Electron micrograph of enterotoxigenic *Escherichia coli* contacting microvilli on the surface of epithelial cells within pig small intestine. These organisms are a major cause of human diarrheal disease worldwide.

Infections of the Gastrointestinal Tract

SUMMARY OF RESEARCH GOALS

Gastrointestinal (GI) infections can be caused by many types of microbes, including bacteria, viruses, protozoa, and helminths. The Commission recommends research goals that are focused on identifying disease-causing microbes, understanding what distinguishes those organisms from the normal microflora of the human GI tract, and using that knowledge to develop safe, effective therapies to prevent and treat intestinal infections. Developing new, more efficient diagnostic methods to identify specific organisms is critical for rapid treatment and for understanding the epidemiology of infectious disease outbreaks. Research is needed to develop better treatments, including vaccines, that address both the infectious agents themselves, as well as the long-term effects of GI infection in the gut and other organ systems throughout the body. The human GI tract is colonized from birth with microorganisms that are essential for normal growth and digestive function. Research on the nature and function of the human microflora could suggest strategies to manipulate these beneficial microbes to combat pathogenic organisms. Collectively, achievement of these goals has the potential to reduce the public health burden of infectious diseases in the U.S. and globally.

INTRODUCTION AND BACKGROUND

Diarrheal disease is a major cause of morbidity, with incidence rates ranging from two illnesses per person per year in developed countries to 12 or more in developing countries. Intestinal infections continue to exact an unacceptable toll on childhood and adult well-being. This is particularly true in the developing world where diarrhea has significant morbidity and mortality, especially when accompanied by malnutrition. In industrialized countries, deaths from diarrheal diseases are uncommon, but the morbidity and economic costs associated with intestinal infections remain substantial and the burden of lost productivity might exceed $19 billion per year. In recent years, developed countries in North America and Europe have experienced outbreaks of a highly toxic and antibiotic-resistant strain of the bacteria *Clostridium difficile* linked to overuse of antibiotic therapy. Although common enteric infections are not the highest priority for efforts directed at bioterrorism, research advances in infectious diseases of the digestive system could help to provide better solutions to the problem, in the event that enteric infectious agents might be used in the future as agents of bioterrorism.

Viruses, bacteria, and parasites cause protean illnesses, including acute watery diarrhea, bloody diarrhea, persistent diarrhea, chronic diarrhea, and asymptomatic infection. The etiologies of many intestinal infections remain unknown. GI infections and alterations in enteric microflora might also be related to functional GI disorders, either directly or as post-infectious phenomena such as irritable bowel syndrome (IBS). Research is also uncovering the role of specific microbes in GI inflammation, such as the ground-breaking studies linking the bacteria *Helicobacter pylori* to gastritis and peptic ulcer disease—conditions that were not previously thought to have an infectious cause—for which the

2005 Nobel Prize in Physiology or Medicine was awarded (additional information on this advance and related future research goals appear in the chapter on *Diseases of the Stomach and Small Intestine*). In addition, animal studies suggest that some species in the intestinal microflora might precipitate or perpetuate inflammatory bowel diseases (IBD) and contribute to extraintestinal manifestations of autoimmune diseases. Most recently, dramatic studies indicate that the microflora living in the intestine can have a profound impact on overall health and non-diarrheal diseases, such as obesity.

Intestinal infections pose multiple challenges. First, illness etiologies vary by geography, requiring investigative strategies that are location-appropriate. Second, accurate diagnoses are often thwarted by the polymicrobial nature of stool and the limitations of current diagnostic tools. Third, host-microbe interactions show great variability related to host-microbial genetics and host-microbial cross-talk. Enteric infections in people with the acquired immunodeficiency syndrome (AIDS), immune suppression due to organ transplants, and other immune disorders also require unique approaches.

Diarrhea continues to burden communities that lack clean water, safe food, and acceptable waste management. Underlying problems often relate to development, economic, and political issues, which are difficult for the medical community to address effectively. However, the consequences of these illnesses diminish the ability of communities to counteract these non-medical challenges. Globally, poor growth is the most important risk factor for childhood morbidity and mortality, and many children in resource-poor regions have abnormal small bowel structure and function, including crypt hyperplasia, villus stunting, hypercellular lamina propria,

decreased mucosal surface area, and increased intestinal permeability. These changes are plausibly caused by environmental exposure to contaminated food and water, but the roles of specific components of the microflora remain to be determined.

A number of promising advances in vaccination strategies have been made, including the development of effective rotaviral vaccines. Additional vaccines and other novel preventive strategies are critically needed to reduce the impact and burden of GI infections. These approaches are highly effective and provide dramatic return on investment.

RECENT RESEARCH ADVANCES

Emergence of novel agents of GI illnesses

Using the best technologies, many enteric infections remain undiagnosed. *Escherichia coli* is the most common facultative organism in the human gut, and most *E. coli* are non-pathogenic. However, a subset of *E. coli* has acquired sufficient virulence traits to cause severe human disease. Recent data demonstrate the likely etiologic role of enteroaggregative *E. coli* (EAEC) in traveler's diarrhea, persistent childhood diarrhea, and even as a cause of emergency department visits in North America. By identifying such agents, it is now possible to develop diagnostic, prevention, treatment, and complication mitigation strategies. The recognition that EAEC and other diarrheagenic *E. coli* cause disease in multiple human populations with diverse syndromes exemplifies the challenge of pathogen discovery when the agent closely resembles harmless or even beneficial microflora (e.g., commensal *E. coli*).

Emergence of enhanced virulent strains

Well-established pathogens have acquired new virulence traits. *Clostridium difficile*

exemplifies a pathogen with enhanced virulence in nosocomial (hospital or other healthcare unit) and community settings, particularly those located in North America and Europe. These organisms cause increased morbidity and mortality, and development of more effective strategies to prevent and treat *C. difficile* disease should be considered a high priority.

Bacterial-host interactions

There has long been recognition that bacterial-host interactions are a two-way street, and that organisms respond to their milieu. However, specific communication mechanisms, particularly those that up-regulate virulence factors and induce injury, are poorly elucidated. Similarly, bacteria in the gut can be modulated by the host, as exemplified by adrenergic signaling to enteropathogenic *E. coli* (EPEC). This work suggests that such a mechanism might be exploited to diminish host injury.

Environment-pathogen-host interconnection

The inter-epidemic reservoir of *Vibrio cholerae* is a major source of human illness. A strong inverse association between bacteriophage "blooms" and *V. cholerae* presence in water in Bangladesh illustrates the complex interplay between the environment, bacterial control elements (e.g., bacteriophages and *V. cholerae*), and reservoirs that serve as vehicles to transmit this pathogen to humans. The intersection between reservoirs and hosts will need to be addressed to control enteric pathogens.

Mechanisms of viral pathogenesis

Novel mechanisms have been discovered for GI viral pathogenesis, including viral enterotoxins and host-pathogen interactions, such as new ways in which viral pathogens counteract the innate host response. Genetic polymorphisms have been shown to increase

non-immune mediated resistance to infection (host susceptibility) and may affect immunity to viral infections and vaccines. New animal models have led to better understanding of pathogenesis and immunity, as well as opportunities for preclinical testing of new vaccines and therapeutics. Such models include rotavirus (RV)-induced biliary atresia, RV-associated intussusception, RV-induced sterilizing mucosal immunity in mice, human RV-induced disease in rats with recurrent infection, and norovirus-induced disease in mice and piglets.

Susceptibility to infection or disease

Data have emerged linking gut disease susceptibility to changes in gut physiology. Alterations in barrier function may give rise to IBD and/or other autoimmune diseases, and acute infectious inflammatory diarrhea may predispose to IBS. Susceptibility to infection is also related to alterations in gut immunology and/or host genetics; for example, *IL8* gene polymorphisms modify clinical diarrheal disease severity.

Antiviral and intestinal parasite vaccines and therapies

The development and licensing of two new rotavirus vaccines is an important advance in the prevention of rotavirus infections, the leading cause of severe diarrheal disease and dehydration in infants and young children in both industrialized and developing countries. Genome sequencing, protein expression, and crystal structures of key proteins and viral structures inform targets for the development of new therapeutics, as well as a molecular understanding of strain diversity that may be critical for vaccine development. Virus-like particles are being studied as safe and efficient immunogens and as tools for environmental and epidemiological studies. Likewise, development and early-stage clinical testing of recombinant

vaccines for hookworm and schistosomiasis, as well as identification of promising vaccine candidates for amebiasis—so-called "anti-poverty vaccine development"—are significant advances with worldwide impact.

Mechanisms of probiotic activity

The demonstration that immune and epithelial cells can communicate and subsequently discriminate between different microbial species has extended the known mechanism(s) of action of probiotics. Probiotics may exert their disease-modifying effects through one or more mechanisms that include: competitive exclusion, antimicrobial activity, enhanced epithelial barrier activity through tight junction modification and mucus production, and stimulation of anti-inflammatory mucosal and systemic effects through a number of complex probiotic-epithelial-immune cell interactions.

The progressive unravelling of these mechanisms of action has led to new credence for the use of probiotics in clinical medicine. Level 1 evidence now exists for the therapeutic use of probiotics in infectious diarrhea in children, recurrent *C. difficile*-induced infections, and post-operative pouchitis. Level 2 and 3 evidence is emerging for the use of probiotics in other GI infections, prevention of post-operative bacterial translocation, IBS, and in both ulcerative colitis and Crohn's disease. Nevertheless, it is clear that not all probiotic bacteria have similar actions or therapeutic effects.

Extraintestinal consequences of GI infection

Campylobacter jejuni is a fairly common cause of bacterial enteric infection. This organism is found worldwide and transmitted through the food supply, most typically poultry. A small subset of infected patients will develop Guillain-Barre syndrome, a severe, debilitating, potentially lethal ascending neurologic

paralysis. The chief pathogenic mechanism is believed to be induction of antibodies to the *C. jejuni* lipopolysaccharide, which also react against peripheral and central nervous system gangliosides. This is an example of autoimmune molecular mimicry following a GI infection and exemplifies the role of host defense (i.e., antibody induction) leading to host injury. It is an important paradigm applicable to a panel of additional pathogens. GI virus infection is not confined to the gut, but can spread extraintestinally; for example, rotavirus causes viremia in children and all animal models tested. Extraintestinal spread may be a general characteristic of human gastroenteritis viruses; this property was not previously appreciated based on studies with animal caliciviruses and astroviruses.

Long-term impact of enteric parasitic infection

The long-term impact on growth and cognitive function of repeated or persistent enteric parasitic infections (e.g., protozoa, helminths) has been recognized. These studies highlight how enteric parasitic diseases contribute to long-term economic and intellectual losses in resource-poor countries with implications for global development.

Bacterial communities in the gut as determinants of non-infectious diseases

The human intestinal microflora are metabolically, genetically, and antigenically diverse. Germ-free mice are protected against obesity when consuming conventional Western-style diets rich in sugar and fat, while colonized animals are not. The gut microflora are an important component of our metabolic output, and metagenomic and biochemical studies demonstrate the critical roles of the microflora in harvesting dietary calories and transferring energy in the form of calories to the vertebrate host. The relative abundance of the two major bacterial intra-intestinal divisions—Bacteroidetes and Firmicutes—is a potential determinant of host adiposity. Alterations in this microflora offer the opportunity for significant influence of extraintestinal health, such as obesity risk—an avenue that has not yet been adequately explored. Many methanogenic archaea—a group of prokaryotic and single-celled microorganisms similar to bacteria—are found in the digestive tracts of humans. The role of this community in human digestive health and disease is not well established.

Research Goal 3.1: Elucidate the etiology, epidemiology, and pathogenesis and improve diagnostic tests for intestinal infections. (See also Goals 1.21 and 9.3.)

In the U.S., an estimated 20-40 million episodes of diarrhea occur annually in children younger than 5 years of age, and diarrheal illnesses continue to be a burden for older children and adults. Approximately 13 percent of all pediatric hospitalizations in the U.S. in this age group are for diarrheal disease. Worldwide, the number of childhood deaths from diarrhea is higher than 2.5 million per year.

Knowledge of diarrheal disease has increased remarkably during the past few decades. Numerous bacterial pathogens and an increasing number of viral pathogens have been demonstrated to cause diarrhea. However, the etiology of diarrheal disease in both developing and developed countries is unknown for 25-40 percent of all illnesses. Although diarrhea morbidity and mortality are largely related to fluid and nutrient losses, the specific etiology of intestinal infection is critically important for designing science-based prevention and control mechanisms, especially vaccination.

Classically, assigning pathogenicity to an organism has depended on identification of candidate pathogens more frequently in infected individuals than in asymptomatic controls. This approach is less informative when considering organisms that contain a repertoire of virulence loci. This is especially pertinent in view of recent data that bacterial chromosomes are mosaics, made up of horizontally acquired elements that may contain genes that are not necessarily found in all pathogens. Clearly, designation of an organism as a pathogen now requires considerably more sophisticated microbial and genomic sampling and statistical analyses than have been used previously.

In addition, genotypic variability among pathogens renders some organisms more virulent than others, and the specific determinants of pathogenicity are often poorly understood for infectious agents.

Objectives:
- Understand the pathogenic mechanisms used by viruses, parasites, and bacteria in causing intestinal infection, including genotypic variability in known and putative enteropathogens.
- Understand how microorganisms interact with their environment to reach critical numbers capable of human infection and to express pathogenic factors in the intestine.
- Conduct epidemiologic investigations using modern tools to define infectious etiologies, establish the incidence of known and novel etiologic agents of diarrheal disease, and characterize the health impact of acute and persistent enteric infection in distinct subgroups of hosts, including normal and immune-compromised populations (e.g., solid organ transplant, bone marrow transplant, oncology patients).
- Develop new point-of-care diagnostics to easily and rapidly detect known microbial enteric pathogens.
- Discern correlates of protective immunity for enteric infections.
- Develop animal models to study the effects of manipulating the microbial community in the gut.

Research Goal 3.2: Improve the prevention and treatment of intestinal infections.

Provision of clean water and sanitary food are important worldwide strategies to prevent enteric infection. However, preventing enteric illness by improved hygiene is currently impractical in most developing countries. For this reason, vaccination

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[9] Research Goals are numbered for ease of reference only; the numbers do not indicate prioritization of scientific topics.

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against the most important GI infections has been aggressively pursued. A new generation of enteric vaccines based on either live or nonliving antigens delivered orally or by injection is in the early phases of evaluation. However, considerable technical barriers need to be overcome related to the large number of pathogens capable of causing disease and the requirement to induce immunity that is effective in the gut. It will be important to advance development of vaccines that are effective and economically accessible to those most at risk for infection and illness.

The use of probiotics (ingested microbes that can modify intestinal microbial populations in a way that benefits the host) has moved from concept to demonstration of unique benefits by specific microorganisms for a given patient population. However, the science of probiotics is still in its infancy, and scientific claims for improved outcomes, such as disease prevention and treatment, are often unsubstantiated. It is increasingly clear that benefits from probiotics are mostly mediated by the effect that intestinal microflora have on gut barrier function and host immune response. Identifying the role and mechanism of action of probiotics for treatment and prevention of diarrhea, reducing the risk of intestinal disease (e.g., necrotizing enterocolitis), and modulating host immune response to extraintestinal disease, such as allergic disease, are important potential areas of future research.

Antibiotic-associated diarrhea and colitis were recognized soon after antibiotics became available, and *C. difficile* was identified as a causative agent in the 1970s. In the last few years, *C. difficile* has been more frequent, more severe, more refractory to standard therapy, and more likely to relapse. This pattern is widely distributed in the U.S., Canada, and Europe and is now largely attributed to a new strain of *C. difficile*, designated BI/NAP1

or ribotype 027. The recent experience with *C. difficile* serves to emphasize the need for better diagnostics, early recognition, improved methods to manage severe disease and relapsing disease, and greater attention to infection control and restraint in the administration of antibiotic therapy. In addition, emerging antibiotic resistance in other GI pathogens and pathogens that use the GI tract as a portal for systemic infection (e.g., *Salmonella typhi*) is an increasing problem.

Objectives:
- Define how common conditions such as age, malnutrition, or diabetes mellitus modify mucosal innate and adaptive immunity and physiology, altering susceptibility to enteric illnesses and vice versa.
- Understand the role of host genetics in the response to GI infections.
- Understand the mechanisms of action of probiotics and prebiotics.
- Conduct appropriately designed and powered large-scale, placebo-controlled, randomized, double blind clinical trials to demonstrate safety and efficacy and substantiate the potential for novel interventions to treat or prevent enteric infections.
- Promote strategies to reduce nosocomial enteric infection, such as handwashing.
- Investigate and promote novel strategies to reduce and treat *C. difficile* infection.
- Develop new strategies for microbial killing or stasis, especially for infections in which there is emerging antimicrobial resistance.
- Advance vaccine strategies for appropriate pathogens to reduce morbidity and mortality of enteric infection. Integrate measures to control enteric parasitic infections, including vaccine distribution and potential mass treatment protocols (e.g., wide-scale de-worming, preventive chemotherapy agents).

Research Goal 3.3: Understand and modulate the long-term intestinal and non-intestinal consequences of GI infection.

Diarrhea is well-recognized as a leading cause of childhood mortality and morbidity in developing countries; however, possible long-term deficits from heavy diarrhea burdens in early childhood include delayed growth and cognitive development beyond the immediate impact of infection. It is unclear whether mechanisms such as disruption of the mucosal barrier account for these long-term sequelae of acute infection.

Similarly, long-term consequences of acute infection in developed countries include persistent IBS. Infection by pathogenic organisms leads to mucosal damage and disruption of the gut's extensive commensal microflora—factors that may lead to prolonged bowel dysfunction. Although many patients improve over the first 6 months, recovery can be slow, with approximately 50 percent still having symptoms at 5 years.

Although it is clearly a goal to reduce the burden of acute enteric infection, understanding the unintended consequences of such a strategy is critical. The hygiene hypothesis suggests that increases in chronic inflammatory disorders (e.g., allergies, IBD, and autoimmunity) in developed countries are partly attributable to diminishing exposure to organisms that were part of mammalian evolutionary history. Crucial organisms, including bacteria, helminths, and saprophytic mycobacteria, are recognized by the innate immune system as harmless or "tolerable." This recognition can trigger development of regulatory dendritic cells that may drive regulatory T cell responses to simultaneously processed "forbidden" target self-antigens of the chronic inflammatory disorders.

Objectives:
- Understand the short-term (and long-term) burden and impact of enteric infections on cognition, development, and health. Comprehensive outcome measurements should be developed to guide and standardize assessments in different populations.
- Identify biomarkers to predict the development of systemic diseases secondary to the exposure to enteric pathogens.
- Attenuate host response to specific human infections.
- Define the relationship between intestinal infection and chronic GI (i.e., IBS, IBD) and non-GI diseases.
- Conduct feasibility studies on developing vaccines or other agents against "non-pathogenic" bacteria that might trigger pathologic intestinal inflammation.
- Identify the consequences of reducing the burden of intestinal infection and/or altering the microflora, such as those anticipated by the hygiene hypothesis.

Research Goal 3.4: Understand the human microflora and microbiome in health and disease and modulate them for beneficial effects.

Microorganisms live in complex environments both *ex vivo* and *in vivo*. Current information about synergy between enteric pathogens and other organisms is limited. Many species of bacteria regulate gene expression in response to increasing cell population density; collectively, this phenomenon is called quorum sensing. Quorum-sensing bacteria produce and release signaling molecules (autoinducers) that accumulate in the environment as cell density increases. Quorum sensing is used by bacteria to communicate within the same species and across species. When a threshold stimulatory concentration of autoinducer is achieved, a signal transduction cascade is initiated that is translated into a change in behavior of the organism. Only recently has the complexity and scope of quorum sensing in bacterial regulation been appreciated. Far from being isolated entities,

bacteria exist in multifaceted and communicating populations. The outcome of this cross-talk can lead to either severe or attenuated infections.

The human intestine contains trillions of bacteria, hundreds of species, and thousands of subspecies. Little is known about the selective pressures that have shaped and are shaping this community's component species, which are dominated by members of the Bacteroidetes and Firmicutes divisions. Obese mice have a different microflora than non-obese mice. In addition, in contrast to mice with a gut microflora, germ-free animals are protected against the obesity that develops after consuming a Western-style, high-fat, sugar-rich diet. These observations underscore the importance of considering the intestinal microflora as an important participant in the metabolome in a supraorganismal context and perhaps as a key driver of such diseases as obesity.

Objectives:
- Develop a comprehensive understanding of the intestinal microbiome and the effect of the host genome on microbial colonization. Understand the consequences of interactions between GI pathogens and normal gut microflora for GI function.

- Develop and make accessible computational approaches and tools to assay the microbiome in the gut in a variety of disease states.
- Determine whether the microflora is altered under pathophysiologic conditions (e.g., infectious diarrhea, IBS, IBD, obesity) in animal models and in people.
- Modulate the GI microflora to prevent systemic and intestinal diseases.
- Determine whether genetically modified microorganisms can deliver therapeutic molecules to the GI tract.
- Define the composition of the gut microflora in development, including during breast feeding, formula feeding, perinatal stress, and illness early in life.
- Determine the role of prebiotics, probiotics, and symbiotics in modulating the long-term composition of the intestinal microflora in the neonatal interval and beyond.
- Define the role of archaea in the GI tract in health and disease.

MAJOR CHALLENGES AND STEPS TO ACHIEVE THE RESEARCH GOALS

Technologies to study intestinal infection: Efforts that replace archaic enteric pathogen detection technology and can identify agents across taxa would accelerate research on intestinal infections. The development of rapid and sensitive diagnostics to detect all known enteric pathogens would facilitate research and improve patient care. Other technologies that could be developed include genetic and replication tools to study viral pathogens and resources to study viral pathogens in human challenges. The development of small mammal models and *in vitro* models to study intestinal physiology, mucosal immunity, and microbial-host interactions will provide fundamental insights before trials in humans. New *in vivo* imaging systems that can be applied in animal models and humans will enhance our understanding of the pathogenesis of infectious agents.

Therapeutic development for intestinal infections: Despite the effectiveness of vaccines in eliminating a subset of infectious diseases, several challenges must be

addressed to develop vaccines for enteric infections. Vaccines for some pathogens (e.g., enterotoxigenic *E. coli*, *Shigella*, *Campylobacter*, and *Norovirus* species) will require multiple or yet-to-be-defined antigens (e.g., enteroaggregative *E. coli* and *Cryptosporidium* species). Also, many oral vaccines are less immunogenic when given to malnourished persons who might need them most; vaccines that target populations in developing countries, such as those for cholera, typhoid, and parasites, will require incentives to bring them to and maintain them in global markets.

The development of new interventions, either vaccines or other methods, is hampered by insufficient knowledge of the pathways triggered by pathogens. It is possible that by establishing correlates of protective immunity for GI pathogens, we would eliminate the need for challenges or field trials to study efficacy and, thereby, speed development of licensed candidate vaccines. A final challenge is that probiotics are classified as food supplements and/or natural health products that do not require rigorous clinical evidence for efficacy; this situation directly conflicts with current and proposed uses of these agents as drugs to treat disease.

Resources for intestinal infection research: Repositories for human biologic samples, research reagents, and animal models are needed to promote progress in the field.

New databases, communication networks, and scientific resources are required to develop and sustain longitudinal, large population field studies and productive international collaborations. Infrastructure and improved technology to more promptly identify infected patients would improve our understanding of the epidemiology of foodborne diseases.

Methods to modulate the human microbiome: Improved understanding of the intestinal microflora and microbiome would enhance our potential to identify novel approaches to treatment and to identify those microbes that result in long-term GI sequelae. Learning how to segregate the important variables and developing adequate bioinformatic/computational power will be required in order to make sense out of the massive data resulting from characterization of the trillions of organisms in the intestine. The formation of multidisciplinary teams of researchers in human studies would enable the field to obtain a complete picture that incorporates immune function, transcriptome, microbiology, and clinical responses. Filling the pipeline by fostering the careers of new investigators in this area is a priority. The resource development and research programs supported by the Human Microbiome Project under the auspices of the NIH Roadmap for Medical Research will be critical for research efforts to prevent and treat human intestinal infections through microbe-based approaches.