Inflammatory Bowel Disease Part II: Crohn's Disease – Pathophysiology and Conventional and Alternative Treatment Options

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Abstract

Crohn's disease, a subcategory of inflammatory bowel disease, contributes to significant morbidity, particularly in industrialized nations. It can affect people of any age, but is more commonly diagnosed in adolescence and young adulthood. Inflammation and ulceration occur primarily in the terminal ileum and colon, although any portion of the intestinal tract can be affected. No etiology has been identified for Crohn's disease, although a number of factors contribute to its etiopathogenesis, including genetic, microbial, inflammatory, immune, and permeability abnormalities. Conventional medications are not curative but can contribute to resolution of acute flare-ups and help maintain remission. Because significant side effects are associated with many of these medications, more natural interventions to help maintain remission should be considered. Associated nutrient deficiencies, dietary interventions, and nutrient and botanical supplementation are discussed.

(*Altern Med Rev* 2004;9(4):360-401)

Introduction

Description

Crohn's disease (CD) is one of two main forms of inflammatory bowel disease (IBD), the other being ulcerative colitis (UC). For a discussion of the pathophysiology and treatment of UC, see *Alternative Medicine Review* 2003;8(3). CD is a chronic, relapsing, transmural (affecting all layers of the intestine) inflammation of uncertain etiology that can affect any portion of the digestive tract from mouth to anus, but is predominantly seen in the terminal ileum and/or colon. Intestinal inflammation and ulceration in CD is asymmetrical and occurs in "patches," with areas of healthy tissue interspersed, and extends deeply into the intestinal wall, forming granulomatous lesions. The disease is named after Dr. Burrill B. Crohn who, with his colleagues Ginzburg and Oppenheimer, published a landmark paper in 1932 describing the features of what is known today as Crohn's disease. Several categories of CD have been described, defined by the portion of the digestive tract involved and the presenting symptomatology (Table 1).

Current statistics indicate 1-2 million Americans suffer from IBD, with approximately half of those cases diagnosed as Crohn's disease. CD affects men and women equally, with a majority of cases diagnosed in adolescents and young adults ages 15-35 years. The disease, however, can affect people at any age and approximately 10 percent of cases are under age 18. Crohn's disease predominantly affects Caucasians; with a prevalence rate of 149 per 100,000, although there has been a steady increase in reported cases of

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Table 1. Subcategories of Crohn's Disease[1]

Subcategory	Area Affected
Ileocolitis	The most common form of CD, affecting the ileum and colon
Ileitis	Affects only the ileum; fistulas or inflammatory abscesses possible
Gastroduodenal Crohn's Disease	Affects the stomach and duodenum; bowel obstruction possible
Jejunoileitis	Patchy areas of inflammation in the jejunum; fistulas possible
Crohn's (Granulomatous) Colitis	Affects only the colon and anus; anal fistulas, abscesses, and ulcers possible

Adapted from Crohn's and Colitis Foundation of America, Inc. 2004
http://www.ccfa.org/research/info/aboutcd

CD and UC among African Americans. IBD is largely a disease of the industrialized world, especially the United States and Europe, and is more common in urban areas and northern climates. CD has a known genetic component, with 25 percent of Crohn's patients having a family member with some form of IBD. Statistics also indicate those with a sibling with IBD are 30 times more likely than the general population to develop IBD.[1]

Signs and symptoms of CD are similar to UC, making diagnosis difficult. For a complete comparison of Crohn's and UC signs and symptoms, see Table 2. Patients diagnosed with CD present with some or all of the following symptoms: frequent diarrhea, abdominal pain in the lower right quadrant appearing soon after meals, fatigue, loss of appetite, weight loss, fever, stomatitis, and perianal fistula or fissures. Some patients also present with rectal bleeding, arthritis, and erythema nodosum lesions on the extremities.[1] In pediatric cases of CD, growth failure is observed in 75 percent of patients.[2]

Risk Factors

Risk factors for CD include smoking, left-handedness, adult appendectomy, and use of oral contraceptives, nonsteroidal anti-inflammatory drugs (NSAIDs), and antibiotics; demographics also affect the risk for CD.

Geographical, economical, educational, and occupational status can impact the risk. Crohn's disease is more prevalent in developed countries and is more common in white-collar workers and individuals with indoor, sedentary occupations.[3] It has been theorized that those with sedentary jobs have delayed intestinal transit time, resulting in increased contact between food antigens and the intestinal mucosa.

Several clinical and case-control studies have determined smoking increases the risk of developing CD, and contributes to earlier disease onset, site of the disease, rate of relapse after surgery, and disease severity.[4-6] Two studies demonstrate an association between childhood second-hand smoke exposure and increased risk for developing CD.[7,8]

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Table 2. Signs and Symptoms of CD and UC

Sign/Symptom	Crohn's Disease	Ulcerative Colitis
Area of intestinal tract affected	Lower ileum most common but can flare up anywhere, including the colon; "patches" of normal tissue between affected areas; can affect entire intestinal wall	Any part of inner most lining of colon, continuous with no "patches" of normal tissue
Diarrhea	Typically four or more episodes per day	Typically four or more episodes per day
Abdominal pain/cramping	Moderate to severe abdominal tenderness in right lower quadrant	Mild tenderness, lower abdominal cramping
Blood in stool	Present; amount depends on disease severity	Present; amount depends on disease severity
Fatigue	Result of excessive blood loss, anemia, and poor nutrient absorption	Result of excessive blood loss and anemia
Fever	Low-grade in severe cases	Low-grade in severe cases
Physical examination	Peritoneal irritation, abdominal or pelvic mass	Rectal exam may show peri-anal irritation, fissures, hemorrhoids, fistulas, and abscesses
Weight loss/anorexia	Weight loss and anorexia common due to poor digestion and intestinal absorption	Weight loss in more severe cases
Appetite	Often decreased during periods of disease exacerbation	Often decreased during periods of disease exacerbation
Risk of colon cancer	Increased	Increased

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Previous antibiotic use appears to be a risk factor for CD. A case-control study of 302 young CD patients (< 25 years) compared to matched controls investigated childhood risk factors for development of the disease. CD patients reported more frequent use of antibiotics in childhood and more frequent upper respiratory infections than control patients. Other factors that appeared to increase disease risk were history of eczema and consumption of a low fiber diet.[9] Another study demonstrated a higher frequency of childhood infections, specifically pharyngitis, as well as more frequent use of antibiotics for otitis media and pharyngitis, in CD patients compared to controls.[10] Utilizing the United Kingdom's General Practice Research Database, researchers at Queen's Medical Centre in Nottingham, England, found a statistically significant association between Crohn's disease and prior antibiotic use in 587 CD cases and 1,460 controls.[11]

Women taking oral contraceptives have twice the risk of developing CD.[12] Use of low-dose oral contraceptives does not appear to significantly influence the activity or course of the disease, although contraceptives compound the risk of thromboembolic events, which is already high due to hypercoagulation characteristic of CD.[13] There is also some evidence of NSAID-induced Crohn's disease in the small and large bowel.[14-16]

Other factors that may increase the risk of developing Crohn's disease are appendectomy in adulthood[17,18] and left-handedness, with left-handed individuals having twice the risk of right-handed persons.[19,20]

Diagnosis

Diagnosis of Crohn's disease is often challenging due to its strong similarity to UC. It is vital, when diagnosing either form of IBD, to obtain an accurate patient history of symptomatology at the time of physical exam. Diagnostic imaging to establish lesion type and extent of involvement include barium enema, small-bowel series, colonoscopy,[21] and capsule endoscopy.[22] Laboratory tests and pathological examination of biopsied intestinal tissue are also important for accurate diagnosis. Tests performed often include complete blood count (CBC) to check for leukocytosis and anemia, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as markers of inflammation, stool cultures to rule out intestinal pathogens, and IgG/IgA antibody levels to *Saccharomyces cerevisiae* (SCA) and *Mycobacterium avium* subspecies *paratuberculosis* (MAP). These antibodies have been shown to be positive in 60 percent and 86 percent of CD patients, respectively, but are less frequently positive in UC patients and rarely positive in healthy subjects.[23]

Perhaps the best tool for establishing and monitoring disease severity and activity is the Crohn's Disease Activity Index (CDAI). The CDAI was developed in the National Cooperative Crohn's Disease Study to provide assessable, uniform clinical parameters with a consistent numerical index of disease status (refer to patient handout at the end of this article).[24,25] The CDAI is a patient assessment form incorporating both objective and subjective information. Using established criteria the physician calculates the CDAI score. CDAI scores > 150 indicate active disease with a poorer prognosis than scores < 150. Since the CDAI is not as accurate for monitoring disease activity in children, a second scale known as the pediatric CDAI (PCDAI) was developed. The PCDAI correlates disease severity and activity to levels of serum albumin. The scoring is easy to perform, reproducible by different observers, and sensitive to changes in clinical status.[26]

Because there is no cure for Crohn's disease, conventional treatment has been aimed at suppression of the inflammatory response and relief of symptoms of fever, diarrhea, and abdominal pain. Once disease symptoms are stable, drug therapy is employed to decrease the frequency of disease flares and maintain remission. Current conventional treatment of CD includes aminosalicylates, corticosteroids, immune-modulating agents, and antibiotics. While reasonably effective in stabilizing disease and maintaining remission, many of these treatments are fraught with side effects and complications. Natural treatment options, as alternatives or complements to conventional therapy, are presented below.

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Etiopathogenesis

The Genetic Component of CD

Several genes have been implicated in the etiology of CD, the most prominent of which are the NOD2/CARD15 located on chromosome 16,[27,28] the OCTN1 gene located on chromosome 5,[29,30] and the DLG5 gene located on chromosome 10.[31] The exact mechanism responsible for NOD2/CARD15's role in the intestinal immune response remains unclear, but mutations of the gene and resultant changes in its function may disrupt the intestinal mucosal barrier and the immune response to the bacterial milieu in the gut.[27,32,33] A recent study of 205 patients with diagnosed CD from northwestern France and 95 ethnically matched healthy controls revealed the R702 mutation of the NOD2 gene demonstrated a significant association with CD and was independently associated with stricturing activity.[34] In a study of 512 German and British CD patients, an insertion polymorphism in NOD2 conferred a significantly increased susceptibility to CD in these patients.[28]

The DLG5 gene has a lesser but significant impact on the risk for developing CD. Although the exact mechanism responsible has not been determined, DLG5 encodes a scaffolding protein important for maintaining epithelial integrity in various organs.[31] DLG5 may interact additively with the NOD2/CARD15 gene to increase susceptibility to CD. Gene OCTN1 is located on chromosome 5q31, codes for an ion channel, and also has a lesser impact on CD risk than NOD2/CARD15.[35] Mutations in this gene may disrupt ion channels via altered function of cation transporters and cell-to-cell signaling in the intestinal epithelium in Crohn's patients.[30]

The TLR4 gene has recently been implicated in CD but is not associated with the chromosomal region previously linked to CD. TLR4 codes for lipopolysaccharide (LPS) signaling, bacterial recognition, and subsequent immune response to bacterial insult. In Crohn's patients, the TLR4 gene is expressed in intestinal epithelial cells, macrophages, and dendritic cells of inflamed intestinal mucosa. Disruption of the LPS signaling pathway could result in an altered immune response to pathogens and a subsequent increase of intestinal inflammation. In two cohorts of 448 Belgian patients diagnosed with CD (n=334, n=114) and 140 controls, it was demonstrated TLR4 polymorphisms enhance the relative risk of developing CD compared to controls. It was also found that polymorphisms of both TLR4 and NOD2 increased this risk.[36] In 2001, a group of Hungarian researchers identified a genetic marker for IBD, the Leiden point mutation, and concluded the prevalence of this mutation was significantly increased in 49 CD patients compared to 57 healthy controls (27.6% versus 5.3%), specifically in central European patients compared to southern or northern European or American patients.[37,38]

Stress in the Etiology of Crohn's Disease

Research has demonstrated stress can be a contributing factor in Crohn's disease. The mechanisms involved vary widely depending on the animal model studied, but it can be concluded stress and other environmental factors affect both the systemic and local immune status of the intestine. Stress signals are perceived by the central nervous system (CNS), triggering transmission of the signal to the intestine via neuroendocrine mediators. The hypothalamic-pituitary-adrenal axis and the sympathetic-adrenal-medullary axis can modulate secretory, absorption, and barrier functions in the gut.[39]

CD is characterized by increased intestinal permeability and extensive animal research has shown stress significantly influences intestinal permeability.[40] Factors involved in the effects of stress on gut permeability include corticotropin-releasing factor (CRF), the autonomic nervous system, and the enteric nervous system. CRF is produced and secreted by the hypothalamus, but has also been found to be secreted in the colonic crypts during times of stress, resulting in increased intestinal permeability.[41] The CNS also influences the degree of intestinal inflammation via the autonomic[42] and enteric nervous systems.[43]

Stress can also contribute to exacerbations of already existing disease. Two prospective studies demonstrated psychological stress, anxiety, and

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Table 3. Infectious Pathogens Implicated in Crohn's Disease

Escherichia coli
Listeria monocytogenes
Yersinia enterocolitica
Mycobacterium avium subspecies *paratuberculosis*
Measles virus

depression are associated with increased CD activity. In one study, 18 CD patients were followed prospectively at 8- to 12-week intervals for two years. Disease activity was measured using the CDAI, Beck Depression Inventory (BDI), and Beck Anxiety Inventory (BAI). The study revealed a strong association between BDI scores and current disease activity when measured simultaneously. Both BDI- and BAI-score increases were independently associated with increased CDAI scores in a subsequent visit 8-12 weeks later.[44]

The second study involved 47 CD patients in remission (after a documented flare) followed for 18 months and assessed using the same scoring inventories as the first study. These researchers also demonstrated psychological stress, anxiety, depression, and altered quality of life were likely to influence further Crohn's disease activity following a relapse.[45]

Microbial Factors

Although a bacterial etiology of Crohn's disease has been postulated for decades, research has never revealed a specific responsible agent. Several possible mechanisms for a bacterial etiology in the development of CD have been proposed: (1) an immune response to a specific pathogen resulting in intestinal infection;[46] (2) alterations in normal bacterial content of the intestinal tract;[47] (3) a defective mucosal barrier and overwhelming exposure to resident bacteria and their antigens and endotoxins;[48,49] and (4) alterations to the intestinal immune response.[46] Numerous bacteria including *Escherichia coli*,[50] viruses, and parasites (Table 3) have been implicated in CD, but none have been confirmed.

A 2003 study published in *Lancet* examined a potential bacterial etiology. French researchers examined evidence for the onset of increased rates of CD in Europe and North America beginning around 1940 and found a corresponding increase in household refrigeration. It appears certain bacteria, known as psychotropic bacteria, are able to grow at the temperatures maintained inside a refrigerator (-1 to +10 degrees Centigrade, or 30 to 50 degrees Fahrenheit). Referred to as the Cold Chain Hypothesis, two psychotropic bacteria – *Listeria monocytogenes* and *Yersinia enterocolitica* – have been isolated from the lesions of CD patients at higher rates than in controls.[51,52] Both bacteria can be found in a wide variety of foods, including meats, dairy products, and vegetables.[53,54] The researchers postulate consuming refrigerated food containing low levels of these psychotropic, pathogenic bacteria results in an over-active immune response, resulting in CD.[55] Additional research is warranted to confirm or disprove this hypothesis.

Epidemiological data indicate an increased risk of Crohn's disease in children with perinatal exposure to the measles virus.[56] Subsequent studies, however, have failed to detect measles-virus DNA in the intestinal tissue of CD patients.[57]

MAP is perhaps the most researched bacterial agent implicated in CD, with at least 20 studies investigating either its role as a pathogenic agent or the effectiveness of antimicrobial therapy to treat it. Mycobacterium species as an etiological agent for gastrointestinal inflammation is not a new theory. As early as 1895, Johne and Frothingham reported findings from tissue analysis of a cow that had died of Crohn's-like symptoms. They identified a bacillus with much the same staining characteristics as the tubercle bacilli; the disease in cattle became known as Johne's disease.[58] In 1901, a Scottish surgeon postulated, after operating on four patients with chronic enteritis, that the disease he observed in the human intestine might be the same as Johne's disease in

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cattle. He was, however, unable to conclusively culture and identify the organisms from human tissue.[59]

In the 1980s Chiodini successfully isolated MAP from intestinal lesions of a small number of CD patients.[60,61] In 1992, an internationally recognized expert in CD, John Hermon-Taylor and colleagues confirmed Chiodini's findings. The study, involving 40 CD patients, 23 UC patients, and 40 controls (patients without IBD), isolated MAP in 65 percent of patients with CD, 4.3 percent with UC, and 12.5 percent of control patients.[62] Other research has confirmed earlier findings of DNA insertion sequence (IS900) from MAP in Crohn's diseased tissues.[63] Hermon-Taylor, aware MAP is ubiquitous in nature, especially in meats and dairy products, tested retail milk supplies in Great Britain. After culturing for 2.5 years, the IS900 MAP DNA sequence was isolated in 16 percent of retail milk samples, easily facilitating widespread transmission to humans.[64] Researchers in Sweden confirmed this finding in 2002 when they revealed 19.7 percent of bulk milk samples across Sweden tested positive for IS900.[65] A 2000 meta-analysis by Hermon-Taylor and colleagues of 18 peer-reviewed publications found nine other studies reporting the presence of MAP in the intestine of CD patients, as well as several studies in which MAP was not identified in Crohn's patients.[66] Since that time, other researchers have demonstrated MAP DNA presence in intestinal and blood samples in up to 92 percent of CD patients compared to 26 percent of controls.[67,68]

Despite its prevalence in tissue and blood of CD patients, the presence of MAP DNA does not prove causality. The isolation of MAP DNA is often not reproducible with subsequent analysis of the same tissue. Conversely, MAP RNA isolation from Crohn's-diseased tissue samples indicates the organism was viable at isolation and not from a contaminating source such as cow's milk. Isolation of the MAP RNA sequence IS900 is more reproducible in human tissue, possibly because it is a much smaller molecule. In addition, because RNA has a very short half-life (in minutes),[69] its presence cannot be attributed to environmental contaminants.[70]

Antimicrobial therapy should be effective at controlling the disease, if MAP is a contributing agent. Studies examining this premise have yielded negative results.[71-74] Negative results may be because only single antibiotics or antibiotics ineffective against MAP were used. MAP is now known to be resistant to most standard antituberculous drugs. Since 1997 four open-label studies have shown efficacy with a combination of rifabutin and macrolide antibiotics for the treatment of CD.[75-78]

Inflammation/Immune Response

Altered Immune Response

While it is unlikely a specific microbial antigen will be established as a consistent causative factor in CD, an abnormal antibody response seems to be a factor. The inflammation appears to be, at least in part, a result of an overreaction to normal intestinal flora. Experts theorize either a microbial antigen may have precipitated the inflammation when elimination from the mucosa was unsuccessful, triggering ongoing inflammation, or an inherent dysregulation of the mucosal immunity exists, resulting in an overreaction to normal gut flora.[79] In either case, it is thought the antigen persists due to an inability of phagocytes to break down the cell walls of commensal microbes.

The presence of antibodies to microbial antigens in CD supports the theory that one aspect of CD pathology involves an abnormal immune response to otherwise normal intestinal flora. For example, Crohn's disease is characterized by elevations in anti-*Saccharomyces cerevisiae* (brewer's yeast) antibodies in 49-60 percent of cases.[80] In addition, levels of protein-bound IgG (bound to proteins of non-pathogenic bacteria) have been found in the intestinal mucosa of patients with active CD to be significantly higher than patients with UC, irritable bowel syndrome, or non-specific IBD.[81]

Zareie et al found mononuclear cells from the lamina propria of the gut mucosal cells in CD patients were spontaneously activated, apparently by normal gram-negative, luminal bacteria. Activation resulted in secretion of tumor necrosis factor-alpha (TNF-alpha) and subsequent epithelial changes.[82]

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Cytokine Patterns

A T-cell mediated immune response has been identified in the mucosa of CD, in contrast to UC, and is postulated to be the primary precipitating event.[83] The ensuing production of inflammatory cytokines can cause ulceration and increased intestinal permeability.[83]

Animal models confirm the generally held consideration that CD is primarily a T-helper 1- (Th1) dominant condition. In murine models, disease induced by a Th1 over-expression results in lesions histologically compatible with CD (including granulomas), while a T-helper 2- (Th2) mediated response results in lesions more closely resembling ulcerative colitis (including a lack of granulomas).[84,85]

As mentioned, the characteristic granulomatous lesion seen in Crohn's disease is evidence of a cell-mediated immune response. In human studies, while chronic CD appears to involve primarily an overactive Th1 response characteristic of a cell-mediated phenomenon,[86] some researchers have determined divergent cytokine patterns at different stages of the disease.[87] Chronic lesions are associated with high levels of interleukin-2 (IL-2), interferon gamma (IFN-gamma),[87] TNF-alpha, and interleukin-12 and -18 (IL-12 and IL-18).[86] Desreumaux et al, however, found a distinctly different cytokine pattern in early CD. By examining ileal biopsy specimens of 17 patients compared to 11 controls, the researchers determined that early lesions were characterized by elevations in interleukin-4 (IL-4) and decreases in IFN-gamma,[87] a pattern more consistent with an overactive Th2 immune response.

Other researchers have found, at least in animal models, the opposite may be true. In a mouse model of ileitis (a CD-like enteritis), researchers found elevated Th2 cytokines, including IL-4, during the chronic phase of the disease.[88]

IL-18 is up-regulated in Crohn's disease. Although typically considered to be an activator of Th1 responses, IL-18 has actually been shown to be a pleiotropic cytokine capable of mediating both Th1 and Th2 responses, providing more potential evidence for divergent cytokine patterns in the pathogenesis of CD.[88]

Another study examined human colonic tissue samples and found IL-16 protein levels elevated in CD patients but not UC patients. The same study found an anti-human IL-16 antibody could suppress colonic injury in a murine model of Crohn's-like experimental colitis.[89]

Pro-inflammatory cytokines are normally kept in check by immunosuppressive cytokines such as transforming growth factor beta (TGF-beta). It is believed the transcription factor T-bet is integral to controlling the balance between pro- and anti-inflammatory cytokines.[90] T-bet is elaborated by Th1 cells, but not Th2 cells. IFN-gamma is enhanced by T-bet. Neurath et al examined T-bet activity in lamina propria T-cells of patients with CD as well as in animal models. The researchers discovered T-bet over-expression in the lamina propria T-cell nucleus in patients with CD, but not UC or controls. In the animal models, T-bet over-expression was consistent with Th1-mediated colitis (animal model of CD), while a T-bet deficiency was protective.

Tumor necrosis factor appears to play a significant role in the pathogenesis of CD. Mucosal biopsies of children with IBD compared to controls found a significantly greater number of TNF-alpha-secreting cells in patients with CD compared to those with UC or non-specific bowel inflammation.[91] A significant difference between mild-to-moderate and severe disease was also noted for the CD subgroup, with severe disease demonstrating a significantly greater percentage of TNF-secreting cells. In animal models of Crohn's ileitis, neutralization of TNF resulted in significant decrease in inflammation.[92] Indeed, suppression of TNF-alpha is the primary mechanism of action of the monoclonal antibody category of CD medications (see Conventional Treatments below). TNF-alpha contributes to gut inflammation in several ways. It induces expression of adhesion factors that allow for inflammatory cells to infiltrate and activates macrophages to promote release of other pro-inflammatory mediators such as IFN-gamma.[93]

TNF-alpha concentrations in the stool can be used to monitor disease activity in both CD and UC. Braegger et al compared 13 children with

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active CD to children with inactive CD, UC, diarrhea, and healthy controls. The average TNF concentrations in the stools of children with active CD ranged from 440-4,322 pg/g compared to a range of 40-84 pg/g in the children with diarrhea, inactive CD, and healthy controls.[94]

A tumor necrosis factor-like cytokine, TL1A, has recently been identified as a co-contributor to IFN-gamma production. Tissue from IBD patients and controls was examined for the presence of TL1A, which was found to be up-regulated in patients with IBD, particularly those with active CD. It appears to be produced primarily in the macrophages and T lymphocytes from the lamina propria in CD patients, with the amount present correlating with disease severity. The result was a four-fold increase in IFN-gamma production. The authors concluded, "Our study provides evidence for the first time that the novel cytokine TL1A may play an important role in a Th1-mediated disease such as CD."[95]

Oxidative Stress

Oxidative stress is thought to play a significant role in the pathogenesis of inflammatory bowel disease, including CD. Endogenous antioxidants such as superoxide dismutase (SOD), glutathione, and catalase are normally able to counteract oxidative stress in the intestinal mucosa. However, inflammation increases the demand for these important antioxidants and results in an imbalance between pro-oxidants and antioxidants, with subsequent mucosal damage.

In an Italian study, subjects (37 CD and 46 UC patients) were compared to 386 healthy controls. Oxidative DNA damage was measured examining the amount of 8-hydroxy-deoxy-guanosine (8-OhdG) present in blood. In addition, evaluation of plasma levels of vitamins A and E and carotenoids demonstrated significant decreases in patients with CD or UC compared to controls. The specific carotene found most significantly decreased was beta-carotene, with plasma levels only 50 percent of controls. No significant differences were noted between CD and UC patients. Levels of 8-OhdG were considerably higher in IBD patients than controls, illustrating increased oxidative DNA damage.[96]

Researchers have studied the connection between oxidative stress and immune-regulated inflammatory factors. Reactive oxygen species (ROS) have been found to be involved in activation of nuclear factor-kappaB (NF-kappaB), which is necessary for encoding genes for TNF-alpha and some of the interleukins involved in inflammation. Antioxidant levels and inflammatory mediators were examined in 26 CD patients compared to 15 healthy controls. Selenium and glutathione peroxidase (GSHPx) activity were both decreased in CD patients, while TNF-alpha levels and ESR were increased and negatively correlated with selenium and GSHPx. Selenium levels decreased in accordance with disease activity, with the most severe disease manifestation exhibiting the lowest levels. These findings occurred in subjects who did not have evidence of malabsorption, indicating malabsorption is not the sole factor contributing to selenium deficiency. The researchers conclude, "Öselenium supplementation in deficient patient groups [should be] regarded as a potential protecting factor against oxidative burst, NF-kappaB activation and excessive inflammatory and immune response."[97]

A study examining indices of oxidative stress and plasma levels of vitamins A and E in 20 CD patients found higher peroxidative status and lower vitamin A and E levels compared to controls. Conservative surgery to remove bowel obstructions resulted in improvements in vitamin A status and oxidative stress measured by thiobarbituric acid reactive substances (TBARS).[98]

A similar study found significantly higher levels of breath-pentane and -ethane and F2-isoprostane (measurements of oxidative stress) in 37 non-smoking CD patients compared to matched controls. At the same time, plasma levels of vitamin C and the carotenoids alpha- and beta-carotene, lycopene, and beta-cryptoxanthin were significantly lower in CD patients.[99]

Pediatric patients also demonstrate signs of increased oxidative stress. In a study of 22 pediatric CD patients, malondialdehyde (MDA) levels were 70-percent higher than controls. Antioxidant levels were measured and only vitamin A was found significantly low, while alpha- and gamma-tocopherol and beta-carotene were no different

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than controls. Red blood cell levels of glutathione were higher in CD patients than controls. The researchers speculate these higher levels may be due to an attempt to compensate for increased oxidative stress.[100]

Although oxidative stress is a factor in the pathogenesis of both UC and CD, the parameters may vary between the two. Both UC and CD are associated with increased levels of MDA, a sign of lipid peroxidation. However, one study found elevated levels of MDA in CD were associated with levels of the antioxidant metallothionein (a hydroxyl radical scavenger) and manganese-dependent SOD (active in the mitochondria); whereas, in UC the MDA levels were associated with catalase, GSHPx, and myeloperoxidase. Based on this data, the researchers suggest the likelihood of the ROS hydroxyl radicals and superoxide anions in the pathogenesis of CD, while hydrogen peroxide and hypochlorous acid may be more associated with UC.[101] Antioxidants for the treatment of CD are discussed below in the treatment section.

Intestinal Permeability

Considerable evidence supports the presence of increased small intestinal permeability (SIP) in Crohn's disease. However, whether it is a contributing factor to the pathogenesis or a consequence of inflammation is not entirely clear.

Evidence for Genetic- and/or Environmentally-Induced Intestinal Hyperpermeability Preceding Disease Manifestation

Evidence that SIP precedes actual disease manifestation has been put forth by a number of researchers. Studies of CD patients and their first-degree relatives point to the possibility of a genetic- and/or environmentally-induced defect in intestinal permeability preceding the onset of full-blown disease. A case report in *Gastroenterology* describes a woman with a positive family history of CD and a positive personal history of increased SIP since age 13; at age 24 she developed CD. In this case, SIP appeared to precede disease manifestation by at least 10 years.[102]

Examination of intestinal permeability in relatives of CD patients sheds light on the potential genetic influence of increased intestinal permeability in the pathogenesis of CD. A study examined intestinal permeability in 16 CD patients and 26 first-degree relatives with whom they lived, compared with 32 healthy controls and their family members. Increased SIP was found in 37 percent of patients and 11 percent of first-degree relatives, significantly greater than controls. Because the patients were living with the relatives tested, it is impossible to know from this data whether the increased permeability was genetically or environmentally induced. However, it does provide evidence of a defect in SIP, possibly preceding the onset of inflammation.[103] Interestingly, another study found a small increase in SIP in spouses of CD patients, pointing to a possible environmental cause.[104]

Another group of researchers, studying the familial connections in SIP and CD, tested SIP in 39 CD patients, 34 healthy first-degree relatives, 22 spouses, and 29 healthy controls, using the lactulose:mannitol test (Table 4). SIP was tested at baseline and then after dosing with acetylsalicylic acid (aspirin) to induce increased permeability. The baseline SIP results found elevations in 36 percent of CD patients, 23 percent of spouses, 18 percent of relatives, and three percent of controls, indicating environment may play a greater role than genetics. On the other hand, after aspirin provocation, all participants experienced an increase in permeability with 32 percent of patients, 41 percent of first-degree relatives, 14 percent of spouses, and three percent of controls demonstrating an abnormally high response. This latter data seems to point to genetic factors. The researchers suggest that baseline permeability may be determined by environmental factors, whereas reaction to provocation by gut toxins such as aspirin may be genetically determined.[105] A similar study found exaggerated response to provocation by ibuprofen ingestion in first-degree relatives of pediatric CD patients, with a greater increase in SIP in relatives than in healthy controls.[106]

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An *in vitro* study provides a potential mechanism by which increased SIP could contribute to the etiology of CD. Mucosal samples from non-inflamed portions of the ileum of CD patients had increased permeability to the common food allergen ovalbumin. The authors suggest this could increase the antigen load in the mucosa leading to disease initiation.[107]

Table 4. The Lactulose:Mannitol Test for Small Intestinal Hyperpermeability

Patient swallows a solution of 5 g mannitol and 5 g lactulose
Urine is collected for six hours
Assay for total lactulose and mannitol
< 14% mannitol = carbohydrate malabsorption
>1% lactulose = disaccharide hyperpermeability

From: Bralley JA, Lord RS. *Laboratory Evaluation in Molecular Medicine.* Norcross, GA: Institute for Advances in Molecular Medicine; 2001:222.

Evidence for Inflammation as Causative Factor of Increased SIP

Other research points to inflammation as the causative factor for increased SIP in CD, implying the disease itself caused the increased permeability. TNF-alpha appears to cause a rearrangement in key proteins associated with the tight junctions in the intestines of CD patients, resulting in increased permeability.[108] Inhibition of TNF-alpha has been found to reverse increased tight junction permeability.[109]

Common medications used to treat CD, including prednisone[110] and infliximab,[111] decrease inflammatory cytokines including TNF-alpha and normalize gut permeability.

SIP as a Predictor of Disease Activity and Potential for Relapse

Several studies indicate the permeability of the small intestine seems to be reflective of disease activity and potential for relapse in CD. One study of 39 CD patients found the effect of an elemental diet on SIP was similar to SIP seen during disease remission – in both instances significantly lower than during active disease.[112]

Wyatt et al followed 72 patients with inactive Crohn's disease for one year, evaluating SIP using the lactulose:mannitol ratio, and found 26 of 37 patients with increased permeability experienced relapse, compared to only six of 35 with normal permeability.[113]

Another study examined absorption of macromolecules (horseradish peroxidase) as a reflection of increased intestinal permeability in moderate-to-severe CD compared to mild disease and controls. The researchers found significantly increased permeability in moderate-to-severe CD but not mild disease or controls. The authors conclude disease activation seems to be associated with increased permeability, which they hypothesize is secondary to the disease process.[114]

Another study examined 50 patients with inactive CD; defined as a CDAI < 150. SIP was assessed via the lactulose:rhamnose ratio. Of 18 patients with increased permeability, eight relapsed during the one-year study, while only one of 31 with normal SIP experienced a relapse.[115]

Evaluation of 132 CD patients in remission who were followed every four months for two years supports the use of intestinal permeability as a predictor of relapse. Forty percent of patients relapsed during this period and increased lactulose:mannitol ratio (signifying increased permeability) and decreased serum iron were associated with relapse. There was no association between tendency to relapse and other disease parameters such as white blood count, ESR, CRP, or other signs of inflammation.[116]

Another study of 27 CD patients in remission, compared to 22 healthy controls, failed to conclude intestinal permeability was a good predictor of relapse.[117]

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A Generalized Permeability Defect in CD?

Whether SIP typically precedes disease manifestation or is a result of inflammation remains to be determined. It is probable increased SIP precedes disease manifestation, but inflammation associated with active disease exacerbates the problem. However, the permeability defect may be more generalized, as increased permeability has been noted in other tissues of CD patients. One group of researchers found increased pulmonary permeability in patients with Crohn's disease. Unlike intestinal permeability, pulmonary permeability did not seem to be affected by disease activity.[118]

Gastroduodenal permeability (GDP), tested by sucrose excretion, has been found by two groups of researchers to be increased in CD patients. One study of 100 patients found GDP was significantly higher in CD patients than controls, and increased GDP was predictive of gastroduodenal involvement.[119] Another study found similar results in a group of 50 CD patients. The researchers also found higher gastric and intestinal permeability were associated with a greater likelihood of granulomatous involvement.[120] Nutrients for the treatment of increased intestinal permeability in CD are discussed below in the treatment section.

Other Abnormalities Contributing to the Etiopathogenesis of CD

Platelet Abnormalities

Increased platelet count is a common feature of active Crohn's disease and contributes to the increased incidence of thromboembolism seen in both CD and UC.[121] In addition to increased platelet count, CD is characterized by increased platelet activation in the mesenteric vessels.[122] Although platelet function has historically been considered to involve primarily blood clotting, there is considerable evidence for platelet involvement in inflammation. Platelets from inflammatory bowel-diseased tissues have been found to express a number of inflammatory mediators, including CD40L, a substance similar to tumor necrosis factor that directs platelets toward inflammation instead of aggregation.[121] A sequence of events has been postulated in which platelets trigger chemokine-mediated adhesion of white blood cells to the endothelium, causing leukocyte migration and subsequent focal inflammatory lesions.[121]

Elevated Homocysteine

Higher than normal incidence of hypocoagulation states and subsequent thrombosis led investigators to examine homocysteine levels in IBD patients, since high homocysteine levels are known to contribute to risk for thromboembolism. Total homocysteine, vitamin B12, and folate levels were tested in 64 IBD patients (25 CD patients) and 121 controls. Seventeen of 64 patients (26.5%) compared with three of 121 controls had hyperhomocysteinemia (defined as homocysteine•e 12.8 microM/L). Folate levels were significantly lower in the IBD group, while there was no statistically significant difference in B12 levels between the two groups. There was also no statistical difference between patients with CD or UC.[123]

In another study of 65 IBD patients (56 with CD; 9 with UC), using 12.0 microM/L as the cutoff point, 10 patients (15.4%) demonstrated hyperhomocysteinemia compared to 3/138 (2.2%) in the control group. In this study, a vitamin B12 deficiency was associated with high homocysteine.[124]

Yet another study found elevated levels of homocysteine in CD patients correlated with both low folate and vitamin B12 levels, although they were more strongly associated with low folate.[125]

Vitamin B6 is essential for the catabolism of homocysteine to cysteine, taurine, sulfate, and glutathione. Thus, deficiencies of vitamin B6 can also result in hyperhomocysteinemia. The *Journal of Neurological Sciences* reports a case of a 39-year-old female CD patient with a history of ischemic stroke associated with a vitamin B6 deficiency and hyperhomocysteinemia along with inflammatory factors associated with hypercoagulation.[126]

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Mitochondrial Dysfunction

Research on mitochondrial dysfunction as a cause of chronic disease is in its infancy. Preliminary investigations point to the possible involvement of mitochondrial dysfunction in the pathogenesis of Crohn's disease. As early as 1985, *in vitro* examination of rectal biopsy specimens provide evidence of mitochondrial damage in CD.[127] More recently, researchers elucidated a potential mechanism for the possible mitochondrial dysfunction in CD. They determined TNF-alpha could enhance mitochondrial NF-kappaB expression, down-regulating mitochondrial RNA expression.[128] A recent case report of a young girl with CD demonstrated impaired oxidative phosphorylation, with abnormalities in Complexes III and IV. In this case, the only medication that provided therapeutic benefit was an anti-TNF-alpha antibody, infliximab.[129] This child had numerous other health problems besides CD. Whether mitochondrial dysfunction plays a central role in the pathogenesis of CD remains to be determined.

Conventional Treatment of Crohn's Disease

Conventional therapies utilized for the treatment of Crohn's disease include NSAIDS, corticosteroids, aminosalicylates and their derivatives, antibiotics, immunomodulatory drugs, and numerous cutting edge therapies including monoclonal antibody preparations, anti-sense nucleic acid drugs, mitogen-activated protein kinase inhibitors, integrin antibody therapy, recombinant growth factors and hormones, and macrolide combination antibiotic therapy. Many of these drugs show promise but are fraught with complications and side effects.

Anti-inflammatory Agents

NSAIDS and COX-2 Inhibitors

NSAIDS, such as aspirin, ibuprofen, and naproxen sodium, act by inhibiting cyclooxygenase and blocking prostaglandin synthesis. Historically, they were used to treat the pain and intestinal inflammation of IBD, until studies demonstrated they caused gastrointestinal erosion and bleeding, protein loss enteropathy, bile acid malabsorption, perforation, and strictures, worsening the course of IBD.[130-132]

Although still currently contraindicated in IBD, cyclooxygenase-2 (COX-2) selective inhibitors have been investigated as therapeutic agents because they appear to cause less gastrointestinal injury than regular NSAIDS. There is some evidence COX-2 inhibitors actually may be involved in preserving intestinal mucosa and promoting healing of gastrointestinal ulcers.[133,134] One COX-2 inhibitor, rofecoxib (Vioxx®) has recently been removed from the market due to safety concerns. A three-year clinical study revealed an increased relative risk for serious cardiovascular effects (including strokes and heart attacks) in patients taking Vioxx longer than 18 months – about twice that observed in the placebo group. The trial investigating the effect of rofecoxib in preventing the recurrence of colorectal polyps was halted two months early. An increased risk of cardiovascular events was not observed in patients taking rofecoxib for less than 18 months.[135]

Aminosalicylates

Other anti-inflammatory agents widely utilized for decades in treating IBD are the aminosalicylates, particularly sulfasalazine (comprised of sulfapyridine, an antibacterial agent, and 5-aminosalicylic acid, also known as mesalamine) or mesalamine alone. Research has shown sulfasalazine to be effective only in mild-to-moderate disease of the colon, but not for isolated small bowel disease,[136] and its use often results in folate deficiency.[137] A recent meta-analysis of slow-release mesalamine (Pentasa®) demonstrated it to be superior to placebo for reducing the CDAI in mild-to-moderate CD.[138] The slow-release forms of mesalamine, Asacol® and Pentasa, are released into the small bowel as far as the distal ileum and tend to have fewer side effects than sulfasalazine.

Corticosteroids

Oral corticosteroids reduce inflammation and suppress the immune system. They comprise the standard conventional treatment of moderate-to-severe CD or disease that is refractory to other

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treatment. While useful for achieving remission, long-term use of steroids can have numerous side effects, ranging from edema, weight gain, insomnia, night sweats, increased facial hair, acne, and mood disturbances, progressing to more serious complications such as hypertension, osteoporosis, diabetes, increased risk of infection, depression, cataracts, and glaucoma.[139] For many years the steroid most frequently used to treat CD was prednisone; however, because of the extensive side effects, other options were explored. Budesonide is a topically active corticosteroid with low systemic bioavailability. When given orally it decreases mucosal inflammation and then undergoes extensive first-pass metabolism in the liver, resulting in far fewer side effects than other oral corticosteroids, providing a safer option for children with CD.[140] Budesonide has also been shown to be more effective than mesalamine in maintaining remission in adults with steroid-dependent CD.[141]

Other Immunosuppressive Agents

Azathioprine, 6-mercaptopurine (6-MP), and methotrexate are widely used immunosuppressive drugs for IBD. The latter two are antimetabolite antineoplastic agents normally used to treat cancer, but have also demonstrated effectiveness in treating refractive CD. Azathioprine has been shown to be effective for inducing remission in steroid-dependent CD, but has also been shown to cause side effects in significant numbers of patients.[142] According to the International Agency for Research on Cancer, this substance is listed as a known carcinogen.[143] The immunosuppressive agent 6-MP has demonstrated effectiveness in maintaining remission in CD patients when administered without concomitant steroids.[144] Low-dose methotrexate is sometimes used in Crohn's patients who have not responded to other drug treatments and is reasonably effective in place of steroids.[145] Side effects can range from nausea, diarrhea, and skin reactions,[146] to more serious problems such as bone marrow suppression, lung lesions, kidney dysfunction, and hepatotoxicity (including liver fibrosis).[146] Methotrexate has also been shown to cause folic acid deficicieny.[147] All of these medications can take up to several months to begin working, although methotrexate seems to work more rapidly than the others. They can be useful therapies for fistular disease and maintaining remission, but have little value in treating acute flare-ups of CD.[148]

Antibiotics

When a bacterial etiology was suggested for CD, numerous studies investigated the effectiveness of antibiotic therapies, mostly with negative results.[71,74] Recent research has suggested MAP as a potential pathogenic agent for CD and research utilizing combination anti-mycobacterial antibiotic therapy has been conducted for up to 46 months. A long duration of therapy is indicated because Mycobacterium species are very slow growing, making long-term therapy necessary. Two studies published in 2002 demonstrate the effectiveness of a combination of rifabutin, a broad spectrum anti-tubercular agent and clarithromycin, a macrolide anti-mycobacterial antibiotic.[77,78]

Biologic Therapies

Numerous promising biologic therapies are emerging, such as anti-TNF-alpha monoclonal antibodies, antibodies to integrins alpha4 and alpha4-beta 7, interleukin antibodies, mitogen-activated protein kinase inhibitors, anti-sense nucleic acids, recombinant growth factors, and colony stimulating factors.

Perhaps the most promising are the anti-TNF-alpha monoclonal antibodies, including infliximab, etanercept, adalimumab, CDP870, CDP571, and onercept. Exploring the mechanisms and efficacy of these drugs is beyond the scope of this article; for a brief description see Table 5. Infliximab has received the most attention in clinical trials and appears to be the most effective. Infliximab is a mouse-human IgG1 chimeric monoclonal antibody to TNF-alpha administered intravenously. Infliximab is used to achieve clinical improvement and induce remission in patients with moderate-to-severe luminal and fistular CD refractory to other treatments. Infliximab exerts its beneficial effects by TNF-alpha neutralization in mononuclear inflammatory cells, thereby inducing apoptosis.[149] A single infusion of 5 mg/kg has

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Table 5. Conventional Medications and their Mechanisms in Crohn's Disease

Medication	Mechanisms of Action
Aminosalicylates	Anti-inflammatory (slow-release topically to small bowel)
Corticosteroids	Anti-inflammatory; immunosuppressive
Other Immunosuppressive Agents	Suppress the immune response in Crohn's
Antibiotics	Damage cell wall of pathogenic agents; reduce bacterial load
TNF-alpha Monoclonal Antibodies	TNF-alpha neutralization and apoptosis
Anti-sense Agents	Modulate lymphocyte migration to gut mucosa
Anti-interleukins	Decrease inflammation by inhibiting inflammatory cytokines
Mitogen-activated Protein Kinase Inhibitors	Indirectly inhibit TNF-alpha
Somatatropin	Decreases intestinal permeability; decreases mesenteric fat; increases amino acid and electrolyte absorption in intestine
Sargramostim	Possible immunostimulation of neutrophils

been shown to induce short-term (four-week) remission in 48 percent of patients[150] and infliximab appears to have the greatest degree of efficacy in maintaining remission for at least one year when dosed at 10 mg/kg body weight every eight weeks.[151] Infliximab is fairly well-tolerated in most patients, although serious side effects include acute infusion reactions,[152] serum sickness-like disease, drug-induced lupus, infectious events attributed to infliximab therapy, pneumonia, reactivation of latent tuberculosis,[153] and even death.[154]

Other anti-TNF-alpha monoclonal agents under investigation are CDP571, CDP870, and onercept, but all have failed phase 2 and 3 clinical trials despite showing a short-term clinical benefit.[155-157] Future research on these drugs is uncertain. Adalimumab is a human monoclonal antibody administered subcutaneously and appears to be well-tolerated in most CD patients, particularly those with reactions to infliximab. Phase 2 and 3 trials with CD patients are currently underway.[158]

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Natalizumab and MLN-01 are other monoclonal antibodies to gut glycoproteins, currently in phase 2 and 3 trials.[159-161] Several other agents are in clinical trials.[162]

Nutrient Deficiencies in Crohn's Disease

A variety of nutrients have been found to be deficient in CD patients. Causes include malabsorption in the small intestine, increased nutrient need because of disease activity, low nutrient intake, nutrient loss due to chronic diarrhea or increased transit time, or effect of medications. Several studies have examined the specific nutrient deficiencies associated with CD.

Studies Examining Multiple Nutrient Deficiencies

A study measured serum, blood, and red blood cell levels of various nutrients in 24 CD patients and 24 healthy controls. CD patients demonstrated significantly lower levels of vitamins A and E, thiamin, riboflavin, pyridoxine, and folic acid compared to controls. Blood levels of pantothenic acid were significantly higher in CD patients, and there were no statistically significant differences in levels of vitamins B12 and C, nicotinic acid, and biotin. No differences were noted on the basis of disease activity, duration, or location.[163]

Another study examining multiple nutrient deficiencies found 85 percent of 279 CD patients had deficiencies. Nutrients most frequently found deficient were iron and calcium, with zinc, protein, vitamin B12, and folate deficiencies noted less frequently.[164]

Nutrient status, body composition, and dietary intakes were analyzed in 32 CD patients with longstanding disease in remission and compared to 32 matched controls. Regarding body composition, bone mineral content was significantly lower in patients; percent body fat was significantly lower in male CD patients. Patients had significantly lower dietary intakes of fiber and phosphorus, while no other nutrient intakes were significantly different. Serum levels of beta-carotene, vitamins C and E, selenium, magnesium, and zinc, and glutathione peroxidase activity were also significantly lower in patients than controls. As noted in a previous study, there was no correlation between nutrient status and duration of disease or extent of bowel resection.[165]

These same researchers conducted a study with the same design on newly diagnosed patients with IBD, 23 with CD. Even at diagnosis, bone mineral content was significantly lower in patients compared to controls. While all nutrients tested demonstrated slight decreases in patients, only vitamin B12, serum albumin (a reflection of protein status), and glutathione peroxidase activity (a reflection of antioxidant status) were significantly decreased.[166] These two studies seem to indicate that, although nutrient status is negatively impacted at the time of diagnosis, longstanding disease increases the extent of derangement.

A study published by the American Dietetic Association examined the effect of dietary counseling on nutrient status in CD. Subjects (n=137) were randomly assigned to one of two groups. The treatment group received dietary counseling monthly for six months, while the control group received no counseling. Iron, vitamin B12, and folate levels were found to be low in a significant portion of patients, with no significant differences between groups at study onset. Although dietary counseling was associated with normalization of serum folate and total iron binding capacity and moderate increases in intakes of vitamin B12, folic acid, and iron, the laboratory values as reflection of nutrient status did not change significantly.[167]

Vitamin D Status in Crohn's Disease

A study of young CD patients (ages 5-22) found low vitamin D (defined as serum concentrations of 25-hydroxyvitamin D < 38 nmol/L) in 16 percent of 112 subjects. Interestingly, the low levels did not significantly correspond to low bone mineral density (BMD) or dietary intakes. Factors associated with hypovitaminosis D included winter season, African-American ethnicity, extent of glucocorticoid medication, and disease confined to the upper gastrointestinal tract.[168]

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Levels of serum vitamin D considered "low" have been inconsistent from study to study. Another study considered low plasma 25-hydroxyvitamin D to be less that 12 nmol/L. In this study, plasma vitamin D levels were examined in 37 CD patients and levels were found to be significantly lower in patients with active disease compared to those with inactive disease.[169]

Studies on vitamin D status have also examined active vitamin D levels. Recent research found circulating levels of 1,25-dihydroxyvitamin D were high in a large percentage of CD patients (42 percent of 138 subjects) compared to UC patients (seven percent of 20 UC patients) and were positively associated with disease activity. Low levels of 25-hydroxyvitamin D result in low serum calcium that in turn stimulates parathyroid hormone and a subsequent rise in 1,25-dihydroxyvitamin D levels to enhance calcium resorption from bone. High levels of active vitamin D were associated with significantly lower BMD in CD patients compared to UC patients, independent of glucocorticoid use. The researchers examined colonic biopsies of patients with UC and CD and found higher levels of 1alpha-hydroxylase in CD mucosa. This enzyme converts 25-hydroxy- to 1,25-dihydroxyvitamin D. Thus, it appears over-expression of this enzyme in the inflamed mucosa may be a cause of low BMD in CD.[170]

Vitamin K in Crohn's Disease

Serum vitamin K status was assessed in 32 CD patients and compared to reference ranges from 384 healthy controls. Levels were significantly lower in CD patients. Vitamin K is a co-factor for carboxylation of the protein osteocalcin, necessary for calcium binding to bone. Thus, deficiencies of vitamin K can contribute to osteoporosis and measurements of free osteocalcin (uncarboxylated) reflect bone-vitamin K status. Levels of free osteocalcin were higher in CD patients, while binding capacity of osteocalcin to hydroxyapatite was lower. High levels of free osteocalcin were associated with low BMD in the lumbar spine.[171]

Water-soluble Vitamin Deficiencies

B-complex Vitamins

As discussed previously, deficiencies of vitamins B6 and B12 and folate in CD have been associated with increased homocysteine levels. Deficiencies may be associated with impaired absorption or decreased dietary intake. A study of folate absorption in patients with Crohn's disease compared 100 patients with 20 healthy controls. Serum folate levels were assessed after a loading dose of folate and deemed normal in the 20 healthy subjects but in only 75 percent of the CD patients. Of the 25 patients with impaired folate absorption, nine demonstrated almost no increase after an oral dose, while 16 experienced an increase but still below normal.[172] In addition to impaired absorption, CD patients frequently have diets devoid of fresh leafy green vegetables and fruits – dietary sources of folate – because of fear that these foods will exacerbate symptoms.

Vitamin C in CD

Several older studies focused on vitamin C status in CD. A 1986 study of 137 patients with CD found low serum ascorbate levels in 11 percent of males and 37 percent of females; leukocyte ascorbate levels were low in 26 percent of males and 49 percent of females. The deficiencies were not associated with disease activity. In this study the deficiencies were due in part to low intake and were remedied by diet counseling.[173] Leukocyte ascorbate levels were found significantly lower than controls in two other studies.[174,175]

Two studies examined ileal tissue levels of vitamin C. The 1974 study found depressed tissue levels of ascorbate in CD patients with fistulas compared to CD patients without fistulas or healthy controls.[176] The 1987 study found tissue ascorbate levels higher in both fistulizing and non-fistulizing CD patients compared to controls. However, the levels in the patients with fistulas were significantly lower than CD patients without fistulas. The authors speculate ascorbate is concentrated in the tissues because of vitamin C's importance in collagen formation. Those subject to fistulas appear to be less efficient at mobilizing

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ascorbate.[177] One of the researchers conducted a subsequent study and found absorption of vitamin C was not impaired in either fistulizing or non-fistulizing CD patients compared to controls.[178]

Fat-Soluble Antioxidants: Vitamin A, Vitamin E, and Carotenoids

As mentioned previously, oxidative stress plays a significant role in the pathogenesis of CD and several antioxidants, including vitamins A and E, have been found to be low in CD patients. Studies support the contention that vitamin E,[179] vitamin A,[180,181] and a combination of vitamins E and A,[182] are low in CD patients. Low vitamin A appeared to be associated with low protein that contributes to a deficiency of retinol binding protein,[181] and both A and E were normalized when active disease was brought under control.[182]

Carotenes, precursors to vitamin A, have been shown to be low in the CD population. A 1987 study examining vitamin A/carotene levels over a six-month period in 137 CD patients (70 percent with inactive disease) found normal vitamin A status in all patients, while 20-25 percent of patients demonstrated low total serum carotenoids.[183] A more recent study confirms low serum vitamin A and carotenoid (zeaxanthin, alpha- and beta-carotene, and lutein) levels in CD patients compared to controls.[184]

Mineral Deficiencies

Zinc

Several studies note zinc deficiencies are common in CD. A study of 54 CD patients found significant deficiencies in serum zinc, vitamin A, and retinol binding protein levels compared to healthy controls. Zinc levels decreased in accordance with disease activity, patients with active disease having significantly lower levels than those with inactive disease. Zinc deficiency is associated with impaired metabolism of retinol binding protein, resulting in a vitamin A deficiency.[185]

Other researchers have corroborated the tendency of zinc deficiency to parallel disease activity in CD. A small study compared five patients with active CD to five patients with inactive disease and found serum zinc levels significantly lower in those with active disease. Furthermore, low zinc levels seemed to be due to increased body clearance, rather than malabsorption. Reasons for the increased zinc clearance were not pursued.[186]

Another study found serum zinc levels deficient (defined as < 75 mcg/dL) in 17 of 50 CD patients (34%). Low zinc levels were associated with an increased tendency toward fistula formation, with 65 percent (11/17) of CD patients with low zinc levels experiencing fistula formation.[187] Thus, both low zinc and vitamin C levels have been implicated in a tendency toward fistula formation in CD patients.

Colonic mucosal biopsies of tissue from CD patients found abnormally low levels of zinc from uninflamed but not from inflamed tissue.[188] Increased levels in involved tissue may be due to the need for more zinc as a co-factor for superoxide dismutase.

Other Trace Mineral Deficiencies

Serum levels of copper, zinc, and selenium were examined in 47 CD patients and compared to 123 healthy controls. The patients had significantly lower selenium and higher copper levels than controls,[189] supporting the premise of increased oxidative stress in the pathogenesis of CD. There were no differences in serum zinc concentrations between patients and controls. A similar study examined children with Crohn's disease (n=36) and found significantly lower levels of selenium, copper, and zinc compared to controls.[190]

Iron Levels in CD: A Double-edged Sword

Iron deficiency is common in CD and is thought to be due to decreased dietary intake or chronic gastrointestinal bleeding. Lomer et al investigated the seven-day diet diaries of 91 CD patients in remission compared to 91 controls. Only 32 percent of CD patients compared to 42 percent of controls consumed the recommended daily intake of iron. Patients tended to eat less fiber and iron-fortified cereal than controls.[191]

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Table 6. Nutrient Deficiencies Associated with Crohn's Disease

Nutrient	Status	Daily Recommendation (adult)
Vitamin A	Deficient	5,000-25,000 IU; not to exceed 7500 IU in pregnancy
Beta-carotene	Deficient	25,000-100,000 natural mixed cis/trans beta-carotene
Vitamin D	Deficient	400-800 IU
Vitamin E	Deficient	400-800 IU
Vitamin K	Deficient	500 mcg-1 mg
Vitamin C	Deficient	500-1,000 mg
Vitamin B1 (thiamine)	Deficient	100 mg
Vitamin B2 (riboflavin)	Deficient	100 mg
Vitamin B3 (niacin)	Deficient	100 mg
Vitamin B12	Deficient	1 mg
Folic Acid	Deficient	400-800 mcg
Biotin	Deficient	300 mcg-1 mg
Calcium	Deficient	1,000 mg
Magnesium	Deficient	500 mg
Iron	Deficient in serum; elevated in gut mucosa	Supplement with 50-75 mg daily well-absorbed form only if anemic; otherwise avoid supplementation
Zinc	Deficient	15-30 mg
Copper	Elevated	Avoid unless supplementing with > 15 mg zinc for long period of time; then supplement with 1-2 mg
Selenium	Deficient	200-400 mcg

Supplementation with iron, however, may not be a prudent recommendation in Crohn's disease because it can exacerbate intestinal inflammation and contribute to oxidative stress. While circulating iron may be decreased in patients with IBD, mucosal levels may actually be

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increased.[192] A small study examined the effect of 120 mg/day ferrous fumarate in 10 patients with CD (eight with active disease; nine with iron deficiency anemia) compared to controls. Assessment after one week found eight of 10 patients experienced gastrointestinal side effects of diarrhea; the opposite effect occurred in the control group – fewer stools. Seven of 10 patients experienced increased abdominal pain and six of 10 reported nausea, compared to none in the control group. After one week of ferrous fumarate supplementation, levels of reduced cysteine and glutathione (endogenous antioxidants) were significantly decreased in patients.[193]

Table 6 summarizes the nutrient deficiencies associated with CD.

Due to extensive side effects associated with conventional medications and significant nutrient deficiencies in CD, the effort to maintain remission with dietary changes, nutrients, and botanicals should be considered.

Dietary Interventions in CD

Pre-illness dietary habits may increase the risk for developing Crohn's disease in susceptible individuals.[194] Research has demonstrated high sugar and carbohydrate intakes significantly impact the development of inflammatory bowel disease. While researchers did not differentiate between CD and UC, both di- and monosaccharide consumption increased the risk of developing IBD in general.[195] Sucrose was consistently associated with increased risk for IBD, and the trend was statistically significant in CD patients. Patients with IBD had a significantly lower intake of fruit, fiber, and vegetables. Another study confirmed a higher intake of total carbohydrates, starch, and refined sugars in 104 patients, immediately prior to diagnosis of CD.[196] A population-based, case-controlled Swedish study examining 152 CD cases found a significant 3.4-fold increase in relative risk for developing CD with consumption of fast food 2-3 times weekly.[197]

Once Crohn's disease has manifested, dietary disturbances result from significant loss of appetite and contribute to weight loss and nutrient deficiencies. Dietary rehabilitation of CD patients depends on the extent of disease, presence of intestinal stricturing, obstruction, or short bowel (due to surgery), and ability to consume food orally.[198] When strictures, obstruction, or short bowel are present it may be necessary for the patient to use enteral nutrition.

Enteral and Parenteral Nutrition

Enteral nutrition involves the provision of liquid-formula diets by mouth or tube into the gastrointestinal tract.[199] It is suggested that partial bowel rest, a restoration of nutritional status, and a reduction in immunological stimulation caused by whole protein can induce remission.

Parenteral nutrition, administering of nutrients by a route other than the alimentary canal (e.g., intravenously, subcutaneously), is now seldom used for CD treatment, except in the most extreme cases involving significant impairment of absorption or intestinal obstruction.[200]

Several diets of potential benefit to CD patients are administered enterally, but all include protein, carbohydrates, electrolytes, vitamins, and minerals. In elemental diets the protein source is from amino acids or short-chain peptides; such diets were once considered the best form of enteral feeding.[201] Polymeric diets containing whole protein have a higher energy:osmolarity ratio than elemental diets and have been shown to be especially beneficial in treating children with CD.[202] Oligopeptide diets contain short-chain peptides of 4-5 amino acids.[203] Numerous studies have compared the benefits of enterally-fed elemental and polymeric diets to steroid therapy for Crohn's disease (Table 7).[204-209] The results are varied, with some showing elemental diets superior to steroids and vice versa. In general it can be concluded, at least in the short-term, that an oral elemental diet is at least as effective as steroids in achieving or maintaining remission of mild-to-moderately active CD in adults. Because of the potentially devastating effects of steroid therapy in growing children, enteral dietary therapy is almost always recommended as a first-line treatment.[210,211]

In certain countries, epidemiological data show an association between high dietary intakes of omega-6 polyunsaturated fatty acids (PUFAs)

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Table 7. Diet Therapies Compared to Steroid Medications in CD

First Author	Study Duration	Diet Patients	Steroid Patients	Diet	Steroid Dose	Results
Gonzalez-Hiux	4 weeks	15	17	Polymeric Diet	Prednisone 70 mg/day with tapering	12 of 15 diet patients achieved remission compared to 15 of 17 patients on steroids; relapse at one year = 66% in steroid patients versus 42% in diet patients.
Gorard	4 weeks	22	20	Elemental Diet	Prednisolone 52.5 mg/day with tapering	Disease activity (SEM) decreased from 4.8 to 1.7 in diet patients vs. 5.3 to 1.9 in steroid patients.
Malchow	6 weeks	51	44	Semi-elemental Diet	6-methyl Prednisolone 48-12 mg/day (tapered)	32 of 44 patients on steroid therapy experienced decreased CDAI vs. 21 of 51 patients on dietary therapy.
O'Morain	4 weeks	11	10	Elemental Diet	Prednisolone 52.5 mg/day with tapering	Similar improvement in clinical score and ESR was experienced by both groups. Diet group showed more improvement in hemoglobin and albumin values.
Riordan	12 weeks	40	38	Elemental Diet	Prednisolone 40 mg/day with tapering	Diet group showed median remission length of 7.5 months vs. 3.8 months in steroid group. Relapse rate at 2 years was 79% for steroid group and 62% for diet group.
Zoli	2 weeks	10	10	Elemental Diet	Prednisolone 0.5 mg/kg/day	Diet group had significant improvements in CDAI, ESR, intestinal permeability, body mass index, and prealbumin levels. Steroid group improvements were noted in disease activity and fat free body mass.

and increased rates of Crohn's disease.[212] The role of fatty acids in inflammation is well established and several studies have examined the therapeutic benefit of different types and amounts of dietary fat in nutritional therapy. Fish oil studies are discussed below. Regarding other types of fats, research indicates enteral feeds or other diets low in fat are more effective in treating CD patients and maintaining remission than diets high in fat.[213] There is also some evidence that fat type can impact the effectiveness of enteral nutrition. Due to its pro-inflammatory effect, high amounts of linoleic acid (an omega-6 essential fatty acid) in an elemental diet would be expected to show less benefit, a conclusion supported by a meta-analysis conducted by Middleton et al.[214] Conversely, Gassull et al found an enteral diet high in linoleic acid and low in oleic acid actually resulted in better patient remission rates (52%) than a high oleic acid/low linoleic acid diet (20%). The use of medium-chain triglycerides (MCTs) in varying amounts has also been studied. MCTs in enteral nutrition do not seem to show any detriment or benefit over long-chain triglycerides, regardless of amount used.[215]

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Dietary Fiber

The results of studies investigating the beneficial effects of fiber in Crohn's patients are inconclusive. In an Italian study, 70 CD patients were randomly assigned to follow a low-residue diet or a normal Italian diet for 29 months. No difference in outcome was observed in the two groups, and a lifting of dietary restrictions did not result in symptom exacerbation.[216] In a second, controlled trial in which 20 CD patients were given an unrefined carbohydrate, fiber-rich diet or an exclusion diet (of foods they were intolerant to), 70 percent (7 of 10) of patients on the exclusion diet remained in remission for six months compared to zero percent (0 of 10) at six months on the unrefined carbohydrate, fiber-rich diet.[217]

In one study, 162 patients with active CD were assigned to a diet unrestricted in sugar and low in fiber, and were compared to 190 active CD patients given a low-sugar, high-fiber diet. Patients were followed for approximately two years. Assessment via history, physical exam, and laboratory testing revealed no significant differences between the two treatment groups, indicating the high-fiber diet did not benefit patients with active Crohn's.[218] In another study 32 patients with CD were placed on a fiber-rich diet in addition to conventional treatment. Another 32 matched CD patients acted as controls and received no specific dietary instruction. After 52 months the treatment group had significantly fewer and shorter hospitalizations and required less intestinal surgery than the control group.[219]

Elimination Diets

Research on elimination diets in CD has yielded inconclusive results. Elimination diets are difficult to follow with high drop-out rates and patients seem to have difficulty in identifying foods that trigger symptom exacerbation. Food sensitivities often are not persistent and are difficult to validate with subsequent blinded challenge. Remission rates in CD patients on elimination diets do not appear to be significantly better than those observed in patients on unrestricted diets.[220]

Probiotics in the Treatment of CD

Alterations in the bacterial milieu of the gut are common in Crohn's disease. The use of various probiotic bacteria to promote a balance of appropriate intestinal flora has yielded mixed results. Mechanisms associated with the beneficial effects of probiotic therapy in CD include: (1) inhibition of pathogenic bacteria via growth suppression or epithelial binding;[221] (2) improved epithelial and mucosal barrier function;[222] and (3) altered immuno-regulation via stimulation of secretory IgA or reduction in TNF-alpha.[223,224]

Saccharomyces boulardii

Plein et al demonstrated the efficacy of *Saccharomyces boulardii* (Sb) in a randomized, double-blind, placebo-controlled study of 20 CD patients. Patients were given 250 mg Sb three times daily for 10 weeks and evaluated via bowel movement frequency and the CDAI index. Patients receiving Sb experienced a significant reduction in frequency of bowel movements (from 5.0 to 3.3 per day) and CDAI index (193 to 107) by week 10 of treatment.[225]

Another study utilizing Sb therapy in 32 CD patients demonstrated a significant benefit of a combination of Sb and mesalamine compared to mesalamine alone. Relapse in the mesalamine-only group was 37.5 percent at six months compared to only 6.25 percent in the mesalamine-plus Sb group.[226]

E. coli *(Nissle strain)*

Pathogenic *E. coli* that adhere to and invade intestinal epithelial cells (IEC) have been isolated from ileal lesions of Crohn's patients.[47] Boudeau et al demonstrated the *in vitro* ability of a non-pathogenic *E. coli* strain (Nissle 1917) to prevent pathogenic *E. coli* strains from adhering to and invading IEC. When IEC were co-infected with probiotic Nissle strain and pathogenic *E. coli*, the Nissle strain exhibited a dose- and time-dependent adhesion to IEC, which prevented adhesion of various pathogenic *E. coli* strains by 78.0-99.9 percent. When IEC were pre-incubated with Nissle strain *E. coli* and pathogenic strains were added later, adhesion and invasion of pathogenic strains was inhibited by 97.2-99.9 percent.[221]

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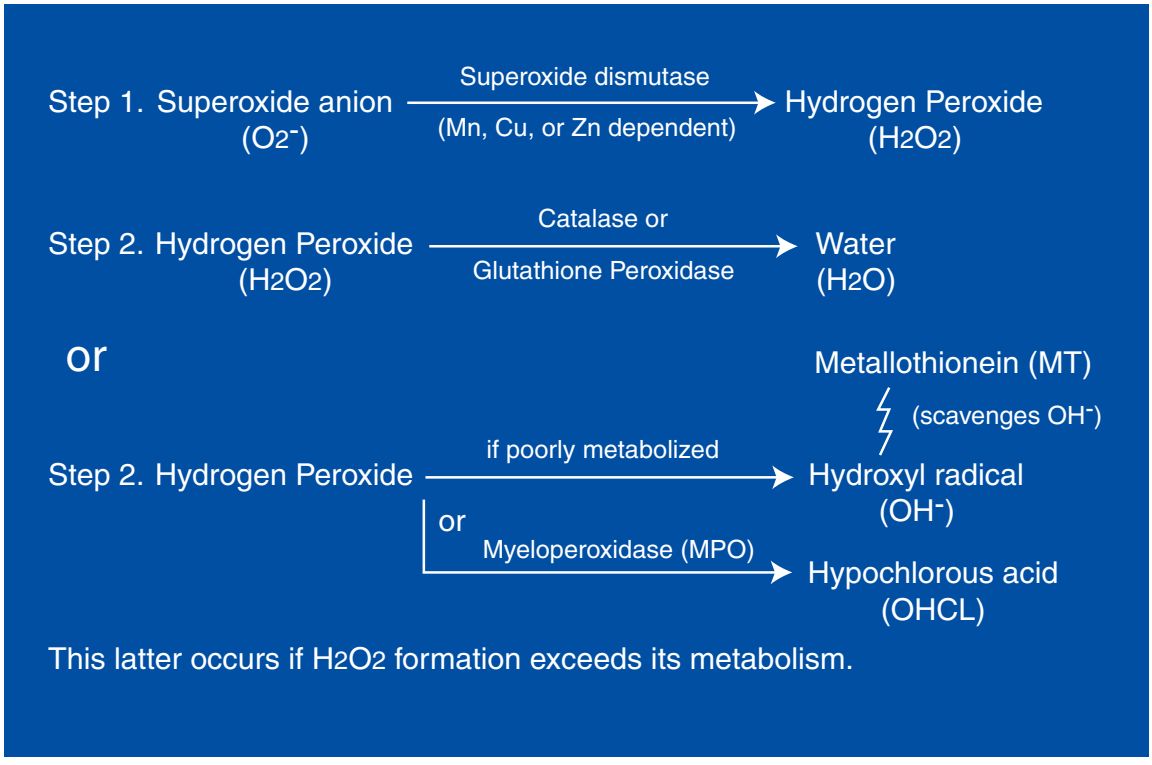
Figure 1. Endogenous Neutralization of Free Radicals



Malchow et al conducted a double-blind, randomized, placebo-controlled trial investigating the efficacy of *E. coli* Nissle strain 1917 for inducing and maintaining remission in 28 patients with colonic Crohn's disease. Patients were randomized to either 60 mg prednisolone daily (with a standard tapering schedule) plus twice daily doses of 2.5 x10^{10} probiotic Nissle strain *E. coli* (treatment group) or identical prednisolone therapy plus placebo (placebo group). The rate at which remission was achieved was comparable in both groups (85.7% for treatment patients versus 91.7% for placebo patients), but only 33.3 percent of patients in the *E. coli* treatment group relapsed at one year, compared to 63.6 percent in the placebo group.[227]

Lactobacillus GG

Malin et al investigated the effect of oral Lactobacillus GG on the intestinal immunological barrier in a small study of 14 children with CD and seven control patients (hospitalized for investigation of abdominal pain but with no evidence of intestinal disease). Lactobacillus GG was administered to patients and controls at 10^{10} colony forming units mixed in liquid twice daily. Lactobacillus GG therapy significantly increased the IgA immune response in Crohn's patients compared to controls, resulting in an improved mucosal barrier.[223]

Another study of Lactobacillus GG demonstrated that administration in children with mild-to-moderate stable CD improved gut barrier function and clinical status after six months of therapy.[228] However, a randomized, double-blind, placebo-controlled trial of 45 post-surgery Crohn's patients given Lactobacillus GG for one year did not show it to be more effective than placebo in preventing disease recurrence.[229]

Antioxidants in CD Treatment

As discussed previously, oxidative stress is one of the pathogenic mechanisms involved in the intestinal damage of CD. Figure 1 illustrates

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normal and deranged endogenous responses. A study examined mucosal tissue SOD levels and found manganese-dependent SOD (Mn-SOD) was significantly higher in IBD patients, including those with CD – 1.6-fold higher in non-inflamed tissue and almost 3-fold higher in inflamed tissue.[230] These same researchers, in a follow-up study, found metallothionein levels were significantly lower in inflamed mucosa from CD patients compared to non-inflamed mucosa and normal controls. Catalase activity was increased 1.5-fold in non-inflamed and 2.5-fold in inflamed mucosa; glutathione peroxidase activity was increased in both inflamed and non-inflamed mucosa; and myeloperoxidase levels were increased 1.5-fold in non-inflamed and 2.3-fold in inflamed mucosa. The researchers hypothesize an increase in the ratio of step 1 of endogenous free-radical quenching activity in relation to step 2 (Figure 1), resulting in an over-abundance of hydrogen peroxide, hypochlorous acid, and hydroxyl radicals in inflammatory bowel disease.[231] Exogenous antioxidants in the form of supplementation may help relieve the burden.

A study using synthetic antioxidants (e.g., BHA; butylated hydroxyanisole) in tissue culture found antioxidants decreased inflammatory cytokine production, although the effects were more dramatic in tissue from UC patients than CD patients.[232]

Glutathione

Reduced glutathione (GSH), an endogenous quencher of hydrogen peroxide, may be of benefit as an exogenous supplement. GSH was deficient and oxidized glutathione (GSSG) elevated in tissue samples from 12 CD patients – demonstrating increased oxidative stress in patients compared to seven controls. Another arm of the study examined the effect of oral supplementation of glutathione to rats and found 1.3-fold and 3.5-fold increases in tissue GSH after 0.4 mM/kg and 4.0 mM/kg, respectively.[233] Nutrients that enhance glutathione levels, either as co-factors for glutathione reductase (e.g., riboflavin and niacin) or because they spare glutathione or contribute to its synthesis (e.g., N-acetylcysteine, lipoic acid), may prove of benefit in CD as well.

Vitamins C and E

In a study of 57 CD patients in remission but demonstrating oxidative stress, subjects were randomly assigned to receive 800 IU vitamin E and 1,000 mg vitamin C or double placebo for four weeks. Oxidative stress significantly decreased in the supplemented group as measured by breath-pentane and -ethane and plasma F2-isoprostane. Disease activity remained low in both groups during the one-month study.[234]

Vitamin A

Because vitamin A enhances epithelial cell differentiation and CD is characterized by disruptions in intestinal epithelium, supplementation was tried in a CD patient at a dose of 50,000 IU three times daily along with vitamin E 100 mg three times daily for two weeks. Diarrhea she had been experiencing disappeared. After experimenting with each of the vitamins alone, it was concluded vitamin A was responsible for the improvement in bowel movements.[235] Based on this case report a small pilot study examined clinical and laboratory parameters in eight patients with severe CD who were supplemented with 50,000 IU vitamin A three times daily for two weeks. No changes in bowel habits, abdominal pain, or gut permeability were noted.[236] Although it may have been sufficient time to note clinical improvement, structural improvements in gut permeability would likely take longer than two weeks.

A more extensive study involving 86 CD patients examined the effect of 50,000 IU vitamin A acetate twice daily or placebo for an average of 14.1 months. All patients had been in remission for at least three months prior to study onset. No differences were noted between the two groups regarding disease activity, rate of relapse, or laboratory parameters. Serum levels of vitamin A were not significantly higher than the placebo group until the fifth month of the trial. Discontinuation of vitamin A at the end of the trial did not result in clinical deterioration.[237]

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Table 8. A Summary of Omega-3 Fatty Acid Studies in Crohn's Disease

Authors	Subjects	Duration	Intervention/Design	Dosage	Outcome
Tsujikawa et al	20 CD patients	1 month	Open trial using diet containing n-3:n-6 ratio of 0.5	Not given	Decreased CRP, improved remission rates
Lorenz et al	39 IBD patients (29 CD patients)	7 months	Double-blind, placebo-controlled crossover of fish oil	1.8 g EPA and 1.3 g DHA daily	Decreased inflammatory mediators TXB2 & LTB4, improved morphology, no change in disease activity
Hillier et al	10 IBD patients	12 weeks	Open label – fish oil versus olive oil	18 g per day, containing 3.2 g EPA, 2.2 g DHA	Decreased inflammatory mediators PGE2, TXB2, & LTB4
Lorenz-Meyer et al	204 CD patients in remission	1 year	Fish oil supplementation compared with placebo or low-carbohydrate diet	6 g daily (containing 3.3 g EPA, 1.8 g DHA)	No difference in relapse rate in fish oil vs. placebo
Belluzzi et al	78 CD patients in remission	1 year	Double-blind, placebo controlled study of fish oil	4.5 g daily (containing 1.8 g EPA, 0.9 g DHA)	41% fewer relapses in fish oil group; 33% more patients in remission at 1 year
Arslan et al	10 IBD patients (5 CD, 5 UC)	10 days	Open label pilot study of seal oil	30 mL daily (containing 1.8 g EPA, 2.6 g DHA, 1.0 g DPA)	Decreased disease activity, decreased joint pain

Fatty Acids for the Treatment of CD

Omega-3 Fatty Acids

A Japanese epidemiological study found a correlation between high omega-6:omega-3 fatty acid (FA) ratios and incidence of CD.[212] In patients with diagnosed CD, eicosapentaenoic acid (EPA) and total polyunsaturated fatty acids were significantly decreased, while the ratio of omega-6:omega-3 FA was increased compared to controls. There was also a negative correlation between EPA levels and disease activity, with lower levels observed in active disease compared to disease remission.[238]

High omega-6:omega-3 FA ratios can contribute to inflammation. The omega-6 linoleic acid has been found *in vitro* to enhance arachidonic acid-induced inflammatory cytokine production in intestinal tissue from CD patients.[239] On the other hand, the anti-inflammatory effects of omega-3 fish oils have been well established. Thus, increasing the intake of omega-3 fatty acids in the diet or by supplementation seems prudent. Table 8 summarizes intervention trials of omega-3 fatty acids for CD.

Elemental diets used in enteral nutrition often consist of only small amounts of fat in the form of soy oil, which can result in a deficiency of essential fatty acids (EFAs). A Japanese study examined the effect of a special Crohn's disease diet (CDD) enriched with omega-3 fatty acids in a ratio of 0.5 omega-3:omega-6 FA that also included a simple orally-ingested diet of rice gruel, progressing to steamed rice and small amounts of meat and noodles. Patients used the elemental diet for breakfast and dinner and the CDD for lunch, and were gradually weaned off the elemental diet as their disease activity permitted. Use of the

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omega-3 enriched diet resulted in a decrease in CRP, a measurement of inflammation.[240]

A study examined the anti-inflammatory effects of omega-3 fatty acids from fish oil on 39 IBD patients (29 with CD). In this double-blind, crossover trial, patients were given either a fish oil supplement containing 1.8 g EPA and 1.3 g docosahexaenoic acid (DHA) daily or an olive oil placebo for three months. After a one-month wash-out period they were switched to the other protocol. Clinical disease activity was determined by the CDAI and endoscopy at the end of each phase. Laboratory evaluation consisted of determination of fatty acid composition of biopsy specimens, urinary excretion of the inflammatory mediator thromboxane B2 (TxB2), and plasma leukotriene B4 (LTB4) levels. Levels of TxB2 and LTB4 decreased by one-third in patients taking omega-3 supplementation. Endoscopy revealed a small morphological improvement in 13 CD patients on fish oil compared to placebo. On biopsy exam, EPA content of phospholipids from colonic mucosa increased three-fold with fish oil supplementation, whereas DHA increased only slightly. During the control period arachidonic acid levels were significantly higher in inflamed than non-inflamed gut mucosa. These differences diminished during fish oil supplementation. No significant difference in disease activity between fish oil and placebo was noted in the CD patients (unlike the UC patients in the study). Thus, although the fish oil seemed to have anti-inflammatory effects, they were not enough to contribute to decreased disease activity in CD.[241]

Another study examined incorporation of fatty acids into the colonic mucosa of IBD patients. Similar to the foregoing study, supplementation of fish oil (18 g daily) resulted in significantly increased incorporation of EPA (seven-fold) and DHA (1.5-fold) into gut mucosa and decreased inflammatory eicosanoid production, compared to olive oil that did not suppress eicosanoid production.[242]

Two treatment protocols were compared against a placebo in 204 CD patients in remission (CDAI•d 150) – 5 g concentrated fish oil daily, low carbohydrate diet (84 g daily), or placebo consisting of 5 g corn oil daily. There was no significant difference between fish oil and placebo groups regarding percent of patients experiencing relapse within one year. Corn oil, a source of linoleic acid, could have provided anti-inflammatory effects via conversion to gamma-linolenic acid (GLA) and ultimately the anti-inflammatory prostaglandin-1 series, thus confounding the results. The low carbohydrate diet provided significant benefit.[243]

A study published in the *New England Journal of Medicine* examined the effect of enteric-coated (to resist gastric acid for 30 minutes but to allow disintegration by 60 minutes) fish oil capsules for the prevention of relapse in CD. Seventy-eight patients were randomly assigned to receive three 500-mg fish oil capsules three times daily or placebo. Fish oil capsules contained 40-percent EPA, 20-percent DHA, and a 40-percent mixture of fatty acids, for a total daily dose of 1.8 g EPA and 0.9 g DHA. Placebo consisted of 60-percent caprylic acid and 40-percent capric acid. Requirements for admission in the study included remission for at least three months (defined as a CDAI score•d 150) but less than two years and avoidance of conventional medications for at least three months. Thirty-four of 39 patients in the fish-oil group and 37/39 in the placebo group completed the one-year study. Eleven patients in the fish oil group (32%) experienced relapses compared to 27 patients in the placebo group (73%). The authors speculate the improvement with fish-oil capsules may be attributed to anti-inflammatory effects of omega-3 fatty acids, including inhibition of leukotrienes, thromboxanes, and TNF-alpha.[244]

A pilot study examined the effect of seal oil instilled into the duodenum in five patients with CD and five with UC. Seal oil (10 mL) was administered three times daily directly into a nasoduodenal feeding tube. Seal oil provided 1.8 g EPA, 2.6 g DHA, and 1.0 g docosapentaenoic acid (DPA; an omega-3 fatty acid not found in fish oil that affects other anti-inflammatory pathways). According to the researchers, the omega-3 FAs from seal oil are more easily hydrolyzed to free fatty acids than those from fish oil. Treatment for

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10 days resulted in significant decreases in disease activity and IBD-associated joint pain, and increases in omega-3:omega-6 ratios in mucosal tissue and blood.[245]

Short-chain Fatty Acids

Short-chain fatty acids, such as butyric acid, provide the main fuel for colonocytes and have been examined in the form of enemas for UC. Butyric acid may also provide benefit for CD. Intestinal biopsies from CD patients were examined with and without exposure to butyric acid. The researchers found butyric acid resulted in decreased NF-kappaB-stimulated TNF-alpha, providing a mechanism for its potential use in CD.[246]

Glutamine

The amino acid glutamine is the preferred fuel for small intestinal enterocytes and can decrease intestinal permeability, a potential benefit for CD patients. Duodenal biopsies from healthy volunteers were cultured in the presence of the inflammatory cytokine-enhancing IL-1beta and increasing amounts of glutamine. Glutamine inhibited IL-1beta-induced production of pro-inflammatory cytokines IL-6 (found to be high in the serum and mucosa of CD patients[83]) and IL-8, and enhanced production of the anti-inflammatory IL-10.[247]

Despite theoretical indications for glutamine supplementation in CD, several small intervention trials have not yielded benefit. In a double-blind study to determine if glutamine decreases gut permeability in Crohn's disease, 14 CD patients were randomly assigned to receive 7 g glutamine or placebo (glycine) three times daily along with their conventional treatment. There were no significant changes in permeability during the four-week trial in either the glutamine or placebo groups. In addition, no significant changes were seen in CDAI, CRP, or plasma glutamine/glutamate levels.[248]

Two studies investigating high-glutamine diets in children with CD also found no significant benefit. Eighteen children received either a low-glutamine (4% of amino acid content) polymeric diet or a glutamine-enriched (42% of amino acid content) polymeric diet to determine whether glutamine enhances rate of remission. After four weeks, 5/9 in the low-glutamine group and 4/7 (two patients dropped out of this group due to diet intolerance) in the high-glutamine group achieved remission. Pediatric CDAI was significantly lower in the low-glutamine group, indicating greater response to the low- than high-glutamine diet.[249] In a second arm of the study, glutamine did not affect intestinal permeability.[250]

Growth is often stunted by childhood CD and low serum levels of insulin-like growth factor-1 (IGF-1) have been implicated. In double-blind fashion, 15 children with CD were assigned to receive one of two diets (same amino acid content as previous study) and the effect on IGF-1 assessed. No significant changes in IGF-1 were noted after four weeks of supplementation.[251]

N-acetyl Glucosamine

The protective mucus in the gastrointestinal tract consists of glycoproteins – a protein backbone with carbohydrate side chains. Half of these carbohydrate side chains consist of N-acetyl glucosamine (NAG). Glucosamine synthetase is the rate-limiting enzyme in NAG synthesis. Decreased glucosamine synthetase activity has been noted in biopsy tissue from IBD patients – both CD and UC – only in inflamed tissue with loss of epithelial cells. In patients with CD, elevated levels of glucosamine synthetase were found in non-diseased tissue, providing a diagnostic tool when involved tissue is not viewable on colonoscopy.[252]

A phase 1, open-label trial was conducted on 12 children with IBD (10 with CD; 2 with UC). Oral doses of NAG ranged from 3-6 g daily in three divided doses. The children had severe disease, some refractory to conventional treatments. Six CD patients and two UC patients responded favorably to treatment and were followed for as long as three years. Improvements included marked clinical, histological, and stricture improvements, with establishment and maintenance of remission.[253]

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Botanicals in the Treatment of CD

Curcumin

TNF-alpha elevation is part of the inflammatory process involved in the pathogenesis of CD. Curcumin, a flavonoid from *Curcuma longa* (turmeric) is a known inhibitor of TNF-alpha. An *in vitro* study found TNF-alpha increased intestinal permeability and curcumin inhibited the NF-kappaB-induced-TNF-alpha-stimulated increase in intestinal permeability.[109]

An interesting text-mining experiment, a Medline search that uses a specific algorithm to make discoveries, was used to determine medical uses for *Curcuma longa*. The goal was to determine possible connections between substances and conditions warranting research, rather than to locate research already conducted. For instance, curcumin's influence on TNF-alpha might be connected to the increased TNF-alpha associated with CD, providing possible mechanisms for its usefulness in that disease. Analysis of the "mining expedition" resulted in the suggestion of evidence for the beneficial role of curcumin in Crohn's disease, as well as spinal cord and retinal diseases. Curcumin inhibits several of the cytokines and genes involved in the pathogenesis of CD.[254] Clearly, clinical trials are necessary to confirm curcumin's benefit in CD.

Boswellia serrata

Boswellia serrata is a botanical with significant anti-inflammatory activity. *In vitro*, this botanical inhibits the 5-lipoxygenase product LTB4, which has been implicated in CD.[255] A randomized, double-blind, clinical trial examined the effect of a Boswellia extract (n=44) or mesalamine (n=39) in 102 CD patients. The primary outcome measured was change in CDAI between baseline and end of the study (article was in German; abstract only in English – which did not note length of study or dosages used). Patients treated with the Boswellia extract exhibited an average 90-point decrease in CDAI, while those on mesalamine averaged a 53-point decrease.[256] Although the difference between Boswellia and mesalamine was not considered statistically significant, Boswellia appeared to perform better than mesalamine. A larger study with more statistical power is warranted.

Berberine

The inflammatory cytokine IL-8 is increased in CD. An animal study of trinitrobenzenesulfonic acid-induced colitis (an experimental model for Crohn's disease) found the alkaloid berberine (7.5-15 mg/kg/day), administered orally for one week, inhibited decreased colonic tissue damage measured histologically and macroscopically. Cultured tissue exposed to berberine also demonstrated inhibition of IL-8.[257] Berberine is an active constituent of several botanicals, including goldenseal, Oregon grape, Coptis, and barberry.

DHEA

Dehydroepiandrosterone (DHEA) is low in patients with CD. In a study of 115 CD patients compared to 66 healthy controls and 64 UC patients, both CD and UC patients had low serum DHEA-sulfate (DHEAS) levels compared to controls. In CD patients, but not UC patients, low DHEAS levels were correlated with high ESR, while high cortisol was associated with high ESR and CRP.[258] Another study found a shift in the ratio of cortisol:DHEA in CD patients with active disease, with higher cortisol and lower DHEA levels.[259]

Because DHEA can be deficient in patients with IBD and has also been shown to inhibit pro-inflammatory cytokines, a phase 2 pilot trial was conducted to evaluate its effect in IBD patients. Twenty patients (seven with CD; 13 with UC), ages 18-45, were given 200 mg DHEA orally once daily for 56 days. All patients were experiencing active disease, defined as CDAI > 150, refractory to other medications. All medications remained the same for two weeks prior to and during the study. One patient with CD (and four with UC) dropped out because of disease exacerbation or noncompliance. In the CD group, six of seven responded to treatment with a decrease in CDAI of 70 points or more. In all six responders, the CDAI dropped below 150, putting them into

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remission. The one patient who did not respond dropped out during the first week. Number of liquid stools, bloody diarrhea, abdominal pain, and CRP all decreased. One CD patient relapsed on day 56. Patients were followed for eight weeks after the end of the study and no further CD relapses were reported.[260]

Potential Sequelae of Crohn's Disease

Osteoporosis

Osteoporosis is perhaps the most significant sequela to Crohn's disease and several aspects may contribute to its development. Nutrient deficiencies of vitamins D and K result in low BMD.[170,171] Corticosteroid therapy contributes to osteoporosis in Crohn's patients.[139] Hypogonadism in men, resulting from CD-related stunted growth has also been shown to decrease bone density.[261]

Numerous studies confirm that Crohn's disease[262,263] and some of the commonly used conventional therapies[264] can result in low BMD, increased fracture risk, and osteoporosis. A study of 95 patients (31 treated predominantly with corticosteroids, 33 treated with dietary manipulation, and 31 not treated with diet and with a history of low life-time steroid exposure) demonstrated those in the steroid-treatment group had significantly lower BMD than those in either of the other two groups, indicating steroids were the cause of the osteoporosis.[265]

A recent study of subjects from the UK's General Practice Research Database examined the incidence of hip fractures among IBD patients compared to controls. IBD cases (n=16,550) were evaluated, and after adjustment for confounding variables, it was determined the relative risk for hip fracture in CD patients was 1.68 (compared to 1.41 for UC patients). These results indicate hip fracture risk in CD patients is increased by approximately 70 percent. However, statistics also indicate less than half of hip fractures in CD patients are not the result of steroid therapy,[266] but may be attributable to other factors, such as altered intestinal absorption resulting in nutrient and vitamin deficiencies,[170,171] or overproduction of TNF-alpha-mediated bone loss.[267]

Cancer and Crohn's

Epidemiological studies evaluating the risk of colorectal cancer in Crohn's patients have yielded contradictory results. A 2004 population-based study of 374 Danish CD patients reported no increased risk of colorectal cancer, but a more than 60-fold increase in small bowel cancer.[268] Conversely, another 2004 publication reported a relative risk of 5.6 for colorectal cancer in Crohn's colitis (a subcategory of Crohn's).[269] Because some conventional drug therapies for CD, including sulfasalazine, have carcinogenic potential, prudence dictates regular cancer screening of Crohn's patients when they are being treated with these drugs.[270]

Infertility caused by Crohn's

Several studies investigated the effect of Crohn's disease on fertility in both men and women. A study examining the medical records of 103 women noted a substantial degree of infertility and a slight increase in spontaneous abortion compared to the general population. Exact numbers and statistics for the general population were not reported.[271] Other research indicates CD patients who have had intestinal surgery are more likely to have unresolved fertility problems than CD patients who have had no surgery.[272] Several studies have shown men with CD have an increased incidence of oligospermia,[273] problems with sperm maturation not caused by sulfasalazine therapy,[274] and poor sperm quality, reflected by decreased motility and density.[275] Current studies are investigating the link between proven zinc deficiencies in men with CD and infertility and sperm function.[276]

Miscellaneous Sequelae

Other conditions reported in Crohn's patients include liver abcesses,[277,278] life-threatening bacterial infections due to immune deficiencies or Crohn's drug therapy,[279] and ischemic stroke, probably due to B-vitamin deficiency and the hypercoaguable state observed in CD.[126]

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Conclusion

Crohn's disease results in significant morbidity with potential life-threatening sequelae. Conventional treatments have been only partially successful in curbing acute flare-ups and extending remission. When these drugs fail, surgical intervention is often employed. Surgery removes localized inflammation but does nothing to address the causes of CD, including abnormal gut immunity, increased intestinal permeability, systemic inflammation, and deranged colonic milieu, or the nutrient deficiencies associated with the disease. Natural therapeutics in the form of dietary modification, nutrient repletion, probiotics, omega-3 fatty acids, antioxidants, anti-inflammatory botanicals, and other nutrients can provide benefit in bringing balance to a severely imbalanced system.

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Crohn's Disease Activity Index (CDAI)

(Sum Multiplied by Factor = Subtotal)

Variable	Description	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Sum	X	Factor	=	Subtotal
X1	Number of liquid or very soft stools									X	2	=	
X2	Abdominal pain 0 = none 1 = mild 2 = moderate 3 = severe									X	5	=	
X3	General well-being 0 = well 1 = slightly under par 2 = poor 3 = very poor 4 = terrible									X	7	=	
X4	Number of associated conditions the patient now has: 1. Arthritis/arthralgia 2. Iritis/uveitis 3. Erythema nodosum/pyoderma gangrenosum/aphthous stomatitis 4. Anal fissure, fistula, or abscess 5. Other fistula 6. Fever over 100 degrees F during past week									X	20	=	
X5	Taking lomotil/opiates for diarrhea (0 = no, 1 = yes)									X	30	=	
X6	Abdominal mass (0 = none, 2 = questionable, 5 = definite)									X	10	=	
X7	Hematocrit: ____ (47 minus Hct, males; 42 minus Hct, females) =									X	6	=	
X8	Body Weight (BW): ___ Standard Weight (SW): ___	(SW-BW)/SW) =								X	100	=	
Sum of X1 through X8 = CDAI												=	

Adapted from: Best WR, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index. Gastroenterol 1976;70:439-444.

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