Redefining IBDs: Toward Molecular Classification

Traditionally, inflammatory bowel disease (IBD) has been divided into 2 distinct entities: ulcerative colitis (UC) and Crohn’s disease (CD). An innovative and nuanced view presents IBD as an immunoinflammatory spectrum of chronic and recurring diseases of the intestines. This newly gained perspective holds the promise of moving treatment in a more proactive direction—toward targeting molecules, rather than treating symptoms.

In the vast majority of patients, the traditional diagnosis of IBD is established by clinical history, examination, endoscopy, pathology, and radiology. Just as the advent of the purified protein derivative (PPD) initially defined IBD by distinguishing it from tuberculosis and chronic appendicitis, molecular diagnostic tests (antibody profiling) will redefine the pathobiology of IBD by characterizing the diversity of mucosal immune responses to luminal bacteria.

The evolution of diagnostic antibody profiling offers physicians the ability to stratify patients and correlate the clinical and immunologic patterns of IBD based on the heterogeneity of mucosal inflammation. The foundation of this new correlative science is predicated on the idea that genetic, bacterial, and immunologic diversity may allow physicians to individualize therapy.

The human genome is 99.9% identical in all people. Individual differences are determined by only 0.1% of the genome. This very small fraction of the genome displaying DNA variations (polymorphisms) is responsible for individual therapeutic response and disease susceptibility. The emerging role of pharmacogenomics is increasingly recognized in optimizing drug therapy. Serologic markers do not replace traditional diagnostic modalities; rather, they serve as clinical adjuvants, potentially enhancing our ability to optimize medical therapy.
Challenging the Traditional IBD Diagnosis

One of the major questions facing clinicians treating IBD is whether the disease is a single entity or a spectrum of multiple disorders. This distinction becomes particularly difficult when one is attempting to classify CD. Three distinct manifestations of CD have been described—inflammatory, fistulizing, and fibrostenotic. However, Crohn’s colitis has not been well defined in the literature. Some patients present with CD-like features—such as UC with rectal sparing or UC with nonepithelioid granulomas (Figure 1). Other manifestations of the heterogeneity of colitis are a superficial mucosal CD involving left-sided refractory colitis with rectal involvement that may actually represent a type of mixed colagenous colitis or vascular collagen disorder still undefined, rectal disease with rectal sparing or UC with nonepithelioid granulomas (Figure 1).

The many forms of UC (ulcerative proctitis, left-sided colitis, and universal colitis) left Brooke to suggest that, rather than a single disease, UC represents a pathologic state with many etiologies. Indeterminate colitis (IC) might represent part of an immunologic continuum, rather than a well-defined clinical subset of UC and CD. Molecular diagnostics, such as antibody profiling, hold the promise of enhancing our understanding of IC and stratifying all IBD patients on the basis of immunophenotypes.

Molecular Classification of IBDs

IBD nomenclature does not accurately reflect the complexity of clinical phenotypic behavior. Although the role of serum antibody markers remains controversial, combining markers enhances their accuracy and specificity in classifying IBD. Genotyping and immunophenotyping are more consistent with a molecular phenotype (Table 1). The emerging role of molecular diagnostics is vital in characterizing the immunologic heterogeneity of IBDs, and will be a bridge linking clinical immunophenotypes with genotypes.

Differentiation between types of IBD becomes important in determining appropriate treatment strategies. Poor therapeutic response is an indication for surgery in nearly 25% of UC patients and approximately 50% of CD patients. Patients with refractory left-sided colitis or IC may benefit from serologic testing, in addition to documentation of clubbing and oral aphthae. In these patients, if the markers are more consistent with a molecular pattern of CD, physicians may consider infliximab therapy as an option rather than total colectomy.

Serologic profiling has already proved helpful in patient stratification. High levels of perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) have consistently correlated with postoperative pouchitis. Anti-Saccharomyces cerevisiae antibodies (ASCA) positivity (expression of both immunoglobulin subtypes IgA and IgG) correlates with a younger age at onset and more aggressive fibrostenotic disease.

Serologic diagnostic testing provides a molecular snapshot of people with IBD. New markers and prospective trials are required to correlate immunologic and clinical patterns of IBD, and will advance the science of IBD.

Treatment of IBD Subtypes

IBD depends on the interaction of luminal antigenic triggers, which initiate an uncontrolled immune response in a genetically susceptible host. In the absence of bacteria, there is no immune-mediated intestinal inflammation (Figure 2). The goals of IBD treatments are universal: induce remission as quickly as possible, maintain remission as long as possible, facilitate mucosal healing, improve the patient’s quality of life, avoid toxicity, and minimize cost.

For patients with UC, oral and rectal 5-aminosalicylic acid (5-ASA)—free 5-ASA (Oral mesalamine: Asacol, Proctor & Gamble; Pentasa, Shire US. Topical mesalamine: Rowasa, Solvay Pharmaceuticals; Canasa, Axcan Scandinapharm), 5-ASA prodrugs (sulfasalazine: olsalazine [Dipentum, Celltech Corporation]; and balsalazide [Colazal, Salix]), corticosteroids (including topically active, rapidly metabolized budesonide [Entocort, AstraZeneca]), immunomodulators (6-MP, azathioprine, methotrexate), and infliximab (Remicade, Centocor) are used to induce remission; for maintenance of remission, immunomodulators, 5-ASA, and antibiotics can be used. Probiotics and novel antibiotics (rifaximin [Xifaxan, Salix]) have the potential to revolutionize our approach to IBD. However, greater understanding of gut microbiology and further clinical trials into these agents are needed.

5-ASA First-Line Therapy:
Mesalamine (Free 5-ASA) and Prodrug (Azo-bonded 5-ASA)

The specific goals of 5-ASA therapy are to quickly induce a complete remission, facilitate mucosal healing, and minimize steroid use and toxicity. One proposed mechanism of action of 5-ASA is the inhibition of the cyclooxygenase and 5-lipoxygenase pathway of arachidonic acid metabolism, resulting in a decrease of proinflammatory prostaglandins and leukotrienes (LTB4). Although clinical trials with leukotriene inhibitors have been disappointing, the role of the cyclooxygenase pathway and prostaglandin biosynthesis in IBD remains to be elucidated. Attention has shifted from the arachidonic acid cascade to nuclear factor kappa B (NFkappaB). The recent discovery of the interaction between Nod2, the IBD gene discovered on chromosome 16, and NFkappaB emphasizes the importance of NFkappaB in the inflammatory signaling cascade and interaction with luminal bacterial antigens (Figure 3).
Studies in cell cultures demonstrate that sulfasalazine inhibits NF-κB, supporting direct biologic efficacy of 5-ASA. The question clinicians ask is: Does the site of 5-ASA release matter in terms of optimizing and individualizing therapy? The tale of 2 therapeutic strategies exposes jostling views. One view is that all 5-ASA preparations are the same and equivalent: both mesalamine (free, unconjugated 5-ASAs) and prodrugs (azo-bonded 5-ASA) have similar modes of action. The other view is that the subtle differences in 5-ASA distribution translate into clinical efficacy, allowing for optimizing strategies. Often overlooked is the distribution of UC; more than one-half of patients have left-sided disease alone.17 (Figure 4, page 44).

Sulfasalazine, the archetypal azo-bonded 5-ASA-containing designer drug, is engineered to release free 5-ASA (mesalamine) in the colon, protecting it from proximal absorption. Intolerance and hypersensitivity to the sulfapyridine moiety limit the dose of sulfasalazine, and have led to the development of new 5-ASA-containing analogs. The newer topical and oral 5-ASA agents are released at different anatomic sites, ideally corresponding to the sites of active disease (Table 2, page 44; Figure 5, page 45). Although these agents are less toxic than sulfapyridine, mesalamine allergies and intolerance may occur, and interstitial nephritis has been reported with the 5-ASA moiety alone. Although rare, the potential for this adverse event mandates periodic renal function monitoring.18

Whereas much of the pharmacology of the 5-ASA formulations is similar, pharmacogenetics emphasizes that different patients metabolize medications differently. Responders to 5-ASA may be distinguishable from nonresponders on the basis of differences in metabolism, anatomic distribution (ileal, ileocolonic, and colonic), and clinical characteristics (mucosal or transmural inflammation, fistulizing, and strictureting).

Until the introduction of balsalazide, all of the newer 5-ASA agents had been shown to induce and maintain remission in UC patients nearly as well as sulfasalazine and, more for more, usually as well as each other. The advantage of the newer preparations is that patients can tolerate higher doses of the agents (Figure 6, page 45). The first head-to-head trial comparing an equimolar dose of balsalazide (6.75 g) with a pH-dependent mesalamine (2.4 g) showed superior efficacy of balsalazide in patients with new-onset left-sided UC disease (62% vs 57%) and shorter time to response (10 days vs 25 days). Right-sided UC also responded more favorably to balsalazide, although the difference was less significant than with left-sided disease.19 A stratification study confirms that among patients with new-onset left-sided UC, more than 60% of those treated with balsalazide were in remission at 1 month, compared with 40% of those treated with a pH-dependent mesalamine. Patients with right-sided UC did better with balsalazide in regard to rectal bleeding, sigmoidoscopic-evident healing, and stool frequency.20

Levine et al conducted a randomized, double-blind study comparing 2 doses of balsalazide (6.75 g and 2.25 g) and mesalamine 2.4 g.21 The investigators concluded that at week 8, rates of remission were similar for all 3 treatment groups, as were the safety profiles. The primary difference between equimolar doses of balsalazide (6.75 g) and mesalamine (2.4 g) appears to be the time to symptom resolution (10 days vs 25 days). The most rapid onset of action of balsalazide might be related to greater 5-ASA delivery to the colon from the azo-bonded delivery system, based on the observation that patients receiving mesalamine 2.4 g per day had significantly higher steady-state plasma levels of 5-ASA and its N-acetylated metabolite at 2 weeks than did patients receiving balsalazide 6.75 g per day (N-acetylated and 2.5-fold, respectively). This finding suggests precociously (ileal) 5-ASA absorption from the pH-dependent delayed-release formulation of mesalamine. Further dose-ranging studies are required to establish the efficacy of high-dose mesalamine (4.8 g) and balsalazide (13.5 g).

Because of the predominance of left-sided disease, the combination of oral and topical aminosalicylates is critical in inducing and maintaining remission.22 Saffidi et al elegantly demonstrated that whereas topical mesalamine was more effective than oral in left-sided UC, the combination of oral mesalamine 2.4 g and mesalamine enemas produced earlier and more complete cessation of rectal bleeding.22 For maintenance of remission for UC patients, D’Albasio et al found that the combination of oral mesalamine 1.6 g with twice-weekly mesalamine enemas was superior to oral therapy alone (61% vs 31%, respectively).23 Topical mesalamine (enemas and suppositories), used as infrequently as 2 times per week, are effective in maintaining remission in patients with distal colitis.

In another study, Biddle et al established that 75% (9 out of 12) of patients randomized to receive mesalamine enemas remained in remission at 1 year, while 85% (11 out of 13) of patients on placebo had relapsed by 16 weeks.24 Similarly, mesalamine suppositories maintained long-term remission in patients with ulcerative proctitis.25 By 12 and 24 months, respectively, 86% and 89% of placebo-treated patients had relapsed, compared with 52% and 46% of patients treated with mesalamine suppositories. A meta-analysis established that in patients with left-sided UC and ulcerative proctitis, topical mesalamine showed greater efficacy and fewer side effects than...
oral therapies and topical steroids.

Campieri et al demonstrated that mesalamine suppositories were effective in inducing remission in patients with ulcerative proctitis (distal colitis up to 20 cm). In that study, 74% of patients receiving mesalamine suppositories (1.5 g) achieved clinical remission at 4 weeks, compared to only 59% of patients receiving placebo.

The pH-sensitive 5-ASA drugs were evaluated in a placebo-controlled trial in patients with mild to moderate UC. Of patients receiving 4.8 g of mesalamine (Asacol), 24% showed complete remission, compared to 9% of patients receiving 1.6 g of mesalamine, and 5% receiving placebo. Partial response was noted in 50% of patients in the high-dose mesalamine group, compared to 18% for the low-dose group and 13% for placebo.

### Combining Oral Agents

Although there are no studies evaluating the combination of oral 5-ASA drugs, we can begin to debate the virtues of combinatorial strategies in an individual patient who fails to respond to mono-5-ASA monotherapy. 5-ASA nonresponders may benefit from a combination of pH-polymer-coated mesalamine formulation (Asacol), moisture-dependent mesalamine (Pentasa), and azo-bonded 5-ASA preparations (sulfasalazine, olsalazine, and balsalazide). A flexible dosing schedule, in which the patient actively modulates the combination therapy based on clinical improvement, may shorten the time to response. Lastly, a flexible dosing schedule combining oral and topical 5-ASA agents is an effective therapeutic strategy that should not be overlooked. With the variety of 5-ASA preparations available, optimization of 5-ASA therapy may be viewed as a dynamic rather than a static process. In the individual patient, the combination of oral 5-ASA drug therapy and topical 5-ASA may be an effective therapeutic strategy.

Table 2. 5-ASA–Containing Drugs Release via the Following Mechanisms (see Figure 5)

<table>
<thead>
<tr>
<th>Type</th>
<th>Mechanism</th>
<th>Advantage</th>
<th>Indication</th>
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<tbody>
<tr>
<td>Diffusion-Dependent</td>
<td>Time-released, moisture-dependent ethylcellulose-encapsulated mesalamine (Pentasa, Shire US); allows free 5-ASA-mesalamine to diffuse out of the ethylcellulose beads and begin releasing in the upper intestines and continue throughout the small and large intestines.</td>
<td>Independent of pH or bacteria; mucosal delivery of mesalamine is less affected by rapid intestinal transit time (ie, diarrhea).</td>
<td>Pentasa is indicated in patients with proximal disease activity, severe diarrhea, stenosis (1-mm ethylcellulose microspheres offer advantages).</td>
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<tr>
<td>pH-Dependent</td>
<td>The pH-dependent mesalamine preparations are coated with an acrylate resin, which is released at variable pH levels between 6 and 7 in the distal ileum and colon. Eudragit S-coated mesalamine (Asacol, Procter &amp; Gamble) is released at a pH level of 7. The pH in the ileum and ascending colon is 7.</td>
<td>Asacol dosage can be maximized to 4.8 g to 6.0 g qd, equivalent to 3 triple dose of sulfasalazine (4.8 g Asacol is equivalent to 12 g sulfasalazine) with significantly less toxicity.</td>
<td>A pH-dependent delivery system is indicated in ileocolonic disease.</td>
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<tr>
<td>Colonic Flora-Dependent (Azo-Bonded)</td>
<td>There are currently 3 variations of colonic-releasing, azo-bonded 5-ASA; osalazine (Dipentum, Celltech Corporation) consists of 2 5-ASA molecules linked to each other; balsalazide (Colazal, Salix Pharmaceuticals) links an inert polymer of 4-aminobenzoylalanine to 5-ASA; sulfasalazine consists of 5-ASA bonded to sulfapyridine.</td>
<td>In these azo-bonded 5-ASA forms, the molecule reaches the colon primarily intact, and the azo bond is cleaved by colonic bacterial azoreductase, thereby releasing free, unconjugated 5-ASA (mesalamine).</td>
<td>Indicated for patients with universal and distal colitis.</td>
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<tr>
<td>Topical/Rectal Formulations</td>
<td>Rectal preparations include 5-ASA suspensions: 4-g mesalamine enema (Rowasa, Solvay Pharmaceuticals) and 500-mg mesalamine suppositories (Cansa, Ascan Scandinap) instilled directly into the rectum.</td>
<td>Advantages of topical preparations include direct exposure to diseased mucosa.</td>
<td>Indicated for patients with left-sided colitis and proctitis.</td>
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Figure 4. Disease distribution of ulcerative colitis at presentation to the physician’s office.

may optimize biologic and clinical efficacy for individual patients.

**Optimizing 5-ASA Remission: Induction and Maintenance**

Infliximab treatment strategies and the NOD2 gene are revolutionizing our understanding of the pathobiology of IBD, providing a prism for understanding the biologic efficacy of 5-ASA. While we develop pharmacogenetic markers for targeted IBD therapy, we can begin to debate optimizing 5-ASA therapy for individual UC and CD patients by targeting the differences in delivery systems to differences in disease location. Studies evaluating combinatorial oral and topical therapy suggest that 5-ASA efficacy is increased when the site of release correlates with the site of disease. Therefore, 5-ASA may work better in mucosal disease processes and less effectively in transmural fistulizing disease or obstructing fibrostenotic CD. If we can select the subgroup of IBD patients in whom a specific 5-ASA works most effectively, perhaps we can decrease the NNT and conduct much needed well-designed clinical trials. In maintenance of remission of CD, the lack of proof of clinical efficacy does not preclude lack of biologic efficacy.

**Conclusion**

With the variety of 5-ASA preparations now available, optimizing strategies for individual patients is viewed as dynamic rather than a static process, allowing physicians to maximize dosing and to use combinatorial strategies (oral-oral and oral-parenteral preparations) until the emerging role of pharmacogenomics allows us to more scientifically individualize IBD therapy.

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### References


### Figure 5. Oral aminosalicylate delivery in small and large intestine.

- **5-ASA**, 5-aminosalicylic acid

### Figure 6. Drug doses needed to provide equal amount of 5-ASA.

- **4-ABA**, 4-aminobenzoyl-8-alanine
- **5-ASA**, 5-aminosalicylic acid
- **SASP**, sulfasalazine
- **Balsalazide**

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