Past and Current Theories of Etiology of IBD

Toothpaste, Worms, and Refrigerators

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■ *"The conservative physician will recognize that much of what happens is transient, but sometimes as he matures, he forgets unfortunately that his own methods of therapy are trembling on the same shifting sands that cover the treatment and alas also the bones of those who have gone before."*
—Howard M. Spiro, Introduction to Clinical Gastroenterology, 1970.

Abstract: While tremendous advances have improved the understanding of inflammatory bowel disease, with regard to environmental risk factors as well as the biochemical nature of the inflammatory process, a determination of primary etiology remains elusive. Numerous theories have been proposed in the past century concerning the cause of Crohn's disease and ulcerative colitis with implications for specific therapies. On further study, most of these ideas and therapies have failed to be accurate in theory or therapeutic approach. Others remain untested or are the focus of current investigation and controversy. This paper reviews the dominant theories of primary etiology. These hypotheses include infectious causes such as *Mycobacteria paratuberculosis* and measles. Allergic and nutritionally related causes have been the focus of considerable research. Microparticles, which is part of the concept behind toothpaste as a cause, have been suggested more broadly to be the principal factor initiating Crohn's disease. Several of these concepts rely on the idea that there is an increased intestinal permeability that is the central defect leading to Crohn's disease. Rather than being an excessive T cell driven process, Crohn's has been suggested to be an innate immune deficiency, leading to the use of colony stimulating factors to augment the intestinal barrier function and innate immunity. A variety of changes in the gut flora, ranging from a basic dysbiosis to the absence of helminths, have been proposed as the root cause of inflammatory bowel disease.

Key Words: Crohn's disease, ulcerative colitis, etiology, history

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The current understanding of inflammatory bowel disease (IBD) has evolved from a description of gross pathology to a detailed biochemical profile of the inflammatory process. Both Crohn's disease and ulcerative colitis (UC) are viewed as predominantly T cell-driven diseases with a complex set of interacting cytokines, chemokines, and other mediators orchestrated by a variety of cell types. The prevailing theory of IBD suggests that the T cell is inappropriately activated due to an unfortunate confluence of genetic and environmental factors, which generate an immune imbalance, leading to the inflammation characteristic of these diseases. While critical to understanding and treatment of IBD, this increasingly sophisticated picture fails to provide information concerning the proximal events, which set the process in motion and would be considered the fundamental etiology of the disease.

This paper reviews past and current hypotheses concerning the etiology of IBD, defined narrowly as theory of primary cause concerning the initiator or instigator of the disease, as group A beta-hemolytic *Streptococcus* would be considered the cause of rheumatic heart disease. While extensive epidemiologic investigations have identified risk factors and provided numerous clues to the development of IBD, an understanding of primary, proximate etiology remains elusive. Many immunologic investigations have proposed various mechanisms of IBD, such as IBD being an autoimmune phenomenon, as an example. This article is not a review of different conceptions of pathophysiology. Instead, this partial survey of ideas includes some of historical interest or curiosity, some hypotheses, which are flat out wrong but may provide insight into these diseases, while other concepts are likely of critical importance in the understanding and treatment of Crohn's disease and UC. Proposed and tested therapies, most discarded, have developed from these theories and offer an appreciation of the theoretical underpinnings of past and current approaches.

INFECTIOUS ETIOLOGIES

The similarities between infectious colitis or enteritis and Crohn's disease or UC are sufficiently evident that numerous specific agents have been proposed over the years. Dalziel, whose description of a new entity, an ileitis, in 1913[1] may represent the first case series of what was subsequently known as Crohn's disease, wrote "I can only regret that the aetiology of the condition remains in obscurity but I trust that ere long further consideration will clear up the difficulty." Because of striking similarities between intestinal *Mycobacteria tuberculosis* (MTb) and this novel ileitis, Dalziel suggested that *Mycobacteria paratuberculosis* (MAP), which had recently been described, may have been the cause of the

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intestinal disease he had characterized. Since then, numerous investigators have seized on this idea, and a considerable literature has developed, both in support of and against this concept (see below).

In the early decades after the Dalziel description, an infectious etiology appeared sufficiently obvious that vaccines, antiseptics, and subsequently antibiotics were attempted to treat these presumed chronic persistent infections. Other earlier ideas proposed that a protective factor was absent in the intestines, leading to unusual therapies of pig stool extract and "slop diets," which included three pints of milk soured with lactic acid.[2,3] Numerous bacteria and viruses were proposed though none fulfilled Koch's postulates. *Diplostreptococci* emerged as a possible agent for UC in the 1920s due to the work of J.A. Bargen of the Mayo Clinic, in which the bacteria was cultured from a high percentage of rectal ulcers in patients with UC[4] and intravenous inoculation of rabbits with these isolated organisms led to colonic lesion.[5] Based on this work, Bargen subsequently tested an autologous vaccine of fecal *Diplostreptococci* in patients with UC without significant success.[3] Other early "vaccines," based on a specific infectious etiology of IBD, included injections of polyvalent antidysentery vaccine (AF Hurst, Guy's Hospital, London) and typhoid vaccine.[2] With the advent of antibiotics, the idea of a bacterial etiology was pursued with great optimism but only temporary benefit at best.[3] Numerous other specific bacterial agents have been advanced as the causative agent of IBD, including *Bacteroides necrophorum,*[6] *Bacteroides fragilis*, *Pseudomonas maltophilia, Helicobacter hepaticus* or pylori species and, *Shigella, Chlamydia, Listeria, pathogenic E. coli, Wolinella*, Coxsakie A, B, Reovirus, Polio virus, Norwalk virus, Influenze B, herpes virus, Paramyxovirus. In addition, atypical forms such as L-forms (cell wall defective bacteria), protoplasts, or spheroplasts, have been suggested.

Mycobacteria Avium Paratuberculosis

Those specific infectious agents, more recently, which gained support based on some evidence includes *Mycobacteria avium paratuberculosis* (MAP) and measles. MAP has been proposed by numerous groups as the causative agent of Crohn's disease.[7–9] The initial case for MAP as a causative agent in Crohn's relies on the similarities of Johne's disease, a chronic MAP infection occurring in ruminants and infecting perhaps as much as 6% of U.S. cattle, but can also infect primates.

Johne's disease is typically an ileitis in which organisms are easily identified when staining for acid-fast bacilli, though not always. Important distinctions occur between Johne's and Crohn's disease. Johne's does not share some of the cardinal features of Crohn's disease. Unlike Crohn's disease, Johne's disease tends not to be segmental. Unlike Crohn's disease, fistulous activity does not occur in Johne's disease, although it does typically cause diarrhea and weight loss in infected animals. Ulcerations, bleeding, stricturing, adhesions, perforations and abscesses, classic features of Crohn's disease, are not features of Johne's disease.[10,11] However, immune and inflammatory responses to the same organisms might reasonably differ in different organisms and produce a different disease phenotype.

MAP survives pasteurization[12] and has been suggested to be transmitted from dairy cows in milk, with 7% of commercial retail milk cartons in U.K. testing positive in one survey,[13,14] but transmission through water contaminated by agricultural runoff has been proposed.[9] The identification of MAP in breast milk of individuals with Crohn's has been suggested as further proof of etiology as well as a source of transmission.[15] MAP can be a human pathogen and was reported as a specific pathogen in a young boy with scrofula who later went on to develop ileitis, indistinguishable from Crohn's, and in whom the infection fully resolved after 32 months of rifabutin and clarithromycin.[16]

The identification of MAP in Crohn's tissue has been difficult, unlike Johne's disease. Cell-well free forms have been suggested to be the agents that induce the human disease.[17] Antibodies have been positive in high percentage of Crohn's patients, though with negative studies as well,[18–23] but studies using PCR for the IS900 sequence have been similarly mixed with positive results above 50% as well as negative results equal to a control population.[24–30] Culturing organisms have also been difficult.[27] Recently, MAP has been identified by PCR from the buffy coat of Crohn's patients' peripheral blood. More importantly, MAP was cultured on 8/15 with Crohn's disease but in no controls.[31] The significance is unclear and, while it adds dramatic information, this finding does not establish MAP as the causative agent. Therapeutic trials have produced mixed results,[8,32–39] using a variety of regimens though a meta-analysis,[40] suggested a benefit in maintaining remission though only after a course of steroids. If MAP is central to Crohn's disease, one would expect that immunosuppression and infliximab would initiate a more evident infection in at least some cases, as can occur with MTb, though this has not been reported.

MAP still remains an unproven etiologic agent, even in a subgroup of patients. Additional randomized, placebo-controlled trials are underway to validate MAP directed therapy, although these are likely not specific for MAP alone. Whether MAP is a benign epiphenomenon, an important disease modifier, or the immediate causal agent for Crohn's remains to be established.

Measles

Wakefield et al proposed that Crohn's results from a chronic infection of submucosal endothelium of the intestines with the measles virus.[41] This infection was proposed to generate a granulomatous reaction and a microinfarct pathologic process leading to the characteristic inflammation identified in Crohn's disease. This theory was buttressed with a number of pathologic and epidemiologic investigations. Microvascular casts of intestinal resections from patients with Crohn's disease suggested a microvascular injury[42]; granulomas were identified as being found in association with endothelium; elevated titers to measles were found in some studies of Crohn's patients, viruses were identified in granulomas from Crohn's patients.[41,43–45]

Epidemiologic studies found that perinatal exposure to measles virus increased the risk of the development of Crohn's disease[46,47]; the attenuated measles vaccine was suggested to be associated with an increased risk of the development of Crohn's disease.[48] This led to considerable media interest and

public concern over use of live measles vaccine as well as other vaccines. A number of researchers countered these claims, with other studies finding that titers to measles were not increased in Crohn's patients, granulomas were not associated with endothelium,[49] measles were not in granulomas[50] and the measles vaccine is not associated with an increased risk of Crohn's disease.[51–55] Subsequently, measles has been proposed to be a cofactor, increasing the risk of Crohn's, though perhaps not the primary causative agent.

THEORIES OF A PSYCHOSOMATIC ORIGIN

In the early and middle decades of the 20th Century, UC and Crohn's to a lesser extent (though little distinction was made until after 1960 between Crohn's colitis and UC) were considered ideal paradigms of psychosomatic diseases.[56] The psychologic theories developed, the certainty with which they were espoused and the therapies, which issued from them present a self-evident critique of their methods and approach.

The theories of a primary psychosomatic etiology of colitis developed out of retrospective studies grounded in an association between "well-marked time relationship between emotional disturbance and symptoms."[57] A psychologic profile or personality type was thought to predispose to the development of UC, in which patients with UC "couldn't cope, giving up [with] diarrhea is substituted for real accomplishment, [having a] … childish, dependent personality … [with the] degree of difference so gross as to make a control group unnecessary."[58] This impaired personality was presented as having been shaped by maternal dominance, loss of a loved one, and social rejection. Studies of the effects of stress on colonic mucosa[59] reinforced this concept of a direct link between psychology and physiology.

Treatment that developed out of these concepts of UC relied on stress control therapy and other attempts at psychoanalytically oriented therapy.[3] More radically, lobotomies were performed on some patients with the attempt of ablating a neurotic focus.[60] These theories were refuted with a number of studies in which a psychologic predisposition was not identified, and any identified character traits were suggested to be a consequence not a cause of the disease. Psychoanalytically based therapy was also demonstrated not to have any influence on surgical rates or recurrences.[61] These concepts died slowly with better methodology putting it more thoroughly to rest in the early 1980s.[62]

DIET AND ALLERGY

IBD, as a gastrointestinal disease, has directed consideration to the role of diet as a central etiologic factor. In the early part of the 20th Century, in part due to undernourishment evident in patients with IBD, nutritional deficiencies were suggested to be causes of IBD.[63,64] The apparent development or at least increase of IBD in the 20th Century and predominance of IBD in the industrialized world have further focused interest on diet as the initiating cause of IBD. Several dietary risk factors have been suggested with regard to infant feeding practices, including breastfeeding as well duration of breast feeding as being protective against the development of Crohn's and UC,[65–69] an increase in carbohydrates intake, simple carbohydrates in particular being a significant risk for development of Crohn's,[70–72] and the intake of fast food as a risk for Crohn's disease.[71] An increased intake of each major component of the diet has been advanced is being critical to the development of IBD, although carbohydrates, consistently demonstrated as being increased in Crohn's disease, simple carbohydrates in particular, has received most attention.[72,73] Trials of low carbohydrate intake have not been useful in maintaining remission or controlling flares,[74,75] although a diet high in complex carbohydrates appeared promising.[76,77] Anecdotal accounts of a low fat intake being successful and pathologic demonstration of fat have led to a hypothesis that increased fats, in conjunction with bacterial antigens, play the primary role in activating the immune system and initiating Crohn's disease.[78,79]

Allergy was advanced as the underlying cause of IBD.[76,80] This concept persisted, although elimination diets and dietary challenges were not found useful. An allergy to cow's milk has been suggested as causative, particularly with regard to UC.[81–84] Breastfeeding was suggested as being protective by delaying an infant's encounter with cow's milk. Individuals later developing UC had a high incidence of milk intolerance as infants.[85] Increased antibodies to cow's milk were found to be associated with earlier onset of UC. Specific antibodies to proteins in milk have been found to correlate with disease activity in adults particularly in UC, although these findings are not consistent and may reflect increased intestinal permeability, rather than a primary allergic phenomenon.

Sulfur Intake and UC

Dietary intake and luminal metabolism of sulfur compounds by sulfate-reducing bacteria have been suggested as the etiology of UC by Roediger et al.[86] One significant change in the diet in the 20th Century in areas with high incidence of UC is the high intake of sulfur-containing food. Sulfur not recycled by an intestinal sulfur salvage pump passes into the colon where it is metabolized, in some, by sulfate-reducing bacteria, a group of bacteria identified as being much more common in individuals with UC and associated with flares of UC, in particular.[87,88]

These bacteria yield compounds, which in turn interact with other luminal substrates to generate sulfoxides, which can be highly injurious to the colonic mucosa. It has been suggested that these compounds can also act on colonocytes to impair uptake of butyrate and other short chain fatty acids (SCFAs),[89] causing a relative mucosal starvation suggested by Scheppach and others as a fundamental aspect of the pathogenesis of UC.[90,91] The use of SCFA enemas to treat IBD have yield mostly negative results, although it could be argued that increased topical SCFAs may still not overcome the defect present.[91] A recent dietary study found individuals with a sulfur-containing diet had an increased risk of flares of UC, which may give a renewed interest to this hypothesis.[92]

Toothpaste and Microparticles

Diet, more broadly defined, has been implicated as a potential etiology of Crohn's disease, in the hypothesis that ingestion of toothpaste causes IBD and Crohn's in particular.[93]

A variety of substances in toothpaste have been demonstrated as harmful in animal studies, in particular some of the particulate substances used as abrasives, such as tricalcium phosphate and quartz, have been identified as being capable of penetrating the epithelium and creating enteric lesions similar to Crohn's disease. Similar findings have been shown when talc has been injected into intestinal lymphatics of animals. Other components of some toothpastes such as carageenan, which has been used to induce an animal model of colitis, and other abrasive agents such as silicates and calcium pyrophosphate could also play a role in initiating Crohn's. The hypothesis claims to fit into the epidemiologic evidence by suggesting other known risk factors are linked to the use of toothpaste: smokers brush more often, children less often, even increased intake of sugar has been suggested to be linked to toothpaste (increased need to brush teeth). This hypothesis has not been rigorously studied and has little supportive data but maintains itself as an oddity as such an innocuous presumably health-maintaining habit could pose such a hazard to some.[93]

The concept developed in the toothpaste hypothesis has been broadened more recently to be inclusive of a large range of microparticles ingested, as part of the diet, increasingly over the past century. Powell et al have proposed that billions of microparticles, mostly titanium, aluminum, and silicon oxides, are ingested principally from food additives.[94–96] These microparticles are taken up by the specialized M cells but are undegradable and accumulate in lymphoid tissues. While not leading to inflammation in themselves, they are proposed to act as adjuvants, permitting the absorption of other antigens and preventing their appropriate disposition by the immune system, altering the normal intestinal immune tolerance, and stimulating an immune response. In addition, these microparticles, calcium conjugates (calcium phosphate) in particular, cause apoptosis of macrophages and release of interleukin-1β.[97] This theory proposes that it is not a particular antigen that induces the inflammatory response seen in Crohn's disease but the way in which the antigen is processed and encounters the immune system.[94]

The therapeutic implications of this hypothesis led to a trial of a diet specially treated to be very low in these particulates. In a randomized, double-blind controlled trial, 20 patients were randomized equally to a normal diet or a comparable diet specially prepared to be low in microparticulates for 4 months.[98] While similar at baseline, those receiving the low particle diet decreased their Crohn's Disease Activity Index (CDAI) from 392 ± 25 at entry to 145 ± 47 at month 4 ($P = 0.002$ vs control group) with 7 patients achieving remission (CDAI <150). The control group had little change from baseline CDAI of 302 ± 28 to 295 ± 25 at month 4, with none in remission. However, outside of this study, when Crohn's patients were compared with a control group for their intake of microparticulates, no differences were observed.[99]

Permeability

A possible common element that has been advanced as a fundamental cause of Crohn's disease is increased intestinal permeability. The increase in permeability or intestinal epithelial barrier dysfunction leads to greater antigen movement across the intestinal epithelium and consequently increased immune exposure to these antigens. Numerous studies have documented increased intestinal permeability in patients with Crohn's though not in those with UC. Similar permeability abnormalities have been identified in asymptomatic relatives of patients with Crohn's, suggesting it may be an early, genetic predisposition, which initiates the cascade of events leading to Crohn's disease. Patients with Crohn's as well as their relatives also have an exaggerated increase in permeability in response to nonsteroidal anti-inflammatory drugs. These observations are the underpinnings of the "leaky gut" hypothesis, which asserts that patients with Crohn's have increased exposure to antigens normally excluded by the intestinal barrier leading to an immune mediated inflammatory response.[100]

Crohn's Disease: An Innate Immune Deficiency

Crohn's has been proposed to be a deficiency of the innate intestinal mucosal barrier, which is inclusive of both the epithelial layer as well as the innate immune system, acting as a second line of defense to protect the host from organisms that breach the epithelial barrier.[101] This hypothesis is based on several lines of evidence. Individuals with genetically defined syndromes of neutrophil and monocyte dysfunction, such as chronic granulomatous disease and glycogen storage Ib,[102] among others, can develop an intestinal phenotype virtually indistinguishable from Crohn's disease. Other genetically defined defects, as has subsequently been identified in the NOD2/CARD15 variant associated with Crohn's disease, may lead to an increased susceptibility as a similar, though milder, defect of the innate immune system.

Several environmental hits may further impair innate immune function. In particular, a change in the gut flora in industrialized countries compared with places in which Crohn's disease is rare, such as rural Africa, result in an increase in certain bacteria, such as *Bacteroides,* and a decrease in *Bifidobacteria*. This shift in bacteria, better studied in association with periodontitis, has greater concentrations of bacteria, which can penetrate the epithelium and produce leukotoxins capable of impairing function of neutrophils and other innate immune cells. Identified environment risks such as smoking and nonsteroidal anti-inflammatory drugs may contribute further insults to the innate immune function as well, as has been described. As has been proposed for periodontal disease, the intestinal innate immune barrier may have a serial mucosal response, utilizing initially protective elements such as the mucous layer, the intestinal epithelium, and complement to thwart a potential bacterial invasion; if inadequate, a neutrophil response would ensue; in turn, if insufficient, monocytes/macrophages would be further activated. If these elements of the innate immune system are unable to contain or repel a potential invader, subsequently a T and B cell response would be activated and predominate. Consequently, innate immune dysfunction could set the process in motion, although the end results appear to be a T cell-driven process. The T cell response may therefore be a secondary phenomenon to a primary innate immune dysfunction resulting from a combination of genetic and environmental factors.

The therapeutic implications of this suggest that, rather than suppressing the end-stage inflammatory process, immune stimulation, as with granulocyte macrophage-colony stimulating

factors[103] (GM-CSF, sargramostim, Leukine, Berlex Inc) acting on the intestinal epithelium as well as augmenting neutrophil and monocyte function, may provide an alternative therapeutic approach for Crohn's disease. Open-labeled studies of granulocyte-colony stimulating factors[104] (G-CSF, filgrastim, Neupogen, Amgen, Thousand Oaks, CA) and GM-CSF were suggested a benefit in Crohn's disease. The study of GM-CSF/Leukine has been followed up with a multicenter, placebo-controlled trial, which confirmed a significant benefit of this treatment in Crohn's.[105]

Helminths

The loss of helminths has been suggested as another relatively recent change in the intestinal flora and as the fundamental permissive factor, enabling the emergence of IBD. Weinstock et al[106] have proposed that, in our zeal to rid the intestines of parasites, we have eliminated a T cell regulatory mechanism that our immune system expects to be present. Helminths have been an integral component of intestinal flora and have acted through T regulatory cells to prevent excessive T cell activation as occurs in IBD as well as perhaps in a variety of other illnesses such as asthma, multiple sclerosis, and allergies. Evolution has engineered human behavioral patterns to pick up soil-borne helminths as infants, during the oral phase. The urban dwellers, wearing shoes and taking on other hygienic practices, have interfered with the acquisition of helminths, causing the loss of this important regulatory function, integral to the healthy functioning of the immune system. Animal models have confirmed a significant impact of helminth colonization on a variety of immune functioning, in particular, augmenting several immune regulatory cytokines, including interleukin 4 and 13, inducing regulatory T cells, and attenuating the T_H1 type inflammatory response.[107–109]

The substantial burden of morbidity and mortality caused by helminths in the world suggests that their adaptation to the human organism does more than only good for its host and that these mechanisms have evolved to permit the organism to thrive by manipulating the immune system of its host. Regardless of the evolutionary details of the theory, harnessing the immune effects of helminths may yield a potent therapeutic force in Crohn's and UC. Preliminary results of therapeutic studies using *Trichiuris suis*, or pig worm, appear extremely promising with a response rate in a recent open-labeled trial in active Crohn's of 80% (in 26 patients) and a remission rate more than 70%.[108]

Dysbiosis, Cold Chain Hypothesis, and Other Alterations of Intestinal Flora

Other recent propositions rest on the concept that the intestines are encountering either new bacteria or concentrations of bacteria, which would have been unlikely before the 20th Century. A fundamental dysbiosis,[110,111] or imbalance between harmful and protective bacteria, has been proposed to be the root cause of IBD, although whether this is a primary causative factor or a secondary epiphenomenon is uncertain. The cause of this dysbiosis is uncertain. Antibiotic use has been proposed to be the central factor leading to an altered gut flora and IBD. The "cold chain hypothesis"[112] posits that IBD results from chronic exposure to organisms which can survive at low temperatures, known as psychrotropic bacteria, such as *Listeria monocytogenes, Yersinia enterocolitica, Clostridium botulinum*, and *Bacillus cereus.* This chronic ingestion of these bacteria, new in the past century, results from the development and widespread use of refrigeration in food preparation, culminating in the domestic refrigerator, the final step in the cold chain. Crohn's has been proposed to develop in genetically predisposed individuals who are chronically exposed to these psychrotropic bacteria, which have been provided an expanded niche in the industrialized world through the use of refrigerators.

In summary, as the understanding of the inflammatory process in IBD progresses, the medications available to treat these diseases, growing out of this knowledge, have improved in parallel. The new therapies being studied at present hold great promise. However, optimal medications may only emerge when a more comprehensive understanding of the primary etiologic factors is established. IBD likely represents more than its two primary subgroups of Crohn's and UC, but each may be a common end pathway, which is arrived at through a number of different routes. The theories surveyed above may all be wrong but may contain at least a kernel, which accurately applies to only a subgroup of individuals. Once this complex knot of multiple subgroups, each with possibly different etiologies, is untangled will ideal therapies be developed for these diseases.

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