in the liver, as well as identification of specific gene products will enable the development of more targeted therapies for all forms of calcium oxalate stone disease.

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Poore RE, Hurst CH, Assimos DG, Holmes RP, "Pathways of Hepatic Oxalate Synthesis and their Regulation," American Journal of Physiology 1997;272 (Cell Physiology 41):C289-94.

Holmes RP, Assimos DG, Leaf CD, Whalen JJ, "The Effects of (L)-2-oxothiazolidine-4-carboxylate on Urinary Oxalate Excretion," Journal of Urology 1997;158:34-7.

Hoopes RR, Hueber PA, Reid RJ, Braiden GL, Goodyer PR, Melnyk AR, Midgley JP, Moel DI, Neu AM, VanWhy SK, Scheinman SJ, "CLCN5 Chloride-Channel Mutations in Six New North American Families with X-Linked Nephrolithiasis," Kidney International 1998;54:698-705.

Scheinman SJ, "X-Linked Hypercalciuric Nephrolithiasis: Clinical Syndromes and Chloride Channel Mutations," Kidney International 1998;53:3-17.

IX. TITLE: Recurrent Urinary Infections in Women: Contributing Molecular and Bacteriological Factors

BACKGROUND: Women with recurrent urinary tract infections (UTIs) often have persistent vaginal colonization with *E. coli*. Evidence suggests that alterations of the normal, *Lactobacillus*-dominant vaginal flora may predispose to colonization with *E. coli*. *In vitro* studies have demonstrated that strains of hydrogen peroxide-producing *lactobacilli* inhibit the growth of *E. coli*. Similarly, it has been previously demonstrated that women with recurrent UTIs are significantly more likely to be nonsecretors of blood group antigens than are women without such a history. The vaginal epithelial cells from these nonsecretors (women with recurrent UTIs) enhance adherence of uropathogenic *E. coli* isolates more often than the secretors.

RECENT FINDINGS: Recently published clinical data from women with and without recurrent UTIs demonstrates that women without recurrent UTIs were significantly more likely to have vaginal colonization with these H₂O₂-forming

lactobacilli, and that spermicides were associated with increased vaginal *E. coli* colonization and absence of the H₂O₂-forming *lactobacilli*. The same investigators studied women with recurrent *E. coli* UTIs, and separated them into secretors and non-secretors of blood group antigens. They have now demonstrated two specific glycosphingolipids (GSLs) of the vaginal epithelial cells (VEC) from these women. One of these, sialosyl galactosyl globoside (SGG), was expressed in human kidney tissue. SSG selectively binds certain uropathogenic *E. coli*. Researchers concluded that SGG expression plays an important role in the pathogenesis of UTIs.

SIGNIFICANCE: These studies suggest that the total microbial ecology of the vagina influences *E. coli* colonization, and that depletion of H₂O₂-forming *lactobacilli* could reduce the risk of recurrent UTI. Avoiding factors such as spermicides that decrease the concentration of H₂O₂-forming *lactobacilli* could also effectively reduce UTI recurrence. In addition, novel strategies to prevent UTIs may be developed using carbohydrate-based compounds that competitively inhibit bacterial attachment.

FUTURE DIRECTIONS: The mechanisms whereby H₂O₂-forming *lactobacilli* prevent colonization of the vagina with uropathogens needs to be further examined and characterized. Further studies are needed to define the expression of SGG in epithelial tissues throughout the urogenital tract. Data from these bladder tissue studies will increase our knowledge of bladder glycobiology.

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Gupta K, Stapelton AE, Hooton TM, Roberts PL, Fennell CL, Stamm WE, "Inverse Association of H₂O₂-Producing *Lactobacilli* and Vaginal *Escherichia coli* Colonization in Women with Recurrent Urinary Tract Infections," Journal of Infectious Diseases 1998;178:446-50.

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Tissues and is a Preferred Binding Receptor *in vitro* for Uropathogenic *Escherichia coli* Expressing *pap*-Encoded Adhesins," Infection and Immunity 1998;66:3856-61.

X. TITLE: Novel Use of Knockout Mice and Genetic Engineering in Bladder Injury and Repair

BACKGROUND: The factors that influence bladder growth, development, and response to injury are not well delineated. Response to bladder injury involves cellular proliferation, migration, and differentiation; removal of damaged tissue; and production of extracellular matrix. Many of these actions also occur during bladder development and growth, and all may be controlled by growth factors. Studies have shown that epidermal growth factor (EGF) is expressed in bladder urothelium, and this family of peptides may mediate urothelial growth. A related substance in skin, keratinocyte growth factor (KGF), is induced after an injury.

RECENT FINDINGS: The role of growth factors in bladder pathophysiology has been an area of intense investigation. Whole bladders transplanted from EGF knockout mice into adult nude rats have normal bladder regeneration after injury. Such findings suggest the EGF pathway is not necessary for bladder regeneration. On the other hand, KGF has a direct effect on urothelial proliferation. In addition, both KGF and transforming growth factor " (TGF") are involved in bladder wound healing and have direct effects on urothelial proliferation. NIDDK-funded researchers have also reported that bladder urothelial cells can be transfected with various genes, and then transferred to a biodegradable polymer scaffold from which a neo-genetically engineered organ develops.

SIGNIFICANCE: These findings provide significant new pathways for the development of novel, organ specific therapies for bladder reconstruction after resection for disease. For example, it is possible that a bladder could be constructed *de novo* to replace a diabetic neuropathic bladder. A tissue-engineering approach might allow transfected cells to be confined and localized, and ultimately removed, if there is evidence of mutagenesis or immunogenicity. Tissue could also be engineered to express specific growth factors which have been demonstrated to be effective in urothelial growth, differentiation and repair.

FUTURE DIRECTIONS: These studies are opening an entirely new concept--engineering urothelial cells--to treat bladder diseases and disorders.

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DiSandro MJ, Baskin LS, Li YW, Werb Z, Cunha GR, "Development and Regenerative Ability of Bladder in the Transgenic Epidermal Growth Factor Receptor Gene Knockout Mouse," Journal of Urology 1997;158:1058-65.

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XI. TITLE: Risk Factors for Urinary Tract Infections (UTI)

BACKGROUND: UTIs are common, especially in women. Controversy over the association between UTIs, sexual activity, and spermicides remains.

RECENT FINDINGS: A large study in HMO patients identified sexually active young women with acute UTIs and compared them to population-based control patients from the same HMO. Sexual activity and contraceptive practices were identified during interviews. Younger age, intercourse frequency, prior UTI, and frequency of use of spermicide-coated condoms were independent predictors of *staphylococcus saprophyticus* infection, the second most common cause of UTI in young women. These investigators previously demonstrated that spermicide-coated condoms were associated with 41 percent of UTIs from *E. coli*, the leading cause of UTIs in young women.

SIGNIFICANCE: This study identifies correlates of UTI in a population of young women receiving care in an HMO setting and identifies factors that may be remediable.

FUTURE DIRECTIONS: Changing behaviors regarding use of spermicides may

decrease the incidence of UTIs in susceptible women.

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KIDNEY PROGRAMS

Renal Epidemiology Program

XII. TITLE: Barriers to Renal Transplantation

BACKGROUND: There is a serious shortage of organs for transplantation, and questions about equitable distribution have arisen.

RECENT FINDINGS: Two NIDDK-funded studies assessed the odds of transplantation for women and African Americans compared to men and Caucasians. The researchers found that female gender and African American race are barriers to transplantation, not only in trying to get on a waiting list but also in obtaining a transplant once on a list. The researchers suggest that these hurdles are unrelated to the separate issue of matching donor organs to recipients.

Because women had more difficulty getting on waiting lists and receiving transplants once there, they were 25 percent less likely to be transplanted compared to men, according to one study. A second study defined four stages to transplantation: 1) being medically suitable and willing to consider transplantation; 2) being definitely interested in transplantation; 3) completing the pre-transplant work-up; and 4) being on a waiting list and receiving a transplant. At each stage, women were less likely than men to complete the stage, and African Americans were less likely to complete stages two through four.

SIGNIFICANCE AND FUTURE DIRECTIONS: These studies will help increase awareness about access to kidney transplantation and support efforts to ensure equitable access regardless of gender or race.

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RO1DK49531	Bloembergen, W.E.	University of Michigan

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Alexander GC, Seghal AR, "Barriers to Cadaveric Renal Transplantation Among

Blacks, Women, and the Poor," Journal of the American Medical Association 1998;280:1148-52.

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XIII. TITLE: Renal Vascular Disease in Different Ethnic Groups

BACKGROUND: Previous studies reported that renal vascular disease was uncommon in African Americans, suggesting a low need to screen this population for the disease.

RECENT FINDINGS: Using duplex sonography, researchers examined the kidneys of people in the Forsyth County group of the Cardiovascular Health Study. Surprisingly, the authors found significant renal vascular disease in 11.8 percent of hypertensive African Americans, a rate comparable to that expected in the general population. After surgery for the vascular disease, kidney function improved in many of these patients, especially among those with poorer renal function.

SIGNIFICANCE AND FUTURE DIRECTIONS: This study suggests a need to screen for renal vascular disease in African Americans, especially those who have high blood pressure. Further efforts must be made to study outcomes in different populations after screening for renal vascular disease and instituting various therapeutic plans.

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Hansen KJ, Deitch JS, Dean RH, "Renovascular Disease in Blacks: Prevalence and Result of Operative Management," American Journal of the Medical Sciences 1998;315:337-42.

XIV. TITLE: High Mortality Among Dialysis Patients After Heart Attack

BACKGROUND: Cardiovascular disease is the most common cause of death in dialysis patients in the United States. Outcomes of dialysis patients after myocardial infarction have not been studied before.

RECENT FINDINGS: Patients who were hospitalized between 1977 and 1995 for a first heart attack were identified by the investigators using NIDDK's U.S. Renal Data System. Overall mortality after a heart attack was 59.3 percent at 1 year and 89.9 percent after 5 years. More recent enrollees had higher overall death rates from all causes, probably because they had a greater number of other health problems at the start of ESRD therapy.

SIGNIFICANCE: These studies have helped to identify poor outcomes in the ESRD population that may be remediable.

FUTURE DIRECTIONS: Efforts to improve cardiovascular outcomes in end-stage renal disease (ESRD) patients, regardless of other health problems, should be undertaken. Investigating outcomes for ESRD patients with other illnesses may lead to improved outcomes for all patients.

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Renal Transplantation Program

XV. TITLE: Tolerance to Non-Inherited Maternal HLA Antigens and Survival of Renal Transplants

BACKGROUND: Kidney transplantation is the most important and physiologic renal replacement therapy. However, only a small proportion of patients with ESRD are able to receive a kidney transplant, and many transplanted kidneys do not survive. The 90-day graft survival is 91 percent, and 1 and 2-year graft survival is 87 and 79 percent, respectively, according to NIDDK's U.S. Renal Data System. These relatively short-term survival probabilities have shown remarkable improvement over the past decade, but long-term survival has not. In 1986, 10-year graft survival was only 33 percent.

Most failed transplants have been attributed to chronic rejection. The T cells of the recipient seem to play the critical role in the rejection process by recognizing histocompatibility antigens from the donor. Although immunosuppressive drugs help in suppressing the T cell response to these foreign antigens, naturally induced tolerance of the recipient to the foreign antigens should help with improved graft survival. During pregnancy, exposure of the fetus to maternal cells and antigens induces tolerance, in some cases, to non-inherited maternal histocompatibility antigens. Theoretically, therefore, transplants between siblings would show improved survival if the differences in HLA antigens were limited to the non-inherited maternal histocompatibility antigens.

RECENT FINDINGS: In this NIDDK-funded study, investigators analyzed data on graft survival in 205 people who received a first kidney transplant from a living-related sibling mismatched for one HLA haplotype. The appropriate immuno-suppressive regimen was used in all patients. The results show that when a patient receives a kidney from a sibling with maternal HLA antigens he/she did not inherit, the long-term graft survival is improved. The 10-year graft survival (where such data is available) in such transplants was 77 percent, which was not significantly different from the 10-year graft survival between HLA-identical siblings during the identical period.

SIGNIFICANCE: This study should help in selecting sibling pairs for transplantation. To enhance graft survival, transplants should be encouraged between siblings who differ only at the non-inherited maternal HLA antigen locus whenever possible.

FUTURE DIRECTIONS: Other databases should be analyzed to confirm the observation made in this study, as well as to identify other HLA mismatches that are not particularly disadvantageous. Conceivably, such observations will extend similar "beneficial mismatches" in cadaveric renal transplantation.

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Chronic Renal Diseases and Pediatric Nephrology Program

XVI. TITLE: Pathogenesis of Polycystic Kidney Disease

BACKGROUND: Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder affecting an estimated 1 in 400 to 1 in 1000 people. ADPKD consists of at least three genetically distinct disorders characterized by bilateral renal cysts and progressive enlargement of the kidneys. About 50 percent of disease-gene carriers progress to kidney failure. Mutations in the PKD1 gene on chromosome 16 account for about 95 percent of clinically recognized cases, while the remainder of cases are largely from mutations in PKD2 on chromosome 4, the second gene for ADPKD. Autosomal recessive polycystic kidney disease (ARPKD) constitutes a very serious renal disease in children, often leading to ESRD in childhood. The ARPKD gene has not been cloned.

Dramatic progress has been made over the past 2 to 3 years, regarding the etiology, pathogenesis and clinical course of PKD. Research continues to focus on the genetics, mechanisms of cyst formation and growth, identification of risk factors for susceptibility to ESRD, and the development of animal models.

RECENT FINDINGS: Somatic inactivation of PKD2 results in PKD. Germline mutations in PKD2 cause ADPKD. A mutant exon 1, in tandem with the wild-type exon 1, was introduced at the mouse PKD2 locus. This unstable allele undergoes somatic inactivation by intragenic homologous recombination to produce a true null allele. Mice heterozygous and homozygous for this mutation develop PKD and liver lesions indistinguishable from the human phenotype. These studies establish that somatic loss of PKD2 expression is both necessary and sufficient for renal cyst formation in ADPKD, suggesting that PKD2 occurs

by a cellular recessive mechanism.

- Gene conversion, identified as a mechanism of mutation for a number of human genes, is a likely cause of mutation in PKD1. About 70 percent of the gene responsible for ADPKD is replicated in several highly homologous copies located nearby on chromosome 16. A novel strategy for finding mutations in the duplicated region of ADPKD was recently described. The technique uses one gene-specific primer from PKD1 as an anchor, in combination with a primer from the duplicated portion to amplify ADPKD1-specific templates. Using changes in restriction digest patterns, investigators showed that sequence substitutions were present in unrelated patients with a nearly identical cluster of base pair substitutions involving exon 23. This was also shown in a rodent-human somatic cell hybrid that contains only PKD1 homologues. These changes were also detected in total DNA from several affected and unaffected individuals who did not harbor this mutation in their PKD1 gene copy. This is the first example of gene conversion in PKD1. These findings highlight the importance of using reagents proven to be locus-specific in identifying and defining *PKD1* mutations that may reflect sequence differences also present in homologous loci.
- Researchers recently reported identifying PKDL, a novel PKD2-like gene whose murine homologue is detected in mice with kidney and retinal defects. Polycystin-1 and B2 are the products of PKD1 and PKD2 genes that are mutated in most cases of ADPKD. It has been suggested that polycystin-2 may function as a subunit of an ion channel regulated by polycystin-1. Investigators report the identification of the homologous human PKDL gene, which encodes a new member of the polycystin protein family called polycystin-L. The full-length transcript of PKDL is expressed at high levels in fetal tissues, including kidney and liver, and down-regulated in adult tissues. PKDL may be an excellent candidate for as-yet-unmapped cystic diseases in people and animals.
- Previous data indicated that renal cyst development in PKD1 is likely to require somatic inactivation of the normal allele coupled to a germ-line PKD1 mutation. The same group of investigators used unique reagents and reported that intragenic somatic mutations are common in liver cysts. All pathogenic mutations altered the previously normal copy of the gene. These new data extend the “two-hit” model of cyst development to include a second focal manifestation of the disease.
- PKD1 mutations on chromosome 16p13.3 account for 85 to 95 percent of all PKD cases. The gene has an unusual bipartite structure, with 70

percent of its 5' end duplicated in other places on chromosome 16. Another important feature of the human PKD1 gene is a cluster of two long polypyrimidine tracts in adjacent introns in the center of the gene. Polypyrimidine tracts can form triple helices with diverse biological effects, affecting gene expression and enhancing mutagenesis in a variety of ways. To test the hypothesis that the differences in the genomic structure of the murine and human PKD1 genes might be responsible for the different rates of mutation observed, these polypyrimidine tracts were sought in the mouse. The hybridization and sequence data from these studies clearly show that the mPKD1 (murine PKD1) does not contain the polypyrimidine tracts found in the human gene. A previous study also reported that the mouse genome, in contrast to that of humans, has only one copy of PKD1. An important question is whether the PKD1 homologues or the pyrimidine tracts play a role in the pathogenesis of human ADPKD. Differences in the genomic structure of murine and human PKD1 genes may be responsible for their different mutation rates. The PKD1 homologues in humans are likely to account for at least some of the increased mutability of human PKD1, since having more than one copy of the gene can promote mutation. Polypyrimidine tracts in human PKD1 may significantly contribute to the gene's overall instability. Additional studies are attempting to further characterize the role of these structures, including their presence and/or absence in regulating the gene's activity and mutability in humans.

SIGNIFICANCE: New opportunities in genetics, as a result of advances in cellular and molecular biology, and selected clinical and animal studies should help elucidate the etiology and pathogenesis of PKD and the related cellular and molecular mechanisms that determine kidney structure and function in general.

FUTURE DIRECTIONS: Work should focus on using pathogenic principles to design and test specific therapies in animal models, with the prospect of ultimate relevance in human disease.

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XVII. TITLE: Renal Disease Progression

BACKGROUND: Renal fibrosis resulting from progressive extracellular matrix accumulation is a main cause of chronic renal disease and the putative final common pathway of renal injury in animals and humans. Fibrosis of the glomerulus and the tubulointerstitium impairs kidney function and ultimately leads to organ failure. Mechanical factors and other mediators, including cytokines, growth factors, and eicosanoids derived from circulating or glomerular cells, have been implicated in initiating or maintaining sclerosis. Considerable evidence has accumulated showing that overproduction of the cytokine transforming growth factor β (TGF- β) plays a key role in the development of renal fibrosis. Diverse actions of TGF- β on collagen turnover may be either immediate or mediated by synthesis of regulatory molecules.

A possible explanation for the kidney's particular susceptibility to fibrosis in response to injury may be the recent discovery of biologically complex interactions between the renin-angiotensin-aldosterone system (RAAS) and certain cytokines and other biologic systems. RAAS has broad impact on progression of sclerotic vascular diseases, by both hemodynamic and non-hemodynamic mechanisms.

RECENT FINDINGS: New information about the interaction of RAAS with cytokines and other factors is emerging regarding powerful effector molecules that preserve systemic and tissue homeostasis. Of particular relevance is the role and interaction of RAAS and TGF- β in the kidney and the molecular mechanisms involved.

- Angiotensin II (Ang II) infusion strongly stimulates the production of TGF- β in the kidney, and Ang II-blockade reduces TGF- β over-expression in kidney and heart. TGF- β has been shown to be a powerful fibrogenic cytokine which acts to simultaneously stimulate the synthesis of extracellular matrix, to inhibit the actions of proteases that degrade matrix, and to increase the expression of cell surface integrins that interact with matrix components.
- In addition, the role of the plasmin protease system in turnover of the mesangial matrix was recently shown. Plasmin has been long recognized as a fibrinolytic enzyme, important in dissolving clots after injury. This molecule, like Ang II and TGF- β , is rapidly increased at the site of a wound, where it stabilizes the fibrin clot, serving as scaffolding for platelet aggregation and temporary matrix production. A relatively new role for this system has been characterized as highly relevant to fibrotic renal diseases. In addition to degrading fibrin, plasmin acts on a

wide range of extracellular matrix proteins, cleaving some procollagenases to produce active molecules that degrade collagens.

- Aldosterone overproduction has been linked to hypertension and glomerulo-sclerosis. These data suggest that aldosterone may have fibrogenic effects, independent of Ang II.
- Finally, an ex-vivo gene transfer system to deliver cytokines into the kidney and circulation was constructed, using genetically modified renal tubular epithelial cells (TEC) infected with recombinant retroviruses expressing macrophage growth factors. These TECs are capable of secreting stable, sustained amounts of cytokines for long periods *in vitro* and *in vivo*. Implanting these TEC-secreting macrophage growth factors under the kidney capsule initiates severe local renal injury in a murine model. This system offers a novel and powerful approach to probe for the impact of sustained cytokine expression in progression of kidney disease.

SIGNIFICANCE: Fibrotic diseases are characterized by the accumulation of extracellular matrix, or scar tissue. A better understanding of the intricate mechanisms leading to matrix accumulation and scarring in the glomeruli and tubulointerstitium should help identify strategies to ultimately control progression of chronic renal diseases.

FUTURE DIRECTIONS: Studies are needed to develop experimental models of progressive renal disease to further characterize the biological events involved in glomerular and tubulointerstitial extracellular matrix accumulation leading to renal scarring; to define biological interactions between the RAAS and the fibrinolytic system, and the impact of common genetic polymorphisms in angiotensin converting enzyme (ACE) and plasmin on the progression of renal disease; to identify and test strategies to influence expression of factors whose actions/interactions have a central role in mediating renal fibrosis.

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R01DK33665	Abboud, H.E.	Univ of Tex-SA

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XVIII. TITLE: The Immune Basis of Lupus Nephritis

BACKGROUND: Systemic Lupus Erythematosus (SLE) is a chronic disease with a broad spectrum of life-threatening complications, affecting mostly women of childbearing ages. Most people with SLE have some degree of renal disease, and many have kidney failure. The etiology remains unknown. However a consistent manifestation of immune dysfunction is the hyperactivity of the immune system, both *in vitro* and *in vivo*. Family, twin and ethnic studies suggest a genetic component in the pathogenesis of SLE. Patients with specific HLA genes and genes that encode for antigen-presenting proteins are associated with differential susceptibility to developing autoimmune disease.

RECENT FINDINGS: Autoantibodies (auto-Ab) target a diverse group of tissue antigens in human and experimental autoimmune kidney disease. Subpopulations of auto-Ab produce different disease profiles with distinguishable histologic and clinical patterns. The initial event and the site are determined by the location of the autoantigens and the direct interactions with the auto-Ab.

- A unique subset of anti-DNA-Ab that entered cells and localized within the nuclei *in vivo* was identified and studied. Following the administration of anti-DNA Ab fragments into normal mice, these immunoglobulins were detected within the cell nuclei of various organs. In the kidney, this was associated with hypercellularity and protein in the urine. The abnormalities appeared to be mediated by a direct effect of the intranuclear antibodies within the glomerular cells. Nuclear localization depended on the unique antigenic binding properties of a subset of lupus auto-Ab. Cellular entry was initiated by the binding of the nuclear-localizing anti-DNA Ab to the myosin-1 cell surface receptor. These data provide insight into the behavior of Ab within the cytoplasm en route to the nucleus. Evidence from other laboratories suggests that myosin 1 forms a DNase-1 and calmodulin complex, potentially interfering with intracellular enzyme activity.
- Another NIDDK-funded laboratory indicates that lupus-like auto-Ab are readily generated in subjects of normal genetic background by random recombination absent mutations. Auto-Ab may contribute to disease if normal immune system regulation is disturbed. In a transgenic murine model, experimental findings supported the hypothesis that autoreactive B cells capable of producing pathogenic immunoglobulins are generated *in vivo* in normal individuals and may contribute to disease if normal immunoregulation is disturbed.

- In the MRL-*Fas lpr* murine model of human SLE, renal disease progresses rapidly, leading to predictable 50 percent mortality at 6 months of age, and is regulated by a single gene mutation. Renal injury involves glomerular, perivascular, and interstitial pathology, and is mediated by macrophages, T cells, and cytokines. Nephropathy is determined by the MRL+/+ background genes, interacting with a single gene mutation in *Fas*. The MRL genes are responsible for autoimmune kidney disease and the *Fas lpr* (*Fas* deficiency) mutation converts a latent, mild nephritis into a rapid, fulminant disease. Colony stimulating factor (CSF)-1 and tumor necrosis factor (TNF)- α are cytokines implicated in developing renal injury. CSF-1 is responsible for eliciting autoimmune kidney destruction and is expressed in the kidneys before disease-development, increasing with advancing kidney damage. Gene transfer of CSF-1 into MRL-*Fas lpr* kidneys elicits renal injury. Gene transfer of TNF- α fails to elicit autoimmune kidney injury; dual gene transfer of TNF- α and CSF-1 amplifies the renal damage produced by CSF-1 alone. Similarly, injecting TNF- α fails to incite renal injury, but accelerates pathology in mice with nephritis.
- T cells are required for autoimmune kidney disease in this model, since during renal injury these cells infiltrate the glomeruli, interstitium, and perivascular compartments, secreting interferon (IFN)- γ . Blockade of IFN- γ signaling prevents glomerulonephritis and prolongs survival. An IFN- γ R-deficient strain was constructed to ascertain whether IFN- γ is responsible for cytokine-, macrophage-, and T-cell-dependent kidney damage, and whether IFN- γ is responsible for programmed (apoptotic) cell death, directly or indirectly. IFN- γ was required for cytokine production and renal parenchymal cell apoptosis. The MRL-*Fas lpr* mice lacking the IFN- γ R are protected from fatal lupus nephritis.
- Using a retroviral gene transfer strategy, researchers established an association between renal expression of RANTES (a β -chemokine, chemoattractant for macrophages and T cells) and renal inflammatory injury. Tubular epithelial cells, genetically modified to secrete RANTES and infused under the renal capsule, incited interstitial nephritis in MRL-*Fas lpr* mice. In addition, delivery of RANTES and CSF-1 into the kidney of this murine model caused an additive increase in pathology.

SIGNIFICANCE: The observations identifying a previously unrecognized means of cellular entry and transit of proteins into and within cellular compartments should lead to a better understanding of the events underlying renal injury in patients with SLE. It should be possible to take advantage of this information to design carrier proteins for transit and targeting of other molecules to the

nucleus, interfering with injury. This approach should lead to potential fruitful therapeutic applications.

FUTURE DIRECTIONS: Because cytokines influence immunity and inflammation, interventions that modify cytokine pathways or destroy cytokine receptor-bearing cells may be effective for modulating harmful inflammatory responses. Further studies with murine antibodies or antagonist molecules that block nephro-pathogenic substances would be highly desirable. Strategies that interfere with the expression of such deleterious molecules and their targets could lead to promising therapeutic interventions and prevention of autoimmune renal injury.

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XIX. TITLE: Pathogenesis of Glomerulonephritis/Tubulointerstitial Nephritis

BACKGROUND: Immune mechanisms are the predominant cause of most forms of glomerulonephritis (GN) and tubulointerstitial nephritis (TIN). Research on GN and TIN has benefited greatly because of advances in cellular and molecular biology and the ability to create and test genetic mutations in animal models. The new tools of molecular biology have been very useful to this field of research, and the emphasis on transgenic and knock-out models producing genetic mutations continues to enhance the study and testing of experimental therapies in preparation for future human research.

RECENT FINDINGS:

- Endogenous fibroblast growth factor-2 (FGF-2) is released from mesangial cells in experimental mesangioproliferative GN. Anti-FGF-2 therapy led to significant reductions in glomerular injury secondary to expression of α -smooth muscle actin, mesangial cell proliferation, matrix accumulation, and platelet influx. Conversely, injections of FGF-2 augmented glomerular inflammation. Studies of mechanisms underlying the amplification of cellular injury by FGF-2 showed that anti-FGF-2 therapy considerably reduced cell death after disease induction. These data suggest that release of constitutively expressed FGF-2 after immune-mediated cell injury contributes to glomerular cell damage and implicates FGF-2 as a novel mediator of toxicity.
- Many forms of human glomerular disease are autoimmune in nature,

characterized by glomerular deposits of immunoglobulin and complement. A significant protective effect of CD59 (cell membrane-bound complement regulatory protein on glomerular cells), which inhibits C5b-9 (membrane attack complex) assembly and insertion, was demonstrated in a new rat model of immune thrombotic microangiopathy. These data confirm that C5b-9 formation has a critical pathogenic role in the mediation of the disease. CD59 may be important in protecting the glomerular endothelium from other complement-mediated injuries.

- NIDDK-funded investigators have characterized the cellular events that occur in the glomeruli and tubulointerstitium in a complement-independent murine nephritis model, characterized by glomerular crescents, progressive glomerulosclerosis and tubulointerstitial fibrosis. Early crescent formation in this model appeared to be due to proliferation of intrinsic glomerular (parietal or visceral) cells and was associated with local platelet-derived growth factor (PDGF) receptor expression. Despite the local expression of osteopontin, a potent monocyte chemoattracting and adhesive factor, neither infiltrating macrophages nor T cells could be identified before Bowman's capsule ruptured.
- The role of osteopontin expression was investigated in a rat model of accelerated anti-GBM GN. Osteopontin was expressed with one of its ligands, CD44, in intrinsic renal cells, and its expression was associated with signs of progressive nephropathy. *De novo* osteopontin mRNA expression was evident in glomerular visceral and parietal epithelial cells; preceded the development of hypercellularity, focal and segmental lesions, and crescent formation; and correlated with macrophage infiltration. Up-regulation of osteopontin by tubular epithelial cells also preceded and correlated with interstitial macrophage and T-cell infiltration.
- Tubulointerstitial fibrosis is one of the most important histologic features predicting progression of kidney disease. Thrombospondin 1 (TSP1) is an extracellular matrix protein that activates latent TGF- β . NIDDK-funded researchers examined the expression of TSP1 in several animal models of GN-associated tubulointerstitial disease. TSP1 mRNA and protein were transiently increased in tubular cells, myofibroblasts and macrophages in areas of tubulointerstitial injury; its expression always preceded the development of fibrosis and correlated quantitatively and spatially with the development of interstitial fibrosis. TSP1 expression also predicted the severity of tubulointerstitial fibrosis better than the degree of macrophage or myofibroblast accumulation, or increased TGF- β 1 expression. These data are consistent with the possibility that TSP1

may be an endogenous activator of TGF- β .

- Cyclosporin A (CsA) nephropathy is associated with a marked increase in apoptosis of tubular and interstitial cells, mediated in part by Ang II and nitric oxide inhibition, suggesting a role for renal ischemia in this process. CsA-induced apoptosis correlated with interstitial fibrosis. The increase in apoptosis along with the increased production of growth factors such as TGF- β 1 and PDGF may act in concert to promote tubulointerstitial fibrosis. Accelerated apoptosis could account for the host's inability to effectively remodel tissue, thereby playing a role in the pathogenesis of fibrosis and chronic transplant rejection.

SIGNIFICANCE: Many advances in understanding disease mechanisms may lead to the development of new therapies for humans, including cytokine and growth factor antagonists and receptor blockers, and agents that suppress the effects of oxidants and proteases produced by both circulating and glomerular cells. Common to these areas is the enormous potential of modern technology to continue to provide new insights into mechanisms of glomerular disease and to generate new techniques and reagents for the study and treatment of disease in humans.

FUTURE DIRECTIONS: Studies using specific peptides to block TGF- β 1 activation by TSP1 and other mediators may prove important. Studies are needed to determine how the proposed pathway of ischemia and apoptosis can be interrupted to prevent the toxic effects of CsA and possibly other nephrotoxins. Research on renal inflammatory processes should focus on the development of transgenic models, structural protein chemistry, cloning biologic mediators, and establishing renal cell lines in culture. Special efforts should be focused on gene therapy and establishing a national consortium to treat patients with GN and TIN.

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Renal Cell Biology/physiology Program

XX. TITLE: Physiologic Roles of Sodium/Hydrogen Exchangers in Health and Disease

BACKGROUND: The proximal tubule reabsorbs more than 60 percent of the sodium chloride (NaCl) and water filtered by the glomerulus. Defining the molecular mechanisms of Na⁺ reabsorption in this nephron segment is therefore of great importance for understanding disease states resulting from abnormal renal NaCl homeostasis, such as hypertension and edematous disorders. In the proximal tubule, the most important apical membrane pathway for Na⁺ reabsorption is sodium/hydrogen (Na⁺/H⁺) exchange.

Na⁺/H⁺ exchangers mediate the electroneutral exchange of Na⁺ and H⁺ across

plasma membranes of all cells in the body. Inhibited by amiloride and its analogs, Na^+/H^+ exchangers participate in diverse cellular functions, including intracellular pH regulation, transepithelial ion transport, cell volume regulation and cellular responses to mitogens and growth factors. Molecular cloning studies by the laboratories of Shull and Donowitz identified five distinct isoforms of Na^+/H^+ exchangers expressed in mammalian tissues (NHE1-5). A major focus of research during recent years has been to identify the sites of expression, physiologic roles, and mechanisms of regulation of NHE isoforms along the nephron in general, and in the proximal tubule in particular.

RECENT FINDINGS: By using isoform-specific antibodies, considerable progress has been made in defining the sites of expression of NHE isoforms along the nephron.

- Aronson found that the relatively ubiquitous "housekeeping" isoform NHE1 is expressed on the basolateral membrane of tubular epithelial cells throughout the nephron. In contrast, the epithelial-specific isoform NHE3 was localized to the brush border membrane of proximal tubule cells, as well as the apical membrane of cells in the loop of Henle and was found to be a basolateral isoform in renal tubular cells. Studies have also begun to define the topology of NHE3 with respect to the membrane bilayer.
- The physiological roles of NHE isoforms have been most thoroughly studied in the proximal tubule. Studies by Aronson made use of known differences in inhibitor sensitivity among NHE isoforms to demonstrate that virtually all Na^+/H^+ exchange in the brush border membrane of proximal tubule cells is mediated by NHE3. A novel approach has been the use of knockout mice with completely deficient expression of NHE isoforms. Mice with a homozygous null mutation in NHE1 were found to have no detectable effect on plasma electrolytes or acid-base status, consistent with its basolateral expression. In contrast, NHE3 null mice developed a profound defect in Na^+ and HCO_3^- reabsorption in the proximal tubule. The use of mice with deficient expression of individual NHE isoforms will be an important tool for understanding the physiologic roles of NHE isoforms in more detail.
- The efforts of many investigators have focused on defining the mechanisms for regulation of NHE3 in the proximal tubule. Indeed, there is new evidence that acute pressure natriuresis causes redistribution of NHE3 from the apical membrane to an intracellular membrane compartment in proximal tubule cells. Studies have also defined the important role of nonreceptor tyrosine kinases in regulating NHE3 activity.

SIGNIFICANCE: There has been enormous recent progress in defining the molecular mechanisms mediating and regulating Na⁺ transport in the proximal tubule. These studies enhance our ability to understand the pathophysiology of hypertension and edematous disorders, and provide novel insight into new therapeutic targets for management of these major clinical problems.

FUTURE DIRECTIONS: An important future direction is to develop a more complete understanding of the molecular mechanisms regulating NHE3 activity in the proximal tubule and other nephron segments. Given emerging evidence that variants in genes governing renal NaCl homeostasis predispose to hypertension and fluid and electrolyte disorders such as Liddle, Bartter, and Gitelman's syndromes, an important future direction will be to test whether variants in NHE3 or its regulatory proteins cause similar clinical disorders. Transgenic and knockout mice will serve as important models for clinical disorders resulting from mutations in genes encoding NHE isoforms or regulatory proteins.

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XXI. TITLE: Role of Aquaporins in Kidney Water Transport and as Carriers for Gases Across Cell Membranes

BACKGROUND: Most molecules that cross cell membranes move through special membrane proteins that act as channels or transporters. Until recently it was generally believed that gases such as carbon dioxide (CO₂) and such small uncharged molecules as water (H₂O) and urea did not need such special carrier proteins, and instead moved freely through the lipid bilayer. However, this issue is being extensively reappraised. The transport proteins for water and urea are

particularly active areas of investigation in our kidney disease program. The existence of a specialized protein named aquaporin-1 (AQP-1) that mediates water movement in red cells was first discovered in 1992. Subsequently seven members of the AQP family have been identified. Studies of these proteins are providing information about their functions and distribution, as well as the mechanisms regulating their activities. Little has been known about the permeability of these channels to small molecules other than water. The role of aquaporins in kidney water transport has also been unclear. AQP-1 was also found to be heavily expressed at sites of rapid water transport in the kidney (proximal tubule and descending limb of Henle's loop), but the initial reports suggested that its absence did not change kidney function.

RECENT FINDINGS: Recent results by an NIDDK grantee indicate that the over-expression of AQP-1 significantly increases the permeability of CO₂, and are consistent with the possibility that AQP-1 may act as a gas channel. These experiments were performed in frog eggs provided with an enzyme carbonic anhydrase, which results in acidification of the cell interior when CO₂ enters the egg. The investigators measured CO₂ permeability using pH-sensitive microelectrodes to measure the initial rate at which CO₂ entry decreased intracellular pH. The increase was reversed by a mercurial compound that blocks water movement through AQP-1.

Other related studies examine the regulation of water transport in kidneys of mice made congenitally deficient in AQP-1. The animals were found to have markedly altered kidney function and enhanced susceptibility to dehydration.

SIGNIFICANCE: Membrane transport proteins are very important for many biological functions, since they are critical to maintaining the cell interior. These proteins are important candidates for drug development, and understanding the relationship between their three-dimensional structure and their small molecule permeability may help in the development of new drugs to protect renal and vascular cells from stress and to protect red cells in the hypertonic kidney medulla. In addition, understanding the biological roles of membrane proteins in gas permeability may open new possibilities to understand disease processes.

FUTURE DIRECTIONS: Early studies of patients lacking AQP-1 did not uncover any kidney defects, but the methods used were insensitive, and this issue will need reappraisal in view of the mouse studies. Gas permeation through protein channels is an understudied topic, and these studies raise the possibility that channels may also be important in movement of another biologically important gas, nitric oxide.

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XXII. TITLE: Functional Roles of ROMK Channels in Renal Handling of Potassium

BACKGROUND: Stability of body potassium balance requires the kidney to match the amounts excreted in the urine to those that are in the diet. Two developments have contributed significantly to understanding renal potassium homeostasis. First, the introduction of patch-clamp techniques has permitted characterization and elucidation of the functional behavior of renal potassium (K⁺) channels in apical and basolateral membranes of defined tubule segments of the nephron. Second, the recent cloning of several K⁺ channels led to progress in establishing important relationships between molecular structure and function of renal K⁺ channels.

New insights into the mechanism of potassium secretion during the past 4 years have been provided by cloning of several potassium channels from renal tissue. The first of these to be cloned was the Inwardly Rectifying K⁺ channel (K_{IR})

(rectification means changing conductance with voltage), a prototype of which is the renal K⁺ channel or ROMK 1 (K_{IR} 1). This was the necessary breakthrough that has led to isolating a family of related channels,

including ATP-sensitive K⁺ channels, and muscarinic receptor-activated K⁺ channels.

RECENT FINDINGS: Recent studies clarify the mechanisms that regulate potassium channel activity. Regulation is achieved by protein kinase A-induced phosphorylation on the kidney's ROMK channels, an effect that depends upon a newly described anchoring protein. Antenatal Bartter syndrome is a variant of an inherited renal tubular disorder associated with hypokalemic alkalosis beginning in the developing fetus. The syndrome is due to a defect in this potassium channel, which leads to a deficit in thick ascending limb (TAL)-Na-K-2Cl cotransport activity because luminal potassium is lacking. Marked hypercalciuria and, as a secondary consequence, the development of nephrocalcinosis and osteopenia are characteristics of this variant. Recent reports demonstrate 14 novel mutations in people with antenatal Bartter syndrome. Also, this channel appears to be unusual in requiring normal interaction with an unrelated but interesting class of membrane proteins, the ABC transporters. Tools are now available to examine the regulation of these proteins that can affect primary mechanisms of ion transport.

SIGNIFICANCE: Potassium balance is critical for maintenance of life. Recently, potassium loss has been implicated as critical in the deaths of several young athletes using diuretic drugs to lose weight. Disordered potassium balance may also contribute to the increased myocardial instability seen in people taking diuretics for hypertension. Understanding potassium channel regulatory mechanisms may lead to improved strategies for developing newer diuretic drugs. Since the ROMK1 channel has been shown to be mutated in some patients with Bartter syndrome, a condition of sodium and potassium wasting, future studies should also provide insight into the pathology of this syndrome.

FUTURE DIRECTIONS: The challenge of future studies in the area of K⁺ secretion will be to characterize further the behavior of K⁺ channels in physiological and pathophysiological conditions, to investigate the interaction of messengers with the channel proteins, and to elucidate the way channels are synthesized and targeted to specific membrane sites. We anticipate that the development of ROMK-deficient mice will also provide insights into the function of these molecules.

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XXIII. TITLE: Role of Cytochrome P-450s in Hypertension

BACKGROUND: About 50 million adults in the United States suffer from high blood pressure, a major risk factor for cardiovascular, cerebrovascular and renal diseases. Hypertension and its complications are major contributors to the national budget for health care. Although the cause of essential hypertension is unknown, data from segregation and twin studies suggest genetic factors could account for an estimated 45 percent of interracial differences in blood pressure. The study of renal mechanisms that control fluid volume and composition, the characterization of the molecular basis of hypertension, and the identification of relevant candidate genes are of great interest and importance.

A role for the microsomal P-450 in the metabolism of arachidonic acid (AA) was first shown in 1981. P-450-derived AA metabolites modulate renal Na⁺ and water transport, are vasoactive, and are regulated by dietary salt intake. P-450-derived AA metabolites are involved in the onset of hypertension in a rat model of genetically controlled spontaneous hypertension (the SHR/WKY model). These observations provide functional and biochemical bases for a role of the

renal P450 AA epoxygenase and ω/ω -1 hydroxylase in the pathophysiology of experimental spontaneous and salt-sensitive hypertension.

RECENT FINDINGS:

- Three research avenues have been applied to study the mechanism of action of the products of the AA epoxygenase (EETs) and ω/ω -1 hydroxylases (19- and 20-OH-AA): 1) administration of synthetic, metabolically stable, eicosanoid analogs; 2) cell transfection studies using cDNA coding for selective AA epoxygenase or ω/ω -1 hydroxylases; and 3) the development of murine strains containing disrupted P450 genes. Importantly, mice carrying mutated P-450 4a14 alleles do not synthesize P450 4a14 and are hypertensive (mean arterial blood pressures of 105 ± 10 ; 140 ± 20 ; and 155 ± 20 mm of Hg, for the (+/+), (+/-) and (-/-) genotypes, respectively). This study found that a blood pressure differential of approximately 50 mm of Hg is associated with a single gene deletion. This important finding: 1) confirms many of the proposed renal roles for the P-450 AA monooxygenase; 2) establishes a solid link between blood pressure and a unique P-450 gene; and 3) underlines the physiological and/or pathophysiological importance of this enzyme system.
- Studies by an NIDDK grantee demonstrated that CoCl_2 -treatment of rats both attenuates the inhibition of proximal tubule Na^+ reabsorption and diuresis and abolishes $\text{Na},\text{K-ATPase}$ inhibition and NHE3 redistribution during acute hypertension, thereby providing further evidence that these responses may be mediated by cytochrome P-450 arachidonate metabolites.
- Growth factors such as epidermal growth factor (EGF), synthesized in the kidney, are highly concentrated in urine and have been implicated in various aspects of renal function, including recovery from acute renal injury, hypertrophy, and inflammation. In addition to their effects on renal ion transport and circulation, the mitogenic properties of the 14,15 form of epoxyeicosatrienoic acid (EET) have been documented in several renal cell lines. However, the mechanisms underlying EET-induced mitogenesis remained unclear. Administration of the sulfonimide analog of 14,15-EET to cultured renal epithelial cells results in activation of a tyrosine kinase phosphorylation cascade, rapid increases in DNA synthesis, and cell proliferation. Furthermore, cell transfection studies using cDNA coding for a region- and stereoselective 14(S),15(R) AA epoxygenase demonstrated unequivocally that the epoxygenase pathway

plays a role in mediating EGF signaling and mitogenesis. These EGF-receptor-dependent EET actions involve phospholipase A₂ activation, P450 oxidative metabolism of AA, and the activation of the PI-3 kinase and MAPK cascades. The sulfonimide analog of 11,12-EET was used to demonstrate that the renal hemodynamic effects of this EET are associated with a protein kinase A-mediated vasodilation of the afferent arteriole.

SIGNIFICANCE: With the exception of a few rare diseases affecting ion channel activity or aldosterone biosynthesis, these studies of the 4a14 knockout mouse provide one of the first demonstrations that high blood pressure can result from alterations in a single gene. Perhaps the most important implication of these observations is that they provide strong evidence that a similar genetic component could be responsible for a yet-to-be-defined subset of human hypertension. The presence in humans of P450 4A isoforms has been demonstrated, and two human 4A isoforms have been cloned (P-450s 4A9 and 4A11). Initial characterization shows that these enzymes are the human homologues of rat 4A and murine 4a P450 isoforms. Inasmuch as the human P450s 4A are also functional homologues of rat and mouse P450s 4A, these results point to a potential role for these genes in the pathophysiology of human hypertension, and suggest them to be strong candidate genes for hypertension in the human population. Identification of the genetic factors responsible will provide a better understanding of the molecular basis of human hypertension and lead to improved diagnosis, risk-assessment, and treatment.

FUTURE DIRECTIONS: Gene-targeted methods need to be developed that will provide a mouse strain containing a disrupted P-450 4a14 gene. Future research needs to address: 1) the use of recombinant adenovirus vectors for studies of EGF-dependent, epoxygenase-mediated cyto-protection; 2) the characterization of the roles played by dietary salt and animal age in the hypertensive phenotype of 4a14 (-/-) mutant mice; 3) hemodynamic and tubular effects resulting from deletion of the 4a14 gene and their relationship to blood pressure regulation; 4) the identification, cloning, and enzymatic characterization of the human homologues of rat P-450 4A2 and mouse P-450 4a14; and 5) the genomic structure of the human P-450 4A gene subfamily.

ACKNOWLEDGMENTS:

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<u>Grant or Contract #</u>	<u>Principal Investigator</u>	<u>Institution</u>
P01DK38226	Capdevila, J.	Vanderbilt University

Publication Data:

Helvig C, Dishman E, Capdevila JH, "Molecular, Enzymatic, and Regulatory Characterization of Rat Kidney Cytochrome P-450 4A2 and 4A3," Biochemistry 1998;37:12546-58.

Reddy KK, Ye J, Falck JR, Capdevila JH, "Intracellular Second Messengers: Synthesis of L- α -phosphatidyl-D-*myo*-inositol 3,4-bisphosphate and Analogs," Bioorganic and Medicinal Chemistry Letters 1998;7:2115-6.

Chen JK, Falck JR, Malla K, Capdevila J, Harris RC, "Epoxyeicosatrienoic Acids and Their Sulfonimide Derivatives Stimulate Tyrosine Phosphorylation and Induce Mitogenesis in Renal Epithelial Cells," Journal of Biological Chemistry 1998;273:29254-61.

Zhang YB, Magyar CE, Holstein-Rathlou N-H, McDonough AA, "The Cytochrome P-450 Inhibitor Cobalt Chloride Prevents Inhibition of Renal Na,K-ATPase and Redistribution of Apical NHE-3 During Acute Hypertension," Journal of the American Society of Nephrology 1998;9:531-7.

XXIV. TITLE: Altered Renal Responses in Pregnancy

BACKGROUND: Normal pregnancy is associated with water retention and dilution of the plasma. The mechanisms of altered water metabolism have yet to be fully elucidated. The fact that the collecting duct water channel aquaporin 2 (AQP2) plays a pivotal role in renal water regulation is the basis for the hypothesis that AQP2 expression could be modified during pregnancy. Preeclampsia is a poorly understood disorder in pregnancy associated with edema, hypertension, and proteinuria. Alterations in renal function play a prominent role in this disorder.

RECENT FINDINGS: NIDDK-funded researchers have demonstrated that upregulation of AQP2 in the papilla contributes to water retention in pregnancy. The expression of AQP2 mRNA early in pregnancy, and AQP2 protein was also increased. Plasma vasopressin concentrations in pregnant rats were no different from those of non-pregnant rats. When a V2 vasopressin antagonist was administered, expression of AQP2 mRNA was suppressed. These results indicate that AQP2 expression could indeed be modified during pregnancy.

SIGNIFICANCE: The results of these studies indicate that upregulation of AQP2

contributes to water retention in pregnancy through a V2 receptor-mediated effect. These data may eventually lead to insights into the treatment of preeclampsia.

FUTURE DIRECTIONS: To better understand the physiology and pathophysiology of preeclampsia, future studies are needed to examine whether nitric oxide synthase (NOS) isoforms are involved in the hemodynamic, neurohumoral and sodium and water retention of pregnancy. Approaches will include the development of knockout mice in which vasoactive or vasoconstrictor systems have been modified, by either disrupting a gene responsible for the synthesis of a vasoactive peptide or coding for a receptor of a vasoactive peptide.

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P01DK19928	Schrier, R.W.	Univ of Colorado

Publication Data:

Ohara M, Martin P, XU D, St. John J, Pattison TA, Kim JK, Schrier RW, "Upregulation of Aquaporin 2 Water Channel Expression in Pregnant Rats," Journal of Clinical Investigation 1998;101:1076-83.

Diabetic Nephropathy Program

XXV. TITLE: Evidence of Pancreas Transplantation Providing Long-Term Benefits for the Kidney

BACKGROUND: Much evidence suggests that the kidney disease of diabetes is related to long-term control of high blood sugar. Some data have also suggested that renal damage may actually be reversed if blood sugar is tightly controlled.

RECENT FINDINGS: Pancreas transplantation, when successful, results in long-term correction of hyperglycemia. NIDDK-supported research published in the *New England Journal of Medicine* demonstrated in patients that after 10 years of normal blood sugar following pancreas transplantation, glomerular and tubular basement membrane thickness and mesangial and mesangial matrix-

fractional volume returned toward normal levels.

SIGNIFICANCE: These data highlight the beneficial effects of successful pancreas transplantation in people with type 1 diabetes mellitus and early kidney disease. A normal blood glucose level is an important goal to protect the kidney, and reversal of damage is possible. Practically, it should be noted, the beneficial effects of pancreatic transplantation on kidney disease must be balanced against the toxic effects of the current immunosuppressants, the risks of surgery, and the adverse consequences of lifelong immunosuppression.

FUTURE DIRECTIONS: Work is needed to increase blood sugar control in people with diabetes and to increase access to pancreas transplantation for those who have the kidney disease of diabetes.

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Sources of Support:

<u>Grant or Contract #</u>	<u>Principal Investigator</u>	<u>Institution</u>
R01DK13083	Mauer, M.	Univ of Minn Sch of Med
RO1DK43605	Mauer, M.	Univ of Minn Sch of Med

Publication Data:

Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M, "Reversal of Lesions of Diabetic Nephropathy after Pancreas Transplantation," New England Journal of Medicine 1998;339:69-75.

XXVI. TITLE: Insights into Therapy and Hyperglycemic Injury To Renal Cells

BACKGROUND: High blood sugar, or hyperglycemia, is associated with renal injury. Various angiotensin converting enzyme-inhibiting (ACEI) drugs have been implicated in improved outcome in patients with diabetic and non-diabetic renal disease. The action of angiotensin II on renal cells has been implicated in pathologic processes, including increased matrix synthesis, thought to be central to the pathogenesis of diabetic nephropathy. A role for transforming growth factor- β 1 (TGF- β 1), a mediator of increased matrix synthesis, has been suggested in the kidney disease of diabetes.

RECENT FINDINGS: NIDDK-funded researchers have demonstrated that

increased concentrations of D-glucose in murine mesangial cell cultures were associated with increased TGF- β 1 synthesis, showing increased TGF- β 1 supernatant bioactivity and increased transcription of TGF- β 1 mRNA in cells exposed to high concentrations of glucose. A possible glucose-responsive element in the TGF- β 1 promoter was identified.

NIDDK-supported scientists have also demonstrated that both enalapril (an ACEI) and losartan, an angiotensin II receptor antagonist, decreased levels of TGF- β 1, fibronectin, and plasminogen activator inhibitor-1 (PAI-1) in treated rat models of glomerulonephritis compared to untreated animals. In addition, the expression of these injury mediators in the treated animals was also reduced, as was glomerular histologic damage.

SIGNIFICANCE: These data show ambient glucose concentration is enough to cause maladaptive cellular reactions that can be associated with the development of diabetic kidney disease, and that available medications that inhibit the renin-angiotensin axis are active at the cellular level to blunt such responses.

FUTURE DIRECTIONS: Studies are needed to establish downstream events associated with TGF- β activation and molecular mechanisms involved in TGF- β inhibition using drugs inhibiting angiotensin at the tissue level. The results of ongoing trials of angiotensin receptor antagonists in people with kidney disease of diabetes will be of great interest.

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<u>Grant or Contract #</u>	<u>Principal Investigator</u>	<u>Institution</u>
R01DK44513	Ziyadeh, F.N.	Univ of Penn
R01DK45191	Ziyadeh, F.N.	Univ of Penn
R01DK49342	Noble, N.A.	Univ of Utah
R01DK43609	Border, W.A.	Univ of Utah
R01DK49374	Border, W.A.	Univ of Utah

Publication Data:

Hoffman BB, Sharma K, Zhu Y, Ziyadeh FN, "Transcriptional Activation of Transforming Growth Factor- β 1 in Mesangial Cell Culture by High Glucose Concentration," Kidney International 1998;54:1107-16.

Peters H, Border WA, Noble NA, "Targeting TGF- β 1 Overexpression in Renal Disease: Maximizing the Antifibrotic Action of Angiotensin II Blockade," Kidney International 1998;54:1570-80.

NIDDK MINORITY TRAINING AND CAREER DEVELOPMENT-1998

Name of Program and Description	Division	# of NIDDK Awards	NIDDK Funding Level	ORMH Collab. Funding
<p><u>Minority Access to Research Careers (MARC) T-34</u> NIDDK Co-funds with NIGMS. Funds predoctoral faculty fellowships, visiting scientists, conferences for minority investigators and minority health issues, and honors undergraduate training in biomedical research. Summer Internship Program in the NIDDK Division of Intramural Research (students-managed by NIDDK-EEO).</p>	DK-wide	6	\$23,236	
<p><u>Minority Biomedical Research Support Program (MBRS)</u> NIDDK co-funds with NIGMS. Provides expanded opportunities for minorities to participate in biomedical research careers. Supports research projects of interest to the NIDDK at Minority and Equal Opportunity Institutions.</p>	DK-wide	25	\$1,985,728	
<p><u>R-13 (Conference Grant) to the American Physiological Society, FASEB</u> Provides support for underrepresented minority students to attend meetings of the Society, and for 36 minority high school science teachers to have summer research training in laboratories of Society members.</p>	DK-wide	1	\$74,315	

<p><u>Initiatives for Underrepresented Minorities in Biomedical Research</u> NIH-wide program initiatives to support minority undergraduate, graduate students, high school students, and faculty members on NIDDK active research grants through administrative supplements.</p>	DK-wide	120	\$4,500,000	
<p><u>Research Training of Underrepresented Minorities on Institutional Training Grants (T32)</u> Highly qualified Minority Investigators are assigned T-32 slots held in reserve for this purpose. DDEMD=5 DDDND=3 DKUHD=6</p>	DK-wide	14	\$175,174 81,437 183,000	\$41,917
<p><u>Pre-doctoral Fellowships (F-31)</u> To provide support to minority students for research training leading to M.D.-Ph.D. in the biomedical sciences. DDEMD=6 DDDND=2 DKUHD=1</p>	DK-wide	9	\$132,269	
<p><u>Cell/Molecular Biology Student/Teacher Learning Center (R-25)</u> Laboratory Research experience for minorities in the District of Columbia (managed by NIDDK-EEO).</p>	DK-wide	1	\$334,767	

<u>Small Research Grants (R-03) for Minority Researchers</u> DDEMD=5 DDDN=n/a DKUHD=1 ORMH Collaboration provides additional support for minority researchers.	DK-wide ORMH	6	\$367,229 84,750	\$466,933
<u>Minority High School Student Summer Research Training Supplement</u> In conjunction with the National Minority Organ Tissue Transplant Program award to Howard University, NIDDK provides meaningful laboratory research experience to minority high school students to stimulate their interest in careers in biomedical science.	DK-wide	1	\$70,138	
Totals		183	\$8,012,043	\$508,850