

### *Advances in Inflammatory Bowel Diseases Research*

NIDDK support for research on genetics and immunology of the inflammatory bowel diseases (IBD) is paving the way to the development of unique and effective therapies for patients who suffer from these diseases.

IBD was described in the medical literature as early as the mid-18th Century, but it was not until the mid-20th Century that the two major subtypes of IBD—Crohn’s disease (CD) and ulcerative colitis (UC)—were identified and distinguished by the area of the intestine they affect. The incidence of these diseases in western, industrialized societies increased dramatically during the 20th century. These are painful and debilitating diseases, characterized by chronic, intermittent intestinal inflammation. In CD, inflammation can occur anywhere in the alimentary tract and sometimes in other sites, but most often occurs in the end of the small bowel and beginning of the large bowel (colon). In UC, the site of inflammation is restricted to the colon, or large intestine. The leading theory for the cause of IBD is that inflammation is triggered by inappropriate immune responses to bacteria that naturally reside in the intestine and that the underlying predisposition to these inappropriate immune responses is caused by multiple interacting genes. Under normal circumstances, most bacteria residing in the gut have a beneficial or benign effect on their host, but an overly active immune system may be provoked by these bacteria in IBD.

#### **Genetic Factors in IBD Uncovered**

Studies of human twins and of animals have confirmed that genetic factors contribute to IBD. Some gene variants are specifically associated with either CD or UC, while others are involved in both diseases. The importance of genetic factors is also reflected in family studies showing the incidence of IBD to be higher among family members.

A major research breakthrough on the genetics of IBD came in 2001, with the discovery of the first IBD-associated gene, called *NOD2*. The *NOD2* gene was found to be associated solely with CD, not with UC. This landmark research, which was supported by the NIDDK, represents one of the earliest, most well-established associations in complex genetic disorders. The product of *NOD2* is a cellular protein found in immune cells, called monocytes, and in cells lining the intestinal wall. Although the mechanisms underlying the relationship of the *NOD2* gene variant to CD are not yet fully understood, the *NOD2* protein is known to activate communication (signaling) pathways in response to components of bacterial cell walls, leading to a variety of immune responses.

Building on this important finding, the NIDDK in 2002 established the Inflammatory Bowel Disease Genetics Consortium (IBDGC). (For more information on the Consortium, see highlights from a Scientific Presentation by Consortium investigator Dr. Judy Cho, which appears later in this chapter).

The Consortium’s efforts were greatly enabled by resources provided by the NIH-sponsored Human Genome Project and the International HapMap Project, which were major drivers in propelling research on human genetics. The Human Genome Project sequenced the 3 billion nucleotide base pairs of the human genome, a monumental effort that concluded in April 2003. Data from this project were made available to scientists around the globe to facilitate the pursuit of medical research. The International HapMap Project, published in 2005, is a catalogue of common small genetic variations called SNPs (single nucleotide polymorphisms) that occur in the nucleotide (or letter) sequences of individuals’ DNA. The Genome and HapMap projects have been accompanied by great strides in the development of new rapid biomedical technologies so that hundreds

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of thousands of SNPs can now be determined in single DNA samples. The genome-wide association scan based on these advances has become the cutting-edge technology for identifying genes that contribute to human disease, and was used by the Consortium to identify genetic factors in IBD.

Recently, members of the Consortium used this genome-wide association technology in a two-phase study designed to identify additional genes that contribute to CD. In this study, blood samples from CD patients and healthy volunteers were scanned for known genetic variants using over 300,000 SNPs. The first phase of the study was very successful in detecting several significant SNP associations, including a variant of a gene encoding a receptor for the cytokine (an immune system chemical) interleukin-23 (IL-23). Surprisingly, one variant of the gene was shown to protect against CD. Additional studies have shown that the IL-23 receptor is required for CD to develop in animal models.

Because the inflammatory bowel diseases are complex diseases involving the contributions of many genes, it was anticipated that genes also existed that had more subtle associations with IBD, the detection of which would require screening much larger cohorts. Therefore, a second, expanded phase of the study was conducted on a larger population of CD patients and healthy volunteers. In this second phase of the study, scientists discovered another CD associated gene, *ATG16L1*, which is involved in autophagy. Autophagy is a process by which cells capture, degrade, and recycle unwanted cellular material into useful molecules. This process has also been associated with the body's early immune response that is activated by the recognition of bacterial components. The involvement of the autophagy process has been verified by two other scientific research groups. One group identified the autophagy gene, *ATG16L1*, using a different protocol in which 72 SNPs, selected through a screening process, were used in a genome-wide association scan of CD patients and

healthy controls. The other group identified a second autophagy gene linked to CD, called *IRGM*, in a major genome-wide association study that scanned 14,000 patients with seven different diseases (2,000 patients for each of the seven diseases) and a shared control set of 3,000 healthy volunteers. The study identified 27 additional disease-related genetic variations, including nine for CD, seven for type 1 diabetes, and three for type 2 diabetes.

### Mapping the Molecular Pathways of IBD Development

Discovery of the IBD gene, *NOD2*, provided the first evidence linking this disease to the immune response to bacteria. The NOD2 protein is an intracellular sensor of bacterial wall components. Upon sensing the bacteria, NOD2 activates multiple molecular pathways associated with initial responses by the immune system. Extensive research continues to clarify the roles that pathways stimulated by NOD2 play in the errant activation of immune response associated with IBD.

Research on chemicals utilized by the immune system, including cytokines such as interleukins, has demonstrated the important role of pathways activated by these molecules in IBD development. Identification of the interleukin-23 receptor gene as being associated with the risk of developing IBD coincided with other research investigating the roles of IL-23 and its receptor in autoimmunity, the immune system's inappropriate reaction to the body's own tissues. Studies exploring the causes of inflammation in autoimmune disease have focused on two cytokines, IL-12 and IL-23, which have related structures, but different functions. These two molecules are dimers that each have one identical subunit, as well as one unique subunit. Antibodies against the common, shared subunit of the two cytokines inhibit inflammation in both animals and in human CD. More recent studies in mice have shown that IL-23, not IL-12, is responsible for inflammation.

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In one study examining the roles of IL-12 and IL-23 in IBD, scientists used a mouse model infected with bacteria known to induce inflammation and then analyzed IL-12 and IL-23 subunit expression in the intestine. The mice responded to bacterial infection with increased production of the common subunit of both interleukins and the unique subunit of IL-23, but not the IL-12 unique subunit, demonstrating that inflammation is dependent on IL-23, not IL-12. Furthermore, when antibody was introduced to block the unique IL-23 subunit, inflammation was markedly reduced, confirming that IL-23 is essential for inflammation in the intestine. Confirmation that IL-23, not IL-12, is required for intestinal inflammation was made in another study using two mice strains with double mutations. Both strains contained a mutation that causes them to spontaneously develop inflammation resembling CD. Additionally, the IL-12 unique subunit was inactivated in one strain and the IL-23 unique subunit was inactivated in the second strain. Mice with mutations in the IL-12 unique subunit developed colitis; however the IL-23 unique subunit mutants remained disease free, confirming that active IL-23 is essential for intestinal inflammation. These results point to selective targeting of IL-23 as a potential new therapeutic approach for human IBD.

Recent research has also refined our understanding of the types of immune cells involved in IBD, and how they interact with key molecular pathways. IL-23 has been shown to induce the production of other inflammatory cytokines by immune cells called monocytes and macrophages. IL-23 also activates a recently identified subtype of helper T cells ( $T_H$  cells), called  $T_H17$  cells. Until recently, only two major subsets of  $T_H$  cells had been identified:  $T_H1$  cells, which secrete molecules that destroy intracellular microbes and are associated with CD; and  $T_H2$  cells, which secrete molecules that destroy extracellular microbes and are associated with UC. The newly-discovered  $T_H17$  cells secrete the inflammatory cytokines TNF-alpha, IL-6, and IL-17, and are thought to be particularly important in causing tissue inflammation in immune

diseases, including IBD. Thus, not only has IL-23 been implicated as an important cytokine in IBD, it appears that the cytokine acts through a very specific type of T cell that has only recently been identified. These discoveries suggest important new pathways to be explored to develop treatments for CD.

### New Treatments for IBD

The elucidation of new disease genes and the molecular responses they initiate is key to developing drugs that prevent and treat IBD. Two examples from recent years involve molecules known as TNF-alpha and PPAR-gamma. The cytokine TNF-alpha is now recognized as a major factor in the inflammatory immune responses associated with IBD. The drug infliximab was the first recombinant antibody designed to bind to TNF-alpha, thereby preventing it from engaging with receptors that activate inflammatory responses. Infliximab was initially thought to be effective only in treating and maintaining remission of CD, but has now been shown to be an effective treatment of UC.

The peroxisome proliferator activated receptor-gamma (PPAR-gamma) regulates gene expression in the nuclei of immune cells and epithelial cells that line the colon and is known for its effects on tumor suppression in the colon and on attenuation of colitis. PPAR-gamma expression was found to be impaired in cells lining the colons of UC patients, indicating a potential role in the treatment UC. Mutant mice with minimal expression of PPAR-gamma in their colon epithelial cells were given a substance that induces colitis, in order to determine if PPAR-gamma plays a protective role against developing UC. Mutant mice exhibited higher levels of molecules that promote inflammation and increased susceptibility to experimental colitis when compared with control mice. Rosiglitazone, a drug used for the treatment of type 2 diabetes, activates the PPAR-gamma receptor. When rosiglitazone was administered to the mice, the severity of the induced colitis was decreased and cytokine production was suppressed

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in both mutant and control mice, demonstrating that PPAR-gamma plays a role in protecting against colitis. Because administration of rosiglitazone was effective in reducing colitis symptoms in mutant mice expressing minimal levels of PPAR-gamma, as well as control mice, it is possible that rosiglitazone may also act independently of PPAR-gamma in suppressing inflammation.

The efficacy of rosiglitazone in treating UC in humans was recently tested in a multicenter clinical trial supported by the NIDDK. Patients participating in the trial had mild-to-moderate UC and had been previously treated with the drug 5-aminosalicylate,

the most common treatment for UC, but had not responded well or were intolerant to the drug. After receiving either rosiglitazone or a placebo, patients were assessed for improvement in their condition. After 12 weeks, 44 percent of patients given rosiglitazone had clinical remission compared to 23 percent of patients given placebo. These data demonstrate that rosiglitazone is effective in the short-term treatment of patients with mild-to-moderate UC who did not benefit from other treatments. Further long-term studies must still be conducted to assess use of this class of drug as a maintenance therapy for UC, and to determine whether they provide an additional new treatment option for patients suffering from UC.