CCFA:
MD ACCOMPLISHMENTS

HANAUER, STEPHEN B

Dr. Hanauer received funds from CCFA from 1992 to 1995 to carry out a multicenter evaluation of the efficacy of methotrexate in chronically active CD.

Methotrexate has been proven effective in moderate to severe CD (1) and to maintain remission in adults with CD (1,2). Hanauer participated in several studies evaluating its efficacy and safety, particularly in maintaining remission.

In a double-blind, placebo-controlled, multicenter trial in patients with active CD who had entered remission, the investigators found that a significant number of these patients were able to remain in remission long-term (40 wk) on a reduced dose (15 mg IM vs 25 mg IM once weekly), and significantly few needed prednisone because of relapse compared with placebo (3).

In a study of the adverse effects of IBD drugs, the investigators found that methotrexate carries a range of adverse effects, including nausea, leucopenia, and, rarely, hepatic fibrosis or hypersensitivity pneumonia (4). It is also contraindicated in pregnancy (5). Because of concerns over hepatotoxicity, a study was designed to determine whether surveillance liver biopsies are warranted in IBD (6). The patients (N=20) had experienced long-term methotrexate therapy. Liver biopsies revealed only mild histological abnormalities (Roenigk's grade I and II) and one case of hepatic fibrosis. Abnormal liver chemistry test results were seen in 30% of patients, none of whom demonstrated Roenigk's grade IIIB hepatotoxicity. The investigators concluded that surveillance liver biopsies were not warranted for such patients. Because of its adverse effects, however, this agent is considered for second-line therapy in patients who are refractory to or cannot tolerate 6-MP/azathioprine (1).

Hanauer also helped evaluate the steroid-sparing effect of methotrexate in CD in a study of patients (N=76) with long-term CD (mean: 9.5 y) and methotrexate therapy (mean: 55 wk; mean dose:20 mg/wk). Improvement was seen in 63% after 9 weeks of therapy and lasted 65 weeks, while remission was seen in 37% after 22 weeks of therapy and lasted 59 weeks. The results were best with parenteral therapy and in younger patients (<40 y) (7).

Continued evaluation of this drug is warranted, given the fact that drugs in this category have already demonstrated their potential for extending the duration of infliximab therapy by keeping the level of infliximab antibodies relatively low (8). This effect, if seen in methotrexate, may indicate the potential for combination therapy.

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KORELITZ, BURTON I, MD

Korelitz received funds from CCFA from 1993 through 1995 to develop a double-blind, randomized trial of 6-MP versus 5 aminosalicylic acid in the prevention of recurrent ileitis after resection in patients with CD.

Judge and Lichtenstein cited studies in which Korelitz participated to indicate that complete 6-MP may be helpful for achieving fistula closure (1) or to complete fistula healing and remission (2). These studies were also used to identify any serious adverse events that can result from 6-MP therapy (3).

Markowitz (4,5) and Dubinsky (6) cited studies by Korelitz indicating that azathioprine and 6-MP are efficacious in patients with CD who develop fistulas. Markowitz also cited studies by Korelitz and colleagues providing anecdotal and trial evidence that 6-MP reduces the rate of postsurgical endoscopic (6 mo) and clinical (12 mo) relapse.

The results of a 1993 study of mesalamine monotherapy in patients intolerant of the parent drug (sulfasalazine), in which Korelitz participated, indicated that the drug was effective in both CD and UC, and that it was more effective than the parent drug in CD (7).

Korelitz participated in a 2-year study comparing 6-MP, 5-aminosalicylic acid, and placebo in preventing the postoperative recurrence of CD (8). The results, reported in 2004, indicated that the recurrence rate was lowest with 6-MP compared with mesalamine and placebo, whether recurrence was evaluated clinically (50%, 58%, and 77%, respectively), endoscopically (43%, 63%, and 64%, respectively), or radiographically (33%, 46%, and 49%, respectively).

Thus, although both agents are safe and effective in CD, Korelitz demonstrated that combination therapy may preclude the need for additional surgery in CD patients with fistulas.

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TARGAN, SR

Targan received funds from CCFA from 1981 to 1982 to study the cytotoxicity of natural killer (NK) cells in normal and IBD intestinal mucosa.

In an early study by Targan and other investigators (1), two systems of antibody (antitetanus toxoid) suppression, one of which appeared to be mediated by NK cells. Shortly thereafter, Deem and Targan (2) delineated the sequence in which an NK-derived cytolytic factor (NKCF) induces cytolysis that indicated that this process is strongly influenced by the presence of gluteraldehyde.

Shortly thereafter, a Targan team found that 6-mercaptopurine (6-MP) could inhibit NK-cell cytolytic activity in patients with CD. Until then, spontaneous cytotoxic activity had not been observed in the human gut. Another Targan team, after identifying NK-positive lymphocytes within the lamina propria of the gut (4), proposed that such activity might not have been recognized or linked with NK-cell activity previously because NK cells in the gut are phenotypically different from those in the peripheral blood.

The cytolytic activity of NK cells was clarified further by 1987, when Targan and colleagues demonstrated that phospholipase A2 (PA2) inhibitors also inhibited NK-mediated cytotoxicity. They suggested that PA2 may also modulate the surface of NK cell targets to uncover a secondary "trigger" that facilitates cytolytic activity (5). Eventually PA2 activity was found to correlate with tumor necrosis factor (TNF)-alpha activity, such that TNF expression was apparently activated by PA2 (6), TNF apparently triggered PA2 activity (7), and substances that inhibited PA2 activity apparently also blocked TNF activity in a dose-dependent manner (8).

In 1997, a Targan team investigated the role of a TNF-alpha antibody in patients with CD (9, 10). This antibody—a chimeric monoclonal antibody known as cA2—was given to 108 patients with moderate to severe CD in a 12-week multicenter, double-blind, placebo controlled trial. A 61% clinical response was seen by week 2 and remained significantly greater than the response in the placebo group throughout the study. By week 4, a third of the active treatment patients were in remission.

This antibody is currently formulated as infliximab (Remicade®), which is now indicated for moderate to severe CD and for fistulizing CD (11).

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MARKOWITZ, JAMES F, MD

Dr. Markowitz received funds from CCFA from 1991 through 1994 to develop a prospective, double-blind, multicenter, placebo-controlled trial of 6-mercaptopurine and corticosteroids in children and adolescents with newly diagnosed CD.

Prior to the start of this project, the long-term efficacy of 6-MP in adolescents with intractable CD was not clearly established. Markowitz and colleagues conducted a study in adolescents (N=36) who had been taking 6-MP for at least 6 months and had been intractable to other IBD agents, antibiotics, and nutrition support for approximately 5 years before starting 6-MP therapy. During the first year of treatment, patients exhibited a higher Lloyd-Still disease activity score and improvements in physical exam, nutrition, laboratory tests, and general activity scores. Annual hospitalization rates also declined (1).

In 2000, the Markowitz team conducted the first controlled trial of 6-MP/prednisone combination therapy versus prednisone monotherapy in children with steroid-dependent CD. They found that 6-MP significantly reduced the need for prednisone and prolonged the duration of remission (3). The adverse events were similar to those seen in adults, including the increased risk for cancer. Concern over this and other adverse effects may be avoided in the future using metabolite tests (the thiopurine methyltransferase genotype/phenotype test and the 6-MP metabolite test) to optimize therapy, detect noncompliance, and reduce the risk for toxicity associated with this drug (4,5).

Despite the apparent efficacy and safety of this drug, continued evaluation is warranted, given the recent controversy over the management of CD in children. American pediatric gastroenterologists appear to be comfortable prescribing immunomodulator drugs for children younger than 5 years (6) and prefer to start therapy in children with steroids and azathioprine (the parent drug of 6-MP), their Western European counterparts prefer to start with nutrition therapy before progressing to budesonide or steroids, at least in children with mild to moderate disease (7). Continued discussions in this area may help the physicians worldwide to reach a consensus.

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ROTTER, JEROME I, MD

Dr. Rotter received CCFA funding from 1992 through 1994 to investigate the role of molecularly defined HLA class II genes in IBD.

At that time, few studies of HLA class II genes in patients with CD or UC were available (1). Those that were available had been carried out using serological techniques and had inconclusive results. When those techniques were replaced with molecular genotyping and allele-specific oligonucleotide hybridization, the investigators discovered a positive relationship between the HLA DR2 allele and UC and a positive association with the HLA DR1 and HLA DQw5 alleles and CD.

Previously, it had been observed that antineutrophil cytoplasmic antibodies (ANCAs) are also associated with UC (3), suggesting a disturbance in immune regulation in UC (2). The investigators in that study also found that patients with UC were likely to demonstrate a link between ANCA and DR2, which suggests that a subset of UC patients may be genetically susceptible to an immune defect that serves as the basis for the disease (2).

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SARTOR, RB

Dr. Sartor was funded by the CCFA from 1987 through 1989 to study the role of bacterial cell walls in the pathogenesis of CD.

The potential contribution of macromolecules crossing normal and injured intestinal tissues to intestinal inflammation had been discussed previously (1-3). Peptidoglycan-polysaccharide (PG-PS) complexes within bacterial cell walls had also been recognized as being responsible for the outcome of inflammation and immunomodulation in granulomas that follow bacterial infection, but their uptake across the intestinal epithelium had not been investigated.

The Sartor team decided to investigate this phenomenon by studying rats in which the induction of colonic injury was followed by injection into the cecum of a small amount of ^{125}I-labeled purified PG-PS fragments obtained from Group A *Streptococcus pyogenes* organisms (4). The results were dramatic: all of the rats developed signs of illness within 24 hours. Illness was indicated by gross evidence (surface hemorrhages near the site of injection and on focal areas of the transverse and descending colon and rectum), microscopic evidence (eg, marked thickening of the lamina propria and dense PMN infiltration), and systemic distribution, indicated by elevated levels of radioactivity in the liver, spleen and mesenteric lymph nodes. Based on these findings, the investigators suggested that PG-PS derived from the normal enteric flora may induce or sustain inflammation within the intestines and in extraintestinal tissue in patients with CD or UC.

Evidence of the systemic spread of PG-PS-induced intestinal inflammation was supported further by a subsequent study of rats in which intestinal injury was induced in the jejunum by means of a surgically created blind loop, within which a proliferation of anaerobic bacteria occurred. The results of these two tests may not be completely equivalent, because immunoreactivity was measured in this study by serological and histological tests, only. However, histological evidence of inflammation within the lamina propria, hypertrophy of the muscle layers of the gut lining, and measured changes in luminal PG-PS and anti-PG antibodies for 3 classes of immunoglobulins (IgG, IgM, and IgA, whose plasma levels did not change) strongly implicate PG-PS as the inducer of the inflammatory process (5).

Based on this evidence and evidence from subsequent studies of the role of bacteria in intestinal inflammation, Sartor has recommended that the goals of IBD therapy include reduced exposure to luminal bacterial antigens (ie, antibiotic therapy) and correction of the abnormal immune response to gut antigens (6). Antibiotics have generally been reserved for infectious complications of IBD rather than as a component of the primary treatment regimen (7). Currently, metronidazole and ciprofloxacin are frequently used (8), despite a lack of rigorous trials (8,9) or evidence of significant benefit over placebo or sulfasalazine (8). Sartor has suggested that evidence from rodent studies provide a rationale for treating human IBD with antibiotics (7). The evidence provided by his CCFA-funded research may have paved the way for developing a rationale for rigorous controlled trials of antibiotics to ensure that they can be safely and effectively incorporated into standard primary therapy for IBD.

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ELSON, CHARLES O, MD

Dr. Elson was funded by the CCFA from 1981 through 1983 to investigate T-cell regulation of immunoglobulin synthesis in IBD.

In an early study, the Elson team sought to determine whether patients with CD have a defect in immune regulation by evaluating suppressor T-cell activity in patients with mild or inactive disease (1). Their in vitro studies indicated that these patients do not have a deficiency in suppressor T cells; indeed, the suppressor T-cell population markedly inhibited IgM synthesis.

Shortly thereafter, the Elson team published a report of their in vitro study of T cell activity during a mixed lymphocyte reaction (2). They found that T cells that had been stimulated by B cells or macrophages were able to suppress proliferation and immunoglobulin synthesis. The B-cell or macrophage-stimulated T-cell activity observed here led the investigators to believe they had stumbled upon a negative feedback mechanism involved in regulating the immune response.

The next step would be to determine whether these early findings—which were performed on elements obtained from peripheral blood—would also be observed in gut tissue. The Elson team evaluated the T-cell immune regulatory effects (suppression or "help") in T cells obtained from intestinal lamina propria tissue that was isolated from patients with CD (3). As in the previous two studies, immunoglobulin synthesis was stimulated by adding pokeweed mitogen to the culture. Additionally, helper T-cell activity was elicited by adding normal peripheral blood cells to the cultures containing lamina propria T cells, and suppressor T-cell activity was elicited by adding B cells to cultures containing irradiated normal T cells (x-irradiation eliminated suppressor T-cell activity in an earlier study [2]). Suppressor T-cell activity was not significant in any of the cocultures, whether the cells were obtained from healthy controls or activity inflamed CD tissue. The investigators concluded that T-cell immune regulatory activity in the gut is carried out primarily by helper T cells, rather than by suppressor T cells, as was seen in the peripheral blood.

Finding that the immune response in gut tissue may allow for immunoglobulin production led Elson to speculate about the possibility of developing an intestinal vaccine (4). The basic requirements for an enteric vaccine are (a) the ability to trigger the production of adequate amounts of intestinal IgA antibodies; (b) the use of antigens that can induce neutralizing antibodies, and (c) the use of an effective antigen delivery system (5).

In their search for the types of helper T cells and cytokines involved in antigen uptake and presentation in the gut, Elson and colleagues found two helper T-cell subsets: Th1 cells—which are involved mainly in cell-mediated immunity and help produce IL-2, IFN-gamma, and TNF-beta—and Th2 cells, which regulate and promote B-cell responses and help produce several interleukins, including IL-5, IL-6, both of which trigger surface B cells to secrete IgA. (6). They also discovered that the GI lamina propria has a relatively high concentration of IL-5-producing Th2 cells and that these cells are stimulated primarily though the oral route (as opposed to Th1 cells, which are stimulated primarily through the systemic route). Additionally, time-course and dose-response studies during this trial indicated that responses to antibody exposure develop according to different sets of kinetics for oral versus serum routes of administration (7).

This led investigators to look for the most effective route of administration. When tetanus toxin (TT) was administered through an indwelling intraperitoneal catheter, high levels of anti-TT antibody-secreting cells were detected in the general circulation and the peritoneal cavity. The predominant immunoglobulin elicited was IgG (80%), rather than the more critically important IgA. Additionally, TT failed to elicit a salivary response. Given that an intestinal antigen is likely to reach the gut by the oral route, the inability to elicit an antibody response in the oral cavity put the patient at risk for prolonged inflammation (8).

Elson was on the first research team to find evidence of a circulation route by which newly activated antigen-specific intestinal T cells return to the gut (10). They also found that activated T cells complete this circuit--through mesenteric lymph nodes, the lymphatics, and blood—and return to the gut guided by cell surface homing receptors (particularly alpha4beta7-integrin). They also discovered that the site of antigen presentation determined whether such homing receptors are expressed or not.

Thereafter, several other investigators searched for a delivery system that could allow oral agents to "survive" the destructive nature of the GI tract, reach the mucosa, and remain there long enough to take effect. Lavelle proposed the use of bioadhesive molecules (eg, lectins) that can recognize epithelial cell surface receptors and thus reach specific regions of the gut (11). Clark and others suggested synthetic delivery particles that can interact with antigen-sampling M cells (12). Zho and Neutra (13) suggested using liposomes (which are quite effective at inducing mucosal IgA responses) that have been modified to withstand the harsh intestinal environment and still interact with M cells. Lo indicated that some investigators are currently looking for genes that might help determine mucosal immunity and thus ensure that immune responses are directed against pathogen-associated targets, only (14). Gene expression studies have led to the discovery of novel receptors of unknown function on the apical membrane of M cells within Peyer's patches. Ligands that are known to trigger pathways used by certain pathogens to invade the intestinal wall are being used to determine the functions of the novel receptors. These ligands may eventually serve as models for developing antigen-loaded nanoparticles capable of binding at these sites to neutralize specific antigens (15). Furthermore, continued investigation of proinflammatory cytokine activity may pave the way to developing a vaccination that takes advantage of host defenses to block TNF-alpha activity and thus modulate the immune response to the bacterial flora in the gut (16).

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CHO, JUDY H, MD

Dr. Cho received funding from CCFA from 1997 through 1998 to conduct genetic mapping studies in IBD.

A substantial amount of epidemiological data had been collected prior to that time suggesting that genetic susceptibility contributes to the development of IBD (1,2). This inspired several investigators to search for specific chromosomal loci that confer IBD susceptibility:

- Mirza and colleagues published the results of their gene-mapping studies, through which they found a CD susceptibility gene (*IBD1*) on chromosome 16 and evidence that this gene may also contribute to UC susceptibility (2).
- Duerr and colleagues (3) attempted to find a link between IBD and chromosome 12, as was done in a British genome screen. They selected 122 white American families that included 208 IBD-affected relative pairs. Given the small sets of affected relatives (ie, relative pairs), they carried out a nonparametric analysis (useful when there is a relative lack of evidence for a Mendelian inheritance pattern or when several genes of low to moderate penetrance may be involved [4]) and a transmission/disequilibrium test (TDT; useful when multi-allelic markers are present [5]) to confirm the British findings.
- Curran and colleagues (6) performed nonparametric analyses of data gathered from a large group of independent European families to demonstrate a link to IBD on chromosomes 12 and 16.
- Neurath and colleagues (7) observed high levels of the transcription factor NK-kappa B in lamina propria macrophages of patients with CD or UC, as well as the consequent increase in the production of several proinflammatory cytokines (IL-1, IL-6, and TNF-alpha) as well as the protein known as p65. They also noted that a specific antisense molecule can downregulate p65 to significantly reduce the production of these cytokines in IBD. These findings suggest the possibility of a molecular approach to patients with IBD.
- Hugot and Thomas (8) reported the findings of several groups who used genome screens (9) not only to confirm the links between IBD and chromosomes 12 and 16, but also to identify additional potential links between IBD and chromosomes 1, 3, 4, 7, 11, 15, and X.
- Hampe and colleagues (10) used a genome-wide search for susceptibility loci to confirm the previously identified link between IBD and 7 chromosomes and to identify 3 additional chromosomes (6, 10, and 22) that might contain genes that predispose individuals to this disease. Of particular interest were the links to chromosome 6p, which suggest an association with human leukocyte antigen and TNF genes, and the suggestion that the link with the X chromosome, which suggests an association with the Ullrich-Turner syndrome.

Others have sought specific genes that confer genetic susceptibility to IBD, including the following:

- Parkes and associates applied the TDT (5) and affected sib-pair test (5) to data from 198 pairs of siblings with IBD and determined that the gene encoding IL-2 may contribute to UC susceptibility (11)

- Noting that an imbalance between IL-1 beta (IL-1 beta) and the IL-1 receptor antagonist (IL-1ra) may play a role in the pathogenesis of IBD, Stokkers and colleagues (12) studied allelic frequencies for IL-1 beta and IL-1ra genes in patients with IBD to determine whether there was a relationship between allelic variants and cytokine production. They found a relationship between UC and several infrequent alleles (Taq1 and Mwo1), but the pathogenicity of this finding was not clear.
- The Stokkers team (13) also studied the role of HLA class II genes in IBD, because the products of these genes play important roles in the immune response. A literature search revealed that UC and CD are each associated with specific HLA class II phenotypes. Additional research may reveal the contribution of these genes to IBD susceptibility.

The search for specific genes responsible for IBD susceptibility expanded exponentially over the following years. By 2001, researchers had identified a strong candidate within chromosome 16—*NOD2.* The gene product normally activates the transcription factor NK-kappa B, thereby allowing the cell to respond to bacterial lipopolysaccharides. Using TDT (5) and case-control analysis, the Ogura team (of which Dr. Cho was a member) determined that same year that *NOD2* undergoes a frameshift mutation through a cytosine insertion in patients with CD. These findings suggest that NOD2 plays a crucial role in CD susceptibility and suggest that a relationship exists between an innate immune response and components of the bacterial cell wall that contribute to this disease (14). In 2003, Cho indicated that a gene on chromosome 16 codes for NOD2/CARD15, a protein that is involved in the immune response to bacterial infection (16), and that three mutations of that gene appear to be independently associated with CD, collectively conferring a 15% to 20% risk for familial CD. Cho also indicated that NOD2 presents an increased risk for ileal disease and an earlier age of disease onset. Subsequently, Drs Cho and Dr Bonen indicated that NOD2/CARD15 is expressed on peripheral blood monocytes (17). They also indicated that three polymorphisms within the gene for this protein complex contain the code for CD, especially in individuals of European descent. Having one copy of a risk allele increases the risk for CD 2- to 4-fold in these individuals; having two copies increases the risk 20- to 40-fold. As a member of the Brant research team (18), Dr Cho helped determine that carrying two of these mutations increased the risk for early onset of disease, ileal involvement, and the development of strictures or non-perianal fistulas. As part of the Ogura team, Cho was part of the effort to find a link between NOD2 mutations and ileal disease. That laboratory developed a monoclonal antibody against NOD2, then used it to detect NOD2 expression in terminal ileal Paneth cells, specifically in the cytosol near granules that contained antimicrobial peptides, and in the epithelial layer of ileal villi and the colon. This protein was found in both patients and controls and, thus, may help regulate Paneth cell-mediated responses to intestinal bacteria (19).

Dr Cho has continued to explore the genetic mechanisms for IBD susceptibility. In 2004, she identified post-transcriptional dependence of IL-1 beta on NOD2/CARD15 suggesting that a signaling defect may be the underlying cause of pathogenesis in CD (20). She also explored several new genes believed to contribute to CD. Her work may bring investigators closer to identifying the earliest pathways involved in IBD pathogenesis which, in turn, may reveal potential novel therapeutic targets.

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DAS KM

Dr. Das was funded to study the pathogenesis of IBD (1981-1983) and the immunopathogenesis of UC (1983-1988).

During the first funding period, Dr. Das worked with Dr. Nagai to clarify the role of a disease-specific colonic tissue-bound antibody (CCA) they identified previously in patients with UC (1). Specifically they improved their methods of extracting and purifying intact CCA-IgG. They then demonstrated that this molecule binds to colonic mucosal tissue in UC but not in CD or in normal colonic tissue from patients with carcinoma (2).

Das and another colleague, Dr. Takahashi, then characterized a colonic protein that is recognized by CCA-IgG. Using immunorecognition studies, affinity-column chromatography, transblot analysis, electrophoresis, and an iodinated CCA-IgG probe on a variety of tissues obtained from patients with UC, Crohn's colitis, or myeloma, they found CCA-IgG was consistently bonded to a 40-kD protein in colon tissue extracts. This bond was found most frequently in tissue obtained from patients with symptomatic UC and never in colonic tissue obtained from patients with Crohn's colitis (3). These findings suggest the existence of an organ-specific colonic "autoantigen" that might be able to initiate an IgG antibody response in patients with UC.

To learn more about this molecule, Das and colleagues developed monoclonal antibodies against it. Antibody studies allowed them to localize the antibody-40kD antigen interaction exclusively to colonic epithelial cells, specifically within the crypt and on the luminal surface of the epithelium in this study (4), primarily along the basolateral surfaces in a murine study (5), and in both membrane regions in another study in human tissue (6). Such studies also allowed these investigators to discover that

- This antibody-antigen interaction occurred more frequently in colon tumor cells and that its frequency was not affected by interferon gamma (IFN-gamma) (7)
- The 40 kDa molecule is involved in antibody-dependent cellular cytotoxicity (ADCC) against colon cancer cells by UC serum (8)
- 40 kDa expression in colonic cells is accompanied by the expression of intercellular adhesion (ICAM) molecules (especially ICAM-1), which may be involved in the localization of leukocytes to the colonic epithelium during UC (9)

Years after the CCFA funding ended, the investigators continued their investigation of the 40 kDa molecule in UC:

- They gave it a name: P40
- They discovered that it can be found in the goblet cells of normal ileal and proximal colonic tissue, as well as in enterocytes, where its concentration increases in a distal direction (11)
- They found it in several noncolonic areas, including the gall bladder, major bile ducts, fallopian tubes, and epidermis (11), as well as nonpigmented ciliary epithelial cells and chondrocytes (12)—all of which suggests potential areas for extraintestinal complications of UC
- They discovered that P40 is a member of the tropomyosin family (10). The most common tropomyosin isoform found in the intestine—human tropomyosin (hTM) isoform 5 (hTM5) (13)—is an intracellular protein that can be externalized in the colonic epithelium but not in the small intestine(14). hTM5 has such a strong

association with a membrane-bound colon epithelial protein that is suspected of being involved in its transport to the cell surface and may serve as a target autoantigen in UC (14)

- They named the anti-P40 antibody—mAb Das-1(15)—and found that it reacts with liver tissue and is expressed in correlation with the expression of specific liver molecules, including glycogen (15)
- They found that B cells in the lamina propria produce IgG against hTM5, most notably in patients with UC (16)
- They identified hTM5 as a colon epithelial cell antigen that can trigger a significant T-cell response in UC (17)

Thus far, Das and colleagues have produced a substantial amount of information about several components of the autoimmune activity in UC. Additional studies of the antibody and its target (as well as the cellular components responsible for its upregulation and presentation on the cell surface) are needed to bring these investigators closer to finding an effective immunologic approach to therapy.

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DUERR, RICHARD H, MD

Dr. Duerr received funding from CCFA from 2001 through 2002 to study linkage disequilibrium patterns with a novel IBD locus on chromosome 3P.

Chromosome 3p was first identified as being likely to contain IBD susceptibility genes in 1996 (1). Genetic screening of a total of 186 sibling pairs affected with CD and UC provided evidence of a link between IBD and 46 microsatellite markers, with 16 of the strongest markers seen in chromosomes 2, 3, 7, 12, and 15. The strongest linkage to a single marker was identified in chromosome 12. By contrast, chromosome 3 contained several markers that lie adjacent to regions containing genes that code for UC complications, eg, carcinoma of the colon and renal cell carcinoma (1), as well as two autoimmune disorders—MS and inflammatory arthritis—which suggests one or more genes involved in the inflammatory response (2). One chromosome 3p marker--D3S1076--lies near several potential IBD susceptibility genes, including the genes for cytokine receptors 2 and 5, both of which play an important role in immunoregulation (2). Neither of these receptor genes appears to play a role in the IBD phenotype, but they both reside near other potential IBD-related genes, including the gene for lactotransferrin (which may play a role in neutrophil and antibacterial activity), the ubiquitin complex (which may be involved in antigen processing), the cathelicidin antimicrobial peptide and the TRAF interacting protein (which play a key role in TNF-alpha signal transduction), the mitogen-activated protein kinase that is activated by protein kinase 3, and the IFN-alpha receptor (receptor 2).

Being closely associated with so many genes creates a serious research-related challenge, however, because it presents the risk of disease-associated disequilibrium (2), ie, the observed frequency of haplotypes may not agree with the frequency predicted by multiplying the frequency of individual markers within each haplotype. Identification of links between markers and disease requires linkage analysis (3), a procedure that is used to determine the distance between the marker and the susceptibility gene. It involves an investigation of pedigree, usually by identifying families with affected sibling pairs or affected relative pairs; genotyping by polymorphisms the entire genome or certain chromosomes; and determining the approximate position of the susceptibility gene within the genome map, which involves calculating the LOD score (a non-parametric measure of distance between the susceptibility gene and marker) for several points. Linkage analysis is then followed by an association analysis of candidate genes (3).

By 2002, Duerr and colleagues were able to report their finding of a specific IBD locus on chromosome 3p26. Evidence of linkage was set at an LOD score of 2 or more in a previous study (4); in the Duerr study, a LOD score of 3.69 was achieved for D3S1297, indicating a strong linkage between marker and disease (5).

A recent study indicates that more than 20 genomic regions have been identified as containing IBD susceptibility loci (6). Continued work in this area may facilitate the development of genetic strategies for preventing or treating this disease.

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CHANG EUGENE B, MD

Dr. Chang received funding from CCFA from 1999 through 2002 to study barrier defects in IBD.

During this period, Dr Chang's team produced numerous reports of their work on epithelial ion exchange in the intestines. Chang was among the first to characterize the intestinal Na^+/H^+ exchanger (NHE) in intestinal tissue (specifically, an intestinal villus-like subclone [C2bbe]) rather than in nonepithelial mutated fibroblasts (as had been the practice until 1999) (1). To measure NHE activity, he monitored the unidirectional apical uptake of $^{22}Na^+$ under basal, non-acid conditions. This approach represented a dramatic change in the process of evaluating NHE activity. Previously, it was evaluated by monitoring intracellular pH, which can only approximate NHE activity and may be altered by buffers and non-NHE contributions to pH. Thus, Chang and colleagues developed a method that could significantly improve the accuracy of research findings. Using this improved technique, the Chang team found that the brush-border NHEs—NHE2 and NHE3—both localize to the C2bbe apical domain. They also found that both NHEs are regulated by second messengers, albeit through different signal transduction pathways.

The precise characterization of such exchange molecules will prove essential in determining the role of anion secretion in IBD, either as a participant in complications (eg, diarrhea) or as a regulatory signal. For example, the Chang team found that an oxidant (monochloramine) could potentiate colonic calcium- and cAMP-stimulated chloride ion secretion through its effect on calcium-activated potassium channel conductance. This could increase the severity of diarrhea in patients with an inflamed colonic mucosa (2). They also found that short-chain fatty acids—produced by fermentation of dietary carbohydrates carried out by the bacterial flora in the colon—enhance apical NHE3 activity (but not NHE2 activity) in a time- and concentration-dependent manner and, thus, may serve as a physiological cue that allows the colon to adjust its sodium absorption rate in response to ongoing changes in dietary carbohydrate and sodium loads (3).

Another Chang team investigated the effect of IFN-gamma on ion transport across the intestinal epithelium (4). This could be a critical component of the pathogenesis of IBD, because IFN-gamma helps regulate and promote B-cell responses and helps produce several interleukins that facilitate B-cell secretion of IgA. Chang and colleagues assessed Na^+/K^+-ATPase activity using the inhibitor ouabain and monitored intracellular Na^+ with the Na^+ ionophore monensin. They found that IFN-gamma acutely reduced Na^+/K^+-ATPase activity and increased the intracellular Na^+ concentration and, consequently, cell volume. These effects suggest that IFN-gamma can trigger signaling events that result in the leaky, dysfunctional epithelium that is characteristic of chronic inflammation (4). The potential role of IFN-gamma in IBD diarrhea was supported by a subsequent study of NHE expression in culture and in adult rats. NHE expression was monitored by unidirectional $^{22}Na^+$ influx and by changes in concentration in rat brush-border membrane vesicles; NHE protein and mRNA levels were assessed by Western and Northern blotting. The investigators found that IFN-gamma triggered downregulation of NHE2 and NHE3 expression and activity, which could result in inflammation-associated diarrhea (5).

The Chang team also investigated the intestine's ability to adapt to new sodium absorption requirements following extensive bowel resection. After removing 50% of the proximal rat bowel, investigators found that brush-border hydrolase activity and total cell protein per DNA was comparable to the length of bowel, but basolateral Na^+/K^+-ATPase activity was increased. NHE2 and NHE3 levels increased in the ileum distal to the anastomosis; their expression in the proximal colon increased only after 80% of the bowel had been removed. The investigators concluded that an increase in luminal sodium concentration in the distal bowel following a proximal resection may trigger a compensatory increase in apical NHE gene transcription and protein expression (6).

After several years of work on transmembrane ion movement, Chang and colleagues expanded their efforts to focus on methods of protecting the integrity of the colonic epithelium. Heat shock proteins (HSPs) had been shown to be effective in this regard in animal models of septic shock (7). HSP expression had not been induced in humans because laboratory induction agents are highly toxic. Chang and colleagues discovered, however, that when glutamine is administered to rats with endotoxemia, it reduces mortality dramatically and protects against end-organ damage (7). This protection appears to be associated with a reduction in the release of at least two pro-inflammatory cytokines: TNF-alpha and IL-1 beta (8). Chang and colleagues also observed this in human peripheral blood polymorphonuclear cells (9). Importantly, it is effective when given as sepsis begins, rather than as a pretreatment (7, 8). Thus, glutamine may prove effective in therapy rather than prophylaxis only.

The gut flora may contribute to the protection afforded by glutamine by continuously inducing the expression of HSPs on the surface of colonic enterocytes. By monitoring *E coli* (10) lipopolysaccharide (LPS) and mouse colonic HSP25 levels, the Chang team found that LPS induced HSP25 induction in colonic epithelial cells and may protect the colon from injury by means of filamentous actin stabilization, both under normal and pathophysiological conditions (11). These findings were confirmed in a subsequent article by the Chang team, which reported that HSP expression in rats that had been surgically altered to achieve continuous colonization within the jejunum resulted in improved protection against oxidant-induced transmural stress (12).

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MAYER, LLOYD F, MD

Dr. Mayer received funding from CCFA from 1998 through 2000 to investigate the mechanism of CD8 suppressor T-cell function induced by intestinal epithelial cells.

As early as 1990, a Mayer research team reported an unusually high proportion of T cells with T-cell antigen receptors containing the gene product V-beta 8 in patients with CD (1). They were unable to correlate this finding with the clinical characteristics of the disease and they were not able to connect the RFLP for V-beta 8 with a specific disease. However, they did find evidence suggesting that these T cells were concentrated in diseased bowel tissues (1). They also found that the monoclonal antibody that detects V-beta 8 interacts strongly with an unidentified antigen on epithelial cells and hypothesized that an autoantigen may exist on damaged epithelial cells.

Five years later, Mayer and associates reported that the key to mucosal epithelial cells being able to trigger CD8-positive suppressor T-cell activity depends on an epithelial cell surface non-class I molecule activating a CD8-asociated tyrosine kinase (p561ck); that activation appears to allow the CD8 molecule to bind with the T cell. This linkage appears to be essential for T-cell activation, but not for T-cell proliferation, which suggests that second signal might be necessary for such proliferation. The authors suggest that the second signal might work through the T-cell antigen receptor (2).

CD8-positive suppressor T-cell proliferation may also require a specific epithelial surface structure. Proliferation is blocked by two epithelium-specific monoclonal antibodies--mAB B9 and mAB L12--both of which recognize a 180-kDa glycoprotein (gp180) on the epithelial membrane. gp180 appears to be capable of regulating mucosal immune responses, as it can bind with peripheral blood T cells and activate p56(lck) (3). Expectedly, gp180 is not as plentiful in inflamed intestinal tissue as it is in normal tissue, as indicated by patchy immunohistochemical staining in UC and faint to absent staining in CD. Additionally, gp180 expression is altered and p561ck activity is reduced in IBD tissue (4). Within another 2 years, Mayer and colleagues had determined that the intestinal epithelium triggers CD8-positive suppressor T cell proliferation in conjunction with p56(lck) and the T-cell receptor-associated kinase p59(fyn) (5).

Suppressor T cells are believed to promote oral tolerance in normal tissue (6). The Mayer team tried to determine whether tolerance could be induced in patients with UC or CD by feeding them keyhole limpet hemocyanin (KLH) and attempting to raise anti-KLH antibodies through subcutaneous and booster immunization. KLH-induced T-cell proliferation was reduced in controls but enhanced significantly in patients with CD or UC. Neither oral tolerance nor antibodies to KLH could be raised in these patients. Active immunity may have been triggered in the patients with IBD, indicating a functional defect in the ability of mucosa to suppress an immune response.

Thus, through their methodical exploration of an unknown protein on the surface of the gut epithelium, Mayer and colleagues eventually found several important keys to a crucial component of autoimmune activity in IBD and the development of oral tolerance. Continued exploration of the latter may pave the way to the development of an oral vaccine against antigens responsible for intestinal inflammation.

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