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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Introduction

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and the Children's Digestive Health and Nutrition Foundation (CDHNF) recognize the importance of addressing current gaps in knowledge and pointing the direction for future research in pediatric gastroenterology, hepatology and nutrition. In addition, both organizations are keenly aware of the vital need to support excellence in the training of those who will form the next generation of investigators in the field.

Therefore, NASPGHAN and CDHNF initiated development of a five-year agenda for research in pediatric digestive and nutritional disorders. At the request of the CDHNF, the Executive Council of NASPGHAN formed the Research Agenda Task Force to identify research needs of the highest priority. The Task Force was charged with the responsibility to define a Research Agenda that clearly enumerates both clinical and basic research requiring support, as well as provides a clear and cogent rationale for these priorities. The Research Agenda would serve as a resource for discussions with funding partners in government, industry and disease-related agencies.

The Research Agenda Task Force was organized into subcommittees, each charged with establishing research priorities in specific areas. The subcommittees focused on the following topics: molecular basis of gastrointestinal diseases, developmental physiology and pathophysiology, secretion and diarrhea, acid-peptic diseases, endoscopy, cystic fibrosis and pancreatic diseases, motility disorders and functional gastrointestinal disorders, hepatobiliary disorders, transplantation, nutrition and obesity, chronic inflammatory bowel disease, and allergy and immunology. In some circumstances, the Research Agenda Task Force employed the following materials:

- Mission Statement of the CDHNF.
- Pediatric Liver Research Agenda 2000, developed by the Children's Liver Council of The American Liver Foundation.
- CD Rudolph, HS Winter. NASPGN Guidelines for Training in Pediatric Gastroenterology. NASPGN Executive Council, NASPGN Training and Education Committee. *J Pediatr Gastroenterol Nutr* 1999;29 (suppl 1):S1-26.
- Challenges in IBD Research: the Clinical Research Agenda for the Crohn's and Colitis Foundation of America.
- Challenges in Inflammatory Bowel Diseases: the Research Agenda—1998.

Other publications that were considered for review included:

- US Digestive Disease National Commission Summary Report (US government source).
- Progress Report from the National Institute of Child Health and Human Development (NICHD).
- American Gastroenterological Association (AGA) Task Force on Training (published in *Gastroenterology* 1996;110:1266-300).

The Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition is presented in this journal supplement. Comprising 12 documents, the report is also available on the NASPGHAN website, www.naspghan.org.

The Research Agenda has been officially approved by the Executive Council of NASPGHAN and by the Board of Directors of the CDHNF. It is the mission of NASPGHAN, founded in 1972, to be a world leader in advancing the science and clinical practice of pediatric gastroenterology, hepatology and nutrition in health and disease. The CDHNF was established by NASPGHAN in 1998 to raise funds to promote research and education that will improve the health of children and adolescents with digestive and nutritional disorders.

The CDHNF is committed to identify, encourage, support and coordinate the scientific research and professional study of gastrointestinal, hepatobiliary, pancreatic and nutritional disorders in children. In addition, the CDHNF aims to strengthen the role of pediatric gastrointestinal and nutritional scientists as leaders in research and education in these medical and health care fields and to evaluate and improve the quality and availability of

medical care for children with digestive disorders. The CDHNF also supports the research and educational programs of NASPGHAN.

We are hopeful that the Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition will enhance research funding, stimulate advances in knowledge and thereby promote the digestive and nutritional health of children.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Molecular Basis of Gastrointestinal Diseases

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

Sophisticated computer software technology has recently been developed to provide data from the human genome project as well as from microarray and proteonomic studies. Initial studies using this technology have demonstrated the potential for major advances in several areas of biomedical research.

Deoxyribonucleic acid (DNA) microarray techniques may be used to characterize profiles of changes in gene expression in unprecedented detail. For example, there have been studies of changes in the expression of thousands of genes in specific cells or tissues in response to aging, tissue injury, caloric restriction, drug treatment or alteration of a specific gene (e.g., mutation, and expression of an oncogenic transcription factor). Novel classification systems for leukemias, which have been generated by gene expression profiles, can potentially lead to optimal, targeted treatments. Gene expression profiles also have been used to characterize pathogenic factors in microorganisms. Together with newly developed laser microdissection techniques, which permit isolation of specific structures and even cell types from a tissue specimen, microarray techniques are beginning to be used for characterization of gene expression profiles within different components of tumors or inflamed tissues.

DNA microarray techniques also may be used for large-scale studies of sequence variation. This type of application has the potential to revolutionize genetic mapping and, in large population studies, to identify genetic alterations that determine disease susceptibility.

In addition, DNA microarray techniques may be used to characterize interactions between gene products. For example, microarrays have been applied to screens designed to identify inhibitors of specific biological response pathways. This type of application will be used in the future to identify substrates of enzymes, substrates for chaperones and ligands for receptors. It may be especially useful for identification of antisense oligonucleotides or antisense ribozymes for pharmacologic interventions.

Finally, DNA microarray techniques have been used to screen for the function of products of disrupted genes in model systems. This type of application will be particularly important for understanding disease pathogenesis and for drug discovery projects.

Proteonomics, or the analysis of complete sets of gene products at the protein level, has not been used widely, but these techniques may prove even more powerful for the identification of interactions between gene products. Thus, they may be designed for identification of substrates, inhibitors, ligands, agonists, antagonists, transcriptional activators and repressors.

Application of these technologies to studies of the molecular basis of gastrointestinal (GI) health and disease in children may produce major advances relatively rapidly. The technology can be applied to almost all disorders affecting the GI tract, liver, biliary tract and pancreas. It can potentially facilitate the elucidation of genes altered in congenital anomalies or in inherited disorders that are monogenic, as well as the elucidation of genes that modify the clinical phenotype of monogenic disorders. Genetic traits that contribute to the development of polygenic diseases or determine susceptibility to infections, inflammatory diseases, toxins, drug reactions and developmentally determined or physiologic stressors also may be more rapidly identified with these technological advances.

Application of these technologies may be particularly useful in understanding host responses to disease and injury, including intestinal adaptation, liver regeneration, responses to intestinal or biliary obstruction, host inflammatory response and host stress response. Indeed, these responses are thought to be critical determinants of the clinical phenotype and severity of many GI disorders.

AREAS OF EMPHASIS

Elucidate the Genetic Basis of Single-Gene Disorders and Genetic Determinants of Polygenic Diseases

Research Goals

Here we need to identify the genes altered in single-gene disorders, such as microvillus inclusion disease,

neonatal iron storage disease, Alpers' disease and Shwachman syndrome. We also need to identify genes altered in polygenic diseases, such as inflammatory bowel disease and gluten-sensitive enteropathy. Finally, the genetic disorders that account for some general syndromes, such as intractable diarrhea of infancy and idiopathic neonatal hepatitis, need to be elucidated.

Research Strategies

This goal will require a combination of genetic mapping, microarray analysis, proteonomic analysis, and classical biochemical studies. A combined strategy was recently used to identify and characterize the ABC1 lipid transporter that is mutated in Tangier disease.

Projected Timetable and Funding Requirements

This area will be optimally addressed by investigator-initiated grants (e.g., RO1 grants). Center grants and program projects also will be effective strategies, particularly because they could permit the establishment of core facilities for computer software and microarray and proteonomic analysis. The initiative should be started immediately to take advantage of the new data being generated by the human genome project.

Analyze Genotype-Phenotype Relationships and Genetic Modifiers of Single-Gene Disorders

Research Goals

Here we need to identify the genetic traits that determine the clinical phenotype of disorders in which the primary genetic abnormalities have already been identified. Cystic fibrosis and α_1-antitrypsin deficiency are examples of monogenic disorders in which there is wide variation in phenotypic expression of target organ injury. Although rare, hereditary hemorrhagic telangiectasia is an example of a disorder with a striking genetically determined phenotypic variation. It is also very important to learn how childhood GI, hepatobiliary and pancreatic diseases can lead to carcinoma during the adult years. Examples include hereditary polyposis syndromes, inflammatory bowel disease and metabolic liver disease. Detailed information on tumorigenesis and cell survival (i.e., apoptosis pathways) is important for our understanding of the pathobiology of these diseases. This information also is important for the development and use of novel therapeutic strategies. For example, in the case of metabolic liver disease, transplanted hepatocytes have a selective advantage for growth and proliferation in the liver of the c14CoS albino mouse (a murine model of hereditary tyrosinemia) and will replace most of the damaged liver. However, it is not known whether a small number of residual dysplastic liver cells will become malignant and thereby limit the potential success of this type of novel therapy.

Proteonomic techniques may be particularly useful for studying how GI diseases can lead to carcinoma. For example, proteonomic analysis was recently used to identify the expression of 43,302 proteins in normal human breast cells in anticipation of a comparison with protein expression in cells from breast cancer specimens.

Research Strategies

This goal will require a focus on basic biologic and pathobiologic work and should utilize genetic mapping, microarray and proteonomic techniques together with classical biochemical and cell biological studies. It will also require large-scale multicenter collaborative studies and registries to carefully characterize patients from a clinical perspective and provide material from these patients for genotyping.

Projected Timetable and Funding Requirements

This research should start immediately and will depend on investigator-initiated grants, program projects and center grants. The development of a registry for specific single-gene disorders, as defined by the other task force subcommittees, is strongly recommended. Funding should be solicited from several institutes at the National Institutes of Health (NIH) as well as from private foundations. A contract mechanism could work particularly well for the registries.

Develop New Animal Models

Research Goals

It is recommended that the most sophisticated genetic engineering strategies be used to generate new animal models of GI disease. These strategies include transgenesis, targeted gene disruption, targeting mutagenesis, and conditional and inducible expression systems. It will be very important to ascertain the effect of genetic background in each animal model so that genetic modifiers of disease phenotype can be identified. Use of inducible or conditional expression systems will permit examination of the effects of developmental stage on disease phenotype. These studies are likely to be particularly informative for animal models of diseases that are affected by development. For example, in α_1-antitrypsin deficiency, there is often clinical evidence of liver injury early in infancy and thereafter most patients enter a period in which there is marked lessening of liver injury. In some of these patients, liver disease recurs during adolescence. Some α_1-antitrypsin-deficient individuals develop liver disease with or without hepatocellular carcinoma later during adult life. An animal model in which the hepato-

toxic condition, retention of the mutant ZZ α_1-antitrypsin molecule in the endoplasmic reticula of liver cells, is induced or abrogated at specific times during development may provide critical information about the mechanism underlying the effects of this disorder on the liver at different stages of development. Use of tissue-specific promoters will permit examination of the role of specific tissues in determining disease phenotype.

Research Strategies

This goal will require a collaborative effort between investigators, genetically altered mouse core facilities and their personnel, anatomists, pathologists, embryologists and physiologists. Characterization of the animal models will require microarrays and proteonomics. In fact, it is likely that at some point in the future, companies will be making the genetically altered mice and supplying them to investigators for this type of characterization. The development of a database for accessing information on all animal models is strongly recommended.

Projected Timetable and Funding Requirements

Program projects and center grants with core facilities would greatly facilitate this type of work. Consideration should be given to encouraging and facilitating collaboration between industry and academic research programs.

Develop Novel Prophylactic/Therapeutic Interventions for GI Disease

Research Goals

It is recommended that proteonomic analytical systems be used to identify novel agonists and antagonists. In addition, three novel strategies for the prevention and treatment of genetically determined conditions should be studied in detail:

Cell transplantation. Recent studies have shown that normal adult hepatocytes can replicate and replace much of the liver parenchyma, but only when transplanted into the background of an injured liver—specifically a liver injured by a metabolic defect, hereditary tyrosinemia. This form of therapy is therefore applicable to liver diseases in which the defect is cell-autonomous (e.g., many of the childhood metabolic liver diseases). In fact, recent studies have shown that hepatocyte transplantation may be effective in the treatment of type I Crigler-Najjar syndrome. There is reason to believe that cell transplantation strategies also can be used for injuries in other organs. Such studies will need to address the changes that occur in the diseased tissues, whether it is regenerative signals that permit cell replication, and how the cells that are to be transplanted can be optimally manipulated *ex vivo* (or *in vivo* after transplantation) using state-of-the-art genetic and molecular techniques.

Chemical chaperones. The use of chemical chaperones for chemoprophylaxis of metabolic liver disease also is deserving of more detailed study. This class of compounds, which includes glycerol, trimethylamine oxide, deuterated water and 4-phenylbutyric acid (PBA), has shown to reverse the cellular mislocalization or misfolding of mutant membrane and lysosomal, nuclear, cytoplasmic and secretory proteins, including mutant CFTRΔF508 and ZZ α_1-antitrypsin. In fact, PBA has been shown to have positive biochemical effects in an animal model of α_1 -antitrypsin deficiency and in humans with cystic fibrosis. Recent studies have also suggested that competitive antagonists may have chaperone effects on mutant enzymes. One drug in this class, 1-deoxygalactonojirimycin, a competitive antagonist of the lysosomal enzyme galactosidase A, mediates partial correction of the defect in localization of this enzyme in one type of Fabry disease. Recent studies have also shown that imino sugar compounds, which inhibit oligosaccharide side-chain trimming of glycoproteins, can partially reverse the mislocalization of mutant proteins such as ZZ α_1-antitrypsin. Taken together, these compounds may have broad applicability in a variety of metabolic and genetic diseases of the liver, biliary tract, pancreas and GI tract, including α_1-antitrypsin deficiency, cystic fibrosis, Wilson's disease, hemochromatosis, Gaucher's disease, Niemann-Pick disease and carbohydrate-deficient glycoprotein syndrome.

Chimeric oligonucleotides. The use of chimeric ribonucleic acid (RNA)/DNA oligonucleotides for the prevention of tissue injury in genetic diseases is deserving of further investigation. Recent studies have shown that chimeric RNA/DNA oligonucleotides, based on the sequence of coagulation factor IX complex with lactose so that it could be taken up by asialoglycoprotein receptor-mediated endocytosis, are delivered to hepatocytes with high efficiency after intravenous administration. Moreover, the oligonucleotide complexes mended the mutation in more than 90% of the liver parenchymal cells in an animal model of factor IX deficiency.

Studies are strongly recommended of genetic polymorphisms in drug metabolism, drug disposition, drug transporters and drug targets.

Research Strategies

This goal will require not only continued innovative cell transplantation gene transfer and pharmacology research, but also continued basic research. The basic research will focus on the developmental biology of the GI tract and liver, the biology of tissue response to injury and the biology of tissue regeneration. Presumably, the success of cell transplantation in treating genetic disease

will depend on the development and differentiation of the cells to be transplanted, the ability to manipulate them *ex vivo* (and presumably *in vivo* after transplantation) using gene transduction techniques, the signals being generated by the response of the affected tissue to injury, and the need for the affected tissues to regenerate. Moreover, one would suspect that the response to injury and regeneration in the native tissue would depend on the developmental stage of the organ. Proteonomic techniques will be particularly useful for the identification of novel agonists and antagonists that bind to key cellular target molecules. This goal will also require large-scale, high-throughput screening methods to identify candidate drugs and genomic screens for the identification of polymorphisms in drug-metabolizing enzymes, transporters and targets.

Projected Timetable and Funding Requirements

This goal will require intensive partnerships with industry; in particular large pharmaceutical companies.

Study the Basic Biology of Intestinal, Hepatic and Pancreatic Development and Responses to Infection, Inflammation, Tissue Injury and Other Physiologic/Environmental Stressors (e.g., Intestinal Adaptation and Liver Regeneration)

Research Goals

An enhanced understanding is required of the molecules involved in development and differentiation of the GI, hepatobiliary and pancreatic systems. In addition, how these tissues specifically respond to conditions associated with the expression of clinical disease is required. The intestinal adaptation response and liver regenerative response to injury are examples of signal transduction systems that are fundamental to determining whether a host will develop clinical disease and how severely the subject will be affected.

Research Strategies

Microarray and proteonomic techniques are particularly well suited to address this type of basic research. These state-of-the-art techniques, together with data from the ongoing genome projects, are likely to produce significant fundamental advances.

Projected Timetable and Funding Requirements

This requires immediate action. Investigator-initiated grants should be used, but sponsorship by industry for this type of research also should be sought.

Develop Tools for Diagnostic and Population Biology Issues (e.g., Screening Programs)

Research Goals

A combination of programs is recommended, including a basic research program designed to identify new methods for diagnostic and population screening assays and a clinical research program in which these assays can be applied. There are many potential applications of such methods, including:

- Diagnostic tests for GI diseases in which the diagnosis currently involves invasive procedures or sophisticated equipment and technology, such as celiac disease, glycogen storage diseases, hereditary fructosemia and disorders of fatty acid oxidation
- Diagnostic tests that can be used in large populations for screening programs and population biology research
- Diagnostic tests to identify markers of tissue injury, such as fibrosis, cirrhosis, graft rejection and risk for cancer
- Assays for modifying genes

Research Strategies

This goal will require basic research to develop diagnostic tools and methods based on DNA, microarray and proteonomic techniques. For example, in cystic fibrosis, genome scanning methods could be used to study the inheritance of microsatellite markers in multigenerational kindreds. The resulting linkage data could lead to identification of additional modifying genes. This goal will also require applied research in the design of screening studies, such as have been undertaken for Tay-Sachs disease and α_1-antitrypsin deficiency, and population biology studies.

Projected Timetable and Funding Requirements

Immediate action is recommended via nationally funded peer-reviewed grant mechanisms. We also recommend partnerships with industry to develop diagnostic assays and sponsor screening studies. A planning committee should be convened to identify key areas and to plan large-scale multicenter collaborative efforts for the development of screening programs and population biology studies. For this effort, multiple NIH institutes as well as foundations should be approached for financial support.

HEALTH AND ECONOMIC OUTCOMES

A greater understanding of the molecular basis of GI disease and improved methods for diagnosis, prophylaxis

and treatment will have a major impact on the clinical outcome for affected children and on the associated costs of health care. This section of the Research Agenda has described the potential use of data from genome projects, as well as use of microarray and proteonomic techniques, to achieve these goals. How the recommended research programs will influence health outcome and cost of care is described in the following sections focusing on specific disorders and organ systems.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Developmental Physiology and Pathophysiology

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

Considerable progress has been made in recent years in elucidating the mechanisms regulating development of the gastrointestinal (GI) tract. Important insights have been gained through the use of model systems, including *Caenorhabditis elegans, Drosophila melanogaster, Xenopus* and transgenic mice. Recent findings have emphasized the common mechanisms and similar genes involved in regulating GI development in diverse organisms. Of necessity, much of this work has focused on individual genes and specific regulatory pathways.

It is becoming clear that an understanding of physiology and pathophysiology requires an integrated approach to the simultaneous interaction of regulatory factors. Such an analysis is now becoming feasible through the use of accumulated genetic data provided by the genome projects currently under way or recently completed. The combination of these data with the rapidly developing technology for synthesizing and screening deoxyribonucleic acid (DNA) arrays should make possible the comprehensive analysis of patterns of gene expression in the developing GI tract as well as of aberrant developments leading to pathophysiologic states.

The availability of data from the human genome project will facilitate the identification of human genes involved in pathophysiology. Support for a major new emphasis on the comprehensive analysis of development should lead to advances in child health. In addition, many promising research directions have been identified recently, for which additional support should be generated. Involvement of clinically trained investigators in these emerging research areas for the study of GI development should be strongly encouraged.

AREAS OF EMPHASIS

Carry Out A Comprehensive Analysis of Gene Expression Patterns in Development and Pathophysiology of the GI Tract

The *hox* genes regulate development in specific regions of the GI tract and likely regulate the overall pattern of development (1,2). The *hox* genes encode transcription factors, but few of the target genes are currently known. Cdx2, a divergent homeobox gene that mediates intestine-specific expression of a number of genes, interacts with a *hox* protein, and its binding site in lactase has been reported to also bind a *hox* protein. Although the *hox* genes are expressed in both developing and adult GI tissues, how they carry out their functions is not understood.

For a number of individual transcription factors and growth factors, specific roles have been established in the development of the GI tract. For example, the transcription factors cdx2, GATA and HNFI regulate a number of genes critical for GI function. Few data are currently available, however, on how they function together either in maturing cells or in the developing GI tract. Similarly, epidermal growth factor (EGF) and transforming growth factor–beta (TGF-β) both regulate enterocyte proliferation, but their integrated function in the organ is not well understood.

Research Goals

With both the technology and an extensive database available, it is now feasible to carry out a comprehensive analysis of gene expression in the GI tract to address these questions. It should be possible to perform such analyses in great detail both spatially and temporally in the GI tract. These data would then serve as a benchmark for analyzing the changes in pathologic conditions. For example, delineation of the changes in expression of cytokines and related immune factors and resulting changes in cellular gene expression during the inflammatory response would enhance understanding of these processes.

A similar approach to analyzing adaptation following resection would also provide important information. Such an analysis of age-related changes and the impact of caloric restriction on these changes in mouse muscle was recently reported (3). Ultimately, such analyses should enable the identification of critical changes during the onset of pathology, and will facilitate the development of therapeutic agents to target critical factors.

Such a possibility is supported by the successful administration of an antisense oligonucleotide to inhibit NF-kappa B and relieve symptoms of inflammatory bowel disease in mice (4).

Research Strategies

This would be an appropriate use of DNA microchip technology. The increasing availability of genetic sequence information and the capability to examine changes in multiple genes allow an evaluation of the integrated physiology of development as well as changes in disease states. This could lead to the development of specific arrays for diagnostic use (e.g., screening for genetic disease) or for the evaluation of disease processes and effectiveness of treatment interventions.

Projected Timetable and Funding Requirements

This research area could be approached both through individual investigator-initiated grants (R01 grants) and through center grants to support the establishment and maintenance of core facilities for computer analysis of microarray data. Some of the necessary reagents are already commercially available. Development of arrays specific for analysis of GI development should be encouraged. The possibility of partnerships with companies interested in commercializing standardized arrays as diagnostic tools should be explored.

Elucidate the Role of Growth Factors in GI Development

Research Goals

In addition to some of the better-studied growth factors, such as EGF and TGF-β, evidence is now emerging for a role in gut development by several less well-studied factors, including trefoil factor and fibroblast growth factor (FGF). One growth factor in particular—bone morphogenetic protein 4 (Bmp4), a member of the TGF-β superfamily—has been shown to be important in early intestinal development. Recent studies demonstrated that several members of the FGF family and their receptors are critical for the process of liver induction (5). The signal transduction pathways and cross-talk among these growth factors in the GI tract should be investigated. More basic work on expression patterns and their effects needs to be done for these less well-understood growth factors. Trophic effects have been suggested, but not definitively established, for many gut hormones, whose involvement has been postulated in the maturation of the GI tract in human newborns.

Elucidation of cellular mechanisms regulating the diverse effects of these growth factors on the GI epithelium, including specific roles and cross-talk, is an important research goal. Another priority area for study is the role of growth factors during epithelial-mesenchymal interactions in the GI tract. Epithelial-mesenchymal interactions have long been known to be critical in GI tract development, but few data are available on the mechanism of these interactions. Evidence is now emerging that growth factors such as Bmp4 mediate these interactions (6). Other soluble mediators, such as sonic hedgehog and several genes of currently unknown function (e.g., Nkx2.3 and Hlx), have recently been shown to be critical for epithelial-mesenchymal interactions during development.

Research Strategies

The use of knockout models and transgenic technology is appropriate. Peptide hormones have been postulated to have developmental effects, especially after the first postnatal feeding. Careful analysis of emerging knockout models for peptide hormones is required. For example, analysis of knockout mice identified a role for gastrin in differentiation of the stomach and proliferation of colonic cells (7). Transgenic technology is a key tool for investigating the trophic effects of these factors.

Projected Timetable and Funding Requirements

Investigations in these areas primarily lend themselves to individual investigator-initiated grants. As our understanding increases, comprehensive analysis as described above would become a priority.

Elucidate the Role of Non-Epithelial Cell Types and Their Interactions With the Epithelium in Development and Pathophysiology of the GI Tract

Research Goals

There is emerging evidence for involvement of dendritic cells, smooth-muscle cells, pericryptal fibroblasts, immune cells and neural cells in pathophysiology of the GI tract. Development of the enteric nervous system clearly is an important pathophysiologic factor, as evidenced by the genetic defects now identified as the cause of Hirschsprung's disease (1). Another example is a study indicating a role for the enteric nervous system in rotavirus-related diarrhea (8).

Similarly, cells of the immune system within the GI tract are emerging as important factors in pathophysiology. Although the gut is the largest immune organ of the body, interaction of this immune system with the luminal environment is not well understood, especially in the human neonate. Much work needs to be done in this area, which has important implications for clinical care.

Although pericryptal fibroblasts were well described some time ago (9), evidence has only recently begun to

be presented on their function. Mice heterozygous for a knockout of the phosphatase PTEN show increased numbers of fibroblasts surrounding hyperplastic crypts, suggesting that part of the abnormality may be due to disordered interactions between these fibroblasts and epithelial cells (10). Recent knockouts of the fkh6, Hlx and Nkx2.3 genes have produced abnormal epithelial development, most likely the result of abnormal epithelial-mesenchymal interaction (1). These findings point the way toward dissecting the mechanisms of GI epithelial-mesenchymal interaction. This is a critical area that until recently has been largely descriptive, but key genes are now being identified.

Research Strategies

These areas are appropriate for investigator-initiated studies, which would benefit from greater support.

Delineate the Relation Between Nutrient Intake and Intestinal Development

Research Goals

Although many of the relevant transporters have been cloned, there are few data on developmental patterns and their regulation in the human GI tract. For example, inherited glucose-galactose malabsorption is due to a defect in the SGLT1 gene, suggesting the potential clinical relevance of additional data in this area (11). Mobilization of transporters to the luminal surface is regulated by nutrient intake. There is also some evidence for the regulation of specific digestive enzyme gene expression by nutrient levels. Some studies suggest a direct effect of nutrients on the expression of transporters and digestive enzyme genes, such as that for sucrase-isomaltase. This is a little-explored field of potential importance to infant nutrition.

Develop Animal Models and Cell Lines to Address Specific Questions in GI Physiology and Pathophysiology

Research Goals

Gene knockout technology is a powerful tool for identifying developmental effects of a specific gene. However, if such knockouts are embryonic lethals, it is impossible to study the role of the gene at later stages, such as in the immediate postnatal period. Recent studies report the development of an inducible gene knockout in cells of the small intestine and colon (12). With this approach, it is now possible to eliminate the gene of choice at any stage of postnatal life. This technology would have obvious applications for a number of questions in developmental physiology.

Another approach that is being developed is the use of immortalized cells from the commercially available "Immortomouse" (sold by Charles River), both to generate cell lines directly and, by crossing to mice, to generate mouse cell lines carrying the desired gene knockout (13). This approach can be used to dissect complex regulatory pathways. Intestinal cell lines lacking the receptor for EGF, for example, can be generated for study.

Another research goal should be to identify stem cells and to develop culture methods to maintain and differentiate such stem cells from the GI tract. A successful effort would not only provide a model for investigation of basic questions in development, but also raise the possibility of using such cells to treat damaged or genetically defective GI tissues. It is known that stem cells exist, for example, in the crypts of the small intestine; their rough location and number within a single crypt are also known (14). Currently, identification and culture of stem cells originating from the gut have not been accomplished. Recent work demonstrated that the transcription factor Tcf-4 is required for the maintenance of stem cells in the murine small intestine (15). Possibly Tcf-4 will provide a marker to successfully identify and culture human small-intestinal stem cells. Stem cell culture is a long term goal, but one of great importance.

Research Strategies

Additional experience with techniques of cell immortalization should make possible the application of these methods to the development of nontransformed human cell lines of intestinal origin, which would provide an invaluable research model. Conditionally immortalized human intestinal cell lines have been reported, but have not so far been widely used. Furthermore, it should be possible to develop and use such cell lines for therapeutic purposes, as was recently suggested for reversibly immortalized human hepatocytes (16).

Projected Timetable and Funding Requirements

The described models obviously would be of use to individual investigators, but might be developed by a core center as part of a center grant, so that funding could support a group of investigators. The model systems might be generated by a core facility and individuals supported to carry out specific projects by pilot project grants, preliminary to obtaining R01 grants.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Secretion and Diarrhea

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

The intestine has four fundamental functions: digestion and absorption of nutrients; vectorial transport of water and solutes; barrier function; and host defense. Failure of any one function typically leads to diarrhea. Diarrheal diseases, which are among the most common pediatric disorders, carry the potential for significant morbidity: children with recurrent disease fail to grow, and parents caring for sick children have diminished economic productivity. In children younger than 5 years of age, diarrhea remains a leading cause of death, accounting for nearly 3 million to 5 million deaths each year. This represents the single greatest worldwide cause of loss of human potential due to disease in the pediatric or adult population. A focus on basic, clinical and epidemiologic research is needed to broaden our understanding of diarrheal diseases.

AREAS OF EMPHASIS: Basic Research

Basic research goals focus on the intestinal epithelial cell and its relation to cellular and noncellular components in the intestinal lumen and subepithelial space. In all transporting organs, such as the intestine, lung and kidney, the epithelial-layer cell defines and maintains a selective barrier separating two physically distinct compartments. The capacity to transport essential solutes, water and cells (such as neutrophils, certain microbes and perhaps macrophages) between these compartments is essential for normal physiologic function of the intestine. Thus, in nearly all instances, the cellular and molecular biology of the polarized epithelium, and its dynamic relationship with the mucosal environment, defines ultimately the physiology and pathophysiology of the intestine.

Study Membrane and Epithelial-Cell Biology of Salt and Water Transport, Epithelial Barrier Function, Epithelial Polarity, and Regulation of Membrane Structure and Function

Research Goals

Such studies should elucidate down-regulatory mechanisms for apical membrane Cl channels and basolateral K channels; the biology of apical membrane Ca^{++}-dependent Cl channels; mechanisms of membrane organization that dictate specificity in signal transduction; and the biology of intestinal crypt cell-cell, cell-matrix and cell-microbe interactions. In addition, mechanisms that couple solute transport with regulation of the intercellular tight junctions, and novel mechanisms of Na-coupled or HCO_3-coupled solute transport (i.e., potential transporters that may be harnessed for oral rehydration therapies) should be studied.

Research Strategies

In our view, the single most important strategy is to increase the number of people engaged in research on diarrheal diseases and to improve access to, and the quality of, the career path these people will follow. It is hoped that funds can be made available to investigators proposing well-founded and hypothesis-driven basic research. The funding of investigators in the early stages of their career path should be considered a priority.

Study Mechanisms and Regulation of Signal Transduction Between and Within Cells and Noncellular Components of the Intestinal Mucosa

Research Goals

Studies should define the interactions between microbes, epithelial cells and subepithelial cells. Examples

of such interactions are the mechanisms and regulation of microbial pathogenesis; inflammatory diarrhea; mucosal immunology; and the sequelae of infection, including hemolytic uremic syndrome, arthropathy and Guillain-Barre syndrome. In addition, interactions between central or enteric nerves and epithelial cells should be defined. Other objectives of these studies include elucidation of the cross-talk between epithelial cells, the extracellular matrix, rnesenchymal cells and subepithelial immune-competent cells. Finally, cytokine and chemokine actions in the intestinal mucosa should be identified.

Research Strategies

Research strategies are as stated above. The single most important strategy is to increase the number of people engaged in research on diarrheal diseases and to improve access to, and the quality of, the career path these people will follow. It is hoped that funds can be made available to investigators proposing well-founded and hypothesis-driven basic research. The funding of investigators in the early stages of their career path should be considered a priority.

Study Mechanisms and Regulation of Macromolecular Transepithelial Transport

Research Goals

An understanding of macromolecular transepithelial antigen transport is important for the development of mucosal vaccines. Knowledge of immunoglobulin transport is helpful in achieving an understanding of host defense mechanisms.

Study Mechanisms of Epithelial Differentiation, Proliferation, Restitution and Repair

Research Goals

Studies should define the determinants, maintenance and development of the crypt/villus axis. What factors regulate and account for the developmental programs of each major cell lineage? A second research goal is an understanding of the regulation of epithelial restitution after infections and ischemic and toxic injuries. Finally, mechanisms of epithelial adaptation should be studied: how epithelial cells respond to hypoxia, nutrient deficiency or excess, and an altered microbial flora.

Delineate the Developmental Biology of the Gastrointestinal (GI) Tract

Research Goals

Studies should search for epithelial stem cells and define the influence of gut flora.

Study the Pathophysiology of Specific Disease States

Research Goals

Studies should characterize the molecular and cellular pathophysiology of congenital diarrheal diseases, including microvillus inclusion disease and tufting enteropathy; autoimmune enteropathy; and congenital transport defects. In cystic fibrosis patients, intestinal consequences should be studied, including effects on the epithelium of secretory defects, malabsorption and pancreatitis. Additionally, the pathophysiology of meconium ileus should be investigated, as well as approaches for overcoming the secretory defect. Finally, the molecular and cellular pathophysiology of intestinal allergy should be characterized.

Focus on Vaccine Development

Research Goals

Studies should characterize vaccines against infections by rotavirus, *Vibrio cholerae, Salmonella, Shigella,Campylobacter,* pathogenic strains of *Escherichia coli*, parasites and human immunodeficiency virus (HIV). Research goals include orally administered vaccines that target other mucosal surfaces, such as the respiratory and genitourinary tracts.

AREAS OF EMPHASIS: Clinical Research

Evaluate Promising New Technologies and Medications

Research Goals

Emerging modalities with high therapeutic potential include prebiotics, probiotics and small and macromolecules exhibiting potent antidiarrheal or anti-inflammatory activity. The treatment of *E. coli* O157:H7 colitis and prevention of the hemolytic uremic syndrome are high priority. Novel nutritional or hormonal therapies for the short-gut syndrome should be targeted.

Research Strategies

Multicenter networks need to be developed well as for information-sharing.

Conduct Outcomes Research on Existing Technologies

Research Goals

Outcome research should evaluate access to, and use of, available health services in the management of acute

and chronic diarrheas. The impact of managed care is an important research goal. In addition, studies should determine the value of available therapeutic approaches, such as improved oral rehydration solutions and non-antimicrobial therapy for diarrheal disease. Finally, cost-effectiveness studies to be considered in specific diseases include the treatment of diarrhea in patients with HIV infection and nutritional management of necrotizing enterocolitis.

Research Strategies

Multicenter networks need to be developed to ensure adequate pediatric study populations as well as for information-sharing.

AREAS OF EMPHASIS: Epidemiologic Research

Epidemiologic research goals focus on disease surveillance and assessment of the health and economic impact of existing technologies and delivery systems.

Measure the Distribution of Disease (Surveillance) to Detect Emerging Pathologies and Determine the Economic Burden of Diarrheal Diseases on Children and Their Families

Research Goals

These studies provide surveillance of diseases not addressed by the Centers for Disease Control and Prevention (CDC), which cover infectious diseases only if they are "reportable."

Projected Timetable and Funding Requirements

Studies are ongoing.

HEALTH AND ECONOMIC OUTCOMES

Diarrheal disease is a major cause of morbidity, with incidence rates ranging from 2 to 12 or more illnesses per person per year in developed and developing countries. GI and nutritional diseases are among the most common causes of death worldwide, causing an estimated 3 million to 5 million deaths each year. Most of these fatalities occur in children younger than 5 years of age (1). An estimated 12,600 children die each day in Africa, Asia and Latin America due to diarrheal diseases (2).

In the US and other industrialized countries, deaths from GI diseases are less common, but the morbidity associated with both acute and chronic intestinal diseases remains substantial. All children are infected with rotavirus during the first few years of life. Rotavirus infections account for 30% to 50% of hospitalizations for diarrhea in children younger than 5 years of age and for 40% to 77% of hospitalizations in those aged 5 years and older in Europe and the US (3). Each year, rotavirus infections result in more than 3 million cases of diarrhea, 400,000 outpatient visits, 160,000 emergency department visits, 50,000 to 70,000 hospitalizations, and 120 to 200 deaths (4,5). Rotavirus admissions at one referral hospital were associated with outlays of $1.5 million annually (6) and $289 per rotavirus episode for outpatient management. More than half of this expense was due to lost productivity of the parents of sick children. Altogether, the annual cost of rotavirus infections in the US alone has been estimated at $352 million for inpatient care (not including lost productivity at work) (6) and up to $1.08 billion for outpatient care (7).

The economic impact of diarrhea due to other intestinal infections is less well documented. One analysis based on community surveys and national interviews placed the annual incidence of acute diarrhea at almost 100 million episodes, with 50% leading to at least one day of restricted activity or missed work (8). Associated medical costs were estimated at $1.25 billion, with an additional $19 billion in lost productivity at the workplace (8). Food-borne infections also exert a significant impact on heath care expenditures. The total cost for an outbreak of hepatitis A has been projected at more than $800,000 (9). Direct medical expenditures during food-borne epidemics are often dwarfed by the additional cost to society of disease prevention efforts, public health responses, insurance and legal expenses.

With respect to chronic diarrheal diseases, the lifetime cost of medical care for a person with Crohn's disease was estimated recently at $39,000 to $125,000 (10). Total direct and indirect costs for all patients in the US with inflammatory bowel disease (IBD) may reach $2 billion dollars annually (11), with nearly 10% to 25% of adults with IBD unable to work full time.

CONCLUSIONS

Of the recommendations in basic research, selected priorities include studies of a) microbe-epithelial-subepithelial interactions; b) the mechanism and regulation of vectorial solute transport and barrier function, including epithelial restitution after injury; and c) the pathobiology of, and potential therapies for, disease caused by *E. coli* O157:H7.

Of the clinical research recommendations, selected priorities include a) clinical trials of probiotic therapies and other antidiarrheal or anti-inflammatory agents, and b) vaccine development for enteric pathogens.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Acid-Peptic Diseases

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

Research priorities in acid-peptic diseases encompass clinical studies in children with gastroesophageal reflux disease (GERD) and those with peptic ulcer disease. Gastroesophageal reflux (GER) is the passive retrograde movement of gastric contents into the esophagus above the lower esophageal sphincter (LES). Physiologically, in both children and adults, reflux occurs as a result of transient relaxations of the LES and inhibition of lower esophageal body peristalsis. Reflux is not primarily related, as previously thought, to lower LES pressure. At least one episode of GER occurs daily in two thirds of infants younger than 4 months of age; by 1 year of age, only 5% of infants have daily reflux. It is clear that in addition to more subtle clinical presentations, toddlers will often manifest the classic signs and symptoms of GERD (1). Based on a study by Nelson et al, 7% of children between the ages of 3 and 9 report symptoms such as heartburn, epigastric pain and regurgitation (2). Approximately 35% of adults have heartburn on a regular basis. In a recent study, up to 50% of adults in Canada and the US have self-reported GERD symptoms weekly. In most adults, GERD is a chronic condition that waxes and wanes during their lifetime. Little information is available regarding the relationship between GER that presents during infancy and early childhood and the development of chronic heartburn and esophageal disease, both macroscopic and microscopic, in an adult.

The gold standard for diagnosing and quantitatively assessing the severity of gastroesophageal reflux and GERD in children remains to be determined. Prolonged intraesophageal pH study, performed by placing a pH probe in the distal esophagus, identifies and quantifies acidic gastric fluid within the lumen of the esophagus. This technique determines not only the frequency but also the duration of reflux episodes. Newer approaches, such as impedance techniques, need to be validated in pediatric patients. Ultimately, such studies may provide vital new information to help guide physicians in managing patients with GERD.

Although much progress has been made in understanding GERD in children, many questions remain unanswered. Based on single-center studies of small cohort size, the prevalence of GER in infants under the age of 3 months is estimated to be in excess of 85%. Premature infants, in particular, are susceptible to GER. The overwhelming majority of infants younger than 3 months have physiologic reflux and do not require medical intervention. Approximately 10% have pathologic reflux, with esophageal manifestations (irritability, feeding refusal, arching, crying during feeding) and extraesophageal manifestations (apnea, bradycardia, reactive airways disease/asthma, impaired growth). Additional research is needed to more fully understand the natural history of GERD to enable the identification of children at risk for serious sequelae, including erosive esophagitis, Barrett's esophagus and esophageal carcinoma. Studies also are needed to determine the optimum dosing regimens for currently available acid-suppressive and prokinetic medications.

Peptic ulcer disease affects approximately 4 million people annually, with some estimates as high as 10 million per annum. For much of the past century, it was thought that gastroduodenal ulcers are caused by multiple predisposing factors, the most important being hypersecretion of gastric acid. Based on this paradigm of peptic ulcer disease, mucosal ulceration develops as a result of an imbalance between acid and cytoprotective mechanisms. Factors such as diet, medications toxic to the gastric mucosa and genetic predisposition were also thought to contribute to the development of peptic ulcer disease. Since the discovery of *Helicobacter pylori* and its relationship to gastritis and peptic ulcers, dramatic changes have occurred in our understanding of the pathobiology, diagnosis and treatment of peptic ulcer disease (3–5).

Since 1983, many studies have confirmed the presence

of *H pylori* in association with antral inflammation. It is now clear that *H pylori* infection is primarily acquired during childhood. In most children, the infection will persist for most of their lifetime. Ultimately, a subset of *H pylori*-infected children are at risk for the development of peptic ulcer disease and possibly even gastric cancer in adulthood. However, most individuals infected with *H pylori* do not experience symptoms or manifest signs of disease and are unaware of the infection over the course of their lives.

The diagnosis of *H pylori*-associated diseases can be reliably established only through the use of gastroduodenal endoscopy with biopsy. Commercial serologic and other noninvasive tests that are currently available have not been proven sufficiently reliable for use in screening children for the presence of *H pylori*. Serologic testing is not recommended to diagnose *H pylori*-associated diseases. Primarily because of a striking paucity of well-controlled studies, eradication therapy is recommended only for infected children with duodenal ulcer, gastric ulcer, gastric lymphoma and atrophic gastritis with intestinal metaplasia. This approach may be too conservative in light of recent data that continue to link gastric carcinoma in adults to the acquisition of *H pylori* early in childhood. Thus, a number of important study objectives remain.

AREAS OF EMPHASIS: GERD

Clarify further the pathophysiology of GERD

Refine our understanding of the natural history and epidemiology of GERD to allow for the identification of children predisposed to developing significant GERD as an adult

Identify cost-effective methods for the diagnosis of GERD

Perform studies of upper GI motility and gastric acid physiology and establish correlations with reflux signs and symptoms. Determine whether there is a correlation between endoscopic and histologic evidence of GERD.

Establish clinical correlates for signs and symptoms of GERD

Determine appropriate and efficacious therapies for GERD

Evaluate the role of lifestyle therapies at various ages. Determine optimal dosages of acid-suppressive and prokinetic medications for use in children.

Establish the role of, and appropriate indications for, antireflux surgery

Evaluate long-term outcomes of antireflux surgical procedures.

Determine the relationship in children between H. pylori infection, gastritis and GERD

Determine whether eradication therapy alters the natural history of gastric and esophageal mucosal disease, as well as reflux signs and symptoms.

Research Goals

The pathophysiology, natural history and management of GERD in children have not been well studied (6). Investigations focusing on upper GI tract motility and gastric acid secretion will enhance our understanding of pediatric GERD. Longitudinal and cross-sectional studies are needed to determine which children with physiologic reflux go on to develop significant sequelae as teenagers and adults. Clinical trials are needed to determine pediatric dosing requirements for antacids, histamine-2 (H_2) receptor antagonists and proton pump inhibitors for the successful resolution of GER symptoms and disease. Objective criteria need to be established for when to recommend an antireflux surgical procedure in children. Finally, it is critical to determine if *H pylori* eradication in children influences the signs and symptoms as well as natural history of GERD.

Research Strategies

To address these critical questions, a number of methodologies must first be developed and validated for use in children. These include a) a method for localization of the LES; b) standardization of diagnostic testing, using either noninvasive methods, 24-hour pH studies or endoscopy with biopsy; and c) an instrument to assess the severity of GERD symptoms. All these methodologies are to have reproducible results. The symptom severity instrument should assess signs and symptoms related to a) regurgitation or vomiting and b) pain, as well as include endoscopic and histologic findings. As part of this methodology, subjective assessments may be correlated to results of a validated 24-hour pH probe analysis.

A multicenter collaborative approach would be most appropriate for ensuring that thorough longitudinal and cross-sectional studies are performed. Family cohort studies and population-based epidemiologic studies may also be useful in answering the above questions.

Projected Timetable and Funding Requirements

These studies may be funded by industry; the National Institutes of Health (NIH), in particular the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), National Institute of Child Health and Human Development (NICHD), and National Institute of Allergy and Infectious Diseases (NIAID); and private foundations. Partnerships with companies currently marketing H_2-receptor antagonists, proton pump inhibitors and prokinetic agents are to be considered.

AREAS OF EMPHASIS: H pylori

Determine whether *H pylori* eradication is effective in resolving upper gastrointestinal (GI) symptoms (e.g.,

chronic or recurrent abdominal pain) in infected children
Determine the relationship between *H pylori* infection and esophageal disease
Identify specific patient groups at increased risk for infection and serious sequelae
Determine the mode of transmission of *H pylori*

Research Goals

Well-controlled studies can expand the therapeutic options for a number of prevalent pediatric conditions, including upper GI symptoms (e.g., chronic or recurrent abdominal pain), extragastrointestinal manifestations (e.g., short stature, growth disturbances, co-infection by other enteric microorganisms such as *Salmonella*) and esophageal symptoms.

Research Strategies

Multicenter, randomized, placebo-controlled trials are recommended for the first two questions. The treatment trial in abdominal pain will evaluate infected children with gastritis and abdominal pain with or without associated peptic ulceration. Follow-up is to include both endoscopy and urea breath testing at 3 months and breath testing at 1 year.

To study the role of *H pylori* infection in esophageal disorders, the recommended protocol will enroll children with gastric and esophageal disease and compare them with children having only gastric disease. Initial follow-up is to include endoscopy and urea breath testing at 3 months. Subsequent follow-up with breath testing at 1 and 2 years in conjunction with esophagogastroduodenoscopy will assess the development or resolution of esophageal disease.

The question of transmission may be studied by means of a family cohort protocol utilizing an infected child as the index case and then evaluating immediate family members. A randomized controlled trial could then be initiated with treatment of family cohorts and just index cases and then reinfection rates evaluated by urea breath testing. It is recommended that the study involve multiple centers in different countries, thereby enhancing catchment of populations with different prevalence rates for *H pylori* infection.

Projected Timetable and Funding Requirements

As with investigations in GERD, these studies may be funded by industry, the NIH (in particular, NIDDK, NICHD and NIAID), and private foundations. Partnerships with companies currently marketing H_2-receptor antagonists, proton pump inhibitors and prokinetic agents are to be considered.

HEALTH AND ECONOMIC OUTCOMES

The economic consequences of acid-related disorders in infants and children have not been well studied. While health care costs for adult patients with acid-related disorders cannot be extrapolated to children, the adult experience nevertheless provides a framework for assessment. In adults with GERD, a prospective study reported that open antireflux surgery accounted for >90% of direct medical costs (7). In this 1998 cost analysis from Scandinavia, direct medical costs represented 53% of the total cost, with loss of productivity and other indirect costs accounting for the remainder. In a retrospective analysis of 1,550 adults enrolled in a US managed care organization, health care costs associated with peptic ulcer disease were reported to be higher than costs for GERD, with inpatient charges representing a significant cost factor (8).

In the pediatric population, uncomplicated GER is associated with a benign course and favorable prognosis. However, GER in infants may lead to apparent life-threatening events (ALTE), and there is a high association with apnea, feeding intolerance and chronic respiratory disorders. Indeed, pediatric GERD exerts a significant impact on health care in Canada and the US, and fully 10% of all pediatric hospital admissions are related to GERD. US hospital data for 1997 estimated that 77,560 patients aged 17 years or younger were discharged with a diagnosis of esophageal reflux (representing 22% of all discharges for esophageal reflux), after a mean hospital stay of 3.9 to 5.7 days and accumulating a mean charge of $7,032 to $13,507 per person (9).

In the same US survey, a total of 51,871 patients aged 1 to 17 years were discharged in 1997 for gastroduodenal ulcer (except hemorrhage) (9). In that year, pediatric patients accounted for only 1% of all hospital discharges for gastroduodenal ulcer (except hemorrhage). However, when hospitalization was required, the mean hospital stay was 6.3 days and mean charges totaled $15,899 per person.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Endoscopy

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

The Committee recognized that endoscopy is a service and not a disease (or a related group of diseases). This dictated that a wide, panoramic "lens" be employed to view all the possible avenues of endoscopic research.

The endoscope is a vital tool for the pediatric gastroenterologist. Endoscopic studies enable visualization of the mucosa of the upper and lower gastrointestinal (GI) tracts. Tissue sampling and fluid collections broaden the diagnostic capability of the pediatric endoscopist. Endoscopy also provides an opportunity to perform therapeutic procedures.

Upper GI tract endoscopy, with appropriate tissue sampling and fluid collections, can establish the diagnoses of esophagitis, Barrett's esophagus, gastroesophageal varices, hiatal hernia, focal and diffuse gastritis (e.g., *H pylori*-related gastritis), gastric outlet obstruction, duodenal ulcer disease, duodenitis (acid-mediated, infectious or nonspecific) and diffuse enteropathies (e.g., celiac disease). Colonoscopy allows for the diagnoses of infectious and nonspecific inflammatory processes of the distal ileum and colon. Obstructing lesions, vascular malformations and intraluminal masses can be identified at the time of colonoscopy. Endoscopic retrograde cholangiopancreatography (ERCP) provides a means of identifying obstructing and inflammatory processes in the hepatobiliary and pancreatic ducts of infants and children. Diagnostic ERCP also identifies congenital abnormalities and sites of duct disruption or leakage after injury from trauma or surgery.

Therapeutic endoscopy enables dilation of esophageal strictures, ligation of bleeding esophageal varices, percutaneous placement of feeding gastrostomy tubes, direct control of intragastric and duodenal bleeding lesions, insertion of nasojejunal or gastrojejunal feeding tubes, removal of upper and lower GI polypoid masses, ablation of upper and lower GI tract vascular malformations, placement of percutaneous cecostomy buttons (for antegrade colonic enemas) and dilation of colonic strictures. ERCP enables dilation of biliary tract and pancreatic duct strictures, with or without stenting; removal of common bile duct or pancreatic duct stones; sphincterotomy; pseudocyst drainage; and stenting to divert biliary leaks.

AREAS OF EMPHASIS

Develop a North American Pediatric Endoscopic Database System

Research Goals

Many endoscopic practices in pediatric gastroenterology are modeled after adult patterns. This is recognized as being suboptimal. Currently, there are no available data on pediatric endoscopic activities at a national level. There are no outcome data regarding the medical effectiveness or cost-effectiveness of endoscopic intervention in pediatric patients.

Research Strategies

Seed money is expected for a *pilot* study of pediatric endoscopic outcomes data, referred to as PEDS-CORI (*P*ediatric *E*ndoscopy *D*atabase *S*ystem—*C*linical *O*utcomes *R*esearch *I*nitiative). This system will provide not only a simple and accurate endoscopy report but also a comprehensive, computerized database for research inquiries. Recently, information from the CORI database, which is virtually identical to the PEDS-CORI template, was published in a review of patterns of adult endoscopic use in the US (1). A pediatric endoscopic database can be an important resource for future ongoing research in endoscopy by documenting current practice patterns and changes over time.

For a data bank to be effective, however, we must establish uniformity in the conduct of procedures, interpretation of endoscopic findings, interpretation of histologic data, and correlation of endoscopic and histologic findings. For example, Hetzel's classification of mucosal findings of esophagitis (2), commonly used in both adult and pediatric management effectiveness studies, does not truly fit the findings in pediatric patients and thereby illustrates the need for relevant pediatric endoscopic

data. A system like PEDS-CORI would generate photographic images, included in the report, which allow objective review of the mucosal findings and provide reproducible research results. Establishment of a data bank of this type will also have a positive impact on endoscopy education. The PEDS-CORI project will serve as a template for national collaborative research in pediatric GI diseases.

Projected Timetable and Funding Requirements

The projected time frame is 3 years. Funding requirements are estimated at $150,000 per year. Ongoing funding for the PEDS-CORI project is already in place.

Critically Examine Indications, Findings and Outcomes of Endoscopic Procedures

Research Goals

Studies would critically examine the indications, findings and outcomes of procedures, and then assess a final value for each procedure. The data generated could be employed in health economic modeling to determine whether the cost of endoscopy is positively balanced by the establishment of a precise diagnosis. Such data are also useful in the development of evidence-based management strategies. This study should also enable generation of accurate data on the relevant cost of doing business.

Research Strategies

Longitudinal multicenter collaborative studies are appropriate. Multicenter protocols for the endoscopic assessment of common pediatric conditions would encompass endoscopic findings, results of pre-set histologic studies, assessment of professional and patient satisfaction, and total costs.

Multicenter studies are also recommended to compare the use of conscious sedation versus general anesthesia. Protocols would include a) predesigned objectives for each clinical indication and b) clinical outcomes, whether assessed by the study group or by patients. A study comparing total costs would permit identification of clinical scenarios in which conscious sedation is equally effective with general anesthesia and those in which general anesthesia is preferred.

Projected Timetable and Funding Requirements

The projected time frame is 3 years.

Develop Tools for Endoscopy Training and Education

Research Goals

There is a need for efforts in three areas: assessment of Fellows, continuing medical education (CME) and the testing of clinical endoscopic competency. North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN)-wide instruments are needed to assess trainees after each endoscopic procedure in order to document improvement over time and eventual competency (3–5). Consideration should be given to the concept of "areas of excellence" for advanced training. This "research in education" project should be accomplished in pediatric endoscopic units, although some training may have to be done in adult endoscopic units.

Consideration should also be given to the development of tools for the testing of pediatric clinical endoscopic competency. This might eventually lead to certification (and possibly recertification) of pediatric clinical endoscopic competency.

Research Strategies

Through the Children's Digestive Health and Nutrition Foundation (CDHNF), NASPGHAN can create CME-accredited training programs (e.g., videos, CD-ROM and workshops) for pediatric endoscopists (6,7). An effort must be made to bring about uniformity in pediatric endoscopic expertise.

Projected Timetable and Funding Requirements

The projected time frame is 5 years.

Apply Newer Technologies in Pediatric Endoscopy

Research Goals

Increased clinical experience with the following technologies would yield additional information and facilitate medical decision-making:

Endoscopic ultrasonography (EUS) is rarely performed in pediatric patients, yet it would allow further investigation of subepithelial lesions and lesions in areas adjacent to the upper and lower GI tracts. EUS combines the diagnostic imaging capability of ultrasonography with the access afforded by endoscopy. Composite instrumentation permits the placement of a piezoelectric transducer directly adjacent to the target tissue, thereby obviating the need to transmit sound waves through media that attenuate sound, such as air in the lungs, gas in the bowel or bone, as is the case with standard transcutaneous ultrasonography (8) This technology also en-

ables biopsy of these lesions. EUS can delineate the dimensions of pancreatic pseudocysts and afford an opportunity to drain these lesions. EUS is currently underused in pediatrics.

ERCP permits anatomic investigation of the biliary and pancreatic ductal systems. Other capabilities include aspiration of bile and pancreatic fluid for culture, biopsy of the biliary mucosa and cholangioscopy (9,10). Ultrathin ERCP scopes allow investigation of the biliary tree in infants with neonatal cholestasis (11). In patients with postprandial epigastric pain, ERCP with manometric measurements of the pancreatic or biliary sphincter can reveal high sphincter pressures, which can be reduced by sphincterotomy (9).

Endoscopic spectroscopy is a means of evaluating the colonic mucosa for early neoplasia in patients with longstanding inflammatory bowel disease. It can also facilitate identification of flat adenomatous lesions in patients with adenomatous polyposis coli. Further evolution of this tool might one day allow macroscopic identification of different types of inflammatory infiltrates (e.g., eosinophilic versus neutrophilic versus lymphocytic).

The primary indication for *enteroscopy* in children is the identification of small-intestinal bleeding and obstructing lesions. This is currently accomplished by collaboration with pediatric surgery and combined laparotomy/enteroscopy (12). It is conceivable, however, that newer equipment will have wider application for the evaluation of small-bowel lesions that are distal to the ligament of Treitz and proximal to the distal ileum (13). The new technology of capsule endoscopy should be explored for use in children.

Biodegradable stents for children. Self-expanding metallic stents are now available that can be compressed into a narrow device and placed with minimal or no prior esophageal dilation (14). These stents are used to palliate intrinsic and extrinsic obstructive lesions in the esophagus. In the future, expandable stents may be constructed of biodegradable materials. Biodegradable stents could be used in the management of infants and children with stenotic lesions in the esophagus (e.g., post-tracheoesophageal fistula repair, caustic injuries and peptic strictures) to reduce the number of repeat dilations.

In addition to the technologies described above, there is a need to continue the search for a child-friendly, safe and cost-effective bowel preparation regimen that permits thorough colonoscopy in all pediatric patients (15,16). Possible future technologies include robot-controlled capsule endoscopy and high-magnification endoscopy for better evaluation of mucosal detail.

Projected Timetable and Funding Requirements

The projected time frame is 5 years (but ongoing).

HEALTH AND ECONOMIC OUTCOMES

Currently there are no data to properly assess the economic impact of pediatric endoscopy on direct health care costs. Properly designed studies will clarify the impact of endoscopic procedures on the overall care of infants and children with GI dysfunction.

CONCLUSION

The studies outlined above will establish an evidence base for the appropriate use of endoscopy in the management of infants and children with GI tract pathology. Moreover, these studies will promote the standardization of endoscopic interventions in children and facilitate multicenter collaborative research programs.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Cystic Fibrosis and Pancreatic Diseases

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

Although pancreatic disease has traditionally been viewed as a problem in adults, exocrine pancreatic insufficiency and pancreatitis can occur in children as well (1–3). Pancreatitis is a clinical diagnosis. In many suspected cases, no single test or series of tests reliably confirms the diagnosis. The lack of a definitive diagnostic test for pancreatitis hampers epidemiologic and clinical studies.

Just as there is no definitive diagnostic test, there is no specific therapy for pancreatitis. Current therapy consists of supportive care (1). No intervention effectively alters the course of pancreatitis or prevents recurrent episodes. Efforts to develop new therapies will be advanced by the development of suitable animal models for pancreatitis.

Recent studies provide a potential direction for developing models of pancreatitis. Two genes and another locus have been linked to recurrent pancreatitis (2). Mutations in the gene encoding cationic trypsinogen are associated with hereditary pancreatitis. Linkage studies suggest that another gene predisposing to hereditary pancreatitis resides on chromosome 12. A large proportion of patients with idiopathic chronic pancreatitis have mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (2,4). Even though several gene mutations have been identified, the role of gene products in the pathophysiology of chronic pancreatitis remains to be elucidated and the genes have not been exploited to create animal models of pancreatitis.

Exocrine pancreatic dysfunction is present in a number of diseases in children (5). Another organ may be primarily affected, such as the liver in Alagille syndrome, but the pancreatic dysfunction contributes to clinical symptoms. In Shwachman-Diamond syndrome, pancreatic insufficiency presents in infancy and is a major clinical problem (6). For unexplained reasons, the pancreatic insufficiency improves with age. The improvement may be related to the developmental program of the pancreas. An understanding of the mechanisms contributing to the improvement in pancreatic function in Shwachman-Diamond syndrome may lead to the development of novel therapies for patients with pancreatic insufficiency.

The most common cause of pancreatic insufficiency in children is cystic fibrosis (CF) (5,7). Up to 90% of patients with CF develop pancreatic insufficiency and do not digest food adequately. The resultant malabsorption of nutrients affects growth and health. Most patients require replacement therapy with fat-soluble vitamins and pancreatic enzymes, which often do not restore fat absorption completely. Since most patients with CF do not have exocrine pancreatic insufficiency at birth, therapies potentially could be developed to arrest the process if the pathophysiology is better understood.

Although pancreatic insufficiency and pulmonary disease predominate in CF, intestinal and liver disease also can cause significant morbidity and mortality (8). Intestinal obstruction with thickened secretions can present 1) as meconium ileus or peritonitis in 10% to 15% of newborns with CF or 2) as distal-bowel obstruction at any age in about 10% of patients. Approximately 5% of patients with CF have hepatic cirrhosis, which can progress to liver failure and require a liver transplant.

AREAS OF EMPHASIS

Define the Molecular Basis of Pancreatic Disease in Cystic Fibrosis

Research Goals

Although the CFTR gene has been identified, the molecular events leading to pancreatic complications in CF have yet to be elucidated. Studies should be encouraged that analyze the effect of CFTR mutations on pancreatic function. Also to be encouraged are studies in healthy individuals that detail CFTR function at the molecular level.

The recent demonstration of increased phospholipid-bound arachidonic acid and decreased docosahexaenoic acid in the pancreas of patients with CF raises questions about the role of CFTR in fatty acid biosynthesis (9). The consequences of an imbalance of fatty acids on the function of other membrane proteins in the pancreas also should be evaluated.

CFTR dysfunction results in increased levels of some cytokines and decreased levels of others, leading to excessive inflammation in the pancreas and lungs. Investigations are recommended to determine the precise mechanisms by which CFTR affects the inflammatory process.

Up to 15% of patients with CF do not have exocrine pancreatic insufficiency, even though they may have CFTR mutations (5,7). Studies comparing patients with and without pancreatic insufficiency will provide insight into the mechanism of pancreatic insufficiency. The objectives of such studies include the identification of a) CFTR alleles associated with pancreatic insufficiency, b) differences in CFTR function between the two patient subgroups, and c) other genes that may modify the effects of mutant CFTR.

Research Strategies

To achieve these goals, clinical and epidemiologic studies are needed, as well as molecular, biochemical, and genetic studies of CFTR function. Clinical research studies will require multicenter participation.

Projected Timetable and Funding Requirements

These research goals will require two to four separate research groups and may take 5 to 10 years to make significant progress. Potential funding sources would include government agencies.

Develop New Therapies and Diagnostic Tests for Pancreatic Insufficiency

Research Goals

Current therapies for pancreatic insufficiency generally do not restore fat absorption to the normal range, resulting in significant loss of calories and fat-soluble vitamins (10). Tests for pancreatic insufficiency are imprecise or invasive and specialized. The widely available fecal fat analysis requires adequate dietary fat during the test, may not be suitable in young infants, and is unwieldy as a routine screening test.

Studies are recommended to:

Develop and validate a noninvasive test of pancreatic function. A simple, accurate measure of pancreatic sufficiency would improve both diagnosis and the monitoring of treatment

Evaluate alternatives to standard enzyme replacement therapy, such as gastric lipase and bacterial or fungal lipases

Identify parameters that affect the efficacy of current enzyme preparations

Develop potential therapeutics that may halt or reverse the progression to pancreatic insufficiency

Develop new vectors that have potential applications in gene therapy for pancreatic disorders and

Develop screening programs to identify newborns with CF

A promising new therapy is docosahexaenoic acid, which has been shown in CFTR-deficient mice to reverse pathologic changes (9). Future evaluation of this therapy would hasten clinical trials in humans.

The ability to reliably identify newborns with CF will be critical if potential therapies are developed that prevent progression to pancreatic insufficiency.

Research Strategies

Both laboratory and clinical studies are needed to develop and validate noninvasive tests of pancreatic function. Clinical trials of alternatives to standard enzyme replacement therapy are important. Several of the proposed research goals require multicenter clinical studies. Further studies in CFTR-deficient mice are warranted to confirm the safety and efficacy of docosahexaenoic acid in reversing pathology. In addition, studies are needed to develop a formulation that is well absorbed in humans.

Projected Timetable and Funding Requirements

The development of diagnostic tests and therapies for pancreatic insufficiency will require two to four separate research groups and may take 5 to 10 years to make significant progress. In addition to government funding agencies, industry sources could contribute to implementation of several research strategies, including studies aimed at improving therapy for pancreatic insufficiency.

Characterize the Pathophysiology of Liver and Intestinal Disease in Cystic Fibrosis

Research Goals

Liver and intestinal disorders cause significant morbidity and mortality in patients with CF (8). However, little is known about the mechanisms underlying these complications. The prevention and treatment of hepatic and intestinal disorders in patients with CF can be accomplished only through a better understanding of the underlying pathophysiology.

An important research goal is to understand the contribution of hepatic and intestinal function to the malabsorption seen in patients with CF. Studies may reveal important insights into the mechanism of malabsorption and advance the development of new therapies.

Risk factors, including genetic factors, for the development of hepatic or intestinal complications need to be identified. Patients in whom risk factors are present can be given appropriate anticipatory guidance. Therapies to prevent or ameliorate these complications can be evaluated only if high-risk patient populations are identified.

Another research goal is to understanding the function of CFTR in the intestinal and biliary epithelium and its relationship to liver and intestinal disease. Studies should include, but not be limited to, defining the transport properties of CFTR, the relationship of CFTR to intestinal mucins, and the regulation of CFTR function.

As the lifespan of patients with CF increases, additional complications may develop. There is evidence to suggest that patients with CF have an increased cancer risk, but the actual risk is unclear. Studies are needed to determine the risk of liver cancer associated with cystic fibrosis.

Research Strategies

Laboratory, epidemiologic, and clinical studies are encouraged to understand the contribution of hepatic and intestinal dysfunction to the malabsorption seen in CF. These studies could provide important insights into the mechanism of malabsorption, which may translate into new therapies. Several clinical studies will require multicenter participation. Epidemiologic studies are needed to determine whether patients with CF are at risk of liver cancer.

Projected Timetable and Funding Requirements

As with the other areas of pancreatic disease research, it is estimated that the research goals described above will require two to four separate research groups and take 5 to 10 years to make significant progress. Funding sources potentially include the pharmaceutical industry and government agencies.

Characterize the Clinical Features of Childhood Pancreatitis

Research Goals

The identification and treatment of children with pancreatitis will be greatly aided if the clinical features of the disorder are well documented. Our current knowledge is based on retrospective studies and there are critical problems with case definitions. Studies are needed that:

- Characterize the epidemiology and clinical presentation of childhood pancreatitis, with investigations addressing incidence, natural history, risk factors (environmental and genetic), optimal diagnostic criteria (biochemical and/or radiographic measures), and prognosis
- Identify genes that act alone or interact with known trypsinogen or CFTR mutations to predispose patients to acute or recurrent pancreatitis
- Determine the role of endoscopic retrograde cholangiopancreatography and magnetic resonance imaging in the diagnosis of pancreatitis

Research Strategies

Multicenter epidemiologic studies are encouraged to characterize childhood pancreatitis. Genetic studies can identify genes or CFTR mutations that predispose an individual to acute or recurrent pancreatitis (2,4).

Projected Timetable and Funding Requirements

As with the other areas of pancreatic disease research, it is estimated that the research goals described above will require two to four separate research groups and take 5 to 10 years to make significant progress. Funding sources potentially include the pharmaceutical industry and government agencies.

Determine the Pathogenesis of Acute and Chronic Pancreatitis

Research Goals

The lack of a suitable animal model for pancreatitis has greatly hampered investigations into its pathophysiology, particularly in defining the early events of pancreatitis. Current animal models do not faithfully recapitulate the characteristics of human pancreatitis and often produce fulminate pancreatitis, which makes studies of early events impossible (11). A greater understanding of the genetics of pancreatitis should guide the creation of new animal models that mimic the events of the disease in humans and provide an opportunity to delineate the pathophysiology.

Research Strategies

Basic science studies are needed to define the molecular basis of gene regulation and to describe the biologic mechanisms for known gene mutations that predispose to pancreatitis. Animal models are required in which pancreatitis is induced using transgenic or gene ablation technology.

Projected Timetable and Funding Requirements

As with the other areas of pancreatic disease research, these research goals will require two to four separate research groups and may take 5 to 10 years to make significant progress. Funding sources include government agencies.

Further Clarify the Molecular Events Governing Development of the Pancreas

Research Goals

A detailed examination of the molecular events governing pancreatic development should lead to novel therapies to improve nutrition in premature infants and to restore pancreatic acinar tissue in patients with chronic pancreatitis. Studies are recommended to:

Identify genes that govern the differentiation of pancreatic stem cells into the various cell types in the pancreas

Identify genes that determine the development of the ventral and dorsal pancreas and that regulate the fusion of the two glands and their ducts

Define the molecular regulation of pancreas-specific genes. In particular, studies are needed to determine the factors regulating the temporal expression of these genes and means of altering that expression.

Research Strategies

Genetic studies are needed to achieve these research goals.

Projected Timetable and Funding Requirements

Each research goal may require two to four separate research groups and take 5 to 10 years to make significant progress. Funding sources potentially include the pharmaceutical industry and government agencies.

Determine the Mechanism of Pancreatic Dysfunction in Disorders Other than Cystic Fibrosis

Research Goals

Characterization of pancreatic dysfunction in disorders other than CF will provide insight into the onset of pancreatic insufficiency in more prevalent diseases and could provide clues to the development of novel therapies that may retard or even reverse the progression to pancreatic insufficiency. Studies are recommended to:

Identify the gene(s) responsible for Shwachman-Diamond syndrome

Define the clinical features of Shwachman-Diamond syndrome

Determine the mechanisms of pancreatic dysfunction in Alagille syndrome, celiac disease and other illnesses in which pancreatic complications are not the primary manifestation

Determine if CFTR contributes to pancreatic dysfunction in these disorders

Research Strategies

Genetic as well as multicenter epidemiologic studies are needed to characterize these disorders.

Projected Timetable and Funding Requirements

As with the other areas of research emphasis, these research goals will require two to four separate research groups and may take 5 to 10 years to make significant progress. The potential for development of targeted therapies suggests that the pharmaceutical industry as well as government agencies may be interested in providing funding.

HEALTH AND ECONOMIC OUTCOMES

Cystic fibrosis affects approximately 30,000 children and adults in the US (12). One in 31 Americans, or about 10 million people, carry the gene for CF (13,14). If two carriers of this recessive gene marry, 1 in 4 of their children will have CF. In 1995 the cost of medical care for a patient with CF was about $40,000 a year. Total medical costs related to cystic fibrosis approached $900 million annually. The burden to the family extends beyond the medical costs. For example, frequent visits to the physician and frequent hospitalizations cause parents to miss work. For any family with a chronically ill child, the emotional and social effects can be overwhelming and potentially lead to a breakup of the family.

The economic and social impact of other pancreatic diseases in childhood is less well documented. From the 1960s to the mid-1980s, the incidence of pancreatitis in adults increased about tenfold (1). The precise incidence in children is not known. Among pediatric gastroenterologists, there is an impression that the incidence is increasing, but this has not been documented. As with families of children with CF, the families of children with recurrent pancreatitis face significant burdens socially and emotionally. Acute pancreatitis accounts for 3% to 5% of hospital admissions, and mortality from pancreatitis can reach about 10% in adults (1). Acute pancreatitis is a significant cause of hospitalization in children, and as a frequent complication of systemic ill-

nesses, pancreatitis can result in prolonged hospitalization and associated morbidity.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Motility Disorders and Functional Gastrointestinal Disorders

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

Motility disorders and functional gastrointestinal (GI) disorders are interrelated. The first category refers to disordered esophageal, gastric, intestinal and colonic motility and encompasses achalasia, gastroparesis, Hirschsprung's disease and chronic idiopathic intestinal pseudo-obstruction (CIIP). The second category encompasses disordered swallowing, vomiting, diarrhea, defecation and functional abdominal pain syndromes such as functional dyspepsia and irritable bowel syndrome (IBS).

Both motility disorders and functional GI disturbances are exciting and fertile areas of current research. New Rome diagnostic criteria for *pediatric* functional GI disorders were published in 1999 and require validation and correlation with pathophysiologic findings. There has been an explosion of relevant basic science investigations in enteric neurobiology, genetics, brain-gut mapping and receptor physiology.

Clinical research infrastructure. Progress in our understanding of pathophysiology and in the development of effective therapies will be facilitated by establishment of a clinical research infrastructure to support collaborative interdisciplinary research endeavors. In particular, such an infrastructure can provide long-term support for urgently needed prospective multicenter trials that can serve as the evidence base for development of diagnostic and therapeutic algorithms.

In addition, consensus conferences are recommended to establish a) standardized diagnostic criteria for symptom-based diagnoses, b) subgrouping/stratification schemes, as well as c) clinical and pathophysiologic testing protocols for both motility disorders and functional disorders. Efforts in some of these areas have begun with the aid of the American Motility Society, Cyclic Vomiting Syndrome Association, National Institutes of Health (NIH), and the Rome Committee for functional GI disorders.

Diagnostic tests and techniques used to evaluate motility disorders and functional GI disorders would be more widely accepted if technical protocols and methods of interpretation were validated and standardized. Such techniques include electrogastrography (EGG) for gastric dysrhythmias, impedance measurement in gastroesophageal reflux (GER), the barostat for visceral hyperalgesia and antroduodenal and colonic manometry for motility disorders.

Fellowship and post-fellowship training. To develop a cadre of future clinical and basic science researchers, opportunities must be created for young pediatric gastroenterology investigators for training and mentoring in motility, functional GI and laboratory research. Fellowship and post-fellowship training with senior investigators is recommended in the areas of GI motility, sensory evaluation, functional neuroimaging and functional GI disorders.

Centers of excellence. There is a need to develop regional and national centers of excellence with combined clinical expertise and ongoing research protocols in motility disorders and functional bowel disorders.

AREAS OF EMPHASIS

Define Mechanisms Leading to Feeding Intolerance in Preterm Infants and Evaluate Potential Interventions to Improve Feeding

Research Goals

The study of oropharyngeal development and GI motility in preterm infants can identify mechanisms that underlie feeding intolerance and assist in discovering means of optimizing maturation of these mechanisms. In addition, intervention trials are needed to improve feeding in these patients. Studies could determine the optimal

macronutrient composition of feeding regimens, use of bolus or continuous infusion, and nutritional manipulations to enhance gastric emptying and antroanal motility. In preterm infants with comorbid disorders (e.g., congenital anomalies or bronchopulmonary dysplasia with intubation) who cannot tolerate oral feeding, the barriers to normal oromotor development must be defined: Is there a narrow window of normal development? Or does aversion to oral feeding result from use of instrumentation?

Research Strategies

Feeding intolerance can be studied by means of retrospective and case-control trials in which antroduodenal motility and swallowing studies are done. Interventions can be evaluated in prospective randomized controlled trials.

Projected Timetable and Funding Requirements

Single-center studies and multicenter collaborations may be undertaken with NIH funding. Applications can be made for R01 (individual award) grants, investigator-initiated interactive research project grants (IRPG), R03 (small clinical trials) grants, F32 grants (or National Research Service Awards, NRSA, for training fellows) and PHS 6246-2 grants (small business innovation research, SBIR, for innovative technologies and approaches).

Consensus conferences could be convened to establish diagnostic criteria and standardized evaluation and treatment protocols. Efforts may be funded by combined support from the NIH (R13 grants for support of scientific meetings), interested foundations and the pharmaceutical industry.

Research efforts should be encouraged in partnership with societies and foundations such as the American Motility Society, Cyclic Vomiting Syndrome Association and International Foundation for Functional Gastrointestinal Disorders.

Evaluate Oromotor Discoordination and Feeding Aversion in the Older Child and in Neurologically Impaired Children

Research Goals

Studies are needed to evaluate and develop treatments for oromotor discoordination and feeding aversion in these patient subgroups. Key research questions in dysphagia include the role (and reliability) of videofluoroscopic swallowing studies, the role of interdisciplinary evaluation, and whether medical and behavioral approaches affect long-term outcome.

Research Strategies

Antroduodenal motility and swallowing studies are recommended. Potential interventions can be evaluated in prospective randomized controlled trials.

Characterize the Pathogenesis of Post-Nissen Fundoplication Complications

Research Goals

The long-term efficacy and safety profile of fundoplication in children has not been definitively studied. Studies are needed to determine the incidence and underlying mechanisms of intractable retching, bloating and feeding intolerance following fundoplication. Can children at risk be identified prior to surgery through a combination of tests? Which techniques are recommended—esophageal biopsies, prolonged pH monitoring, esophageal emptying studies, gastric emptying tests, EGG, gastric barostat, antroduodenal motility testing?

Research Strategies

Retrospective and case-control studies can be done. Multicenter studies evaluating children with refractory GER prior to antireflux surgery, coupled with long-term clinical outcomes, would be appropriate.

Characterize the Pathophysiology of Chronic Idiopathic Intestinal Pseudo-Obstruction and Evaluate Diagnostic Techniques and Potential Treatments

Research Goals

Further studies are needed in the areas of pathogenesis, subclassifications of CIIP, diagnosis and treatment. The prevalence of extraintestinal (e.g., gallbladder, pancreas and bladder) involvement of smooth muscle; the role of antroduodenal and colonic manometry in diagnosis and subclassification; the efficacy of new prokinetic agents; the utility of jejunal feedings versus parenteral nutrition—all are important clinical research questions. Basic research questions are reviewed below.

Research Strategies

For uncommon disorders such as pseudo-obstruction (and Hirschsprung's disease), a centralized tissue bank of appropriately preserved muscle tissue and blood samples should be available for cellular and histochemical studies.

The clinical research questions can be investigated by means of multicenter and individual investigator studies, using manometry, radiographic and nuclear medicine

transit studies, and intestinal tissue samples (banked). Prospective randomized controlled trials can be performed to evaluate therapeutic agents and feeding approaches.

Characterize the Pathophysiology of Functional Constipation, Hirschsprung's Disease, and Other Neurocristopathies and Neuronal Dysplasias; Evaluate Diagnostic Techniques and Potential Treatments

Research Goals

Studies are needed to define the pathophysiology, long-term outcome, diagnosis and treatment of these conditions. Important research questions include the role of motility testing (colonic manometry, radioopaque marker transit studies, anorectal manometry) in diagnosis; its utility in subclassifying patients; and the efficacy of current and novel therapeutic agents, evaluated in prospective controlled trials.

Research Strategies

For uncommon disorders such as Hirschsprung's disease, a centralized tissue bank of appropriately preserved muscle tissue and blood samples should be available for cellular and histochemical studies.

The clinical research questions can be investigated by means of multicenter and individual investigator studies, using manometry, radiographic and nuclear medicine transit studies, and intestinal tissue samples (banked). Prospective randomized controlled trials can be performed to evaluate therapeutic agents and feeding approaches.

Assess Utility of Rome Criteria for Functional Disorders and Visceral Pain Syndromes

Research Goals

Do the Rome criteria correctly discriminate between organic and functional disorders? Studies are needed to determine the frequency of missed diagnoses of organic disorders and whether the Rome criteria reduces the need for testing to rule out potentially serious disorders, shortens the time to diagnosis, and directs appropriate treatment. An interesting question is whether the Rome criteria apply cross-culturally. Other research questions include the role of the barostat in evaluating and classifying children with visceral hyperalgesia and IBS; the natural history of IBS (is there a progression from toddler's diarrhea to IBS?); a comparison of efficacy of pharmacotherapy versus cognitive and behavioral treatments; and the synchronous and metachronous incidence of different functional bowel disorders.

Research Strategies

A variety of studies can be performed, including case-control and outcome studies, cross-cultural studies, longitudinal studies, interdisciplinary studies and randomized controlled therapeutic trials.

Evaluate Pathophysiologic Mechanisms and Potential Interventions in Patients with Cyclic Vomiting Syndrome

Research Goals

A number of factors may contribute to the onset of cyclic vomiting syndrome, including migraine, mitochondriopathy, autonomic dysfunction and stress axis activation. Studies are needed to determine whether patients can be grouped into clinical phenotypes, with overlapping or distinct pathophysiologic mechanisms. Interventional studies are needed to evaluate the efficacy of current and new pharmacologic agents for prophylaxis and treatment.

Research Strategies

Questions of pathophysiology may be examined in multicenter and individual investigator studies, utilizing mitochondrial deoxyribonucleic acid (DNA) analysis, autonomic function testing and measurement of corticotropin-releasing factor (CRF). Therapeutic agents may be evaluated in prospective randomized controlled trials.

Encourage Expansion of Basic Research in Enteric Neurobiology and Development of the Enteric Nervous System (ENS)

Research Goals

Increased involvement by pediatric gastroenterologists is recommended in areas of basic research related to GI motility and functional disorders in children. Studies are needed to:

Identify factors regulating development of the ENS. Studies can target the role of interstitial cells of Cajal and intestinal stem cells in such disorders as achalasia, pyloric stenosis, CIIP, Hirschsprung's disease and constipation. The effects of actins and gut peptides also should be studied.

Characterize molecular, cellular and physiologic defects in mutant mouse strains and transgenic knockout mouse lines that are relevant to the study of motility disorders in humans.

Identify neuroendocrine interactions, e.g., the effect of mast cells (histamine) on motor function in antigen-

sensitized animal models. Afferent and efferent central nervous system and ENS pathways can be mapped using immunohistochemical stains (e.g., cholera toxin horseradish peroxidase).

Evaluate the role of CRF in gastric stasis and vomiting using specific antagonists.

Identify Histopathologic and Ultrastructural Features of Neurocristopathies and Other Lineage Defects of Intestinal Nerve and Muscle

Research Goals

Histopathology studies can evaluate the role of interstitial cells of Cajal in the pathogenesis of CIIP. In addition, these research findings will help to determine whether full-thickness biopsies are necessary in the evaluation of patients with CIIP.

Research Strategies

Collaboration with pediatric pathologists is recommended to define appropriate guidelines for the histopathologic evaluation of neurocristopathies. By correlating histopathologic findings with clinical patterns and results of functional testing, investigators can determine the subclassification of patients with CIIP and their kindreds.

Identify Genetic Markers of Neurocristopathies and Neuronal Dysplasias

Research Goals

In 20% of patients, Hirschsprung's disease has a genetic component, and some kindreds display a dominant inheritance with mutations identified in the receptor tyrosine kinase gene (ret) and endothelin receptor-B gene (ENDR-B). Studies in mutant mouse strains (piebald lethal and lethal spotted) have demonstrated mutations in the same regions (ENDR-B gene and endothelin-3).

Research Strategies

Two transgenic "knockout" mouse lines have been developed, including the ret-k- (functional-loss mutation of ret gene) and ENDR-B null. Introduction of a Lac-Z reporter gene into neural crest cells of these mice allows study of enteric neuroblasts affected by Hirschsprung-type mutations.

HEALTH AND ECONOMIC OUTCOMES

Disordered GI motility and functional GI disorders cause substantial medical morbidity in a large proportion of the pediatric population. Combined, these disorders constitute more than 50% of a pediatric gastroenterology practice. Recurrent abdominal pain accounts for 25% of pediatric gastroenterology consultations. In one report, irritable bowel syndrome affected 17% of a high school-aged population, matching the prevalence in the adult population. Constipation accounts for 3% of visits to a general pediatrician's office and 25% of referrals to a pediatric gastroenterologist. Less prevalent disorders also have significant economic consequences: health care costs for cyclic vomiting syndrome (2% of school-aged children) can total more than $17,000 per patient annually. Each year, pseudo-obstruction (100 new cases each year) accounts for more than $50,000 in parenteral nutrition costs alone. The medical consequences of prolonged parenteral nutrition in infants and children with long-segment Hirschsprung's disease and pseudo-obstruction include repeated catheter-related sepsis and total parenteral nutrition-related liver disease (with end-stage liver failure and liver transplantation).

Feeding intolerance occurs in 50% to 70% of preterm infants (weighing <1500 g and 1250 g at birth, respectively). Thus, approximately 20,000 infants are affected each year in the US. An advance by 1 week in the placement of preterm infants on full enteral feeding would save an estimated $1 billion annually in parenteral nutrition costs in the US alone.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Hepatobiliary Disorders

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

A significant health problem in children, hepatobiliary disorders include anatomic disorders (biliary atresia, choledochal cyst), autoimmune diseases (autoimmune hepatitis, primary sclerosing cholangitis), viral hepatitis (hepatitis A, B, C, delta agent, E), metabolic liver diseases and drug-induced hepatic toxicity.

Extrahepatic biliary atresia (EHBA), which affects 1 in 8,000 to 1 in 15,000 children, is a leading cause of morbidity and mortality in children with gastrointestinal (GI) or liver disease. EHBA is characterized by fibro-obliterative destruction of all or part of the extrahepatic biliary system. The etiology of EHBA is not known. Putative factors include viral infections, a genetic predisposition, immune-mediated cholangiopathy and developmental defects.

Two consensus conferences have been convened by the National Institutes of Health (NIH) to focus on extrahepatic biliary atresia (1,2). Although significant progress has clearly been made in the care of children with EHBA, the field nevertheless is only "halfway there" (3). EHBA is the leading indication for liver transplantation in the pediatric age group (4). Even though patients have relatively high survival rates as a result of management with hepatoportoenterostomy or liver transplantation (5,6), much remains to be done before the highest quality of care can be attained.

Acute liver failure. In most pediatric series, 10% of liver transplantations are performed in patients with acute liver failure. As such, between 50 and 75 children each year undergo liver transplantation for acute liver failure. In such patients, extensive hepatocyte damage has resulted in diminished liver synthetic function and encephalopathy. The condition is considered fulminant when the patient has no prior history of liver disease. Depending on the definition of acute liver failure, estimates of the incidence of this disorder range from 200 to 1000 cases each year in the US pediatric population. Viral hepatitis and drug toxicity can cause acute liver failure in children, but in many pediatric cases, the cause is unknown. The case-fatality rate for idiopathic fulminant liver failure associated with encephalopathy can reach 90%. Current therapy is limited to supportive care and liver transplantation.

Chronic viral hepatitis affects more than 300,000 children in North America. As many as 150,000 have chronic hepatitis C (HCV); yet we know little about the natural history nor is there effective therapy for pediatric patients.

Worldwide, the most common form of acute and chronic hepatitis is hepatitis B (HBV). In the US alone, 150,000 new cases are estimated each year, and about 1 million people are chronically infected with HBV. Children are much more prone to develop chronic HBV if they acquire an acute infection prior to 1 year of age. Consequently, childhood infection accounts for a disproportionate number of chronically infected individuals with HBV. Most children chronically infected with HBV will not respond to currently available therapy.

The incidence of hepatitis A peaks in children between the ages of 5 and 14 years, reaching 17 per 100,000 population (7). In 1997 a total of 30,000 cases of hepatitis A were reported to the National Notifiable Diseases Surveillance System in the US. About 7% of all children and adolescents with hepatitis A were hospitalized, resulting in an average cost of $1500 per case.

Metabolic pathways in the liver. The liver is not physiologically mature at birth. The metabolism of bilirubin is not fully developed, and numerous pathways for the disposition of drugs and toxins are still immature. While some metabolic pathways are the focus of active research, interactions between the developing liver and exposure to drugs and toxins in the environment have not been well studied. With recent advances in our understanding of hepatic drug metabolism, the stage is set for a developmental approach to investigations of adverse effects of medications and the role of the liver.

A major limitation in addressing key clinical questions has been the relatively small numbers of patients cared for at any given center. Assessment of treatment approaches and development of trial designs are predicated

on the availability of high-quality, prospectively collected data on the natural history of a disease, such as could be provided by a multicenter database. For example, the natural history of portal hypertension, especially variceal hemorrhage, could be defined prior to implementation of a multicenter interventional trial. Ascending cholangitis could be studied and its prevalence correlated with the progression of disease. Growth parameters in biliary atresia could be prospectively assessed and correlated with disease status.

AREAS OF EMPHASIS

Evaluate Efficacy of Corticosteroids After Portoenterostomy for EHBA

Research Goals

To date, no randomized treatment trials in EHBA have been performed. However, recent descriptions of center-specific excellence in the care of children with EHBA suggest that optimal clinical approaches do exist. Important research questions in the area of EHBA include the effect of steroids in post-portoenterostomy patients.

Some centers have advocated the use of corticosteroids in the postoperative care of children with EHBA. The rationale for corticosteroid use is to reduce inflammation in the bile ductules at the level of the porta, thereby enhancing bile flow.

Research Strategies

For any particular hepatobiliary disorder, no single medical center has enough patients to enable detailed study of the etiology and management. It is clear that for pediatric hepatobiliary research to advance, a mechanism for collaborative clinical research needs to be established.

A *Children's Liver Study Group* (CLSG) would provide the infrastructure to conduct multicenter clinical trials and collaborative research investigations. Such an organization would comprise a scientific advisory board, a central data coordination site, and a central biological-materials storage facility. Centers that are CLSG members would be eligible to participate in CLSG-sponsored clinical trials, contribute to a CLSG database and receive funding for a clinical research coordinator.

Members of the scientific advisory board would be selected on the basis of demonstrated expertise and commitment to pediatric hepatology. Physicians, nurses, statisticians and lay persons would be represented on the board. Board responsibilities would include organization of an annual meeting; the review and approval of research proposals, including requests to use either the central database or biological-materials bank; and the review and selection of centers to be funded.

The data coordination site is a fundamental requirement for an effective study group. This site would be a central repository for longitudinal information collected by CLSG member centers. The site would also be responsible for subsequent data analysis and for assisting member centers with research questions. The central biological-materials facility would collect and organize liver, bile, serum, fibroblast and other samples obtained from children with liver diseases. Separate supervisory panels would set up the two sites and initially review requests for materials.

Study design. With a CLSG in place, a multicenter randomized trial is recommended to evaluate the use of corticosteroids after portoenterostomy for EHBA. The proposed study would enroll infants with EHBA in whom portoenterostomy was performed before they were 10 weeks old. Subjects with known hypersensitivity to corticosteroids would be excluded.

Infants could be randomized prior to portoenterostomy to one of three arms of this study: 1) empiric and interventional corticosteroid administration, 2) interventional corticosteroid administration only, and 3) no corticosteroid administration. In the first treatment arm, empiric therapy would consist of prednisolone taper over 4 weeks after portoenterostomy. Interventional corticosteroid administration would consist of 7-day prednisolone pulse for all episodes of cholestasis, including poor postoperative drainage (acholic stools or no reduction in serum bilirubin level) and subsequent episodes of cholestasis (i.e., development of acholic stools or increase in total bilirubin levels to >5 mg/dL).

The primary study endpoint would be liver transplantation or death. Secondary endpoints would include total bilirubin levels at 12 and 24 months of age, blood culture-positive episodes of ascending cholangitis, and linear growth velocity at 12, 24 and 36 months of age.

Projected Timetable and Funding Requirements

Funding of a CLSG would be required to cover costs associated with major activities and administrative expenses in support of a scientific advisory board, central data coordination site and biological-materials bank.

A single source of funding would likely prove insufficient to cover CLSG expenses. Potential funding sources might include the NIH, organizations such as the American Liver Foundation, and pharmaceutical firms.

Evaluate Efficacy of Beta-Adrenergic Blockade in Preventing Complications of Portal Hypertension in Children With EHBA

Research Goals

In a number of adult studies, nonspecific beta-blocker therapy decreased the risk of variceal hemorrhage. Inter-

estingly, the effect of beta-blockade on portal pressure is greatest in patients without varices. Animal models have indicated that beta-blockade may actually prevent the development of portal hypertension.

Children with EHBA universally develop some stigmata of portal hypertension. Thus, they are an ideal study population for the investigation of novel approaches to the treatment and prevention of portal hypertension. Preliminary nonrandomized data suggest that beta-blockade is safe in children and may be useful in preventing variceal hemorrhage (8).

Research Strategies

A multicenter clinical trial would enroll subjects who had had a portoenterostomy for biliary atresia prior to 10 weeks of age. Subjects would be excluded if they have a known hypersensitivity to Inderal (propranolol), known reactive-airways disease, or an underlying heart condition that is a contraindication to beta-blockade.

The infants could be randomized 1 month after successful portoenterostomy to receive either propranolol or placebo. Baseline studies should include electrocardiography and Holter monitoring to assess baseline heart rate. The starting dose of propranolol could be 1.0 mg/kg administered bid or tid, with a 50% dose escalation every week until a 25% reduction in heart rate is achieved.

Primary study endpoints would be a) complications related to portal hypertension, including variceal hemorrhage, ascites, hepatopulmonary syndrome and hepatic encephalopathy; and b) either liver transplantation or death. A secondary endpoint could be the presence of esophageal and gastric varices, as documented by upper GI endoscopy, at the time of liver transplantation.

Prospectively Analyze the Etiologic Role of Reovirus, Rotavirus and Cytomegalovirus in EHBA

Research Goals

Perhaps the most fundamental unresolved issue regarding EHBA is its etiology. Reports have described fetal and perinatal forms of EHBA, which may be distinct diseases. The recent description of an entity akin to biliary atresia in a mouse with a defect in sidedness supports such a contention (9). A number of viruses are thought to play an etiologic role, including reovirus 3, cytomegalovirus and group C rotavirus. Recent advances have indicated that immune responses may also be critical in the pathogenesis of EHBA. Clearly, a better understanding of the etiology is essential for better diagnostic accuracy, improved treatment regimens and ultimately prevention of EHBA.

Research Strategies

Infants younger than 6 months of age would be recruited into a multicenter clinical study. Inclusion criteria would include EHBA, neonatal cholestasis, choledochal cyst and pyloric stenosis (sepsis would be ruled out). In subjects with a qualifying diagnosis, serum and whole-blood DNA samples would be obtained at clinical presentation and 4 months later.

Biliary tissue should be obtained in subjects with a qualifying diagnosis who undergo a clinically indicated procedure in which such biopsies might be routine (e.g., choledochal cyst repair or hepatoportoenterostomy). Liver tissue should be obtained in infants with a qualifying diagnosis who undergo a laparotomy (e.g., exploratory laparotomy for neonatal cholestasis or choledochal cyst repair). Serologic and DNA diagnostic procedures should be performed in an investigative reference laboratory that is blinded to the clinical diagnosis of the subject in question. Similarly, investigations of immune function (e.g., lymphocyte activities, antibody production) should be performed in a blinded fashion by appropriate investigative laboratories.

Analyze Molecular Events Leading to Development of Fibrosis and Cirrhosis in EHBA

Research Goals

Potential research issues of general relevance to children with liver disease include the prevention of variceal hemorrhage, the role of choleretic agents in treatment of cholestatic liver disease, and mechanisms for the prevention of fibrosis and cirrhosis. Over the past ten years, much has been learned about the molecular events contributing to the development of fibrosis (10). This knowledge needs to be examined in a comprehensive and systematic fashion in infants with EHBA. With further advances in our knowledge base, therapies might be developed that can slow or arrest the development of cirrhosis in EHBA.

Research Strategies

Children who have received a diagnosis of EHBA and undergone laparotomy would be enrolled in a multicenter study. Patients would be excluded who have uncorrectable coagulopathy or thrombocytopenia precluding liver biopsy.

Wedge liver biopsy should be obtained in children with EHBA who undergo laparotomy. Similarly, liver tissue should be obtained in children with EHBA who undergo liver transplantation. Molecular and immunohistochemical analyses of these samples would be performed.

Identify Risk Factors for Development of Acute Liver Failure of Unknown Etiology

There is mounting evidence that the causative agent of acute liver failure is a virus. The illness is preceded by a

viral infection-like prodrome, including fatigue, loss of appetite and GI complaints. Episodes of this illness tend to cluster in geographic areas and are more common in the winter months. Between 5% and 25% of patients develop hypoplastic/aplastic anemia, which is a known complication of other forms of viral hepatitis (5). An alternative theory is that the liver injury is caused by an atypical host response to a common viral pathogen. Thus far, attempts to define the epidemiology of this rare disorder have been unsuccessful.

Research Goals

Potential areas of investigation include the identification of host factors that predispose an individual to the development of severe liver injury. Studies of potential host factors might include, for example:

Screening for HLA subtypes that are more prevalent in patients who develop acute liver failure
Testing for specific genetic defects that precipitate a metabolic crisis following periods of fasting
Identifying subtle immune deficiencies that cause the host to be more susceptible to virus-mediated liver injury

Collection of serum and liver specimens from affected individuals would allow analysis of novel viral particles and future testing of the frequency and clinical relevance of newly discovered hepatotrophic viruses.

Research Strategies

A large collaborative research group focused on studying acute liver failure in children would be essential to accomplishing these goals. A multicenter case-control study of this condition in the pediatric population has yet to be accomplished.

For a matched case-control study, it is recommended that patients between the ages of 6 weeks and 18 years be enrolled who have acute liver failure, defined as progression of acute hepatitis within an 8-week period to liver dysfunction. The inclusion criteria would include a) prothrombin time > 20 seconds or international normalized ratio (INR) > 2, b) hepatic encephalopathy of any grade, c) negative serology for known hepatotropic viruses, and d) a negative workup for Wilson's disease and autoimmune hepatitis. The matched controls could be parents available for a phone interview.

Subjects would be excluded who have a history of toxic ingestion, including acetaminophen use exceeding 150 mg/kg/day, or a known history of illicit drug use. Exclusion criteria for controls would include a history of significant liver disease, toxic ingestion (including excessive acetaminophen use) and illicit drug use.

Cases would be identified by principal investigators at participating centers when patients present for management of acute liver failure. Case report forms, including demographic and exposure data, would be completed by family interview. The family would be asked to identify two peer children as control subjects. Controls would be matched for age, area of residence and school (if applicable). The parents of control subjects would be interviewed by telephone to collect the same demographic and exposure data collected for the case subject.

Blood would be collected at enrollment for a bank of serum, genomic DNA and lymphocytes. In those children who undergo liver transplantation of postmortem analysis, liver, bile and skin fibroblasts would be collected. These samples would be available for future analysis of potential etiology.

Evaluate Efficacy of Prostaglandin Infusions for Severe Acute Liver Injury

Research Goals

Prostaglandins play an important role in regulating cell growth and immune function in the liver. The therapeutic use of prostaglandin E (PGE) infusion in the setting of serious liver injury has yielded conflicting results. There appears to be some benefit, particularly in patients with drug toxicity who are treated early during the course of the illness. However, the benefit of PGE infusion has never been carefully studied in a pediatric population with acute liver failure.

Research Strategies

A multicenter randomized trial would enroll infants and children (up to 18 years of age) who have severe acute liver injury, based upon the inclusion criteria listed above. Potential participants would be excluded if they had contraindications to PGE infusion, such as congenital heart disease or demonstrated hypersensitivity.

Children meeting the inclusion criteria would be enrolled and stratified according to age group, cause of liver injury, and degree of coagulopathy and encephalopathy. Enrollees would be randomized to treatment with PGE infusion or placebo.

Primary study endpoints could include hospital discharge, liver transplantation and death. Potential secondary endpoints include number of days in the intensive care unit, need for dialysis, length of hospital stay and blood product requirements.

Screen for Presence of Fatty Acid Oxidation Defects in Patients With Acute Liver Failure of Unknown Etiology

Research Goals

The association of fatty acid oxidation defects with isolated liver failure is a relatively new observation (11).

Previously healthy children presenting with acute liver failure frequently are not screened for fatty acid oxidation defects. By measuring fatty acid uptake and metabolism in cultured fibroblasts of patients who present with acute liver failure, the frequency of fatty acid oxidation defects as a cause of acute liver failure in children can be defined.

Research Strategies

Study subjects would meet the inclusion criteria of acute liver injury listed above. In addition, subjects would be negative on serologic testing for known hepatotrophic viruses, autoimmune liver disease, and other common forms of metabolic liver disease, including Wilson's disease, tyrosinemia and α_1-antitrypsin deficiency. Exclusion criteria would include a history of toxic ingestion, including acetaminophen use exceeding 150 mg/kg/day.

Data and specimens to be collected from study subjects should include a urine sample for organic acid analysis, a serum sample for carnitine profile and free fatty acid analysis, a whole-blood sample for DNA analysis of known genetic defects in fatty acid oxidation, a skin biopsy specimen for fibroblast culture and subsequent measurement of fatty acid oxidation, and liver tissue (biopsy, explant or autopsy specimen), if available, for analysis of fatty acids and carnitine content.

Prospectively Analyze Acetaminophen Hepatotoxicity Related to Therapeutic Misadventure in Children

Research Goals

Because acetaminophen is so widely given to children, a determination of its role in liver injury would have important public health implications. A recommended research topic is the prospective analysis of the frequency of acetaminophen hepatotoxicity related to therapeutic misadventure in children. Reliable indications of acetaminophen-induced liver injury include positive staining of fixed liver tissues and serum immunoassays for acetaminophen/protein adducts (12,13).

Research Strategies

Study subjects would be children identified with acute liver failure as described above. There would be no specific exclusion criteria as patients with toxic acetaminophen ingestion would serve as a positive control.

Careful historical data would be collected for each study subject to ascertain any acetaminophen use in the 3 months prior to enrollment in the study. Serum for analysis of 3-(cystein-S-yl)-acetaminophen protein adducts (3-cys-A) would be collected at enrollment and at 24-hour intervals. Available liver tissue would be fixed in formalin and processed for immunohistochemistry of 3-cys-A. Liver specimens obtained from patients who require liver transplantation or at autopsy would be snap-frozen and subsequently analyzed by immunoblotting to quantify 3-cys-A tissue levels. Levels of 3-cys-A in serum and liver specimens would be correlated with outcome measures such as survival, liver transplantation and time to recovery of normal liver function.

Describe the Natural History of Chronic Hepatitis

Research Goals

Chronic HCV appears to progress slowly. Children who acquire the infection are likely to be at increased risk of long-term complications, including cirrhosis and hepatocellular carcinoma. There is a pressing need to understand the natural history of HCV acquired in infancy. It is possible, for example, that HCV acquired by vertical transmission may be relatively quiescent, and similar to the course of vertically acquired chronic hepatitis B. However, because the immunologic mechanisms underlying liver injury with HCV and HBV do differ, it is also possible that vertical transmission of HCV will result in an inexorable progression to cirrhosis.

Studies are recommended to analyze the immunologic response to hepatitis C in children. What immunologic factors contribute to the clearance of hepatitis C in some patient populations, while the infection persists in those who acquire the virus vertically? Studies could include an evaluation of immunologic competency, tolerance and viral factors.

As with HCV, the natural history of chronic HBV has not been well characterized to date. Factors need to be identified that can lead to adverse outcomes with chronic infection. This information will assist in targeting therapies for children at increased risk of developing end-stage liver disease.

Finally, non-alcoholic fatty liver disease (or non-alcoholic steatohepatitis [NASH]) has recently been identified as one of the most prevalent forms of liver disease in the US. The natural history of this disorder in children is unknown. In fact, diagnostic criteria and standardized evaluations for children have not been determined.

Research Strategies

Multicenter epidemiologic trials are recommended to study the natural history of chronic hepatitis.

Evaluate Potential Antiviral Therapies for Children With Chronic Hepatitis Infection

Research Goals

There is a pressing need for clinical trials of antiviral therapy in children with chronic hepatitis infection. Al-

though antiviral therapy, with interferon or interferon plus ribavirin, is currently approved for use in adults with chronic HCV, no therapy has been approved for use in infected children. Interferon therapy is approved for use in children with chronic HBV, but response rates do not exceed 25% to 30%.14 Effective therapies for HBV and HCV have yet to be developed.

Research Strategies

Multicenter clinical trials are needed because no single center has enough patients to adequately power a study. Long-term follow-up studies must also be designed, especially in the treatment of Hepatitis B. The outcome of hepatitis B e antigen (eAg) seroconversion in adults is just now being assessed and this information is unavailable for children. The influence of seroconversion on long-term risk of the development of cirrhosis and hepatocellular carcinoma will require 10 to 20 years' follow-up.

Evaluate the Natural History of NASH in Children

Research Goals

A multitude of questions exist regarding NASH in children. Many are quite fundamental, including issues of prevalence and natural history.

Research Strategies

As an initial step in understanding this disorder, prospective information would be collected in a multicenter study of the natural history of NASH in children. Data to be collected would include demographic, vital statistics, standard diagnostic investigation, histology and response to empiric therapies (weight loss, vitamin E, ursodeoxycholic acid). Preliminary analysis of this information will permit design of prospective investigations of this important disorder.

HEALTH AND ECONOMIC OUTCOMES

Surveillance studies have not yet been conducted to determine the precise prevalence of liver disease in children. A recent report suggested that 0.2% of all children in the US younger than 12 years of age were infected with hepatitis C virus.15 The economic impact of pediatric liver disease is largely unknown. An evaluation of the cost of liver transplantation in adults concluded that charges for the first year of care routinely exceed $150,000 (16). Assuming similar costs for pediatric liver transplantation, approximately $82.5 million of health care charges are generated each year to provide this therapy for children. The cost of care for patients with chronic liver dysfunction who do not advance to end-stage liver disease is substantially less, but because the number of these patients far outweighs the number requiring transplantation, the financial burdens are still considerable.

The total cost of caring for children with liver disease includes not only the direct cost of health care delivery but also the cost to society of caring for the sick individual. Economic analyses of health care costs frequently include indirect costs of illness, such as absenteeism from work, decreased earning ability and the value of quality-of-life-years lost. Such calculations are difficult in children who have not yet demonstrated their potential societal contribution as adults. Nevertheless, it would appear that indirect costs are substantially greater for children. Caring for a seriously ill child can involve parents, siblings, and extended family members. Many parents must take an extended leave of absence from work. The impact of disease on the family is not routinely measured in adult studies, but should be a central focus of quality-of-life research in the pediatric population.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Transplantation

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

In 1998, a total of 522 liver transplants, or approximately 12% of such operations in the US, were performed in patients younger than 18 years of age (1). For these children as for adults who underwent liver transplantation, 1-year survival rates approached 85%, the result of improvements in surgical technique, immunosuppression and antiviral therapy.

The findings of studies in adult populations cannot be generalized to children because of differences in the indications for transplantation, as well as differences in surgical, infectious and developmental complications. A number of factors hamper pediatric studies. Even at the largest centers, only 30 to 40 children undergo transplantation each year and the population is heterogeneous. Consequently, individual transplant centers do not care for populations of sufficient size to identify interventions that improve outcome. Furthermore, with pharmacotherapeutic and surgical advances, the standard of care has evolved. The interpretation of studies performed over time at any single center is subject to the biases introduced by changes in care practices.

Three major areas of research are considered of primary importance: tolerance induction, evaluation of outcome after liver transplantation, and post-transplant lymphoproliferative disease. In addition, there are three major research areas that may be considered a rank below: nonimmune graft injury, intestinal graft rejection and hepatocyte transplantation.

AREAS OF EMPHASIS

Evaluate Strategies to Induce Tolerance

Research Goals

Tolerance is classically defined as donor-specific immuno-nonresponsiveness and is manifest by long-term allograft function, without evidence of immunologic injury, maintained in the absence of immunosuppression (2). Full immunoresponsiveness to non-donor-derived antigens is preserved: animal studies have demonstrated the ability to fully reject a graft from a different donor as well as acceptance, without immunosuppression, of a second graft from the original donor. To date, true tolerance has been achieved in some small rodent models, but it is proving difficult to achieve consistently in large primate models.

Current immunosuppressive strategies are nonspecific and, as such, associated with significant long-term risks of malignancy and infection. The nonimmunologic toxicities of current therapeutic modalities are substantial. With prolonged use, debilitating and life-threatening complications, including nephrotoxicity, neurotoxicity, bone disorders and cardiovascular disease, may occur. This has spurred research efforts to explore the mechanisms and clinical applications of tolerance induction. Furthermore, the problem of chronic rejection, which most likely is mediated by immune pathways different from those of acute rejection, has not been prevented by current immunosuppressive regimens (3).

With successful tolerance induction strategies, long-term immunosuppressive drugs can be avoided. Such avoidance is of particular urgency in pediatric transplant recipients, who currently face the prospect of many decades of immunosuppressive drug use. Efforts to decrease long-term immunosuppression, or even consider discontinuation of immunosuppressive agents, are severely handicapped by lack of a reliable test to measure the recipient's degree of immunoresponsiveness to the graft. Without such a tolerance assay, random discontinuation of immunosuppressive therapy in stable patients is fraught with uncertainties.

Research Goals

The principal mechanisms of tolerance—anergy or depletion of alloreactive T cells, immuno-regulation and chimerism—are now providing promising strategies for clinical application. T cells are an absolute requirement for the rejection response. Cell surface markers that are capable of initiating co-stimulation are prime targets for new blockade approaches to modulate the immune re-

sponse. Monoclonal antibodies to these targets are being developed, many of which are humanized to avoid induction of a neutralizing human antibody response.

To advance this critically important field, the following areas of research must be targeted and supported:

Increased application of immunomodulatory strategies to induce tolerance in large primate models

Clinical trials in pediatric populations. Even early human trials of tolerance-inducing strategies must include pediatric recipients, while recognizing that the immune response and its regulation may be different in children

Development of biologic markers to measure donor-specific immune response. Such markers will not only assess outcome in studies designed to induce tolerance but also, in the short term, provide important information for the tailoring of current immunosuppressive regimens to individual patients, to avoid over- and under-immunosuppression

Research Strategies

Critical to these efforts are basic science investigations that will further our understanding of T-cell signaling and activation. Clinical trials will require the participation of multiple transplant centers.

Projected Timetable and Funding Requirements

Basic science and clinical research initiatives to achieve tolerance will require a substantial and ongoing financial commitment. These initiatives provide an ideal opportunity for partnering between federal agencies and private groups, including the pharmaceutical industry. This research area is an extremely important frontier for young investigators.

The infrastructure needed to support such studies includes data-gathering and analysis mechanisms to allow multicenter trials to be conducted. Funding for such collaborative multicenter databases, specific to the special outcomes and requirements of pediatric liver and intestinal transplant recipients, is critical.

Prospectively Evaluate Outcome Measures After Pediatric Liver Transplantation

Advances in immunosuppressive therapy and surgical techniques have improved graft and patient survival rates as well as expanded access to donor organs for pediatric liver transplant recipients. The improved rates have, in turn, resulted in greater acceptance of the procedure by patients, parents and physicians. The number of centers performing pediatric liver transplantation has increased as a direct result of greater patient and physician enthusiasm for the procedure, while the number of pediatric transplants has remained constant. Although the increased number of transplant centers likely has improved access to the procedure and proved convenient for patients and their families, the experience at each center has been substantially diluted.

Increasing donor demand adversely affects donor availability for pediatric recipients. In addition, there is increasing pressure at present from public and private payers to raise efficiencies and cut costs. These factors provide a substantial impetus for pediatric transplant centers to examine the outcomes achieved in order to provide adequate stewardship for scarce donor resources and utilize dwindling financial resources most effectively (4). Unfortunately, the steady dilution in pediatric transplant experience precludes all but the crudest analysis of transplant outcomes.

Research Goals

It is recommended that a large multicenter transplant registry be developed that would prospectively collect data from all pediatric transplant centers. Such a database is essential for the accurate analysis of patient outcomes and for the development of innovations that might improve these outcomes in the future.

Data from the multicenter pediatric transplant registry would be analyzed to determine the:

A) Long-term graft and patient survival for pediatric transplantation, stratified by disease. Data on survival are currently available only for a few pediatric liver diseases. However, less common liver diseases that are considered potential indications, such as metabolic diseases, collectively account for about 30% of pediatric transplants. For many of these disorders, only anecdotal experience of short-term outcomes at a single center has been published. Establishment of a registry detailing the results of transplantation would allow for a more accurate assessment of the effectiveness of transplantation. These data, for example, would help determine the optimal timing of transplantation and the appropriateness of transplantation as a treatment option.

B) Best methods of surgical and medical management. With dispersion of the pediatric transplant experience, local variations have developed in both the surgical procedure and postoperative care, including the use of immunosuppression. Some of these local idiosyncrasies may add to the overall cost of the procedure. Since there is currently no method to track the outcome of these various approaches, their effectiveness cannot be determined. The analysis of outcomes resulting from these management strategies, with comparison to outcomes in the entire data set, would be a first step in determining whether there is an optimal approach to operative and postoperative management.

C) Long-term growth potential and long-term development potential of patients undergoing liver transplan-

tation. Information regarding these issues is currently lacking and requires the collection of data to track somatic growth, reproductive capabilities, and educational and occupational achievements relative to the clinical characteristics of patients undergoing transplantation. Acquisition of this information would help to determine the optimal timing of the transplant procedure, and would allow for comparison of transplantation with other management strategies (5). For example, if it were shown that otherwise stable patients with cholestatic liver disease suffered irreversible compromise of growth or intellectual achievement over time, early transplantation would be justified.

D) Long-term effects of immunosuppressive therapy. Several complications of standard immunosuppressive medications have been documented in children and adults. These include increased lipid levels, hypertension, decreased renal clearance, altered glucose metabolism, compromised growth and diminished bone accretion (6–8). The impact of these adverse effects is substantially greater in children than in adults because of the longer period of exposure to the drugs. The incidence of severe injury leading to potentially life-threatening conditions such as renal failure, heart disease or osteoporosis is not known. A determination of the incidence of these adverse effects, and the factors that predispose to their development, would be an important initial effort in devising strategies to reduce drug-induced organ damage.

E) Long-term quality of life of children and their families after liver transplantation. The goal of transplantation is to improve the quality, as well as the quantity, of the patient's life. Recently, accurate, validated tools have been developed to assess pediatric quality of life. A large multicenter study utilizing these tools to evaluate the effectiveness of liver transplantation in reaching this goal is essential. As part of this study, children and their families would be followed over the long term, data would be stratified by specific diseases, and management strategies could be identified that yield the best outcomes.

F) Long-term costs of transplantation. Anticipated costs for individual liver transplant recipients are currently difficult, if not impossible, to assess. Factors influencing cost include regional differences in overall cost of medical care, the United Network for Organ Sharing (UNOS) status of the patient at the time of transplantation, the number of comorbid conditions, and the type of graft. A database that tracks expenditures in addition to medical variables would be a first step in developing an accurate projection of costs for individual patients. Long-term follow-up of patient outcomes and cumulative associated costs would enable development of realistic cost-benefit analyses for pediatric transplantation.

Research Strategies

To achieve the above goals, a registry encompassing the majority of North American pediatric liver transplant recipients needs to be developed. Data must be collected in a prospective, standardized manner and analyzed in a timely and statistically valid fashion. Individual patients must be followed until adulthood. The ultimate goal of such a registry would be to determine the expected outcomes of liver transplantation for specific recipients and to identify factors that would influence the likelihood of achieving these outcomes. Validated tools need to be utilized or developed to assess some of these outcomes. Comparable experience in the pediatric oncology community has demonstrated the value of such a registry for determining therapeutic outcomes and developing new strategies to improve outcomes.

An industry-funded pediatric liver transplant registry called SPLIT (*S*tudies in *P*ediatric *L*iver *T*ransplantation) currently collects data from 34 centers in Canada and the US, representing approximately 25% of the procedures performed annually. We propose expanded funding of the existing database to enable recruitment of additional centers to capture a minimum of 75% of the transplants performed each year. In addition, prospective studies evaluating specific outcomes are recommended. It is only through acquisition and analysis of these data that true measurements of the long-term effectiveness of liver transplantation will be achieved.

Projected Timetable and Funding Requirements

Funding for individual centers is needed to expand the existing SPLIT database to encompass the majority of pediatric liver transplant centers. Funding would largely be directed at support for transplant coordinators who gather the large amount of data required and for data entry personnel responsible for inputting the data. A small part of the funds would be allocated to a central data collection agency.

Evaluate Interventions to Prevent and Treat Post-Transplant Lymphoproliferative Disease

Post-transplant lymphoproliferative disease (PTLD) occurs in up to 11% of pediatric liver transplant recipients and up to 25% of pediatric intestinal transplant recipients. The associated mortality rate can be as high as 20% to 60%. In pediatric patients, more than 85% of PTLD is related to Epstein-Barr virus (EBV) infection and PTLD presents as a spectrum of disease ranging from benign B-cell hyperplasia to malignant lymphomas (9).

Patients who are EBV naive and receive an organ from an EBV-positive donor, especially those being treated with increased levels of immunosuppressive agents for resistant rejection, are at high risk of developing PTLD (10). Infants and toddlers, who constitute 50% of the pediatric liver transplant population, are usually EBV naive. Up to 15% of high-risk liver transplant recipients

will develop PTLD. More than 75% of high-risk patients acquire the virus within the first year of life. For children, especially those younger than 2 years of age, PTLD not only can be lethal but also can critically affect quality of life and graft function.

It has been hypothesized that the outcome of EBV infection in pediatric transplant recipients reflects a balance between EBV-driven B-cell proliferation and the activity of EBV-specific cytotoxic T cells. If this hypothesis is true, then therapy that enhances the EBV-specific T-cell response or decreases B-cell proliferation should prevent PTLD. Prevention and preemptive treatment strategies include polymerase chain reaction (PCR) monitoring of the peripheral blood for the EBV genome, combined with antiviral therapy and reduction of immunosuppression with the first evidence of EBV infection. Treatment requires stopping T cell-directed immunosuppression so that immune surveillance by EBV-specific cytotoxic T cells is restored. Transplant physicians also use medications that inhibit viral replication and high-titer cytomegalovirus (CMV) globulin to prevent and treat PTLD. The efficacy of antivirals and immunoglobulin is difficult to assess because reduction of immunosuppressive therapy is almost always initiated simultaneously. However, the enhanced immune response that results from reduced immunosuppression is nonspecific and may precipitate allograft rejection.

Research Goals

Interventions need to be tested that can prevent or treat PTLD in pediatric liver transplant recipients by shifting the balance between B-cell proliferation and activation of EBV-specific cytotoxic T cells. Basic research is needed to develop:

- A reproducible method to measure EBV-specific cytotoxic T-cell activity; and
- A method for in vitro activation of recipient-derived EBV-specific T cells, including T cells from EBV-naïve recipients. The T cells can then be reinjected into the recipient at the time of diagnosis of PTLD to restore EBV-specific cytotoxic T cell competence (11).

Clinical trials are needed to:

- Evaluate preemptive therapy that enhances EBV-specific cytotoxic T-cell activity and prevents development of PTLD
- Study the role of serial EBV PCR monitoring of the peripheral blood in preemptive therapy
- Determine the efficacy of standard treatment approaches (reduced immunosuppression, antivirals, hyperimmune globulin) in patients with PTLD
- Determine the efficacy of treatment with monoclonal antibodies directed against B cells or chemotherapy in restoring the balance between B-cell proliferation and EBV-specific T-cell response in patients who fail to respond to preemptive or standard therapy.

Research Strategies

An analysis of the SPLIT registry shows that 120 children annually meet the criteria for entry into the registry. It is known that up to 80% of high-risk patients are infected with EBV each year and that as many as 12% develop PTLD. We predict that 50% of patients with PTLD will not respond to standard therapy. A multicenter trial to determine optimal novel treatment is necessary because the number of patients at even the largest transplant centers is too small to achieve adequate power.

Projected Timetable and Funding Requirements

One or two funded investigators will be required to address each basic research goal. The clinical research goals will be aided by creation of a clinical trials network linking multiple centers.

Evaluate Treatment Strategies for Minimizing Nonimmune Graft Injury

Liver allografts are a precious commodity since demand far exceeds supply. To use existing resources efficiently, initial graft function must be optimized. When the graft does not function, or initial function is poor owing to reperfusion injury, there is an increased risk of perioperative morbidity and graft loss, as well as greater health resource utilization. It is increasingly recognized that nonimmune injury to the allograft is associated with significant short-term and long-term consequences. Nonimmune graft injury results from the effects on the donor organ of brain death itself, ischemia/reperfusion injury and preexisting injury in the graft. With a better understanding of the underlying mechanisms, preventive strategies can be designed.

There are several compelling reasons why progress in this area of research is important. First, preventing or ameliorating the injury induced by brain death and ischemia/reperfusion is likely to improve early cadaveric graft function and reduce the need for retransplantation due to primary nonfunction or poor initial function. In view of the ongoing cadaveric donor shortage, advances in this area are of particular importance. In addition, improved graft protection may allow the successful use of more marginal donors, which would further expand the cadaveric donor pool.

Second, it is now known that the nonspecific inflammatory response induced by nonimmune injury itself upregulates the immune response to the graft (12). The risk of acute rejection may be increased, but of greater importance is recent evidence suggesting that chronic rejection may be linked to early nonimmune injury.

Third, an understanding of the regenerative response of the liver after injury is critically important with the increasing use in children of segmental liver grafts, particularly those from cadavers. This research is also relevant to liver donor grafts. Interleukin-6 is a key cytokine involved in activating transcription factors, such as JAK kinase and STAT3, which activate hepatocyte cell division. Current immunosuppressive drugs may be detrimental to some of these responses; for example, steroids inhibit liver regeneration.

Research Goals

Research efforts are needed to develop strategies for minimizing nonimmune injury and thereby optimizing early graft function. Such efforts will improve the cadaveric donor supply by allowing more cadaveric organs to be split. It is also important to promote the regenerative response of the segmental liver graft for a successful outcome after pediatric split transplantation.

Identify Markers and Develop Diagnostic Tests for Rejection Following Intestinal Transplantation

Research Goals

Isolated intestinal grafts constitute about one half of the 50 to 100 intestinal transplants that are performed each year in the US. Recent reports have indicated that acute rejection of the isolated graft is virtually universal and an important cause of graft loss. The incidence and severity of rejection, although somewhat less in cases of combined liver-intestinal transplantation, is greater than occurs with isolated liver grafts. Intestinal allografts are also susceptible to chronic rejection, which occurs rarely with liver grafts.

Rejection of intestinal grafts is difficult to diagnose and treat. Unlike with liver and kidney transplantation, there is no simple blood test to detect when the small intestine initiates the process of rejection. Tissue diagnosis is often difficult as the process can be patchy. A late diagnosis often results in loss of an intestinal graft. Studies are needed to:

Evaluate histologic markers for rejection. Special staining techniques may be used based on an understanding of the mechanisms underlying rejection.

Develop functional tests to assess changes in intestinal function that correlate well with rejection. Permeability studies have been evaluated for this purpose, but results were nonspecific (13).

Identify serum proteins, enzymes, and other markers that might be elevated early during the course of rejection (14).

Research Strategies

Several complementary approaches may be required. Studies can be conducted only at centers where large numbers of intestinal transplant procedures are performed.

Projected Timetable and Funding Requirements

These studies are likely to be an ongoing project. The cost of part-time technical assistance, specialized nursing support for clinical aspects of the research, and data analysis may be significant. Supplemental immunologic studies, if included, could easily raise estimates of the total annual cost.

Broaden the Clinical Applications of Hepatocyte Transplantation

Hepatocyte transplantation (HTX) continues to evolve as a potential therapeutic adjunct to liver transplantation. By providing normal hepatocytes to patients with a liver-related metabolic defect, HTX can improve metabolism (15). HTX can also serve as a lifesaving "bridge" in liver failure patients awaiting liver transplantation, providing a cellular mass sufficient to temporarily carry out metabolic functions (16).

The development of HTX as a treatment modality came after decades of basic research in liver cell biology. Technical issues concerning the isolation of hepatocytes and cryopreservation needed to be resolved. With successful cryopreservation, hepatocytes can be stored and shared among centers for use in the treatment of patients with liver failure.

Research Goals

To broaden the applications of HTX, further studies are critical to determine:

Optimal methods of infusion/transplantation of hepatocytes. Is a threshold number of cells needed for successful transplantation? Is a single infusion sufficient, or are repeated infusions preferable? What is the optimal route of infusion, intrasplenic or intraportal?

Guidelines for patient selection. Are patients with metabolic disease appropriate candidates for HTX? In considering HTX, do criteria differ for patients with acute versus chronic liver failure?

Optimal time of infusion/transplantation in relation to a patient's clinical course. What principles might apply during advanced stages of liver failure? During early stages?

In addition, studies are needed to address the issue of availability of hepatocytes. New methods must be developed that enable hepatocytes to proliferate in culture.

Once that technology has been secured, the number of hepatocytes available for transplantation will increase independent of the availability of new livers.

Research Strategies

To achieve these research goals, multicenter clinical trials as well as basic research are needed. It is important to support the creation of regional centers for the storage/banking of cryopreserved hepatocytes. Future research will also be enhanced by the development of animal models of liver cell transplantation.

HEALTH AND ECONOMIC OUTCOMES

The population of long-term survivors of liver transplantation has grown, exceeding by tenfold the number of transplant procedures performed each year. In adults, the mean cost of the liver transplant procedure and associated hospitalization alone is estimated at about $150,000.17 For each year after a successful liver transplantation, direct health care costs are estimated to reach 7% to 10% of the mean cost of transplantation and recovery. Consequently, after 10 years, the cumulative cost of maintaining graft function and wellness in a population of long-term survivors is equal to the cost of the liver transplantation procedure. Based on these assumptions, it is estimated that $154 million is spent each year on liver transplantation in the pediatric population: $77 million for the procedures and an equal amount to maintain graft function and wellness in survivors. Moreover, the impact of pediatric transplant survivors on total health care costs will become increasingly relevant, since the potential for years of life gained for a 2-year-old undergoing transplantation is much greater than that for a 40-year-old.

The economic and psychosocial cost to the family is more difficult to estimate. There is loss of productivity when patients care for their children through long periods of hospitalization and frequent follow-up visits. Parents may lose their jobs and health insurance while meeting the intense medical needs of children during the peritransplant period. Quality-of-life studies are virtually nonexistent in children and parents after liver or intestinal transplantation. The learning abilities of children who successfully undergo transplant procedures will directly affect their ability to become productive and independent members of society; the impact of liver and intestinal transplantation has not been studied.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Nutrition and Obesity

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

Nutrition is a determinant of health and illness. The nutrition children receive can ensure health, prevent disease, have a positive effect on chronic disease and influence brain development. Nutrition may play an important role in gene regulation. However, suboptimal nutrition can lead to illness, worsen chronic disease, and place children at risk for developing disease in adulthood. There may also be critical times when the nutrition children receive has an important effect later in life. These critical times may vary with the specific nutrient or nutrients and the child's developmental status. The impact of changes in nutrition at these potentially critical times may not become evident for years.

Because nutrition is vital to so many aspects of child health, a comprehensive nutrition research agenda can be overwhelming. Therefore, this discussion focuses on areas in which an advance in knowledge can have an impact on the health of a population and prevent the later development of disease. In addition, research is discussed that may lead to new substances that can ease the lives of, and improve outcome in, children with chronic diseases and that may alter the outcome of disease through the regulation of specific genes.

AREAS OF EMPHASIS

Study the Pathogenesis of Obesity and Potential Interventions to Prevent or Treat Comorbidities

The prevalence rate of obesity is increasing so rapidly that it has been called an epidemic by the Centers for Disease Control and Prevention (1). The National Health and Nutrition Examination Survey (NHANES) III (1988–1994) reported that approximately 14% of children aged 6 to 11 years and 12% of adolescents aged 12 to 17 are overweight. The prevalence of overweight during NHANES II (1976–1980) was 7.6% for children aged 6 to 11 years and 10.9% for 12- to 17-year-old adolescents. Similarly, the Bogalusa Heart Study showed an almost doubling of the prevalence of overweight from 1973–1974 to 1992–1994 independent of changes in height and other covariates. The trend for overweight appeared to be accelerating because larger increases in prevalence were seen during the latter part of the study.

These studies make clear that obesity is one of the most prevalent diseases among children and adolescents in the US. The trend toward an increase in body weight beginning at age 6 years is believed to be related to environmental factors and is neither biologically nor medically desirable. Overweight in adolescents is associated with changes in blood pressure, lipoproteins, and plasma insulin levels. Half of obese children in grade school remained obese as adults and that the risk of obesity in adulthood was at least twice as high for obese children as for nonobese children. Parental obesity has a profound effect on the likelihood that a child will become an obese adult. Obesity in adulthood is associated with increased morbidity and mortality. No reliable method exists to treat obesity, and long-term intervention studies have shown that 80% to 90% of those who achieve weight reduction return to their previous weight. Childhood obesity is associated with other diseases, including type 2 diabetes mellitus, liver and renal disease, and hypertension.

Research Goals

While intuitively appropriate, weight reduction efforts to date have failed to successfully prevent obesity or sustain weight reduction over the long term. While efforts to reduce weight should continue, other approaches may be beneficial. The pathogenesis of comorbid factors needs to be understood and interventions identified to prevent or treat comorbidities, specifically liver, renal and cardiovascular disease, as well as type 2 diabetes.

Research Strategies

Studies of basic pathogenesis as well as of pharmacological intervention are needed. Animal models can be

used initially, with the most promising therapies evaluated in children in carefully designed randomized controlled studies. A combination of environmental modulation and population-based intervention studies is required, as well as studies of substrate regulation in subsets of obese children.

Projected Timetable and Funding Requirements

These different strategies can best be accomplished by investigator-initiated grants. Because the proportion of the population that is obese is increasing dramatically, immediate attention is desirable.

Determine Harmful Effects or Benefits of Dietary Supplements

An understanding of the beneficial and harmful actions of dietary supplements is critical in light of a $21.2 billion industry that targets children (2). Dietary supplements can be considered a form of complementary alternative medicine. These products are "intended to supplement the diet to enhance health" and include vitamins, minerals, amino acids, herbal products or other botanicals, and substances such as enzymes, organ tissues, glandular products and metabolites. Further, a dietary supplement is "not represented as a conventional food or a sole item of a meal or the diet" and is intended for ingestion in the form of a capsule, powder, soft gel, or gelcap. A nutraceutical is a "diet supplement that delivers a concentrated form of a biologically active component of food in a non-food matrix in order to enhance health" (3). An example of a nutraceutical is genistein, which is purified from soybeans and delivered in a pill in dosages greater than can be consumed in soy. Dietary supplements and nutraceuticals are different from functional foods, which are considered to deliver an active ingredient within a food matrix. An example of a functional food is bread or breakfast cereal with added high-dose folic acid. Food additives are substances that enhance flavor or aroma, but not the nutritional value of a food.

Dietary supplements, nutraceuticals and functional foods are designed to supplement the human diet by increasing the intake of bioactive agents that are thought to enhance health and fitness (3). Although some of these products have been used through the ages, their safety and efficaciousness cannot be guaranteed. It should be noted that many important medications (aspirin, atropine and digitalis, to name a few) share a common history with herbs.

Research Goals

A key priority is to identify currently marketed products that are harmful. In addition, it is important to identify substances in marketed supplements, herbs and nutraceuticals that may have previously undescribed beneficial effects. Potentially beneficial substances should be purified and studied in randomized blinded trials to rigorously evaluate effective.

Research Strategies

The evaluation of currently marketed products that are potentially harmful is best performed by a governmental agency. The urgency of this need has been demonstrated repeatedly by published accounts of toxicity (4). The identification of substances in marketed products with possible therapeutic benefit can best be achieved by investigator-initiated studies in conjunction with the industries that produce, package and market these products. It is to the industry's advantage to demonstrate the efficacy of these products, and industry can supply the substances in pure form for study.

Projected Timetable and Funding Requirements

There is a real urgency to evaluate products that may have harmful effects. Activities to identify such products must begin immediately. The evaluation of novel substances in herbs and dietary supplements that may provide therapeutic benefit, if commenced within 1 to 2 years, would likely prove fruitful within 3 to 5 years.

Evaluate Whether Childhood Nutrition Influences Adult-Onset Disease

Whether early childhood nutrition influences adult health and adult-onset disease is a critical public health question as well as a biological question. Data suggest that the nutritional support provided to premature infants likely has consequences for cognitive development, cardiovascular health, atopic disease, bone formation, blood pressure, low-density lipoprotein cholesterol and proinsulin at 13–16 years. Children and adolescents with high cholesterol levels are more likely than the general population to have high levels as adults. Adequate calcium intake during childhood is necessary for the development of maximal peak bone mass. Increasing peak bone mass may be an important way to reduce the risk of osteoporosis in later adulthood. The risk of an obese child becoming an obese adult is twice as high as for a nonobese child.

Thus, what infants, children and adolescents eat has not only immediate consequences, but likely long-term consequences as well. It is probable that a clearly articulated nutrition policy implemented in childhood would have a positive impact on adult health. However, this type of intervention, while seemingly obvious, is not supported by robust data, and many unanswered questions remain. For example, a substantial number of chil-

dren with high cholesterol levels become adults with desirable cholesterol levels without intervention. About half of obese adolescents do not become obese adults (5). Calcium requirements may be affected substantially by other factors, including genetic variability and other dietary constituents.

Research Goals

The hypothesis that early nutrition could have lifetime effects must be tested in tightly designed nutritional intervention studies. These studies needed to be performed in premature infants with special reference to cognitive development. Studies in neonates would permit the development of standardized infant feeding protocols in neonatal intensive care units (NICUs), which will result in positive health outcomes long-term. Similar studies in older children and adolescents will serve to prove or disprove the importance of child health throughout life.

Research Strategies

An evaluation of feeding practices in NICUs is needed immediately. Current practices are highly individualized and variable among physicians and units in different parts of the country. A nationwide study is recommended, during which protocols can be put in place to test the hypothesis that feeding practices have long-term consequences. Randomized controlled interventional studies of both single nutrients (e.g., calcium or iron) and combinations of nutrients (e.g., calcium plus low levels of sodium) are needed to evaluate the effect of nutrient intake during childhood on adult health.

Projected Timetable and Funding Requirements

Because of the public health implications of these studies and the long study periods (years) required, it is important that the work commence immediately. Large numbers of study subjects are likely needed to prove causality; therefore, the work could best be performed by a consortium of geographically diverse centers. Depending on the nutrient to be studied and the specific disease, different foundations will be interested in providing support. However, because of the immensity of the probable public health impact, federal funding through grants or contracts administered to the consortium is highly desirable.

Identify Nutrient-Gene Interactions that Have Therapeutic Potential for Specific Single-Gene and/or Polygenic Disorders

Alteration of gene expression has become a rapidly developing area of research in medicine, particularly in the context of novel therapeutic options. Although current research has focused on the therapeutic benefit of altering gene expression by insertion of genetic material into cells, gene expression can be changed by altering the molecular environment of gene-responsive intracellular elements. Nutrition (food consumption) dynamically alters the cellular environment and serves as a potential stimulus for the alteration of gene expression (6). Thus, the future of nutrition as a therapeutic tool may lie in its potential for influencing gene regulation (7).

The human organism adapts to nutrient perturbations by means of physiological and metabolic responses that are under the influence of genetic control. The mechanisms underlying these responses to nutritional perturbations are poorly understood in humans. Progress in our understanding of nutrient-gene interactions has been slow because the molecular mechanisms controlling gene expression are complex and the direct functional consequence may, in fact, be the effect of nutrient metabolites rather than the nutrient itself.

The physiological importance of the nutritional regulation of gene expression resides in the variety of functional consequences underlying specific clinical considerations. For example, genes may be regulated to better utilize nutrients during periods of scarcity, including upregulation of nutrient transporters or enzymes that metabolize nutrients. Gene expression may be altered in the context of nutrient abundance and, hence, nutrient storage (8). Nutrients regulate the secretion of hormones to achieve metabolic homeostasis and, therefore, indirectly affect genetic mechanisms responsible for the endocrine control of cellular metabolism. Nutrients within the gut lumen may directly alter epithelial cell gene expression or change primarily luminal flora with secondary effects on tissue gene expression. Finally, nutrients may serve as regulatory factors for gene expression during critical windows of cellular development, resulting in a cascade of differential effects (9).

The fundamental regulation of gene expression by nutrients involves two mechanisms:

A) Molecules, either receptors or enzymes within the cell, must sense or recognize the presence of the nutrient;

B) Once nutrient sensing occurs, this interaction initiates a sequence of molecular events, i.e., signal transduction, which ultimately alters gene transcription or translation.

For example, glucose deficiency leads to the upregulation of specific glucose transporter proteins. Transporter protein synthesis usually is the consequence of increased transporter messenger ribonucleic acid (mRNA) owing to increased transcription, although other mechanisms have been observed.

The clinical and therapeutic implications of nutrient-gene interactions have been difficult to ascertain in children. An example is total parenteral nutrition, which has profound alterations on the gut. Although much emphasis has been placed on individual nutrients that may be

responsible for the maintenance of normal bowel morphology and function, no clear answer has emerged. Indeed, the luminal contents of the gut may prove to dominate the molecular control of gene expression. The more important aspect is the role of nutrients as they affect the luminal contents of the gut. How these mechanisms affect the intestinal cell is not clearly understood.

Research Goals

It is important to identify the genes that are altered in single-gene or polygenic disorders for which nutritional interventions are likely to have an impact. These disorders include inflammatory bowel disease, diabetes mellitus, cancer, Rett syndrome, and obesity. It is also important to identify the molecular mechanisms of individual gene functions. Studies are needed to determine the susceptibility of specific gene targets to single or multiple nutrient perturbations, e.g., glucose, folate and vitamin A.

Research Strategies

These goals require a combination of approaches to achieve gene identification and an understanding of gene function. Recommended approaches include basic cell biology research, the development of specific animal (transgenic) models that reflect comparable human abnormalities, and ultimately large-scale multicenter collaborative registries and therapeutic trials that provide the basis for the assessment of favorable clinical and biochemical outcomes in response to individual or multiple nutrient therapies.

Projected Timetable and Funding Requirements

These initiatives should be started immediately. These research questions should be addressed via multiple funding approaches, including investigator-initiated grants, center grants, industry support, and interinstitutional consortium arrangements.

Investigate Antecedents, Mechanisms and Long-Term Outcomes of Nutrition in Chronic Disease

Nutritional comorbidities frequently complicate the clinical course of chronic diseases of childhood. The prevalence of nutritional deficiencies and excesses such as growth failure, osteopenia, vitamin or mineral deficiencies or toxicities, and obesity is estimated to range from 30% to 40% in specific intestinal, hepatic, and pancreatic diseases of childhood. The potential scope of nutritional comorbidities is broad; most, if not all, major pediatric disorders are potentially affected. The future of nutrition as a therapeutic tool in chronic childhood illnesses may be in reversing the cellular dysregulation associated with individual chronic diseases and replacing or removing nutrients, or their metabolites, essential to normal cellular function.

Before a healthy nutritional status can be restored and nutritional comorbidities reversed, the mechanisms that precipitate nutritional comorbidities in individual chronic diseases must be identified. These mechanisms may be associated with:

Decreased dietary intake, particularly in conjunction with altered appetite regulation
Perturbations in nutrient metabolism, particularly in relation to the hormonal milieu and substrate utilization
Increased nutrient losses, particularly from the gastrointestinal tract
Increased nutrient requirements in conjunction with cellular dysfunction underlying the disease entity, inflammation, or growth

The pathophysiological factors contributing to these mechanisms have not been elucidated fully. Progress in our understanding of nutritional comorbidities in chronic diseases has been slow because of difficulties in achieving homogeneity of study populations, the possibility of unrecognized nutrient interactions, and the presence of confounding variables associated with various therapeutic regimens.

A greater understanding of nutritional comorbidities in children afflicted with chronic diseases, it is hoped, will enable interruption of the cascade of associated adverse physiological events and improvement in functional clinical outcome. For example, in conditions such as childhood cancer, inflammatory bowel disease, cystic fibrosis, and chronic liver disease requiring transplantation, the presence of nutritional comorbidities may be associated with metabolic, physiological and hormonal perturbations; increased occurrence of infections; and reduced tolerance to pharmacological treatment or surgical intervention (10). Conversely, in some conditions, including cystic fibrosis and inflammatory bowel disease, aggressive nutritional intervention and reversal of nutritional comorbidities may alter the clinical course of disease, reduce pharmacological toxicities, and improve quality of life (11). Thus, a focus on basic, clinical and epidemiologic research is needed to broaden our understanding of the antecedents, mechanisms, and long-term outcomes of nutritional comorbidities associated with chronic diseases of childhood.

Research Goals

Research goals include:

Identification of the risk factors, such as genetic polymorphisms and critical windows of development, that predispose children to nutritional comorbidities and

lend themselves to preventive or delay-of-onset strategies;

Exploration of mechanisms that account for nutritional comorbidities—e.g., appetite regulation in the context of altered dietary intake; the endocrine-metabolic control of nutrient disposal in the context of the underlying cellular dysregulation associated with the chronic disorder; quantitative and qualitative differences in nutrient requirements in the context of impaired cellular metabolism, inflammation, increased nutrient losses and growth; and

Assessment of short- and long-term outcomes based on novel nutritional intervention strategies, e.g., prebiotics and probiotics (12).

Research Strategies

These research goals require prioritization of chronic diseases of interest, based on frequency of occurrence or the robustness with which potential mechanisms of cellular dysfunction contribute to nutritional comorbidities. Subsequently, studies in this area will require a combination of approaches, including basic cell biology research, the development of specific animal models that reflect comparable human disorders, and ultimately large-scale multicenter collaborative registries and clinical trials.

Projected Timetable and Funding Requirements

These initiatives should be started immediately. These research questions should be addressed via multiple funding approaches, including investigator-initiated grants, center grants, industry support, and interinstitutional consortium arrangements.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Chronic Inflammatory Bowel Disease

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

Both Crohn's disease (CD) and ulcerative colitis (UC) occur frequently in the pediatric age group, with a peak incidence in the second decade of life. Approximately 25% of all patients with these disorders are children. These two disorders, commonly grouped together as idiopathic inflammatory bowel disease (IBD), share many similarities in epidemiologic, immunologic, clinical and therapeutic characteristics. Despite intensive research efforts, the etiology of IBD remains unknown, although emerging data implicate genetic susceptibility as a major pathogenetic factor. Available evidence suggests that IBD results from a genetically conditioned susceptibility to immune-mediated bowel injury triggered by one or more environmental factors. Accordingly, basic research in early-onset IBD should focus on the predisposing factors, triggering events and potentiating mechanisms that lead to and sustain the disease.

Studies have identified the presence of susceptibility loci for IBD on chromosomes 3, 5, 7, 12 and 16. Linkages on chromosomes 2 and 6 have been associated specifically with UC. Linkages with specific HLA haplotypes have also been reported. UC and CD clearly appear to be polygenic disorders with heterogeneous expression. Therefore, future research will require an understanding of genotype/phenotype relationships, modifier genes that influence onset, disease characteristics including extraintestinal manifestations, and response to therapy.

Studies in animal models of IBD support a role for microbial flora in the initiation and propagation of the intestinal inflammatory response. For example, in the interleukin-10 (IL-10) knockout mouse, IBD does not develop in the germ-free state, which indicates that interaction with bacteria is necessary to sustain inflammation. Research is needed to determine whether these reactions, which have a genetic basis, are due to an overexuberant host response to normal flora, to altered flora or to, as yet unidentified, pathogens.

Animal models have shed light on a variety of immunologic defects that can predispose to IBD, but it is clear that the complex factors involved require further elucidation. Interestingly, different immunologic defects can lead to similar pathologic findings. For example, genetic alterations in G proteins and E cadherin can lead to IBD, presumably through distinct immune pathways. Understanding the mechanisms by which these and other disparate pathways produce IBD is an important research goal. In this regard, pediatric-onset disease provides an important model. Elucidation of the immune responses predisposing to early-onset disease will enable the design of more specific therapy. Other immunologic events may involve activation of autoreactive T cells, leading to destruction of target intestinal epithelial cells. Accordingly, future research should focus on an enhanced understanding of the mechanisms of autoimmunity and immune tolerance, including the complex network of cytokines involved.

Over the years, attempts have been made to implicate specific dietary components as triggers of IBD. Studies have also suggested that altered intestinal permeability is a contributing factor. Concerns about dietary and environmental influences arise from the increasing prevalence of IBD. Future research efforts should focus on possible associations with specific food-processing practices, additives, infant feeding practices and the introduction of genetically altered foods.

Although a great deal is known about the characteristics and management of IBD in the pediatric population, important clinical questions remain unanswered. For a comprehensive understanding of pediatric IBD, it is critical to identify epidemiologic trends and correlations, define the economic impact, and better understand treatment outcomes. To accomplish this, an ongoing national data registry is mandatory. Initial support for such a database has been provided by the Crohn's and Colitis Foundation of America, Inc. (CCFA), but strategies for the long-term maintenance of the database need to be developed. Successful tracking of the impact of disease on the health status of children with IBD also depends on the development of validated quality-of-life indices.

Management of pediatric IBD has largely been based on studies in adults. Only a few controlled clinical trials have been performed in children and adolescents with

IBD. Accordingly, a major thrust of future research must be a comprehensive understanding of the pharmacology and pharmacogenomics of relevance to pediatric IBD. For example, basic pharmacokinetic and efficacy data in children are lacking even for the widely used aminosalicylates. Only limited information is available for other therapeutic modalities currently used to treat children and adolescents with IBD, including antibiotics, immunosuppressives, immunomodulators, probiotics and nutritional therapy.

A unique aspect of pediatric IBD is the impact of disease activity on growth and nutritional status. Although uncontrolled disease activity appears to be the major pathogenetic factor for poor growth, the presence of modifier genes affecting stature may also be involved. Nutritional therapy has been associated with restored growth in children with CD, but in most studies, final adult height has not reached predicted levels. Another influence on growth may be the high prevalence of bone mineral abnormalities in children with IBD independent of exposure to corticosteroids.

A well-planned and comprehensive research program addressing the basic and clinical questions described in this chapter will significantly improve the ability to diagnose and treat IBD in the pediatric population. At the same time, more extensive knowledge of early-onset IBD may provide important insights into the treatment of adults with IBD.

AREAS OF EMPHASIS

Elucidate the Genetic Basis of Pediatric IBD

Research Goals

An important goal is to identify the genes that confer susceptibility to early-onset IBD. In addition to known susceptibility loci, linkages to new genetic loci should be sought. Human homologues to genes known to be linked to IBD in animal models should be identified. For example, more information is needed concerning the region on chromosome 5q31-33 that is associated with CD developing before the age of 16 years. Modifier genes that alter expression of genetic abnormalities warrant elucidation. Genotype-phenotype analysis should focus on the type, location, extent and severity of disease. Studies of sporadic cases should be compared with those of familial cases. Large numbers of sporadic cases should be useful in confirming loci identified from familial linkage studies. Genetic information should be sought that is linked to responses to various treatment approaches.

Research Strategies

A combination of techniques should be used, including trio analysis, haplotype analysis and single-nucleotide polymorphisms (SNPs). Microarrays and proteomic analyses should also be applied. Pharmacogenomics will be helpful in identifying drug responses in patients of differing genetic profiles, as well as in predicting toxicity.

Projected Timetable and Funding Requirements

These research questions are of high priority. While the National Institutes of Health (NIH) has instituted a U01 program for Genetics Research Centers for IBD, which includes six centers and a data-coordinating center, additional investigator-initiated research (R01) should be supported. Center grants (P50) and program project grants (P01) are also important, especially as they permit establishment of shared facilities, data and computer cores. Pharmacogenomics may be particularly appropriate for collaboration between NIH and industry.

Evaluate the Role of Enteric Microbial Flora in the Pathogenesis of IBD

Research Goals

The most compelling evidence of a role for resident flora in the intestinal and systemic inflammatory response in IBD comes from studies in animal models. Intestinal inflammation and joint responses are absent in many models in the germ-free state. Goals for the future include identification of the organisms responsible for initiating and propagating the mucosal immune response. A number of classes of bacteria, fungi and viral agents may be involved. Cross-talk between flora and epithelial and immune cells needs to be characterized, and the potential for "molecular mimicry" to trigger autoimmune events should be studied. Studies are need to determine whether host responses in IBD are the result of over-responsiveness to normal flora or a reaction to altered intestinal flora, such as might result from chronic exposure to antibiotics. As yet unidentified pathogens might be involved in pathogenesis, a possibility that could yield novel therapeutic strategies.

Studies should focus on:

Analysis of normal resident flora
Host response to luminal components (bacterial products, toxins, lipopolysaccharides)
The types of immune activation triggered by luminal components
Signaling by resident flora to the epithelial cell and immune system

Research Strategies

Important information will be acquired from studies in animal models, but techniques must be developed to

search for comparable mechanisms in humans with IBD. Investigators studying these pathways need to work in collaboration with scientists interested in the genetics of IBD.

Projected Timetable and Funding Requirements

These research questions are particularly amenable to program projects and other collaborative efforts. Investigator-initiated research is appropriate for generating new ideas. Requests for applications (RFAs) to study the role of flora in initiating and propagating pediatric IBD would spur action in this field.

Define the Role of the Immune System and the Mucosal Barrier in Early-Onset IBD

Research Goals

A careful analysis of early-onset IBD is needed to determine whether there are pathologic features distinctive from adult-onset disease. It is critical to further delineate cell-mediated and humoral-mediated immune events as well as the regulatory mechanisms governing epithelial injury. Both clinical trials and studies in animal models are needed to identify markers of autoimmunity and elucidate mechanisms of tolerance. A key research focus is the cytokine-driven intestinal and systemic response that leads to the establishment of pediatric IBD. Another focus is the immune response driven by resident luminal flora, dietary antigens and specific pathogens. Studies to date of intestinal permeability have been inconclusive regarding a primary or secondary role in pathogenesis; careful analysis in humans and in animal models should be initiated.

Current research is focusing on:

The intestinal response in animal models of mono- and complex bacterial reconstitution
Alterations in response to flora in different genetic models
The implications for pathogenesis using different background strains of mice

Future research should focus on:

The early intestinal immune response in animal models, coupled to similar analysis of the human response
Identification of potential autoantigens and the associated immune responses
Attempts to induce tolerance to reduced intestinal inflammation

Research Strategies

This is one of the largest areas of current research, involving a number of projects in animal models. Clinical studies must involve large numbers of study subjects, carefully stratified patients and a consistent methodology. Pediatric patients may be uniquely suited to such studies because of a lack of confounding factors, such as use of alcohol, comorbid conditions, long-standing subclinical disease and therapy for concomitant disorders. As specific pathways of immune response are confirmed, novel therapies can be defined based on these data.

Projected Timetable and Funding Requirements

This field has already generated interest in investigator-initiated research grants. Additional investigator-initiated projects as well as collaborative initiatives such as program projects are appropriate.

Study the Role of Dietary, Nutritional and Environmental Factors in the Pathogenesis of Pediatric IBD

Research Goals

A major goal is the conduct of large population studies evaluating dietary influences on the prevalence and onset of IBD. Recognizing that large databases to date have focused on adults, the prospective acquisition of data may enable the identification of risk factors not previously identified. Such data could also answer specific questions, such as the impact on expression of IBD of food processing, genetically altered food, specific food substances, infant feeding practices and use of antibiotics in childhood.

Research Strategies

Retrospective reviews of large databases (e.g., the Nurses' Health Study, Physicians' Health Study and Kaiser databases) should be performed for information relating to dietary and nutritional risk factors for IBD. If possible, National Health and Nutrition Examination Survey (NHANES) data should be reviewed for pertinent data. As the large surveys are updated, new questions should be added which will be valuable for identifying factors associated with IBD. The short-term analysis of specific risk factors may be better accomplished by prospective case-control studies.

Projected Timetable and Funding Requirements

These studies may be funded by individual R01 grants, but collaboration between the principal investigators and managers of the large databases will be required. Supplemental support from organizations such as the Children's Digestive Health and Nutrition Foundation (CDHNF), the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and

American Gastroenterological Association (AGA), in the form of seed grants, might be useful in establishing new investigators in this important field. Seed grants from CCFA also might be helpful.

Establish A North American Database of Pediatric Patients With IBD

Research Goals

A pediatric IBD database needs to be established that can be utilized to prospectively characterize disease. The database would contain information on family history, disease expression on presentation, disease characteristics recorded annually, potential predisposing factors, extraintestinal manifestations, treatment course, history of surgery, and outcome. The database can also serve as a reference for investigators studying genetics, drug therapy, health outcomes and the socioeconomic impact of IBD.

Research Strategies

The Pediatric IBD Consortium, which was established under CCFA pilot support, should be expanded beyond the original centers in Boston, Philadelphia, Atlanta, Chicago, Houston and San Francisco. A mechanism for NIH support should be developed to expand the database to include all 50 states. An important advance will be the involvement of Canadian physicians and pediatric IBD centers in Canada.

A focused effort should be made to establish a validated self-reported health status instrument for use in children across the developmental spectrum. In addition, appropriate quality-of-life indices can be developed for children with IBD.

Projected Timetable and Funding Requirements

Initial funding for a pediatric database has been provided by CCFA, but NIH support is needed to sustain and expand the database. The success of the Cystic Fibrosis Registry and existing cancer registries attests to the value of such investments.

Evaluate Therapies and Management Approaches for Pediatric IBD

Research Goals

Are novel approaches needed for the management of pediatric patients with IBD? The traditional paradigm has been to treat mild disease with "mild" drugs and to intensify therapy as the disease intensifies. This paradigm also assumed that the first drug used (e.g., aminosalicylates) could also serve as maintenance therapy. Alternative treatment paradigms do need to be evaluated, such as suppression of early, mild disease by a potent agent, which is quickly replaced by an effective maintenance treatment. For example, initial therapy with budesonide could be followed by early institution of 6-mercaptopurine. Or short-term use of a potent biologic to "turn off" the active immune response could be accompanied by an equally potent maintenance drug suitable for long-term oral administration. Thus, smoldering disease could be eliminated and outcomes in young adulthood altered. Once the role of enteric flora is better understood, a targeted antibiotic could be used as initial treatment, to be followed by a probiotic.

Growth failure persists as a common complication of IBD, particularly CD, and optimal therapy has not yet been defined. Studies are needed to determine the role of nutritional support and the potential of growth hormone, insulin-like growth factor 1 (IGF 1) and pharmacotherapy other than corticosteroids. The concept of control of disease activity at the appropriate time requires further study. Bone mineral status also requires further study. Bone mineral depletion has been reported in pediatric patients with IBD who never received corticosteroids. Optimal approaches for the management of all patients with osteopenia/osteoporosis are needed.

Studies evaluating specific therapies are also needed, including the use of 6-mercaptopurine early in the course of treatment; anti-tumor necrosis factor alpha (TNFα) therapy; nutritional therapy (e.g., prebiotics, eicosapentanoic acid/docosahexanoic acid [EPA/DHA]) both for induction and for maintenance; combination therapies; and both preventive and therapeutic interventions for steroid-induced bone mineral loss.

Research Strategies

The first priority is to understand the pharmacokinetic, pharmacodynamic and pharmacogenomic characteristics in children of commonly used anti-IBD drugs, which were originally evaluated in adult patients. Another priority is the establishment of collaborative controlled clinical trials. These will be facilitated by the large pediatric database described above.

Projected Timetable and Funding Requirements

Support from a broad range of resources can be explored. Investigator-initiated proposals are appropriate for the study of the pharmacology and pharmacogenomics of specific agents. Support also can come from collaboration between industry and independent investigators.

HEALTH AND ECONOMIC OUTCOMES

At present, the health care costs associated with pediatric IBD include not only the direct costs of treatment,

but also the economic impact on families of expenses not covered by insurance and days missed from work. There is a further impact on society as a whole, as the productivity of entire families can be impaired by these chronic diseases. In addition, pediatric-onset disease may alter the capacity of the child to become a successful and productive adult. Advances in therapeutics for pediatric IBD should therefore yield a significant reduction in both individual and societal costs. The successful accomplishment of these research goals will enable us to better quantify the current and future impact of these diseases.

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Research Agenda for Pediatric Gastroenterology, Hepatology and Nutrition: Allergy and Immunology

Report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition for the Children's Digestive Health and Nutrition Foundation

RATIONALE

The gastrointestinal (GI) tract is exposed to a vast array of foreign antigens in the form of ingested foods, environmental toxins and microorganisms. The organized immunological system of the GI tract, or gut-associated lymphoid tissue (GALT), is responsible for defending the host from pathogens while simultaneously remaining unresponsive to food antigens. In addition, several other cell types, such as the epithelial cells that line the intestines, perform important immune functions.

Aberrant activity in GALT is associated with a variety of diseases, such as food hypersensitivity reactions, eosinophilic gastroenteritis, eosinophilic esophagitis, celiac disease, autoimmune diseases and inflammatory bowel disease, but the precise pathogenesis of these conditions is not well understood. As many as one in four American households report the perception of a family member with a food allergy. However, the true prevalence of food allergy is much lower. Approximately 6% to 8% of children in the US are affected with food hypersensitivity reactions in the first year of life, a much higher prevalence than in adults. However, controversy still exists over the true prevalence of allergic diseases of the GI tract because of the limited tests available to accurately diagnose this and other immunologically mediated diseases.

An important area for ongoing investigation is the prevention of disease expression. This can be at the level of primary prevention (inhibiting sensitizing antibody production), secondary prevention (inhibiting disease expression after sensitization), or tertiary prevention (suppressing symptoms despite sensitization and disease onset). It is clear that the prevalence of these food-induced reactions is significantly higher in children with atopic dermatitis or asthma. Strategies aimed at primary prevention will be aided by further studies of the genetic factors associated with food hypersensitivity—in particular, the identification of genes or genetic markers that would identify individuals at risk.

Celiac disease is an autoimmune process driven by ingestion of wheat, rye and barley proteins in genetically susceptible individuals. Celiac disease is unique among the autoimmune diseases in that the inciting agent—the cereal protein gliadin—is well known, a genetic marker (DR3 or DQ2) is known, and treatment with a gluten-free diet is effective. The prevalence of celiac disease may be as high as 1:250 based on cross-sectional studies from Italy and Baltimore blood donors, with emerging evidence of 1:100 based on prospective early childhood data. Certain groups are at increased risk of celiac disease, including those with type 1 diabetes (4% to 10%), Down syndrome (7% to 19%), and other autoimmune disorders. Most diabetes programs regularly screen for celiac disease. It is not known why most individuals with a genetic predisposition and similar environmental exposures do not develop celiac disease. In addition, the factors influencing early versus late onset and disease severity are not known.

The majority of affected individuals have no symptoms or mild symptoms, which are usually not concerning enough to seek medical attention. Over the past few decades, a decrease has been noted in the frequency of the "classic" presentation of celiac disease (diarrhea, malabsorption, growth failure, nutritional deficiency). Instead, there has been an increase in the "atypical" presentation, not obviously related to the GI tract (e.g., dental enamel defects, arthritis, iron deficiency anemia, epilepsy with cerebral calcifications and cerebellar ataxia, infertility, osteoporosis). Why celiac disease is associated with multi-systemic involvement is unclear.

The current, albeit inadequate, definition of celiac disease requires the demonstration of characteristic change in the lining of the small-bowel mucosa, which is reversible with treatment with a gluten-free diet. However, lifelong adherence to the diet is achieved primarily by those with severe symptoms on ingestion of offending proteins. Untreated celiac disease is a risk factor for intestinal lymphoma.

A major advance has been the description in 1997 of tissue transglutaminase (TG) as the antigen recognized by the anti-endomysial antibody test. The TG assay detects the major antigen associated with celiac autoimmunity, is now widely commercially available, and lends

itself to mass screening. Use of new serologic assays has confirmed that most affected individuals have little or no symptoms and are identifiable only by screening programs.

Although the pathogenesis of GI manifestations of immunoregulatory disorders is not well understood, correction of immune deficiencies often corrects or improves malabsorption. This observation is especially true in human immunodeficiency virus (HIV) disease. On a worldwide scale, HIV is the most devastating of the immunodeficiency disorders. According to the World Health Organization, in the year 2000, approximately 36 million adults and children are living with HIV disease, the great majority (25.3 million) in sub-Saharan Africa. A total of 920,000 adults and children are affected in the US and Canada. Over the past two decades, about 21.8 million people have died, including 3 million in the year 2000. Because heterosexual transmission throughout the world has increased in prevalence, the number of congenitally infected children continues to increase, especially in Africa and Asia. The socioeconomic impact of this disease is measured in billions of dollars.

Breaks in mucosal integrity such as mouth ulcers, surface inflammation such as with oral thrush, and a more permeable mucosal surface may be the entry route for HIV infection in the gut. The presence of virus particles in oropharyngeal and gastric aspirates of newborn infants, along with the observation that premature and low-birth-weight infants have a higher risk of mother-to-child HIV transmission, supports a role for the GI tract in the acquisition of this perinatally acquired infection. In addition to transmission of HIV to children, injury and malabsorption in the GI tract may contribute to morbidity and mortality. Effective antiretroviral therapy may reduce GI dysfunction. A better understanding of the relationship between mucosal immune function and absorption will lead to more effective therapy for both primary and secondary immunodeficiencies.

AREAS OF EMPHASIS

Elucidate the mechanisms involved in oral tolerance

Research Goals

Oral antigen is known to induce the down-regulation of immune responses at peripheral sites. This property, known as oral tolerance, is not completely understood at a mechanistic level. However, there have been several attempts to use this property of the GI immunological system as a therapeutic modality for multiple myeloma, rheumatoid arthritis and other autoimmune diseases. To date, this approach has been problematic for several reasons, including a lack of understanding of the fundamental mechanisms of oral tolerance and a lack of knowledge of the specific antigen that might be involved in these conditions.

Studies should be directed at elucidating the mechanisms of oral tolerance. The specific issue of how oral tolerance is induced and maintained needs further investigation. The role of antigen processing in the gut also warrants further study as it impacts on this issue.

Research Strategies

The Immune Tolerance Network (ITN) is an international consortium of clinical researchers dedicated to developing approaches to induce immune "tolerance." The scope of this organization should be expanded to include pediatric-based immunological disorders such as food hypersensitivity and eosinophilic diseases of the GI system.

Projected Timetable and Funding Requirements

These questions are amenable to requests for applications (RFAs) to study specific aspects of mucosal immune function. Many of these basic questions will be answered by research in related areas. Investing in the training of pediatric investigators will enhance the transfer of knowledge to problems relevant to pediatric gastroenterology.

Define the role of the intestinal epithelium in immune responses

Research Goals

Considerable evidence suggests that the intestinal epithelium is not a passive barrier to luminal antigens, but in fact acts as a sensor of the luminal environment and may play a role in initiating immunological responses. For example, intestinal epithelial cells can produce a variety of pro-inflammatory cytokines and chemokines that may influence inflammatory reactions. The intestinal epithelium has also been shown to present antigens in a non-classical, non-major histocompatibility complex (MHC) restricted manner. Most of the research into epithelial immune function has been carried out using transformed tumor cell lines of colonic origin, which may not accurately represent epithelial cell function in vivo. Human and small animal cell lines representative of small-intestinal epithelium must be developed and in vivo approaches to epithelial immune function must be emphasized.

Research Strategies

The mechanisms of epithelial cell antigen processing must be addressed at the molecular level, using approaches such as confocal microscopy to determine antigen uptake, intracellular trafficking and antigen presentation. The signal transduction pathways involved in ep-

ithelial cytokine production need to be elucidated, particularly from the standpoint of ligand/receptor interactions. Development of new cell lines must be a priority to facilitate these studies.

Projected Timetable and Funding Requirements

This area of research has many investigator-initiated research grants and program projects, but additional projects relating to epithelial function in the developing organism are appropriate.

Develop new approaches to diagnose and treat food hypersensitivity reactions

Research Goals

The diagnostic approach for suspected food hypersensitivity reactions was recently reviewed, and it is clear that little has changed over the past decade. The radioallergosorbent test (RAST) and properly performed prick/puncture skin testing have a role in patients suspected of having reactions mediated by immunoglobulin E (IgE). In the case of food hypersensitivity reactions thought not to involve IgE-dependent mechanisms, elimination diets and food challenges remain the mainstay of diagnosis, with the double-blinded and placebo-controlled food challenge considered the "gold standard." While accurate, this approach is costly and can be performed only in a limited number of centers. Similarly, therapy in general remains the avoidance of offending antigens, but this is often difficult when there are reactions to multiple antigens.

Research Strategies

New diagnostic and therapeutic approaches need to be developed. In vitro analysis of T-cell function and development of Th2 responses must be studied at the molecular level to potentially down-regulate or switch-off aberrant T-cell activity. The genetics of food hypersensitivities must be studied from both a diagnostic and patient counseling standpoint. New therapies for other atopic diseases are on the horizon. For example, administration of humanized monoclonal anti-IgE antibodies has been demonstrated to be efficacious in the treatment of asthma and allergic rhinitis. Such novel therapies should be studied in children with food hypersensitivity reactions. In addition, new genetic approaches aimed at "desensitizing" the patient to reactive proteins warrant intense investigation.

Projected Timetable and Funding Requirements

Unfortunately, many in the scientific community have considered research in the area of food allergy a "soft" science. Given the large number of patients that research in this area will impact, more investigator-initiated research grants are necessary to understand these problems. The development of a consortium to investigate the genetics of food hypersensitivity and new diagnostic and therapeutic approaches would greatly enhance our understanding, and the treatment, of these conditions. Such a consortium would coordinate clinical research protocols and share patient materials for basic research efforts.

Clarify the natural history and immunopathogenesis of celiac disease

Research Goals

Develop working definition of celiac disease. The 1990 European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) definition of celiac disease requiring typical alterations of small-bowel mucosa is outdated. A modern definition incorporating newer serologic assays and the extraintestinal manifestations of celiac disease is needed. Many GI societies in Europe and the US have convened expert panels to clarify the diagnostic criteria for celiac disease, and some guidelines have recently been published.

Investigate pathogenesis of celiac disease. The pathway leading to T-cell activation—specifically the interplay between DQ2 and DR3, T cells, gliadin and transglutaminase—is an area of potential importance to understand the immunopathogenesis of celiac disease.

Evaluate screening cost-effectiveness. The cost-effectiveness of mass screening strategies needs to be evaluated. This entails assessing the clinical importance of screening-identified celiac disease and the benefits of early vs. late case ascertainment. The social impact of screening also warrants careful analysis.

Research Strategies

A number of research strategies are recommended. Increased collaboration with diabetes centers can provide an infrastructure and offer additional resources to evaluate potentially effective screening programs. Collaboration with researchers in related fields, including immunology, preventive medicine and social sciences, also is to be encouraged. Multicenter prospective studies are needed to assess the role of environmental factors and genetics in development of celiac autoimmunity and the clinical significance of celiac autoimmunity. New technologies, such as DQ2 or DQ8 tetramers, need to be applied to study the interplay of T-cell activation mechanisms with gliadin and transglutaminase.

Projected Timetable and Funding Requirements

These research questions can be addressed in investigator-initiated studies, but such projects also can

be integrated into large multicenter studies and program projects evaluating other autoimmune disorders.

Develop approaches for the prevention and treatment of celiac disease

Research Goals

Develop animal model. The prevention of celiac disease is a tantalizing goal that should be considered within reach. Development of an animal model for celiac disease would provide a critical tool for this endeavor.

Improve compliance with gluten-free diet. Allocation of resources to the development and management of post-diagnosis management of celiac disease would result in programs that improve compliance. Modified grains, whether bioengineered or created through cross-breeding, may possess nontoxic or less toxic or less abundant offending proteins. Genetic engineering of foods is a hotly debated area of research and ethical discussion. Clearly, patients with celiac disease are one group that might benefit from genetically engineered foods from which gluten is eliminated. In addition, a clearer definition of "gluten-free" is needed for international commerce, to increase availability of gluten-free products to the consumer.

Research Strategies

Collaborative relationships are to be established with the agriculture industry and social science professionals. Development of better treatment options may require novel strategies, such as the alteration of dietary proteins and modification of the intestinal milieu to induce immune tolerance.

Projected Timetable and Funding Requirements

Collaboration with industry to develop gluten-free foods could be supported through Small Business Initiated Research (SBIR) grants.

Define the role of the mucosal and systemic immune systems and HIV in malabsorption and growth retardation associated with congenitally acquired HIV disease

Research Goals

The pathogenesis of intestinal dysfunction is a major question in HIV-infected children. The lack of appropriate animal models has resulted in data primarily from human subjects. Basic physiological data in HIV-infected children are lacking that would enable clinicians to understand the role of malabsorption, viral load and immune function on growth and body composition. Clinical trials on the impact of early nutritional therapy in regions with high transmission rates, such as sub-Saharan Africa, may provide data that will guide future research involving effective nutritional therapeutic interventions. In the less-developed world, maternal-child transmission and malnutrition are the most important areas in which to focus research efforts. Research efforts should be prioritized to these areas in which new knowledge is needed.

Research Strategies

Animal and cellular models should be developed to investigate the potential interrelationships between nutrition, infection, gastric acid production, motility and the effects of the immunological, neurological and endocrine systems on GI tract function as well as mucosal and systemic immunity. In the less-developed world, socioeconomic issues play a central role in the GI and nutritional aspects of HIV disease. Research studies are currently ongoing to evaluate nutritional strategies in HIV-infected children. Integrating studies of intestinal function and mucosal immunity will enhance our knowledge of the pathogenesis of malabsorption.

Projected Timetable and Funding Requirements

In the US, investigator-initiated research projects may be funded through collaborative RFAs from the National Institute of Child Health and Human Development (NICHD), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Institute of Allergy and Infectious Diseases (NIAID). In less-developed countries, agencies such as the World Bank could play a central role in the education of the public and professional sectors of the community.

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