Challenges in IBD Research: Updating the Scientific Agendas

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EXECUTIVE SUMMARY

Crohn’s & Colitis Foundation of America Research Priorities: Joint Agenda for Clinical and Basic Science

Substantial progress has been made since the first “Challenges in IBD Research” white paper was drafted in 1990. Advances in basic science—particularly in immunology, genetics, epithelial cell biology, signal transduction, molecular biology, and other areas—have added greatly to our understanding of disease pathogenesis and have identified many new targets for therapeutic intervention. One important realization that has emerged in the past few years is that a complex and active communication among the bacterial flora, epithelium, and immune cells exists in the intestine and that perturbation of these interactions can result in chronic intestinal inflammation. Thus, the major working hypothesis, particularly coming from basic research in experimental models, is that inflammatory bowel disease (IBD) is due to an abnormal cell-mediated immune reaction—primarily by CD4+ T cells—to the antigens and adjuvants of the enteric bacteria in genetically susceptible hosts. Consequently, many of the priorities and associated resources are directed at gaining a better understanding of the interactions between enteric bacteria and the host as well as the regulation of T-lymphocyte activation and differentiation.

Powerful new technologies are now available to define the molecular and genetic basis of complex diseases such as ulcerative colitis and Crohn’s disease. These technologies must be applied to clinical studies of patients, i.e., to define subsets of patients with common molecular and/or genetic features. One emerging theme of the 2002 meeting in Phoenix was that there must be closer integration of laboratory-based and clinical investigators to take advantage of these powerful new technologies.

A second overriding theme was a focus on disease prevention by both laboratory-based and clinical investigators. One goal for basic research is the achievement of sufficient knowledge and understanding of disease pathogenesis to frame strategies to prevent the onset or recurrence of disease in genetically susceptible individuals, and one goal for clinical research is to develop better treatments for the prevention of relapse in patients who have achieved remission through medical therapy or surgery. Prevention of IBD will require the combined efforts of both laboratory-based and clinical investigators.

Many worthy avenues of research were considered and discussed. The leading basic and clinical research priorities at the present time are the following:

- Identification of major susceptibility genes for Crohn’s disease and ulcerative colitis. While most of these efforts will involve the general population with IBD, efforts should be extended to other specific populations such as African-Americans, Hispanics, and Asians; different clinical populations such as individuals with childhood-onset disease and those with extraintestinal manifestations; and IBD associated with Mendelian disorders such as the Hermansky-Pudlak syndrome.
- Development of surrogate markers of disease activity to serve as the primary end points for clinical trials, identifying subclinical stages of disease, and for determining the prognosis of patients.
- Development of a detailed understanding of regulatory cells in the intestine—including their origin, localization, cell surface markers, activation requirements, antigens/adjuvants/ligands recognized by their receptors, mechanism and targets of their action, and influence of CARD15/NOD2 on their
INFLAMMATORY BOWEL DISEASE: A BRIEF INTRODUCTION

Inflammatory bowel disease, which includes Crohn’s disease and ulcerative colitis, affect as many as one million Americans, most of whom are diagnosed before age 30. Although these illnesses have distinctive genetic and pathophysiologic characteristics, they are frequently considered together because of several pathophysiologic, genetic, clinical, and therapeutic similarities. Both diseases are marked by an abnormal immune response that causes chronic inflammation in the intestinal lining.

The precise triggers of this immune response, as well as the mechanisms of activation of the immune cells and the role of intestinal bacteria, are not clearly understood. Considerable progress has been made toward identifying genetic components that render some people susceptible to IBD. To date, there is still no cure or prevention of disease in high-risk individuals. Treatment consists primarily of suppressing the inflammatory process to control symptoms and prevent relapse of disease.

At the meeting “Challenges in IBD Research: Updating the Scientific Agendas,” held May 2–5, 2002, in Phoenix, Arizona, the following hypothesis for the pathogenesis of IBD was agreed on:

Crohn’s disease and ulcerative colitis are the result of an overly aggressive cell-mediated immune response to discrete antigens and adjuvants from commensal enteric bacteria in genetically susceptible hosts.

BACKGROUND

The goal of this meeting was to update the research agenda of the Crohn’s & Colitis Foundation of America (CCFA). The original Challenges in IBD Research Agenda was released in 1990. It was the first compilation of the state of investigations of the pathogenesis and treatment of Crohn’s disease and ulcerative colitis. A review and update was published in 1993; another was issued 5 years later, in 1998.

With the rapidly moving course of research today, newer discoveries are accelerating the availability of information to IBD researchers. In the past, there were two
distinct (and separate) categories of research: basic science, which deals with physiology, pathophysiology, and underlying mechanisms of disease, and clinical science, which focuses on patients and patient care. However, newer technologies have shortened the time to translate a discovery in the arena of basic research into a practical tool for clinical use, and consequently, the lines between basic and clinical research have become blurred.

A significant body of basic and clinical investigation has been conducted since the first research agenda was formulated, leading to a better understanding of IBD. Of particular note is the identification of the NOD2 gene as a susceptibility gene for Crohn’s disease. Nonetheless, significant questions remain to be answered.

This document summarizes the research priorities that were identified at the consensus meeting in Phoenix in May 2002. It includes both the clinical science agenda and the basic science agenda, and for the first time—in keeping with current research trends—the two groups came together to define a combined research agenda, containing the integrated priorities of both basic and clinical scientists. This is followed by individual sections addressing the issues discussed by the clinical science and basic science groups. In each section, the research priorities are followed by a description of the resources needed to achieve the priorities, including both physical and personnel resources, and recommended requests for research applications (RFAs). Workshops and work groups to better define the priorities and to design studies are also described.

**STEPS TO ESTABLISHING THE AGENDA**

As with previous versions of the Challenges in IBD Research Agenda, the 2002 edition necessitated months of preparation. A three-phase approach (designed by Stephan R. Targan, M.D.) was used to gather as comprehensive a perspective as possible by tapping the expertise of leaders in a broad range of relevant scientific disciplines. Researchers of IBD in each of these disciplines, selected from the foremost scientists and clinicians, were invited to participate as members of a task force. They were charged with preparing a “position paper” specific to their discipline, mapping out what they regarded as the pressing issues of that discipline, and spelling out what would be required to make significant steps in that area during the coming 5 to 10 years. The participants were given brief but specific guidelines. Their papers were to include prioritized, detailed descriptions of the work to be performed, the likely outcome, and the resources necessary to realize those goals.

In phase I, the members of the task force convened subcommittees of three to four advisors (also within their disciplines) to participate in the development of a discipline-specific 5- to 10-year plan, outlining the course and projected needs of research in their area. These subcommittees drafted a document providing specific details of the most promising research, within their discipline, on IBD. The documents produced by their coordinated efforts were distributed to each subcommittee chairperson and his/her advisors. With their advisors, they discussed the latest in available technology, considered potential application to the study of IBD, and prioritized the studies and resources required.

In phase II, the chairs of each task force gathered together at a 3-day meeting in Phoenix, sponsored and organized by CCFA. Charles O. Elson, M.D., moderated the proceedings. R. Balfour Sartor, M.D., served as chairperson of the Basic Science Group; Stephan R. Targan, M.D., and William Sandborn, M.D., served as co-chairs of the Clinical Science Group. All of the position papers were distributed to the participants before the meeting. The chair of each subcommittee presented the results of the subcommittee’s deliberations and proposed areas of high priority for future research. Each report was discussed at length. After all reports were heard and discussed, all the proposed priorities were considered together and voted on by the workshop participants in the basic research and clinical research sections. The two groups then met together to come up with a final list of research priorities.

Phase III consisted of the development of this document. This white paper states the needs and priorities established during phases I and II. The specific details and conclusions of the prioritization are presented here, in Challenges in IBD: Updating the Scientific Agendas.

**SCIENTIFIC DISCIPLINES/AREAS OF INTEREST REPRESENTED ON THE TASK FORCE**

The task force tapped the expertise of 14 different disciplines or areas of interest. They are as follows:

**Epidemiology**

For the purpose of gathering more detailed information about the prevalence, incidence, and etiology of IBD, this discipline has been broken up into four basic subcategories: descriptive epidemiology, time trends of IBD epidemiology, economics of IBD, and the utilization of epidemiologic studies to pursue clues related to disease etiology. Extrapolating preliminary figures indicates that there are approximately 1.3 million people...
with IBD in the United States and Canada. Better databases and better registries are clearly needed, as well as the investigation of “emerging” epidemics.

Clinical Phenomics

Although IBD is used as an umbrella term for two disease subtypes—ulcerative colitis and Crohn’s disease—the two comprise clinically distinct groups or phenotypes. Any classification system for IBD should acknowledge this phenotypic variability. This, in turn, will have major implications for the prognosis and treatment of affected individuals. It will also influence study design, patient selection, and analysis of clinical trials to elucidate the pathophysiology of IBD.

Genetic Evaluation for Diagnosis and Prognosis

Rapid progress in genetics has made the past few years in IBD research exciting. Now, the charge is to apply genotype data to provide diagnostic and prognostic information for patients with Crohn’s disease and ulcerative colitis. Another imperative is to assess the possibility of utilizing genetic patterns to evaluate response to various therapies.

Dysplasia and Cancer

Because dysplasia is an observer-oriented subjective measurement, it would be extremely useful to establish biomarkers that could serve as objective tools to identify epithelial dysplasia. Such molecular biomarkers may ultimately help determine what drives the neoplastic progression.

Outcome Measures for Clinical Trials in Inflammatory Bowel Disease

Randomized clinical trials serve multiple purposes in the development of new treatments for IBD. In addition to providing benchmark information for clinical practice, they can also offer new insights into disease mechanisms. Laboratory data and, more recently, imaging studies are essential components of outcome measures.

Medical Therapy

A cure for IBD appears to be a long way off, but more effective disease control is a realistic goal. An ideal therapy is one that could alter the natural history of disease to prevent complications and the need for surgery. Other important features include appropriateness to condition, method of delivery, acceptability of formulation, and a safe side effect profile. A holistic treatment approach—one that takes into account the patient’s milieu—is also valuable. While drugs for induction of remission have improved over the past decade, better agents are needed to maintain remission, particularly in Crohn’s disease.

Surgery

Although surgery may be characterized as an intervention in the medical treatment of IBD, technical advances over the past 20 years have increased safety and decreased complication rates. In addition to pouchitis, other areas of further study should include fertility issues in women with IBD, as well as pregnancy and delivery.

Genetics

Key to the understanding of why individuals develop IBD is the identification of susceptibility genes and an understanding of how these genes function. So far, one susceptibility gene (NOD2) and one cluster of genes (cytokines) for Crohn’s disease have been identified, but several more appear likely to exist.

Epithelial Cell Biology

The epithelial lining of the intestine is a primary site of host defense and provides a barrier to intestinal bacteria and the first response to enteric pathogens. Developing a better understanding of the interaction between epithelial cells and commensal and pathogenic enteric bacteria, and between epithelial cells and inflammatory and immune cells, could shed light on how this complicated system might be altered in IBD.

Immunoregulation

The immune system plays an important role in the initiation and perpetuation of the inflammatory response in IBD as well as maintaining an active state of suppression of pathogenic responses (tolerance) to resident bacteria in normal hosts. The specific characteristics of the cell-mediated immune response to luminal antigens and adjuvants and how these are regulated require further study.

Signal Transduction

For the first time, the topic of signal transduction is included in the Challenges in IBD Research Agenda.
Signal transduction refers to the transfer of signals from outside the cell to inside, which results in changes in cell function or gene expression. A better understanding of signal transduction in immune, epithelial, and mesenchymal cells could provide fundamental insights into the pathogenesis of IBD.

**Intestinal Microecology**

Normal resident bacteria that are present in large numbers in the distal ileum and throughout the colon elicit a dysfunctional immune reaction in susceptible hosts. The study of intestinal microecology seeks to understand what bacteria are present and which might cause inflammation and conversely how the host might change the intestinal bacteria. Little is known, as yet, about the precise constituents of this bacterial ecosystem, a prerequisite for understanding its significance.

**Host–Bacteria Interactions**

Closely related to the topic of intestinal microecology, the area of host–bacteria interactions delves more deeply into the relation of intestinal flora with the intestinal mucosa—including inflammation, host metabolism, barrier function, and gut function.

**Tissue Injury and Fibrogenesis**

Inflammation, which is a normal part of the process of injury and repair, is amplified and prolonged in IBD. The unchecked activation of inflammatory cells, such as neutrophils, macrophages, T lymphocytes, and mesenchymal cells, leads to tissue injury and eventually to fibrosis, which preferentially occurs in Crohn’s disease. The cellular and molecular mechanisms of tissue injury and repair, including those that lead to fibrogenesis, must be better understood.

**CLINICAL SCIENCE RESEARCH AGENDA: A SUMMARY**

Co-chairs Stephan R. Targan, M.D., William Sandborn, M.D., and members-at-large Stephen P. Hanauer, M.D., and Daniel Present, M.D., assembled with the chairs of the seven clinical science task force committees to review the research priorities proposed by each committee. The Task Force Chairs are:

- Clinical phenotyping—Marla Dubinsky, M.D.
- Dysplasia and cancer—Teresa A. Brentnall, M.D.
- Epidemiology and natural history—Edward V. Loftus, M.D.
- Imaging, laboratory, serologic, and genetic evaluation for diagnosis and prognosis—John D. Rioux, Ph.D.
- Medical therapy—Bruce Sands, M.D.
- Outcome measures for clinical trials—Brian Feagan, M.D.
- Surgical therapy—Robin S. McLeod, M.D.

The working group proposed five strong directives that it believed should be viewed as priorities for research. They are as follows:

**Priorities**

*Development of Surrogate Markers of Disease Activity*

Surrogate markers are essential tools, serving a number of valuable functions. Ideally, they should be divided into markers that are static and those that can track or prognosticate. The static markers will help to identify the heterogeneity of various subsets of patients. The goal is to allow these surrogate markers to serve as the primary end points for proof-of-principle clinical trials and for determining the prognosis of patients.

In addition to traditional laboratory markers, other suggested technologies to identify markers include microarray studies of messenger RNA (mRNA) and/or proteomics revealing specific gene expression and post-translational patterns, as well as clinical features. All of these could be used, possibly in a combination index form.

*Randomized Controlled Trials of Therapy in Important Human Disease Models of the Evolution of Inflammatory Bowel Disease*

The postoperative course of patients with Crohn’s disease represents a major area for study. One randomized controlled trial (RCT) should be implemented to compare azathioprine versus surgical resection in patients with steroid-dependent or steroid-refractory terminal ileal and/or right colonic Crohn’s disease. Another RCT should be a three-armed one: to evaluate placebo versus azathioprine versus azathioprine combined with an antibiotic for the prevention of postoperative recurrence.

These studies would answer some important clinical questions, while at the same time allowing the assessment of the utility of potential surrogate markers. The latter trial could facilitate natural history studies in patients treated with placebo in whom basic science analy-
ses (such as microarray studies of mRNA, proteomics, bacterial flora studies, etc.) could be performed.

Another important area for an RCT is prevention of pouchitis. It would be valuable to compare the effects of placebo with either a probiotic therapy such as VSL#3 or an antibiotic (preferably a nonabsorbable one) for prevention of pouchitis following ileoanal pouch surgery. The RCT would yield important information regarding clinical therapy; the placebo arm would allow definition of the natural history of pouch evolution, including basic science end points such as histology, immunohistochemistry, mRNA microarray studies, proteomics, bacterial flora, etc.

Other proposed RCTs of interest include one that evaluates infliximab monotherapy versus infliximab plus methotrexate versus infliximab plus azathioprine for induction and prevention of relapse in patients with Crohn’s disease. Another recommended RCT would look at azathioprine versus mesalamine for prevention of relapse following steroid therapy in patients with ulcerative colitis.

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**Definition of the Clinical Description of Patients for Potential Use in a Molecular Classification of Inflammatory Bowel Disease**

The primary goal is to deconstruct the current clinical classification system and return to earlier approaches in which patient findings were described in a rigorous, reproducible way. These descriptive data definitions will allow for the possible emergence of molecular classification systems.

**Identification of Novel Epidemiologic and Environmental Risk Factors for Inflammatory Bowel Disease, as Well as Further Classification and Elaboration of the Impact of Smoking on Crohn’s Disease**

Host–environment interaction appears to be the key in IBD. Defining populations at greatest and least risk for developing IBD, and trends in IBD prevalence, may help to identify potential environmental contributors to disease etiology as well as gene–gene factors that lead to IBD. Recent trends suggest a male predominance among newly diagnosed cases of Crohn’s disease; whether changes in cigarette smoking behavior have contributed to these changes must be investigated further.

**Identification of Biomarkers for Dysplasia and Cancer in Patients With Ulcerative Colitis**

Overall agreement between pathologists in detecting and grading dysplasia in the setting of ulcerative colitis is poor, underscoring the need for better diagnostic criteria. This effort potentially could be accomplished within the context of an RCT of chemoprevention with agents such as ursodeoxycholic acid, a cyclooxygenase 2 inhibitor, etc. The first step would be a cross-sectional study of potential biomarkers—both singly and in combination—followed by a longitudinal study (possibly an RCT) to test the potential of these early biomarkers predicting the development of dysplasia and cancer. The study could be designed to assess the efficacy of treatments aimed at slowing or halting the progression of malignancy.

**Resources Needed to Help Advance Research**

The clinical science task force outlined a number of resources that are critical for the further understanding and treatment of IBD. They are as follows:

**Definition Resources**

- Case definitions of ulcerative colitis, Crohn’s disease, and indeterminate colitis for use in epidemiologic studies.
- Definition of outcomes measures for use in clinical trials in Crohn’s disease and ulcerative colitis. The ongoing work by the International Organization of Inflammatory Bowel Disease in combination with the CCFA and Crohn’s & Colitis Foundation of Canada, related to definition of outcome measures for Crohn’s disease, should continue and be completed. A similar process must be initiated for ulcerative colitis.
- Development of a CCFA position statement on the process for drug development in patients with IBD. This should address each step of the clinical drug development process, with emphasis on appropriate sample size for each phase of the development process, and an approach to stepwise decision-making (whether to proceed forward) for advancing from one phase of drug development to the next.
- Definition of the placebo response rate in patients with Crohn’s disease and ulcerative colitis.
- Organization of a 3-day conference on immune-mediated chronic inflammatory diseases including ulcerative colitis, Crohn’s disease, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, etc. To that end, a steering committee should be formed. It should be comprised of a gastroenterologist, a rheumatologist, and a dermatologist—each with the charge of identifying thought leaders from their respective disciplines who would serve as speakers. The conferences would be struc-
tured to ensure adequate blocks of time devoted to etiopathogenesis for each of the diseases, natural history of each of the diseases, response to treatment with various therapies (with an emphasis on therapies that have been tried in more than one of the disease categories), and measurement instruments for disease activity. It is anticipated that such a conference could be underwritten entirely by industry sponsors.

- Development of validated definitions for possible risk factors associated with IBD including smoking, nonsteroidal anti-inflammatory drug use, etc.
- Organization of a workshop to determine the priorities for clinical research in pediatric IBD.

**Patient Cohort Resources**

- A large “moon shot” study of the unaffected family members of patients known to have a marked increased relative risk in developing IBD should be conducted. Potential groups of patients would be the unaffected family members of patients who belong to a multiplex family of Crohn’s disease, or the unaffected family members of patients who are known to be homozygous for one of the three NOD2 polymorphisms that lead to an increased risk of Crohn’s disease. Using NOD2 as an example, it was estimated that approximately 2,000 unaffected family members of approximately 400 patients homozygous for the NOD2 polymorphisms would need to be recruited and followed up for 10 years to yield finally several hundred patients who developed Crohn’s disease during the observation period. These unaffected family members would be followed up prospectively for symptoms compatible with IBD, serologic markers, disease activity markers, fecal flora (fecotype), genetic findings (genotype), etc. It was recognized that this is a large and expensive undertaking (estimated to be $1 million per year for 10 years) but one of great importance, and it was thought to be feasible.
- Studies in identical twins who are discordant for IBD, to carefully examine environmental risk factors that may lead to one twin developing IBD and the other twin remaining free of IBD.
- Development of an incident cohort of cases with ulcerative colitis and Crohn’s disease that is followed prospectively from the time of diagnosis. These subjects would likely be followed up serially with serologic markers, genetic typing, clinical features, environmental factors, possibly bacterial flora, disease activity markers, etc. The goal of this cohort would be to allow a biologic natural history study of ulcerative colitis and Crohn’s disease.
- Identification of one or more population-based cohorts from Scandinavia, Canada, or North America from whom DNA could be obtained, thus allowing population-based studies of the genetics of IBD and the correlation of genotypes with clinical features in patients that are representative of these diseases in the community at large (vs. Academic Medical Centers). One advantage of this approach is that such a study could be performed quickly.

**Request for Applications**

Development of surrogate markers to predict outcomes of disease natural history and for use as end points in proof of principle clinical trials.

Studies to evaluate the interaction of bacteria and IBD in human disease models, such as postoperative Crohn’s disease, pouchitis, discordant identical twin studies, high-risk unaffected family studies, etc.

Prospective studies to evaluate the interaction between genetic and environmental risk factors by studying discordant identical twins and the unaffected family members of patients with Crohn’s disease in multiplex families.

Development and validation of biomarkers for dysplasia and cancer in the setting of ulcerative colitis. It is important to establish biomarkers of dysplasia to identify patients who have or may develop cancer.

Randomized controlled trials evaluating prevention of relapse (maintenance of remission) using therapies for Crohn’s disease.

Studies related to pregnancy and IBD, including fertility issues in patients with previous surgery, pregnancy outcomes, and risk of adverse pregnancy outcomes with various medical therapeutics.

Clinical aspects of pediatric IBD, including population-based natural history studies and evaluation of established and novel treatments in the pediatric setting. One focus should be on growth and bones.

**BASIC SCIENCE RESEARCH AGENDA: A SUMMARY**

Co-chairs Charles O. Elson, M.D., R. Balfour Sartor, M.D. (Chairman), and members-at-large Stephen P. James, M.D., and Richard P. MacDermott, M.D., assembled with the chairs of the seven basic science task force committees to present the research priorities proposed by each committee. The task force chairs are as follows:
Epithelial cell biology/barrier function—Hans-Christian Reinecker, M.D.
Genetics—Jerome I. Rotter, M.D.
Host–bacteria interactions—Richard S. Blumberg, M.D.
Immunoregulation—Lloyd F. Mayer, M.D.
Intestinal microecology—Jonathan Braun, M.D., Ph.D.
Signal transduction—Kim Barrett, Ph.D.
Tissue injury/fibrogenesis—William F. Stenson, M.D.

The group selected the seven research priorities that were considered the most important for enhancing basic knowledge about IBD and that were also thought to be feasible and likely to produce results in the next few years. Each of the seven topics is represented in the top priorities.

The group described some essential resources that would be needed to carry out the stated priorities and identified some potential “requests for grant applications” (RFAs) that would serve the priorities. In addition, a 4-day workshop was proposed that would help to realize some of the goals of the research agenda.

Priorities

Genetics: Identification of Novel Genes in General and Specialized Populations (Ethnic, Pediatric, Extraintestinal Manifestations, Clinical Syndromes)

The identification of the NOD2 gene on chromosome 16 as a susceptibility gene for Crohn’s disease was an important success and has validated the need to delineate more genes that are responsible for the genetic susceptibility to chronic IBD, as well as the concept that genotype determines clinical phenotype.

The identification of susceptibility genes can potentially lead to improvements in disease classification, etiology, and diagnosis. Additionally, gene identification may lead to the identification of new inflammatory, regulatory, and barrier pathways to be investigated (such as innate immune responses involving NOD2). Gene identification can also be used to subclassify disease, define prognosis, and identify those at risk for developing disease. Most importantly, gene identification can lead to the development of new therapies and strategies for prevention that selectively target discrete subsets of patients likely to respond. NOD2 has already demonstrated significant genotype–phenotype correlations (ileal localization).

While the identification of NOD2 was indeed an important advance, it must be noted that NOD2 variants are found in the minority patients with Crohn’s disease and are not increased in ulcerative colitis. Therefore, susceptibility to both Crohn’s disease and ulcerative colitis involves a multitude of genes, most of which are as yet unidentified. The available linkage information suggests that at least six genes (on chromosomes 16, 12, 6, 14, 5, and 19) contribute to Crohn’s disease, and it is reasonable to deduce that a similar number (with possible overlap) contribute to ulcerative colitis. This means that there are at least 8 to 11 more genes left to identify.

Studies on gene mapping and identification, gene function, and genotype–phenotype correlations have focused and will continue to focus on the general population. However, studies should focus also on certain ethnic populations, such as African-Americans, Hispanics, and Asians. They should also cover childhood-onset cases and different phenotypes, such as IBD associated with Mendelian disorders (e.g., the Hermansky-Pudlak syndromes).

Studying each of these groups is important in its own right and may be particularly helpful in the search for additional genes. The interaction between genes and environmental may better be identified in populations undergoing environmental changes (e.g., Hispanics, Asians migrating to North America) or in which the time frame to onset of disease is measured in years (childhood onset) rather than decades (adult onset). The hypothesis that childhood-onset IBD is more likely to be based on genetics makes this population particularly valuable for genetic mapping. The elucidation of biochemical pathways may also be facilitated by study of Mendelian disorders that feature IBD.

Additional resources are needed to accomplish these goals, including central genotyping laboratories, a more complete and accessible genotype/phenotype DNA bank, and increased funding for the international IBD Genetics Consortium. These needs are described in more detail in the Resources section.

Immunoregulation

Understand the mechanisms of activation and function of regulatory cells (T reg/B/dendritic cells), including the influence of NOD2 on stimulation with antigens and adjuvants—signaling, cytokines, costimulatory molecules, etc.

Experimental chronic intestinal inflammation can result from multiple defects in immune system regulation. Mechanisms involved in the process of immunoregulation are not clearly understood. Research is needed to elucidate the specific mechanisms of activation of regulatory cells that, when stimulated by bacterial antigens and adjuvants, lead to tolerance in normal hosts or, when defective, lead to IBD in susceptible hosts.
There are many levels at which the immune system can be regulated. Among them, active suppression appears to be the most relevant for IBD. This is because regulatory cells play a prominent role in peripheral tolerance to auto antigens in that depletion of these cells leads to autoimmunity. Additionally, regulatory cells are critically important in normal gastrointestinal immunologic homeostasis (tolerance).

Recent studies in animal models of autoimmunity and chronic intestinal inflammation driven by bacterial antigens have rekindled interest in the existence of a subset of lymphocytes that inhibit immune responses. In addition to these T cells, other cells that have regulatory activity include NK cells, B lymphocytes, and dendritic cells.

An important area of investigation would describe the mechanisms of development and activation of these regulatory cells. Finding the stimuli that activate differentiation regulatory cells versus effector cells requires identification of the antigens, the antigen-presenting cells, the costimulatory molecules, the cytokine microenvironment required for the outgrowth of regulatory cells versus effector cells, and the chemokines that influence cellular trafficking. It would also be helpful to understand the signals that regulatory cells receive from the epithelium, luminal bacteria, and other cell types. Because NOD2 is involved in activation of the innate immune system, it is also important to decipher its potential role, if any, in regulatory cell function.

Discovering how regulatory cells mediate their inhibitory function and the targets of that inhibition is another important research goal. They appear to function via both cognate and noncognate interactions, which could both be therapeutically relevant. However, precisely how this occurs is not known.

Signal Transduction

Determine signaling pathways and mechanisms of IBD-related genes, especially NOD2. Define context-specific signaling in pathways and cells relevant to IBD (in vivo, in vitro, epithelial/innate/cognate/mesenchymal cells).

In the area of signal transduction, recent advances have led to greater understanding of the mechanisms whereby information is transferred from the receptors on the cell surface to complex cascades of cytoplasmic signaling molecules, resulting in acute changes in cell function or changes in gene expression. However, more work is needed to understand how signaling networks function in vivo and the specific alterations that may occur in signal transduction pathways in the immune, epithelial, and mesenchymal cells in the setting of IBD.

For example, it is important to determine how IBD-related genes (particularly NOD2) signal. In addition, understanding context-specific signaling and pathways relevant to IBD requires deciphering the nuances of signaling within, for example, T cells or dendritic cells. There is also a need for better understanding of the complete set of signaling intermediators that comprise an entire receptor-linked pathway, such as those evoked by tumor growth factor β and interleukin 10. Understanding such signaling has important implications for small-molecule pharmacotherapy.

A more detailed understanding of signal transduction events that pertain specifically to cell types in the intestinal mucosa should accelerate progress toward treating IBD.

Host–Microbial Interactions

Define antigens/molecular specificities of effector and regulatory cell populations. Examine in vivo host responses to colonization of gnotobiotic rodents with defined bacterial stimuli (normal and genetically susceptible hosts, with a focus on effector/regulatory T cells, innate immune cells, and epithelial cells).

The lumenal microbial ecosystem is a highly complex community of primarily anaerobic bacterial microorganisms that communicate extensively both among themselves and with the host. These microbes have major effects on gut morphology, motility, epithelial differentiation, the immune system (innate and adaptive), metabolism, and barrier function. The host must be tolerant to the normal luminal microbes and at the same time be able to respond to intestinal pathogens.

Inflammatory bowel disease is thought to represent an aberrant response to normal intestinal microbes due to immunoregulatory or mucosal barrier defects. Experimental models have shown that an abnormal balance between aggressor and regulatory T cells appears to be central to IBD pathogenesis. An emerging concept identifies defective barrier function as a mechanism by which luminal bacteria could stimulate chronic intestinal inflammation. Altered epithelial/mucosal exclusion of bacterial antigens/adjuvants, defective clearance of invading bacteria, or defective mucosal healing could each lead to chronic immunologic stimulation.

Studies are needed to define the bacterial antigens and that drive effector and regulatory cell populations in the adaptive and innate immune system. In other words, the dominant stimuli activating the mucosal immune response must be identified, particularly in effector and
regulatory cell populations. What are the antigens and adjuvants that stimulate protective cells? Are the same signals that induce pathogenic effector cells in the susceptible population also stimulating the protective regulatory cells in a normal population?

An important technique for gaining a better understanding of the host immune response is to use gnotobiotic rodents that are selectively colonized with defined populations of bacteria. Studies can compare responses in wild-type and genetically modified animals. The bacteria could be a single organism, a cocktail of defined species, or a genetically modified microbe. DNA microarrays can then be used to look for global effects on structure and function of the host, and classic immunologic studies can define the response of the innate and adaptive immune systems. The goal is to identify the physiologically relevant antigens and adjuvants that selectively activate pathogenic and protective immune responses.

Intestinal Microecology

Define lumenal microbial populations (microecology) by examining 16S DNA and trait/expression profiles.

Endogenous bacteria provide the essential proinflammatory stimulation for IBD, and commensal flora in patients with IBD have increased epithelial association and invasion. Bacterial species differ in their ability to induce and prevent inflammation, and hosts differ in their sensitivity to different species. There is extensive gene sharing among the luminal bacteria.

In light of this, there is a need to develop tools and concepts to better understand the intestinal microbiota, including the definition of the microbial populations in normal murine and human hosts using 16S DNA or trait/expression profiles. In this way, it will be possible to establish a baseline for mouse and human microflora, defining what species and strains are present and where in the intestine they are located.

It will be important to redefine microbial populations, not only as taxonomic groups, but more importantly as functional groups pertaining to the responses they elicit in the host. This will require use of newer tools (DNA arrays, microbial genomics, and proteomics) to characterize the products and activities of both bacterial populations and the mucosal tissue with which they interact. Additionally, this work would require the recruitment of environmental microbiologists, who are knowledgeable about microbial population biology, and molecular microbiologists who can apply molecular techniques to study complex enteric bacterial populations.

Epithelial Cell Biology

Identify mechanisms of host defense by intestinal epithelial cells and innate/adaptive immune cell interactions.

The epithelium has a large repertoire of mechanisms by which it protects the host, which can be divided into three broad categories. First, intestinal epithelial cells are joined together by tight junctions, which are responsible for creating a physical barrier that excludes antigens and proinflammatory molecules. This barrier is rapidly restored following injury by both proliferation/differentiation and restitution (cell migration without proliferation).

Second, in addition to creating a physical barrier, the epithelium produces antimicrobial peptides and trefoil peptides and the mucus layer (which is just above the epithelium).

Third, signals are emitted from epithelial cells to the immune system. Intestinal epithelial cells respond to invasive pathogens with production of inflammatory mediators, but they do not respond in the same way to harmless bacteria. One important question is how the epithelium signals to the innate to the adaptive immune system and vice versa. A second key question is defining the mechanisms by which epithelial cells selectively respond to pathogens but not commensal organisms.

Overall, this is a very complicated system that is highly regulated and very dynamic. Studies are needed to more clearly identify bacterial–epithelial interactions such as the proteins, genes, or other substances that regulate the epithelium and how these function. There is a need to identify the mechanisms and mediators that epithelial cells use during host defense, including restitution, tight junctions, epithelial barrier function, pre-epithelial barrier function, antigen presentation capabilities, and innate immune activities.

Tissue Injury and Fibrogenesis

Define the cellular and molecular mechanisms of tissue injury, repair, fibrosis, fibrinolysis, and immunolysis in rodent and human cells/tissues and in murine models with attention to genetic background.

The definition of cellular and molecular mechanisms of tissue injury and repair remain a high priority. In IBD, the inflammatory component of the wound repair process is inappropriately amplified and prolonged. One consequence is fibrosis. Clinically significant fibrosis occurs in a subset of patients with IBD and is a larger problem in Crohn’s disease than in ulcerative colitis. Fibrosis involves increased synthesis of several fibrillar collagens and laminins and an intracellular actin binding protein.
Exposure of submucosal mesenchymal cells to proinflammatory cytokines, growth factors, and tissue damage is probably a major factor leading to fibrosis of the submucosal layers in Crohn’s disease.

There is a need to define more precisely the cellular and molecular mechanisms that lead to the interruption of wound repair and to fibrosis in IBD. For example, is fibrosis merely a consequence of deep penetration of inflammation or is it an intrinsic process that differs fundamentally from inflammation? Is it a secondary or primary phenomenon? How do inflammation and fibrosis interact?

**Resources Needed to Advance Research Priorities**

**Gnotobiotic Facilities**

Gnotobiotic research animals (which are kept in germ-free [sterile] or defined bacterial conditions) are essential to furthering IBD research, particularly in the areas of host–microbial interactions and intestinal microecology. These animals should consist of commonly used strains and genetically engineered (knockout and transgenic) rodent strains. Germ-free rodents can remain sterile or be populated with single or multiple defined bacterial species, or can be reconstituted with pathogen-free normal enteric flora. Such genetic and luminal environmental manipulations provide a unique opportunity to study the interaction between a host and the microbial flora, including a better understanding of the immune response.

**Primary Cell Lines**

An important resource that was agreed on by several task force members was the development of primary intestinal epithelial cell and myofibroblast lines with different genotypes. Both human and murine cell lines are needed, including NOD2 normal and homozygous abnormal. These cell lines should be genetically defined for IBD susceptibility and other genes of interest.

**Development of Inflammatory Bowel Disease/Gastrointestinal–Related Gene Chips**

A valuable research aid would be the development of a gastrointestinal IBD-oriented gene microarray chip, along with an associated bioinformatic resource database and genomic analytic effort. Different scientists use different gene chip systems and database systems, which can be difficult to merge and compare. Therefore, it would be helpful to have a shared data bank of universal and cell/pathway-specific gene chips.

**Bacterial Strains**

To facilitate research into the bacterial factors driving the host response, a useful resource would be a library of defined bacterial strains (commensals, vectors, and mutants). This would require cooperation with molecular microbiologists to provide well-characterized standard and genetically modified bacterial strains. These bacterial strains could be used for studies on colonization of gnotobiotic animal models.

**Central Genotyping Laboratories**

A network of molecular laboratories around the country that contributes to a central, reproducible, and rapidly accessible genotyping effort would provide valuable information that could potentially be used for identifying IBD-related genes in clinical studies.

**Mutant Mice**

In addition to gnotobiotic mice, central access to additional types of mutant mice is also needed, including conditional knockouts, cell-specific knockouts, etc.

**Genotype/Phenotype DNA Bank**

The CCFA has established a Genotype/Phenotype Bank to collect and store information about the genetic variants and clinical characteristics of people with IBD. The Bank contains DNA and cell lines (via blood samples) from patients with IBD with a relatively small number of defined phenotypes. This resource already exists; however, to make it more useful to genetic researchers, it must be more complete and accessible.

**Funding for the International Inflammatory Bowel Disease Genetics Consortium**

This important resource for sharing data and analysis is currently run by Dr. Juleen Cavanaugh in Australia. The Consortium has been formed “for the purpose of examining the influence of genetic variables on IBD to improve the understanding of the pathogenesis of IBD and to use such genetic information to realize progress in diagnostic, management and preventative strategies for individuals affected by IBD." Financial support for this worthy project is warranted to ensure that its data are accurate and to maintain communication with U.S. investigators.
Requests for Applications

Epithelial/Microbial Communication

In the areas of epithelial cell biology and host–microbial interactions, studies are needed to delve further into the bidirectional communication between epithelial cells and microbes. Not only do epithelial cells interact with bacteria, but invasive bacteria cause proinflammatory signals. It will be important to define characteristic differences between the normal and inflamed epithelium. Such studies will require better epithelial models to elucidate microbial pathogenesis.

Definition of Bacterial-Specific Regulatory Cells

When aggressive bacteria are present in the intestine, most people heal because a powerful regulatory healing mechanism becomes engaged, allowing the mucosa to be restored. An important feature in normal hosts is prompt down-regulation of inflammation after the pathogen is cleared. To understand how this works and what can go wrong, studies are needed that focus on the definition and characterization of naturally occurring bacterial-specific regulatory cells.

Phenotypic/Genetic Characterization of Regulatory Cells

To further the aim of understanding the mechanisms of activation and function of regulatory cells, research is needed to characterize the cells more carefully from a phenotypic standpoint.

Genetics of Inflammatory Bowel Disease in Murine Models

Rodents can be superb models of immune function. Studies should be performed with genetically targeted animal models (transgenic and knockout) that develop IBD to look for genes that modify the phenotype. Additionally, animal models (transgenic, knockout, tissue specific, and conditional models) related to IBD-specific genes should be established promptly as these genes are identified in humans.

Identification of Intestinal Microbial Populations and Studies on the Biology of Microbial Communities/Biofilms in the Intestine

Overall, the make-up of the bacterial population in different parts of the gut is stable under normal conditions. An as-yet-unidentified process enforces a consistent composition of the bacterial population. Host genetic factors may influence the luminal bacterial composition.

It is important to know what makes these bacterial populations perpetually consistent and how the different populations interrelate. Several approaches are possible, including 16S DNA or trait/expression profiles to provide definition of the microbiota under specific conditions.

Methods to Measure/Identify Signaling Pathways In Vivo

Thus far, investigations of signal transduction events that pertain to cell types in the intestinal mucosa have occurred in reductionist models. To yield truly useful insights about IBD, methods must be devised to identify and measure activated signaling pathways in vivo in both humans and mice.

Cellular/Molecular Mechanisms of Tissue Injury/Repair/Fibrosis/Fibrinolysis

Studies are needed to define the cellular and molecular basis of tissue injury and repair in both mice and humans. In addition, mouse studies of healing and fibrosis using established models of intestinal inflammation will help to increase knowledge about tissue injury and fibrogenesis.

Workshops

Bacterial–Host Interactions at the Mucosal Interface

The basic science group proposed a 4-day workshop that would pull together some of the overarching themes that came out of the Phoenix meeting and would specifically focus on bacterial–host interactions. Some potential topics for discussion during the workshop would include the following:

Bacterial ecology/population dynamics
Bacterial-specific stimulation and mechanisms of responses of intestinal epithelial cells and innate and cognitive immune cells
Adaptive responses to host commensals
Lessons from animal models
Therapeutic potential of manipulating the luminal microbiota
Future directions
Other Workshops

It was also suggested that workshops could be conducted on each Request for Application topic to define more clearly the parameters and protocols of specific studies.

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