3. REVIEW OF LITERATURE

Inflammatory bowel disease is a collection of systemic diseases involving inflammation of the gastrointestinal tract. IBD majorly includes two diseases: Ulcerative colitis, which affects only the large bowel; Crohn's disease, which can affect the entire gastrointestinal tract; while indeterminate colitis, which consists of large bowel inflammation, shows elements of both Crohn's disease and ulcerative colitis.

3.1 Description of IBD (Barbara et al, 2002):

**Ulcerative colitis (UC):** UC is a condition in which the inflammatory response and morphologic changes remain confined to the colon. The rectum is involved in 95% of patients, with variable degrees of proximal extension. Inflammation is limited primarily to the mucosa and consists of continuous involvement of variable severity with ulceration, edema, and hemorrhage along the length of the colon. The characteristic histologic findings are acute and chronic inflammation of the mucosa by polymorphonuclear leukocytes and mononuclear cells, crypt abscesses, distortion of the mucosal glands, and goblet cell depletion.

**Crohn's disease (CD):** CD, in contrast to UC, can involve any part of the gastrointestinal tract from the oropharynx to the perianal area. Diseased segments frequently are separated by intervening normal bowel, leading to the term "skip areas." Inflammation can be transmural, often extending through to the serosa, resulting in sinus tracts or fistula formation. Histologic findings include small superficial ulcerations over a Peyer’s patch (aphthoid ulcer) and focal chronic inflammation extending to the submucosa, sometimes accompanied by noncaseating granuloma formation. The most common location is the ileocecal region, followed by the terminal ileum alone; diffuse small bowel, or isolated colonic disease in decreasing order of frequency.

3.2 Classification of Ulcerative Colitis (Barbara et al, 2002):

In individuals with ulcerative colitis, a distinct portion of the colon is diseased. Disease starts at the rectum and moves "up" the colon to involve more of the organ. Ulcerative colitis is categorized by the amount of colon involved. Regardless of how...
little or how much of the colon is involved; symptoms can vary from mild to severe in any individual. Types of UC are as follow:

- **Ulcerative proctitis:** If ulcerative colitis is limited to the rectum, it is known as ulcerative proctitis. Symptoms are diarrhea, bloody stool, pain in the rectal area, and a sense of urgency to empty the bowel.

- **Proctosigmoiditis:** If ulcerative colitis affects the rectum and the sigmoid colon, it is known as proctosigmoiditis. Symptoms are diarrhea, bloody stool, cramps and pain in the rectal area, and moderate pain on the left side of the abdomen.

- **Left-sided colitis:** Left-sided colitis affects the entire left side of the colon, from the rectum to the place where the colon bends near the spleen and begins to run across the upper abdomen (the splenic flexure). Symptoms include diarrhea, bleeding, weight loss and loss of appetite, and sometimes severe pain on the left side of the abdomen.

- **Pancolitis:** If the entire colon is affected, the term pancolitis is used.

### 3.3 Classification of Crohn’s disease (Barbara et al, 2002):

The symptoms and potential complications of Crohn’s disease differ, depending on what part of the GI tract is inflammed. The following are five types of Crohn’s disease based on the main area involved:

- **Ileocolitis:** The most common form of Crohn’s, affecting the ileum and colon. Symptoms include diarrhea and cramping or pain in the right lower part or middle of the abdomen. Often accompanied by significant weight loss.

- **Ileitis:** It affects the ileum. Symptoms are the same as ileocolitis. Complications may include an inflammatory abscess (a collection of pus) in the right lower quadrant of the abdomen or fistulas. Fistulas are tunnels leading from one loop of intestine to the other, or between the intestine and another part of the body.

- **Gastroduodenal Crohn’s disease:** It affects the stomach and duodenum (the first part of the small intestine). Symptoms include loss of appetite, weight loss, and nausea. Vomiting may indicate that narrowed segments of the bowel are obstructed.
Review of Literature

- **Jejunoileitis**: It produces patchy areas of inflammation in the jejunum (upper half of the small intestine). Symptoms include abdominal pain, ranging from mild to intense, and cramps following meals, as well as diarrhea. Fistulas may also form.

- **Crohn’s (granulomatous) colitis**: It affects the colon only. Symptoms include diarrhea, rectal bleeding, and disease around the anus (abscess, fistulas and ulcers). Skin lesions and joint pains are more common in this form of Crohn’s than in others.

3.4 Epidemiology of IBD (Cosnes Jacques, 2011; Kelvin Thia, 2008):

The highest incidences of CD and UC have been reported in northern Europe, the United Kingdom, and North America. In those regions, such high incidences may indicate common etiologic factors. The incidence of UC is greater than that of CD, except in Canada and several areas of Europe, although this has been changing over the past 20 years. Canterbury County, New Zealand, has among the highest incidence of CD (16.5/100,000 people). IBD has emerged in countries in which it had rarely been previously reported, including South Korea, China, India, Iran, Lebanon, Thailand, the French West Indies, and North Africa. In these countries, the occurrence of UC preceded that of CD by about 10 years. In some countries, such as Japan, the incidence of IBD was initially low but has recently increased.

The prevalence of CD in North America varies from 44 to 201/100,000, and that for UC from 37.5 to 238/100,000; in Europe, CD prevalence varies from 8 to 214/100,000 and that of CD from 21 to 294/100,000. When values are extrapolated to the European Community, they are estimated to be 1 million persons with CD and 1.4 million with UC in Europe. There are more than 1.3 million patients with IBD in the United States.

In countries that are becoming westernized, the incidence of UC increases first, followed later by CD; Asia had a high ratio of UC/CD incidence in the 1980s and 1990s, but, then in 2000, the incidence of CD increased. The increasing prevalence in Asia is possibly related to growing industrialization and in part, related to increased diagnostic accuracy and increased awareness (Sood and Vandana, 2007). In Japan, Singapore, and South Korea, IBD frequency was initially low but rapidly increased. In South Korea, between 1986–1990 and 2001–2005, the incidence of UC
increased from 0.3/100,000 to 3.1/100,000 and that of CD increased from 0.5/100,000 to 1.3/100,000, respectively. In China, CD incidence and prevalence is estimated to be 0.3/100,000 and 1.4/100,000 respectively. In India, the prevalence of UC in the Punjabi population has been reported to be 44/100,000, and its incidence is 6.0/100,000. Overall, a pattern can be drawn for IBD frequency in the developing world: an initially low UC incidence, followed by an increase in UC while the CD incidence remains low, and eventually a CD incidence that approaches UC levels.

The peak age for CD occurrence is 20–30 years; for UC, it is 30–40 years. Among migrant populations, age at time of migration affects IBD risk; the risk of developing IBD is highest among children that migrate before the age of 15 years. Interestingly, appendectomies were reported to reduce the risk for UC if performed before the age of 20 years. This indicates that environmental factors may remain active in triggering IBD (mainly CD) in children.

All the races and ethnic groups in the world have CD and UC. The highest prevalence is seen in North America and Europe. In contrast to UC, which shows a slight male predominance, CD appears to be slightly more common among women (Khosla et al, 1986). So, this disease appears to have slight gender related differences in incidence. The female predominance, especially among women in late adolescence and early adulthood, suggests that hormonal factors may play a role in disease expression.

Both types of IBD may increase the risk of cancer. The risk of colorectal cancer in patients with IBD is increased 4 to 20-fold compared to the general population, and some malignancies can develop in apparently uninvolved sites. Patients with UC and CD have been reported to develop leukemia, suggesting a potential relationship between IBD and leukemia.

3.5 Etiology of IBD:

The disease is characterized by cycles of clinical exacerbation and remission, with periods of improvement followed by relapse. Although the etiology of IBD remains unknown, the pathogenesis is gradually being unraveled, seeming to be the result of a combination of environmental, genetic, and immunological factors which are depicted below.
3.5.1 Environmental Triggers (Hanauer, 2006; Noel et al, 2004):

Westernization

IBD is most prevalent in developed regions. It is postulated that this is the result of ‘‘westernization’’ of lifestyle, such as changes in diet, smoking and variances in exposure to sunlight, pollution, and industrial chemicals. (Lakatos et al, 2007)

Hygiene and Sanitation

More hygiene is associated with less frequent helminthes infections during childhood. This result in a lack of mucosal TH2/anti-inflammatory or regulatory cytokines or both & leaves poinflammatory effector mechanisms unopposed (Noel et al, 2004). IBD is a disease of cleanliness. It demonstrates an inverse relationship with the degree of sanitation. Poor sanitation appears to protect against IBD. It is postulated that improved hygiene alters the intestinal flora by decreasing exposure to certain critical bacteria. There is also an increased frequency of UC and CD in higher socioeconomic groups.

Occupation

Higher mortality from IBD has been noted in managerial, clerical, and sales positions, which typically involve sedentary and indoor work. In contrast, mortality resulting from IBD is low among farmers and construction workers. Employment involving outdoor air and physical activity is protective against IBD, whereas work in artificial venues confers an increased risk.

Diet

There is some evidence that a higher intake of fatty acids increases the risk for IBD. Similarly, frequent fast-food intake confers a 3- to 4-fold greater risk for IBD. Diets that are low in fiber and high in refined sugars could possibly contribute to the development of Crohn’s disease. High sucrose intake was associated with risk for both UC and CD, while fat (especially animal fat) was associated with increased risk for UC only. The most significant finding was an increased relative risk for IBD associated with fast food consumption. Eating fast food twice weekly resulted in a relative risk of 3.9 for UC (Yehuda et al, 2002).
Tobacco smoking

The strongest environmental risk factor for IBD is tobacco smoking. Current smoking is protective against UC. The decreased risk for UC in smokers appears to be dose dependent. Cigarette smoking is significant risk factor for the development of CD. Smokers with CD have a poorer disease course than nonsmokers, with higher disease recurrence, more frequent surgical interventions, and a greater need for immunosuppressive agents (Zijistra, 1998).

Industrial Dust

Industrial dust particle which directly exposes on workers for a long term of exposure also act as causative factor (Podolsky et al, 2002).

Medications

Non-steroidal anti-inflammatory drugs (NSAIDs) are the drugs that reduce pain, fever, and inflammation. Popular NSAIDs such as ibuprofen, aspirin, and naproxen (if used excessively) destroys the mucus protective lining of the digestive tract thereby promoting IBD.

Microbes and Enteric Flora

Bacteria are considered as an antigen which leads to activation of intestinal immune system & epithelial cells. Activated immune system leads to the secretion of cytokines, reactive oxygen species, nitric oxide, proteases & eicosanoids. Release of these inflammatory mediators cause local mucosal damage.

Gut Permeability

An impaired colonic mucosal barrier leading to increased intestinal permeability has been demonstrated in patients with UC. Local leaks due to apoptosis of colonic epithelium comprise the primary lesion in mild UC. Moderate to severe UC is characterized not only by extensive local leaks but also by highly permeable ulcerous lesions (Gitter et al, 2001). Patients with UC have also demonstrated decreased colonic mucin. An invitro study demonstrated a possible interaction between bacterial peptides and the mucosa in UC, resulting in depletion of mucus secretion by goblet
cells (Leiper et al, 2001). Medical therapy leading to remission not only results in decreased inflammation but also improved gut barrier integrity.

**Appendectomy**

Patients who have been appendectomized have a lesser risk of developing UC. Moreover, in few appendectomized patients who develop UC, disease course is less severe, with a decreased need of colectomy compared to non-appendectomized (Radford-Smith et al, 2002; Cosnes et al, 2002). In Crohn’s disease, the effect of previous appendectomy remains debated. Some series reported an increased risk of Crohn’s disease after appendectomy (Koutroubakis et al, 2000; Andersson et al, 2002) and others did not (Duggan et al, 1998). Increased risk of surgery for Crohn’s disease was observed only in patients with perforated appendicitis (Andersson et al 2002). Appendectomy has no effect on Crohn’s disease severity.

**Stress**

As shown in the fig. 3.1 stress causes neuroendocrine response which decreases the mucus secretion and thereby weakens the mucosal barrier & increases the permeability. Thus increases the risk of developing IBD.

![Figure 3.1: Role of Stress in IBD](image)
3.5.2 Immune responses (Podolsky et al, 2002 ; Giorgos et al, 2005):

As shown in the Figure 3.2 normal epithelium, with its highly evolved tight junctions and products of goblet cell populations, most notably trefoil peptides and mucin glycoproteins, provides an effective barrier against luminal agents (Noel et al, 2004). The integrity of the barrier may be compromised by genetic variations in key molecular determinants, a diminished reparative response to injury, or exogenous agents, such as nonsteroidal antiinflammatory drugs. Chronic, recurrent intestinal inflammation appears to result from stimulation of the mucosal immune system by products of commensal bacteria in the lumen. Antigens from dietary sources may also contribute. Stimulation may occur as a result of the penetration of bacterial products through the mucosal barrier, leading to their direct interaction with immune cells, especially dendritic cells and lymphocyte populations, to promote a classic adaptive immune response (Podolsky et al, 2002).

Alternatively, bacterial products may stimulate the surface epithelium, possibly through receptors that are components of the innate immune-response system; the epithelium can, in turn, produce cytokines and chemokines that recruit and activate mucosal immune cells. Activation of classic antigen-presenting cells, such as dendritic cells, or direct stimulation through pattern-recognition receptors promotes the differentiation of type 1 helper T cells (Th1) in patients with Crohn's disease or, possibly, type 2 helper T cells in patients with ulcerative colitis. The stereotypical products of Th1 promote a self-sustaining cycle of activation with macrophages (Luster et al, 2001).

In addition to producing the key cytokines that stimulate Th1 (interleukin-12, interleukin-18, and macrophage migration inhibitor factor), macrophages produce a mix of inflammatory cytokines, including interleukin-1, interleukin-6, and most notably tumor necrosis factor, which target a broad variety of other types of cells. The latter include endothelial cells, which then facilitate the recruitment of leukocytes to the mucosa from the vascular space, as well as fibroblasts and epithelium, modulating their functional properties. Most important, these functions may be altered either by genetically determined variants, as exemplified by germ-line mutations in the gene encoding NOD2, the product of the IBD1 locus, in some patients with Crohn's
disease, or by environmental factors (Yehuda et al, 2002). Table 3.1 describes the involvement of T-cell in immune responses.

![Figure 3.2: Immune response in IBD](image)

### 3.5.3 Microbial Antigens and Adjuvants (Blumberg et al, 2001):

Studies also demonstrate a role of the presence of bacterial antigens, adjuvants, mucosa-associated and intra mucosal bacteria as the initiating factor in IBD. Mucosa-associated or intra mucosal *E.coli* is present in 43% and 29% of CD, and 17% and 9% of controls, respectively. Table 3.2 describes the role of various bacteria in the pathogenesis of CD & UC.
**Table 3.1: Involvement of T-Cells in Immune response.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Cytokine Stimulus</th>
<th>Master Transcription Factor</th>
<th>Effector Cytokine(s)</th>
<th>Effector Functions</th>
<th>Pathological Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1</td>
<td>IL-12</td>
<td>T-bet</td>
<td>IFN-γ</td>
<td>Intracellular pathogens</td>
<td>Autoimmunity; cell-mediated allergies</td>
</tr>
<tr>
<td>Th2</td>
<td>IL-4</td>
<td>GATA-3</td>
<td>IL-4</td>
<td>Extracellular pathogens</td>
<td>Asthma and IgE-mediated allergies</td>
</tr>
<tr>
<td>Th17</td>
<td>TGF-β plus IL-21 or IL-6 Inhibited by retinoic acid</td>
<td>RORγ</td>
<td>IL-17 &amp; IL-22</td>
<td>Extracellular bacteria; mediates inflammation</td>
<td>Autoimmune diseases</td>
</tr>
<tr>
<td>Treg</td>
<td>IL-2 TGF-β minus IL-6 Stimulated by retinoic acid</td>
<td>Foxp3</td>
<td>IL-10</td>
<td>Immunosuppression; anti-inflammatory</td>
<td>None</td>
</tr>
</tbody>
</table>
**Table 3.2:** List of Microbial Antigens & Adjuvant involved in IBD

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Evidence for association</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium avium</em></td>
<td>May be the causative factor as a subset of patients with CD</td>
</tr>
<tr>
<td><em>Echerichia coli</em></td>
<td>Increased incidence in postoperative CD recurrence</td>
</tr>
<tr>
<td><em>Helicobacter</em> species</td>
<td>The presence of <em>H. Bilis</em> &amp; <em>H. Hepaticus</em> results in more severe colitis in mice</td>
</tr>
<tr>
<td><em>Cytomegalovirus</em></td>
<td>Been proposed as having a potential etiologic role</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>May be the causative factor as a subset of patients with CD &amp; UC.</td>
</tr>
<tr>
<td><em>Mycobacterium paratuberculosis</em></td>
<td>Been proposed as having a potential etiologic role</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>May be the causative factor as a subset of patients with CD</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Increased incidence in postoperative CD recurrence</td>
</tr>
<tr>
<td>Nonmethylated DNA (CpG motif)</td>
<td>Been proposed as having a potential etiologic role</td>
</tr>
<tr>
<td>Flagellin</td>
<td>May be the causative factor as a subset of patients with CD &amp; UC.</td>
</tr>
<tr>
<td>Commensal bacteria</td>
<td>Overall, very strong evidence has led to the “no bacteria, no IBD” theory.</td>
</tr>
</tbody>
</table>
Enteric microflora can stimulate immune responses either by functioning as adjuvants or antigens. As adjuvants they activate innate immune responses, including dendritic cells and other APCs, and as antigens they stimulate the clonal expansion of T cells that selectively recognize the antigen through their T-cell receptor (Cong et al, 2002). Numerous bacterial adjuvants, bind selectively to various TLRs on innate immune cells, intestinal epithelial cells and mesenchymal cells Ligation of these TLRs activates NFκB and the mitogen-activated protein kinases, which stimulate the transcription of a host of pro-inflammatory and regulatory genes (Ulrich et al, 2000).

Resident intestinal macrophages have a limited capacity to respond to bacterial adjuvants owing to down regulation of their bacterial recognition receptors, such as TLR and CD14, the co-ligands for lipopolysaccharide. Similarly, intestinal epithelial cells normally have low levels of TLRs, which allows epithelial cells to reside in the high bacterial concentration of the distal ileum and colon. TLR molecules are expressed on the surface of various effector cells of the innate immune response. Like CARD15, these pattern-recognition receptors selectively bind to specific microbial adjuvants and initiate signaling through NFκB. Although each type of TLR binds a specific bacterial adjuvant (i.e. TLR4 and CD14 bind lipopolysaccharide, and TLR2 binds peptidoglycan), these signals all converge on a single pathway via myeloid differentiation primary response protein MyD88, which activates NFκB.

Finally, the role of bacteria in the pathogenesis of IBD is supported. Although it is not an environmental factor, it has been postulated as an early predisposing per-se factor for the development of this condition.

3.5.4 Genetic Predisposition in IBD:

Genes regulate several important biologic functions, including immunoregulation, mucosal barrier integrity and microbial clearance and/or homeostasis. However, studies have shown evidence for a genetic predisposition to IBD. First-degree relatives of patients with IBD have a 4- to 20-fold increased risk and a 7% absolute risk. Among family members with CD, there is strong concordance within disease category and disease location. Monozygotic twins have significantly higher concordance rate than dizygotic twins. The genetic contribution appears to be greater in CD than in UC. Overall, the genetic predisposition to CD and UC appears to be
multifactorial, as opposed to being linked to one specific gene. Several genes on different chromosomes have been linked to the development of CD and UC. The IBD1 gene, which is located on chromosome 16, has been linked to CD. Early onset CD has been associated with a specific locus on chromosome 5. In another study, the strongest association with the susceptibility locus on chromosome 5 was observed in patients with perianal CD.

The most promising candidate gene is CARD15 (also known as NOD2), which is expressed in macrophages and paneth cells. The variant form of CARD15 results in paradoxically reduced macrophage activation of the NF-κB pathway. One would expect this to result in a diminished inflammatory response. Candidate gene SLC22A4 & SLC22A5 affect the transcription and function of these carnitine and this organic cation transporters have been associated with Crohn's disease in association with CARD15 mutations. Another DLG5 gene, which encodes a scaffolding protein that helps to maintain epithelial integrity, have been associated with Crohn's disease and combined ulcerative colitis. The MDR1 (The Multidrug resistance gene) Mutation, MDR1 encodes P-glycoprotein 170, a transporter that governs efflux of drugs and possibly xenobiotic compounds from cells. MDR1 variants have been associated with ulcerative colitis and Crohn's disease. At last, PPARγ (peroxisome proliferative-activated receptor) gene, PPARγ polymorphisms were found to be associated with human Crohn's disease. PPAR is a nuclear receptor that inhibits NFκB activity: its expression is decreased in patients with active ulcerative colitis and its expression is upregulated by 5-aminosalicylic acid. However, homozygotes for this variant gene have a 20-fold increased risk of developing CD & UC. Table 3.3 shows possible genetic loci influencing the presentation of IBD have already been identified on more than half of all chromosomes.
### Table 3.3: Locations of Twelve major loci showing linkage to IBD

<table>
<thead>
<tr>
<th>Inflammatory Bowel Disease</th>
<th>Locus Chromosome</th>
<th>Identified Genes</th>
<th>Functions</th>
<th>Types of IBD Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD1</td>
<td>16q13</td>
<td>NOD2/CARD15</td>
<td>NF-κB activation and regulation, Killing of intracellular pathogen, Paneth cell function, α-defensin production</td>
<td>CD</td>
</tr>
<tr>
<td>IBD2</td>
<td>12q14 (VDR, STAT6, MMP18, b2-integrin)</td>
<td>Not known</td>
<td>Not known</td>
<td>UC</td>
</tr>
<tr>
<td>IBD3</td>
<td>6p (HLA, TNF)</td>
<td>Not known</td>
<td>Not known</td>
<td>IBD</td>
</tr>
<tr>
<td>IBD4</td>
<td>14q11-12</td>
<td>Not known</td>
<td>Not known</td>
<td>CD</td>
</tr>
<tr>
<td>IBD5</td>
<td>5q31-33 (TCR, LTB4 receptor)</td>
<td>SLC22A4, SLC22A5</td>
<td>Organic cation, carnitine transporters, possibly transport xenobiotic substances</td>
<td>CD</td>
</tr>
<tr>
<td>IBD6</td>
<td>19p13 (ICAM1, C3, TBXA2)</td>
<td>Not known</td>
<td>Not known</td>
<td>IBD</td>
</tr>
<tr>
<td>IBD7</td>
<td>1p36 (TNF-R family)</td>
<td>Not known</td>
<td>Not known</td>
<td>IBD</td>
</tr>
</tbody>
</table>
In conclusion, various factors have been implicated in the pathogenesis of IBD. Recent data indicate that altered NOD2/ CARD15, TLR Receptor mediated bacterial sensing of normal commensal flora in the gut and mucosal permeability changes may be the key mechanisms. At present, most efforts are devoted to better understanding of the genetic changes underlying IBD and to further clarification of how the altered recognition of pathogenic and/or commensal microbial factors by the mucosal immune system leads to inflammation in IBD subjects but not in the general population.
3.6 Pathophysiology of IBD (Yehuda et al, 2002):

3.6.1 Genetic susceptibility:

Advances have occurred in understanding the genetics of human IBD, from studies based on single nucleotide polymorphism (SNP) and candidate gene approaches, and from studies in mouse experimental colitis that used transgenic and deletion (knockout) techniques. The common features of these studies revealed that the implicated genes regulate several important biologic functions, including immunoregulation, mucosal barrier integrity and microbial clearance and/or homeostasis (Giorgos et al, 2005).

A) The CARD15 Gene:

The first gene to be associated with Crohn's disease was CARD15 (caspase recruitment domain family member 15, formerly known as NOD2). There are three mutations causing amino-acid substitutions Arg702Trp and Gly908Arg and the frameshift 1007fs found within the region of CARD15 that encodes a leucine-rich repeat, which is responsible for bacterial recognition. At least one of these mutations is present in 25–35% of Crohn's disease patients of European ancestry, but not in Asian or African American Crohn's disease patients. Mutations in CARD15 are associated with distal ileal Crohn's disease in particular, and have been found in some patients with stricturing disease (Noel et al, 2004).

The leucine-rich repeat region of CARD15 binds muramyl dipeptide (MDP), which is the biologically active moiety of peptidoglycan, an ubiquitous cell wall polymer found in almost all bacteria. The binding of MDP by dimerized CARD15 activates nuclear factor NFkB, which forms part of a central signaling pathway that stimulates the transcription of multiple genes that encode both proinflammatory and protective molecules. The mutations causing Arg702Trp, Gly908Arg and 1007fs cause defective MDP binding, but studies report conflicting consequences of having such mutations.

It is observed that activation of NFkB in patients with active Crohn's disease, rather than decreased activity predicted by a loss-of-function mutation. Their investigations demonstrated that, in CARD15-defective cells, Toll-like receptor 2 (TLR2) was unable to downregulate NFkB activation.
Figure 3.3: Model of NOD2 gene mutation in the pathogenesis of CD.

As shown in the Fig. 3.3 mutations in NOD2 result in diminished immune cell NF-kB activation in the presence of lipopolysaccharide (LPS). The NOD2 gene consists of 2 CARD, amino-terminal effector domains, a central NOD, and multiple leucine-rich repeat (LRR) domains that function as sensors of bacterial infection. Normally, stimulation of NOD2 with bacterial proteins activates the NF-kB pathway via RIP-like interacting CLARP kinase receptor interacting protein 2l (RICK), a serine-threonine kinase that phosphorylates the inhibitor of NF-kB kinase (IKK), thus allowing transport of NF-kB to the nucleus.

These abnormalities could result in defective downregulation of the innate immune response to bacterial adjuvant stimulation, ineffective clearance of intracellular bacterial infection and proliferation of both luminal and mucosally adherent commensal bacteria. Each of these situations has been documented in Crohn's disease patients.

B) SLC22A4 and SLC22A5 (Peltekova, 2004):

Two functional variants of the organic cation transporters OCTN1 and OCTN2 have been associated with Crohn's disease in association with CARD15 mutations. Mutations in the transcribed region of SLC22A4, which encodes OCTN1, and the
promoter region of SLC22A5, which encodes OCTN2, affect the transcription and function of these carnitine and organic cation transporters. These variants are most actively expressed in the intestinal epithelium, macrophages and T cells, and cause decreased carnitine transport.

C) The DLG5 Gene (Stoll et al, 2004):

Two haplotypes of DLG5, which encodes a scaffolding protein that helps to maintain epithelial integrity, have been associated with Crohn's disease and combined ulcerative colitis and Crohn's disease populations like the OCTN1 and OCTN2 variants, the 113G>A substitution in DLG5 is associated with CARD15 mutations in patients with Crohn's disease.

D) The MDR1 Gene (Noble et al, 2005):

The multidrug resistance gene MDR1 encodes P-glycoprotein 170, a transporter that governs efflux of drugs and possibly xenobiotic compounds from cells. P-glycoprotein 170 might also function as a 'flippase' that moves amphipathic substrates from the inner to the outer leaflet of the cell membrane. MDR1 variants have been associated with ulcerative colitis and Crohn's disease. MDR1 is of particular interest, because it has been associated with treatment-refractory IBD and because mice in which MDR1 has been deleted develop colitis.

E) The PPARγ Gene (Lewis et al, 2001):

PPARγ (peroxisome proliferative-activated receptor) variants have been linked with susceptibility in the SAMP1/YitFc mouse model of spontaneous chronic ileitis, and rare PPARγ polymorphisms were found to be associated with human Crohn's disease. PPAR is a nuclear receptor that inhibits NFkB activity, its expression is decreased in patients with active ulcerative colitis and its expression is upregulated by 5-aminosalicylic acid. In addition to a potential role in protecting against intestinal inflammation, treatment with the PPAR ligand rosiglitazone was effective in an open-label trial involving ulcerative colitis patients as well as in mouse experimental colitis. Following are the genes, chromosomes and their functions in IBD (SuCG et al, 1999) (Table 3.4).
Table 3.4: Genes Involved in IBD.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn’s disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CARD15 (NOD2)</td>
<td>16</td>
<td>NFκB activation and/or regulation, killing of intracellular pathogen, Paneth cell function, α-defensin production</td>
</tr>
<tr>
<td>SLC22A4 SLC22A5</td>
<td>5</td>
<td>Organic cation, carnitine transporters, possibly transport xenobiotic substances</td>
</tr>
<tr>
<td>DLG5</td>
<td>10</td>
<td>Epithelial scaffolding protein</td>
</tr>
<tr>
<td>PPARG</td>
<td>3</td>
<td>Intracellular inhibitor of NFκB and cellular activation</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDR1</td>
<td>7</td>
<td>Efflux transporter for drugs and possibly xenobiotic compounds</td>
</tr>
</tbody>
</table>

3.6.2 Microbial antigens and adjuvants (Baumgart et al, 2007):

The bacterial load at the mucosal surfaces in our body has resulted in a need to respond quickly to pathogenic invasion. Most of the invaders are identified and destroyed within minutes or hours by innate immune system. As a tool in the identification of invaders, the innate immune system has developed a set of receptors recognizing common and conserved structures on the microorganism. These structures known as the Pathogen Associated Molecular Patterns (PAMPs) and are molecule of the microbiota that have evolved to perform essential function for microbiota survival.

Toll Like Receptors:

They are transmembrane receptors that are found either on the cell membrane (TLR1, 2, 4, 5 and 9) or on intracellular organelles (TLR3, 7 and 8). TLRs are expressed throughout the gastrointestinal tract on intestinal epithelial cells (IECs), myofibroblasts, enteroendocrine cells, and on immune cells within the lamina propria, such as T cells, and dendritic cells (DCs). Extracellular domains of TLRs consist of leucine-rich repeats (LRRs), whereas their intracellular component contains a TIR (Toll/IL-1 receptor) domain, exhibiting homology with the interleukin-1 receptor (IL-1R) superfamily (Gay et al, 1991).
Most TLR signal via the MyD88 adaptor (TLR1, 2, 4, 5, 6, 7, 8 and 9), whereas TLR3 signaling activates an alternative “MyD88-independent” pathway. TLR4 is the only receptor known to activate both MyD88-dependent and -independent pathways. Ligand binding to TLRs initiates signaling cascades that activate NF-κB and the induction of pro-inflammatory responses as shown in the Figure 3.4.

![Figure 3.4: Mechanism of TLRs activation](image)

MyD88 is a universal adapter for induction of the cytokines TNF and IL-6 by IL-1, TLR-2, TLR-4, TLR-5, TLR-7 and TLR-9. It is also required for activation of the transcription factor NF-kB by IL-1, TLR-2, TLR-5, TLR-7 and TLR-9. Mal is also required for cytokine induction, but only by TLR-2 and TLR-4. It is also essential for NF-kB activation by TLR-2, whereas for TLR-4, both MyD88 and Mal appear to be essential for optimal activation of NF-kB by TLR-4, as the deletion of either leads to delayed activation. However, a double knock-out of MyD88 and Mal will still display NF-kB activation, pointing to an additional adapter. TLR-2, TLR-3, TLR-7 and TLR-
9 all increase the expression of the NF-kB-dependent genes CD80 and CD86, required for T-cell activation by dendritic cells. MyD88 is essential for this response in the case of TLR-9. Whether it is required for TLR-2 or TLR-7 is not known, but seems likely. In the case of TLR-4, however, neither MyD88 nor Mal are required for this response, again pointing to another adapter for TLR-4, which would activate NF-kB but, unlike in TNF production (which does not occur in spite of NF-kB activation being detected), this would lead to induction of CD80 and CD86. Finally, both TLR-4 and TLR-3 have been shown to activate the transcription factor IRF-3, leading to induction of such genes as the chemokine IP10 and interferon-β. Neither MyD88 nor Mal are required for this response, and in the case of TLR-3, neither are required for NF-kB activation, cytokine induction or CD80/86 expression. The latest adapter to be described, TRIF, may be responsible for this response.

Enteric microflora can stimulate immune responses either by functioning as adjuvants or antigens. As adjuvants they activate innate immune responses, including dendritic cells and other APCs, and as antigens they stimulate the clonal expansion of T cells that selectively recognize the antigen through their T-cell receptor (Figure 3.5).

![Figure 3.5: Overview of TLRs activation](image-url)
A) **Adjuvants** (Blumberg *et al*, 2001):

Numerous bacterial adjuvants, most notably lipopolysaccharide, peptidoglycan, flagellin and nonmethylated DNA (CpG motif), can bind selectively to various TLRs on innate immune cells, intestinal epithelial cells and mesenchymal cells (Figure 3.6). Ligation of these TLRs activates NFƙB and the mitogen-activated protein kinases, which stimulate the transcription of a host of proinflammatory and regulatory genes.

Activation of macrophages from susceptible individuals, by enteric lipopolysaccharide, peptidoglycan, flagellin or CpG, stimulates the production of IL-1, TNF, IL-6, IL-8 and other chemokines, IL-12 p40 (and thus IL-12 and IL-23), adhesion molecules, IL-18, reactive oxygen species, nitric oxide and leukotrienes, which can all participate in the inflammatory response. In addition, NFƙB activation of APCs (including dendritic cells) by microbial adjuvants induces the expression of MHC class II antigens, co-stimulatory molecules, IL-12 and IL-23, which can activate T\(_{H1}\) and T\(_{H17}\) cells, respectively, if the appropriate antigen is present (Hanauer, 2006).

Lipopolysaccharide can stimulate IL-12 p40 production by bone-marrow-derived dendritic cells in IL-10-deficient mice, and colonization of previously germ-free rodents with various commensal bacterial species can induce ICAM1 and IL-6 expression by intestinal epithelial cells. *In vivo* studies show that commensal bacteria selectively activate IL-12 p40 in dendritic cells of the distal ileum. Bacterial flagellin is both an antigen and adjuvant. A form of flagellin was shown to be a dominant antigen in experimental colitis and to induce serum antibody production in 50% of Crohn's disease patients. Flagellin binds to TLR5 to activate NFƙB.

In addition to their proinflammatory properties, bacterial adjuvants can induce protective anti-inflammatory responses. For example, lipopolysaccharide stimulates IL-10 production in dendritic cells from normal mice, and certain CpG preparations can prevent the onset of experimental colitis by inducing the production of type 1 IFN (IFN-\(\gamma\)) in plasmacytoid dendritic cells, via TLR9 ligation.
B) Antigens (Blumberg et al, 2001):

Genetically susceptible hosts, including IBD patients, and genetically engineered rodents and mice with spontaneous mutations (C3H/HeJ Bir and SAMP1/YitFc), have aggressive T-cell responses to luminal commensal bacteria. Although B-cell responses to enteric microbial constituents are exaggerated in Crohn's disease, ulcerative colitis and experimental intestinal inflammation, antibodies are not necessary to transmit disease in experimental colitis, and B-lymphocytes seem to be regulatory rather than pathogenic.

It has been shown that persistent luminal antigen stimulation is necessary for the transfer of colitis. CD4+ T cells from rodents with colitis induced colitis when they were transferred into T-cell-deficient recipients colonized with specific-pathogen-free commensal bacteria, but did not cause disease in germ-free recipients. After transfer, T cells in the recipients responded to luminal bacterial products. There is both bacterial and host specificity in these T_{H1} responses to bacterial antigens. Several groups have used molecular approaches to identify the dominant antigens in experimental colitis models.
A specific form of flagellin from commensal *Clostridium* species acts as the dominant antigen in C3H/HeJ-Bir mice and transferred colitis via T-cell clones that reacted to flagellin. Approximately half of Crohn's disease patients, but not ulcerative colitis patients or normal controls, have a selective serologic response to the same form of flagellin, showing the clinical relevance of these experimental model studies. *Bacteroides vulgatus* heat-shock protein 60 has been identified as a dominant antigen in HLA-B27TG rats mono-associated with *B. vulgatus*. Both heat-shock protein 60 and flagellin are strong adjuvants as well as antigens by virtue of their binding to TLR4 and TLR5, respectively.

**3.6.3 T-Cell Responses and NF-κβ In IBD** (Ulrich *et al.*, 2000):

T-Cell Responses are similarly activated in all forms of IBD, T-cell profiles are disparate in Crohn's disease and ulcerative colitis, and so are considered separately. Crohn's disease (*Zetiz et al.*, 1998) involves T<sub>H</sub>1 and T<sub>H</sub>17 while T<sub>H</sub>2 responses are produced in UC. T<sub>H</sub>1 cells secrete their own lymphokines namely Tumor-necrosis factor-beta (TNF-β) (also known as lymphotoxin) and Interferon-gamma (IFN-γ). Th17 cells are subset of CD4<sup>+</sup> T helper cells. They are found at the interfaces between the external environment and the internal environment, e.g., skin and lining of the GI tract. Dendritic cells have the unique capacity to activate naive T cells. IFN-γ, the production of which is stimulated by IL-12, produced by antigen-presenting cells (APCs) or dendritic cells. Major lymphokines secreted by T<sub>H</sub>2 cells are Interleukin 4, Interleukin 13 and Interleukin 5.

Dysregulated cytokine production and signalling mechanisms by epithelial cells, mucosal lymphocytes and macrophages have been implicated in the pathogenesis of IBD. Over the past few years, various murine models of chronic intestinal inflammation resembling IBD have been established. These models have provided important clues as to the nature of such dysregulation and to its possible cytokine based treatment. Although some NF-κβ family members are apparently important in preventing inflammatory responses (e.g. RelB), it was found that nuclear NF-κβ levels are increased in patients with IBD. In particular, the p65 subunit was highly activated in epithelial cells and lamina propria macrophages from patients with active Crohn's disease and ulcerative colitis. These findings are consistent with immunohistochemical data indicating increased expression of NF-κβ in active IBD.
and data from intestinal biopsy samples showing increased p65 in active Crohn’s disease. In addition, it was shown recently that a specific p65 antisense oligonucleotide can block p65 expression and proinflammatory cytokine production by lamina propria macrophages in patients with active Crohn’s disease and ulcerative colitis. Furthermore, in a murine model of colitis p65 antisense treatment led to an abrogation of chronic intestinal inflammation. In particular, there are few data concerning the role of other NF-κβ /Iκβ family members in epithelial cells and T cells in the gut. In addition, the expression of Iκβ family members and their degradation mechanisms in IBD have only been partially characterised. NF-κβ activates in epithelial cells in response to IL-1 and altered regulation of Iκβ degradation in native colonic epithelial cells. Such enhanced resistance of epithelial cells to Iκβ α proteolysis suggested a potentially increased responsiveness to therapeutic blockade. Indeed, adenoviral mediated delivery of a mutant NF-κβ repressing Iκβ alpha protein resulted in inhibition of IL-8 production by intestinal epithelial cells. Furthermore, pharmacological inhibition of Iκβ alpha degradation strongly reduced IL-8 secretion by intestinal epithelial cells. Finally, recent evidence suggests that NF-κβ is important in regulating intercellular cell adhesion molecule expression in the intestine. Preliminary data from the same group also showed a beneficial therapeutic effect of proteasome inhibitors (that block NF-κβ activation) in experimental colitis. Inhibition of NF-κβ activity has been recently suggested as a major component of the anti-inflammatory activity of glucocorticoids that are frequently used for treatment of chronic intestinal inflammation in humans. Although activation of NF-κβ p65 is not specific for patients with IBD, its perpetuated activation makes it a very attractive target for therapeutic intervention. Thus, downregulation of NF-κβ activity emerges as a potential key event in the control of chronic intestinal inflammation in humans and strategies to inhibit NF-κβ activity more specifically are desirable. Such strategies include antioxidants, proteasome inhibitors, inhibition of NF-κβ by adenoviral Iκβ expression vectors, and antisense DNA targeting of NF-κβ. Thus, the above data suggest that targeting of NF-κβ may be a novel molecular approach for the treatment of patients with IBD that could lead to the design of new treatment strategies that have added specificity but reduced toxicity compared with standard immunosuppressive therapy.
3.6.4 Enteric Nervous System and Substance P (Kara et al., 2007):

Intestinal inflammation damages colonic nerves and leads to changes in mucosal innervation and neuropeptide expression. Neuronal changes seen in CD include hyperplasia of the ganglion cells, extensive axonal degeneration, changes in gut neuropeptide content and infiltration of the myenteric plexus with plasma cells, mast cells, and lymphocytes. Patients with long-standing UC have similar but less evident alterations. These changes are seen in both humans and experimental animal models.

The gut is one of the most abundant sources of Substance P (SP) in the body, where it is expressed in the myenteric and submucosal plexi, as well as in the dorsal root ganglia and intrinsic and extrinsic sensory neurons. Immune cells such as monocytes, lamina propria macrophages, eosinophils, and lymphocytes also express SP. The effects of SP are mediated by three receptors belonging to the G-protein receptor superfamily, neurokinin (NK)-1, -2, and -3. The NK-1 receptor is the high-affinity receptor for SP, while NK-2 and NK-3 bind with much lower affinity to this peptide. NK-1 receptors are expressed abundantly in the GI tract and the colon in multiple cell types, including nerves of the Enteric Nervous System, smooth muscle, immune, endothelial, and epithelial cells. Since primarily NK-1, but not NK-2 or NK-3, receptors have been linked with intestinal inflammation and IBD. The expression of NK-1 receptors has also been observed to vary at virtually all levels of the intestine, mostly based on whether samples are taken from inflamed versus noninflamed tissue and from CD versus UC patients. The mechanism of NK-1 receptor upregulation during intestinal inflammation is likely associated with increased cytokine expression, a consistent intestinal response in IBD patients. Table 3.5 describes about various neuropeptides found in the gut and their receptors.
### Table 3.5: Neuropeptides & their receptors location.

<table>
<thead>
<tr>
<th>Neuropeptide</th>
<th>Receptors</th>
<th>Main Neuropeptide Locations in the Gut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance P (SP)</td>
<td>Neurokinin-1*, 2, 3</td>
<td>Myenteric and submucosal plexus of the gut, dorsal root ganglia, intrinsic and extrinsic sensory neurons, monocytes, lamina propria macrophages, eosinophils, lymphocytes</td>
</tr>
<tr>
<td>Corticotropin-releasing hormone (CRH)</td>
<td>Corticotropin-releasing hormone receptor I and II CRH: CRHR1/2</td>
<td>Enterochromaffin cells, myofibroblasts, autonomic ganglia, and extrinsic nerve cells</td>
</tr>
<tr>
<td>Urocortins I, II, III (UI, II and III)</td>
<td>CRHR1 - CRHR2 U11 and 111:CRHR2*</td>
<td></td>
</tr>
<tr>
<td>Neurotensin (NT)</td>
<td>Neurotensin Receptor I* and II</td>
<td>Throughout the intestine: N cells localized among epithelial cells of the jejunum and ileum</td>
</tr>
<tr>
<td>Vasoactive intestinal peptide (VIP)</td>
<td>VPAC1 and VPAC2</td>
<td>All layers of the colon, with highest concentration in the myenteric plexus: primarily localized to neurons, but also in T cells and eosinophils. Also abundant in smooth muscle sphincters of the lower esophagus, ampulla of Vater and the rectum.</td>
</tr>
<tr>
<td>Opioids</td>
<td>Mu-opioid receptor (MOR)</td>
<td>MOR locations: mesenteric and submucosal plexi in the ileum and colon</td>
</tr>
<tr>
<td>Galanin</td>
<td>Galanin receptor 1(Gal1R),*</td>
<td>Enteric nerve terminals</td>
</tr>
<tr>
<td></td>
<td>Gal2R, Gal3R</td>
<td></td>
</tr>
</tbody>
</table>


#### 3.7.1 Psychological Stress:

Psychological stress influences on gastrointestinal tract homeostasis through corticotrophin-releasing factor, a key player in the brain–gut axis. Stress stimulates the release of corticotrophin releasing factor (CRF) from the hypothalamus, causing the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Stress finally stimulates secretion of cortisol from the adrenal cortex. CRF directly induces alteration of gastrointestinal motility. Endogenous CRF mediates the stress-induced inhibition of the upper GI tract and the stimulation of colonic motility. Further, CRF is thought to have the potential to change the production of several cytokines and the function of immune cells, including lymphocytes and NK cells. Interestingly, it has been reported that CRF contributes to the pathophysiology of human UC. CRF levels are increased in lamina propria mononuclear cells from patients with active UC. Thus, both central and peripheral CRF systems are stimulated by stress and may have the potential to regulate gut homeostasis and so influence IBD pathophysiology.
3.7.2 Oxidative Stress (Kathleen et al, 2003; Mckenzie et al, 1996):

Signs of increased oxidative stress are in evidence in the intestinal mucosa of patients with ulcerative colitis and may be secondary to inflammation. One study examined signs of oxidative stress and plasma antioxidant levels in controls compared to patients with UC and CD. Oxidative DNA damage was noted in both IBD groups compared to controls, measured by production of 8-hydroxy-deoxy-guanosine (8-OHdG). Mucosal biopsies of UC patients were analyzed and shown to have increased reactive oxygen intermediates & DNA oxidation products (8-OHdG). Decreased levels of endogenous antioxidant superoxide dismutase were also observed. This supports the theory that free radicals can produce damage to mucosal proteins in IBD. A theory proposed by several researchers involves TNF-α produces reactive oxygen species (ROS); ROS in turn activate NF-kB, which then enhances further TNF-α production, propagating a vicious cycle.

3.7.3 Glycosaminoglycans (Vantrappen et al, 1993):

The gastrointestinal extracellular matrix is composed of the proteins collagen and elastin, and ground substance that include glycosaminoglycans (GAGs). GAGs are abundant in the basement membrane, lamina propria, and submucosa of the GI tract. The composition of GAGs may significantly affect both the permeability of the colon and immune/inflammatory reactions. Analysis of diseased, resected colons yielded altered GAG content in the colon of patients with IBD and colonic neoplasia compared to tissue from undiseased colons. In histologically normal colon tissue the majority of GAG content consists of chondroitin and dermatin sulfate, with smaller amounts of hyaluronic acid and heparan sulfate. Ulcerative colitis yielded a distinctly abnormal distribution of GAGs, with significantly greater amounts of total glycosaminoglycans, heparan sulfate, and hyaluronic acid than control tissue. Colonic neoplasias were also found to contain these abnormal GAG profiles, but to a greater extent than UC tissue.

Other researchers report the alterations are limited to the mucosa in UC, with substantial loss of GAGs from the subepithelial basal lamina. It is hypothesized that alterations in negatively charged sulfated compounds could significantly affect the passage of substances through the colonic mucosa, contributing to leakage of proteins.
and fluids, thrombosis, and extensive remodeling observed in UC and other inflammatory bowel conditions. Some researchers hypothesize these alterations may contribute to the inflammatory process since hyaluronic acid can interact directly with lymphocytes, inhibit macrophage response to cytokines, and enhance phagocytosis. GAG content has been associated with alteration in the distribution of macrophages reactive to TNF-α.

3.7.4 Short Chain Fatty Acid (SCFAs)

SCFA, mainly acetate, propionate and butyrate, which are produced in the large bowel by the anaerobic bacterial fermentation of undigested dietary carbohydrates and fiber polysaccharides, have been proposed to actively contribute to the maintenance of colonic homeostasis, butyrate being considered as the major fuel source for the colonocyte. In fact, it has been suggested that a diminished b-oxidation of luminal butyrate to CO₂ and ketones, which results in energy deficiency within colonic epithelial cells, could contribute to the pathogenesis of ulcerative colitis. In addition, several authors have reported decreased fecal concentrations of SCFA in patients with ulcerative colitis and in idiopathic colitis. All these facts suggest that restoration of luminal levels of butyrate may facilitate the recovery of the inflamed mucosa. Proper ion transfer, mucus synthesis, phase II detoxification, and lipid synthesis for cell membrane integrity in the colonocytes depend on butyrate oxidation (Roediger et al, 1995). Impaired metabolism of SCFAs has been implicated as a factor in UC. Hond et al 1998 compared butyrate metabolism in healthy controls with that of hospitalized patients with severe ulcerative colitis and UC patients in remission. They measured butyrate metabolism after rectal instillation of C-labeled CO in the breath. Patient’s butyrate by measuring with active UC had significantly lower butyrate oxidation than patients in remission (who had normal butyrate oxidation) or controls. Three patients with inactive disease had decreased butyrate oxidation and interestingly, all three relapsed within a few weeks. Perhaps decreased oxidation of SCFAs is a good predictor of possible relapse and occurs before other signs of inflammation. Because normal oxidation was observed in patients in remission, faulty SCFA oxidation is likely to be a result rather than a primary cause of ulcerative colitis.
3.8 Clinical features of Ulcerative Colitis and Crohn’s Disease (Barbara et al, 2002):

UC and CD are associated with both intestinal and extraintestinal manifestations. Extraintestinal manifestations are usually related to intestinal disease activity and may precede or develop concurrently with intestinal symptoms. While UC and CD have a number of similarities in their clinical presentations, characteristic features are emphasized below in Tables 3.6 and 3.7.

**Table 3.6: Intestinal complications**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Ulcerative Colitis</th>
<th>Crohn's Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diarrhea</strong></td>
<td>Recurrent diarrhea is very common, but onset may be very gradual and mild or it may not be present. Feces may also contain mucus.</td>
<td>Recurrent diarrhea is fairly common.</td>
</tr>
<tr>
<td><strong>Rectal Bleeding</strong></td>
<td>Blood is almost always present in stools. It may be readily visible or visible only using a microscope (called occult blood).</td>
<td>Bleeding not as common as in UC, but can occur.</td>
</tr>
<tr>
<td><strong>Abdominal Symptoms</strong></td>
<td>Pain is not prominent symptom, but can vary. May cause vague discomfort in the lower abdomen, an ache around the top of the hipbone, or cramps in the middle of the abdomen. Severe pain can occur during flare-ups. Vomiting and nausea.</td>
<td>Main symptom is recurrent episodes of pain in the lower right part of the abdomen or above the pubic bone. Often preceded by bloating, nausea, and vomiting. In some cases, pain may also occur. Intestinal pain may also be an indication of a serious condition, such as an abscess, or a perforation of the intestinal wall.</td>
</tr>
<tr>
<td><strong>Loss of appetite, weight loss, and impaired growth in children</strong></td>
<td>Often not evident in mild or even moderately severe UC. Occasionally impairs growth in children and teenagers.</td>
<td>Common. Typical weight loss is 10 - 20% of normal. Commonly impairs growth in children and teenagers.</td>
</tr>
</tbody>
</table>
Review of Literature

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal defecation</td>
<td>Increased frequency, a feeling of incomplete evacuation, and tenesmus (a painful urge for a bowel movement even if the rectum is empty). Fecal incontinence.</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Symptoms may be mild or severe.</td>
</tr>
<tr>
<td>Can occur in active stages.</td>
<td></td>
</tr>
<tr>
<td>Anal ulcers and fistulas:</td>
<td>Almost never a symptom.</td>
</tr>
<tr>
<td>Fistulas around the anus</td>
<td>Fistulas and ulcers around the anus may be early symptoms.</td>
</tr>
<tr>
<td>Neurologic or psychiatric symptoms</td>
<td>No.</td>
</tr>
<tr>
<td>Extraintestinal complications:</td>
<td>May be early signs of Crohn's disease when accompanied by gastrointestinal problems.</td>
</tr>
</tbody>
</table>

Extraintestinal complications:

It occurs in approximately 20% of patients with IBD. In some cases, they may be more problematic than the bowel disease itself.

Fevers: Fevers are seen in 40% of patients with IBD at the time of presentation. Fevers can be high spiking on occasion but are usually low grade and chronic and may be unrecognized.

Weight loss: Weight loss may be a feature of IBD in both adults and children. In children, weight loss or a failure to maintain a normal growth velocity is the commonest systemic feature of IBD and is observed more frequently with CD than with UC.

Delayed growth and sexual maturation in children: Growth failure (defined as either reduced growth velocity, in centimeters per year, for age or a fall in height
percentile from the child’s previous level) and delayed sexual maturation occasionally may be the initial presentation of CD in children (Kanof et al, 1988). Patients also may have a concomitant delay in skeletal maturation, which is evaluated by radiologic determination of the nondominant hand. Delayed growth is more common in CD (60 to 88%) than in UC (6 to 12%), with the greatest frequency found in prepubertal children. Growth delay also can occur as a consequence of chronic corticosteroid use. Delayed sexual maturation or arrest of sexual maturation may occur concurrently with growth failure. Some females may also experience secondary amenorrhea due to active disease or weight loss (Barbara et al, 2002).

Other extraintestinal manifestations of IBD are as follow:
**Table 3.7:** Extraintestinal manifestations of IBD

<table>
<thead>
<tr>
<th>Site</th>
<th>Complication</th>
<th>Description</th>
<th>Pictures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin</strong></td>
<td>Pyoderma Gangrenosum</td>
<td>Rare, ulcerating sores most often on legs; usually with extensive and active colon disease. It is a deep severe ulceration of the skin and is an unusual manifestation (&lt;1%) that is usually seen in association with UC.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most common skin manifestation; more common with Crohn disease; may raise suspicion of IBD in previously healthy child. It is characterized by the development of painful, indurated, purplish red, ovoid nodules 1 to 3 cm in diameter, most commonly seen over extensor surfaces.</td>
<td></td>
</tr>
<tr>
<td><strong>Mouth</strong></td>
<td>Recurrent aphthous ulcers</td>
<td>Most common skin manifestation; more common with Crohn disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other assorted mouth lesion</td>
<td>Lip swelling, fissures, gum inflammation (gingivitis)</td>
<td></td>
</tr>
<tr>
<td>Joints</td>
<td>Migratory peripheral arthritis</td>
<td>Involves the large joints; redness, swelling and stiffness; generally non-destructive (as opposed to rheumatoid arthritis); parallels bowel disease</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>Inflammation of the vertebrae; usually begins in the early twenties, most commonly with ulcerative colitis who have a certain human leukocyte antigen (B27); low back pain and morning stiffness; back, hips, shoulders, and sacroiliac joints typically affected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>Episcleritis</td>
<td>Fiery red inflammation of the conjunctivae mimicking &quot;pink eye&quot; eyes red, burn; vision not affected</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anterior uveitis</td>
<td>Inflammation of the iris and accommodation muscle (ciliary body); eye pain, headache, blurred vision; possible glaucoma, cataracts, and permanent visual impairment; may initially be silent</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Primary Sclerosing Cholangitis</td>
<td>Chronic inflammation and obliteration of the bile ducts within and outside of the liver: cirrhosis; nonspecific fatigue, appetite loss, itching, and jaundice</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>Osteoporosis</td>
<td>Mild bone loss affects 2/3 of IBD patients; increased risk of fractures, bone deformities, chronic pain</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>Anemia</td>
<td>Nutritional: deficiencies of iron, B12, folate</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Glomerulonephritis</td>
<td>It is rarely observed: deposition of immune antigen/antibody complexes in filtering tubules; amyloidosis; kidney stones</td>
<td></td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Blood clots</td>
<td>In extremities or brain (stroke)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arteritis</td>
<td>Inflammation of blood vessels in extremities, or brain (stroke)</td>
<td></td>
</tr>
</tbody>
</table>
3.9 DIAGNOSIS of IBD (Noel et al, 2004):

3.9.1 Laboratory Tests

A complete blood count test can detect infection and anemia, as well as monitor for side effects of certain IBD medications. An electrolyte panel measures potassium and other minerals that can be depleted in IBD-associated diarrhea. (A low iron or vitamin B12 level can lead to anemia and may indicate decreased absorption from CD that affects the small intestine.)

✓ Erythrocyte sedimentation rate, C-reactive protein levels imperfectly correlate with inflammation and disease activity.
✓ Albumin, ferritin may indicate absorption or loss of calcium, magnesium, vitamin B12 problems. Serum ferritin can be elevated in active IBD and may be in the normal range even in the face of severe iron deficiency. Transferrin saturation can also be done to evaluate anemia. Also test for soluble transferrin receptor (sTfR) can also be done.

Liver function tests (International Normalized Ratio, bilirubin, and albumin) help screen for liver and bile duct abnormalities seen in some IBD patients. Stool studies determine whether patients have treatable bacterial infections that can trigger a flare-up of IBD. In stool test following are measured:

✓ Routine fecal examinations and cultures to eliminate bacterial, viral, or parasitic causes of diarrhea.
✓ Clostridium difficile (should be considered even in absence of antecedent antibiotics).
✓ Occult blood or fecal leukocytes if a patient presents without a history of blood in stool can heighten indication for lower endoscopy. If lower endoscopy is readily available these tests are rarely indicated.
✓ Cytomegalovirus (should be considered in those on immunosuppressive or chronic steroids).
✓ Calprotectin, lactoferrin, α1-antitrypsin levels
3.9.2 Antibody Tests

Sometimes a definitive diagnosis of ulcerative colitis or Crohn's disease cannot be made. This occurs in approximately 10-15% of patients. These unclear cases are called “indeterminate colitis.” For these patients, there are antibody blood tests.

- The antibodies are "perinuclear anti-neutrophil antibody" (pANCA) and "anti-Saccharomyces cerevisiaeantibody" (ASCA).
  - Positive p-ANCA antigen and negative ASCA test suggests UC.
  - Negative p-ANCA antigen and positive ASCA test suggests CD.
  - These tests are unnecessary as screening tests particularly if endoscopy or imaging is going to be pursued for more definitive diagnoses. p-ANCA can be positive in Crohn’s colitis and hence may not be able to distinguish CD from UC in otherwise unclassified colitis.
  - ASCA is more specific for CD.

- Celiac antibody testing should be pursued unless presentations include obviously no celiac features such as fistulas, perineal disease, and blood in the stool.
- To exclude intestinal TB (in area of high pretest probability) following tests are done.
  - Tuberculin purified protein derivative (PPD) skin test
  - Serum PPD antibody test.
  - Interferon assays.

3.9.3 Endoscopy

Several types of endoscopy are used to determine if the patient has ulcerative colitis or Crohn's disease and how much bowel is affected. A thin, flexible tube with a lighted camera inside the tip is used, which examines the lining of the gastrointestinal (GI) tract. The image is magnified and appears on a television screen. Each procedure is named for the part of the GI tract examined:

**Sigmoidoscopy** — Examines the lining of the lower third of the large intestine (the sigmoid colon). It is a useful test to confirm the presence of inflammation or a source of bleeding (such as hemorrhoids) within the reach of the scope. Sigmoidoscopy helps rule out infectious causes of inflammation, such as disease caused by bacteria, which may mimic IBD. It is also useful in evaluating symptoms that do not respond to
current treatment or that return despite treatment. In these cases, one can take biopsies from any abnormal areas to confirm the diagnosis as well, to exclude treatable infections that can trigger an IBD flare-up.

**Colonoscopy** — Examines the lining of the large intestine (colon), and the small intestine. A full colonoscopy can assess the complete extent of colitis. This is important for determining the type of therapy offered. It is also useful for evaluating and taking biopsies of the distal small intestine (terminal ileum), which is important in the evaluation of CD. Chronic inflammation of the colon increases the lifetime risk of colon cancer compared to the general population. Years of inflammation may lead to gene abnormalities in the cells within the lining the colon (called dysplasia). Dysplasia can only be seen under the microscope. When colonoscopy is performed to look for dysplasia, multiple biopsies are taken throughout the entire colon and rectum.

**EGD (Esophagogastroduodenoscopy)** — Examines the lining of the esophagus, stomach, and duodenum (first part of the small intestine). EGD is a common procedure that is used to evaluate a wide variety of symptoms, such as abdominal pain, nausea, vomiting, and painful swallowing. Unlike UC, CD can affect the esophagus, stomach, and small bowel. If small bowel Crohn's is suspected, radiological tests, such as small bowel X-rays and other scans are recommended.

**ERCP (Endoscopic retrograde cholangiopancreatography)** — Examines the bile ducts in the liver and the pancreatic duct. A small percentage of patients with IBD also may have a liver disease called "primary sclerosing cholangitis" or PSC. PSC is suspected if repeatedly abnormal blood tests results reflect the activity of enzymes in the liver. ERCP is a method that combines X-ray and endoscopy to examine bile ducts and pancreatic ducts. In EGD, a tube is passed through stomach and into small bowel. The papilla, a small bump with a tiny opening in duodenum, leads to biliary and pancreatic ducts. A small catheter is introduced through the papilla into either bile ducts or pancreatic duct and contrast dye is injected. An X-ray then demonstrates the anatomy of ducts as outlined by the contrast dye.

**Endoscopic ultrasound (EUS)** - An ultrasound probe attached to an endoscope is used to obtain deep images of the gut. In IBD, this is most often used to look at fistulas in the rectal area. Fistulas are abnormal channels or loops from the intestine to
another part of the intestine or another organ of the body and are a complication of CD. EUS can determine the depth and extent of the fistula and biopsies can be taken if needed.

**Capsule "Mini-camera" endoscopy** — Patients swallow computerized cameras in vitamin-sized capsules to produce images of sections of the small intestine that are beyond the reach of an EGD. The preparation for the test consists of fasting after the evening meal. The morning of the test, patients are fitted with a belt that contains a recorder. Then swallow the endoscopy capsule. The capsule travels down the small intestine and transmits approximately 50,000 images to the recorder. At the end of the day, images can be downloaded. It takes approximately two hours to review the images. The capsule is excreted in the stool normally and effortlessly. For patients with CD, patients are examined carefully to determine strictures or bowel obstructions before the capsule is used.

### 3.9.3 Radiology Test

Radiologic tests provide information that endoscopy cannot and can image the small intestine.

**Plain X-rays**

X-rays are the oldest way of imaging the inside of the body. Plain X-rays (without contrast) are a quick, inexpensive, and effective way of detecting blockage in the small or large intestine. Patients with Crohn's disease can have inflammation of the small bowel that narrows the intestine and prevents the easy passage of stool and air. This is called a small bowel obstruction. The large bowel can also become blocked and dilated. People with ulcerative colitis can develop a widening of the large bowel called toxic megacolon. This is a serious complication. These conditions are easily visible on plain X-ray.

**X-rays with Contrast**

Contrast X-rays are used together with endoscopy in monitoring and treating IBD. These X-rays track special liquid ("contrast") as it passes through the intestine,
highlighting specific conditions. Contrast X-rays of the small intestine examines the lining of the small intestine that is beyond the reach of the endoscope.

The contrast used for these tests is barium. It is a thick, chalky liquid that can be given by mouth or via the rectum. The preparation for barium enema is similar to that of a colonoscopy and requires a liquid diet the day before, a bowel preparation to purge the colon of stool and debris, and no food or drink beginning at midnight before the test.

**CT scan**

CT scan takes simultaneous X-rays from several different angles to reconstruct a realistic image of the internal organs. Contrast may be given by mouth, by rectum or through the veins to improve the quality of the test. During the test, patient lies on a special table that advances through the scanner so images are taken at each level of abdomen. A CT scan of the abdomen takes five to 20 minutes to complete. Abdominal CT scans are used primarily to evaluate IBD patients who have abdominal pain and fever. Although this test can confirm the inflammation present in the small or large intestine, it is used more importantly to rule out complications of IBD like intra-abdominal abscesses, strictures, small bowel obstructions, fistulas and bowel perforation.

**MRI**

Magnetic Resonance Imaging (MRI) is used to evaluate perianal fistulas and abscesses in patients with IBD. It is useful for viewing internal organs, muscles, soft tissue, and the brain. It works by detecting the signal produced by atoms in response to a strong magnetic field. It converts this signal into a realistic image of the body. During the test, patients lie on a special table inside the MRI scanner while the magnet generates the images.

**Leukocyte scintigraphy ("tagged white blood cell scan")**

Leukocyte scintigraphy or "tagged white blood cell scan" detects white blood cell accumulation in inflamed tissue. It involves drawing blood from the arm, labeling the white blood cells in the test tube with harmless amount of a radioactive substance, and
then reinjecting the blood into bloodstream. The labeled white blood cells travel through the bloodstream and migrate into the inflamed tissue. A special camera detects where the white blood cells accumulate and therefore can assess where and how much inflammation is present. Leukocyte scintigraphy has been used to detect the location of bowel inflammation and to evaluate treatment response in IBD. It is a relatively safe test and entails less radiation exposure than contrast X-ray or CT scan.

3.9.4 Ultrasound

Ultrasound technology is used to study many organs in the abdomen, most typically the liver, gallbladder, or organs in the pelvis. Ultrasound is harmless and relies on the shadows cast by sound waves. These tests are often used in combination with other radiological tests.

3.9.5 Differential Tests (Table 3.8)

Table 3.8: Tests used to differentiate Between Crohn's Disease and Ulcerative Colitis

| Clinical | ✓ In UC, frequent small volume diarrhea with urgency and predominantly bloody diarrhea  
|          | ✓ In CD, diarrhea accompanied by abdominal pain and malnutrition, stomatitis, abdominal mass, perianal lesions |
| Endoscopy | Ulcerative colitis almost always involves the lower left colon and rectum and can be diagnosed using sigmoidoscopy.  
|          | ✓ Diffuse superficial colonic inflammation–Involvement of rectum but can be patchy  
|          | ✓ Shallow erosions and ulcers  
|          | ✓ Spontaneous bleeding  
|          | Crohn's disease may require colonoscopy as well. Endoscopy often reveals ulcers, diseased regions that have a cobblestone-like appearance in Crohn's disease, but not in ulcerative colitis.  
|          | ✓ Discontinuous transmural asymmetric lesions  
|          | ✓ Mainly involving ileum and right- side colon ulcers  
|          | ✓ Cobblestone appearance |
Review of Literature

<table>
<thead>
<tr>
<th>✓ Longitudinal ulcer</th>
</tr>
</thead>
</table>

**X-Rays (Barium Enema) or Computed Tomography Scans**

| ✓ In ulcerative colitis, inflammation is usually evenly distributed on the surface lining of the intestine, and the bowel wall bleeds easily when touched with a swab. The pattern observed in Crohn's disease is usually one of scattered patches of ulcers that are deep, thick and large. |
| ✓ Crohn's disease produces pockets (fissures) or channels (fistulas). They do not occur with UC. |
| ✓ In ulcerative colitis the ileum is often dilated while it is narrowed in Crohn's disease. |

**Histopathological**

| In UC, |
| ✓ Diffuse inflammation in mucosa or submucosa |
| ✓ Crypt architecture distortion |
| In CD, |
| ✓ Granulomatous inflammation |
| ✓ Fissures or apthous ulcers can be seen; often |
| ✓ Transmural inflammation |

**Laboratory Tests**

| ✓ Tissue samples obtained from a patient with Crohn's disease may reveal granulomas, small collections of inflammatory cells. Granulomas may also be present in other conditions, however. Tissue samples should also be examined for the presence of cancerous cells. |
| ✓ About 70% of tests for antibodies in people with UC will show perinuclear-staining antineutrophil cytoplasmic antibodies. Over 50% of Crohn's people have anti-Saccharomyces cerevisiae antibodies. Such tests are expensive and infrequently performed, but they may be useful in cases of uncertainty. |

---

**3.9.7 Problems in diagnosis of IBD in children:**

Children and adolescents with IBD present unique challenges to physicians and all health-care providers. It is not difficult to recognize IBD in children, when it presents with classical symptoms such as bloody diarrhoea, abdominal pain and weight loss. However, some children present with abdominal pain and depression. In addition, several children will be initially diagnosed as having a bacterial gastroenteritis with a proven positive faecal culture. It seems to be the triggering event in these children,
and if adequate therapy fails, colonoscopy is indicated. Children seen for chronic abdominal pain simple routine blood tests including full blood count and erythrocyte sedimentation rate are almost always abnormal in children with IBD. But most importantly, growth retardation is common in children with IBD and is more often found in Crohn's disease than in ulcerative colitis. Therefore, it is essential to follow the growth of children at the beginning and during treatment of IBD. Rarely, extra-intestinal manifestations, particularly arthritis, can be the first and sometimes only initial symptom for months to years in children with IBD. About 2% of all patients with IBD present before the age of 10 years, but 30% present between the age of 10 and 19 years. A significant proportion of young patients with IBD will develop the disease just prior to or during puberty. Adolescent growth is characterized by rapid accumulation of lean body mass and any inflammatory disease occurring at this time is likely to have a major impact on nutritional status and growth. Most of the growth retardation is seen in children with CD, approximately 30%. However, also in UC 15% will show a reduction in growth. The higher percentage in CD could be due to the disease itself or to the relative subtlety of the intestinal manifestations of CD, mainly abdominal pain and general malaise. Not only growth, but also delayed puberty, is a sign of an ongoing disease that most likely needs more intensive treatment. The potential benefit of nutritional therapy should be seriously considered in addition to aggressive medical therapy including steroids and other immunosuppressive agents such as azathioprine. Malnutrition is also primarily responsible for growth failure. The malnutrition is caused by inflammation and loss of appetite. Recommendations for nutritional therapy include an increase in energy and protein intake to 150% of recommended daily allowances for height and age. Some studies have shown the benefit of nocturnal nasogastric infusion as supplements of daily intake. Importantly, nutritional support has been shown to be as effective as steroids in achieving remission of disease in children.

3.10 Treatment for IBD

3.10.1 Pharmacological treatment (Fernando, 2009):

There are five basic classes of medications used in the treatment of IBD. They are:

A. Aminosalicylates
B. Corticosteroids
C. Immunomodulators
D. Antibiotics
E. Biological therapies

Medical treatment for Crohn's disease and ulcerative colitis has two main goals: achieving remission (the absence of symptoms) and, once that is accomplished, maintaining remission (prevention of flare-ups). To accomplish these goals, treatment is aimed at controlling the ongoing inflammation in the intestine—the cause of IBD symptoms.

A. Aminosalicylates

Aminosalicylates are compounds that contain 5-aminosalicylic acid (5-ASA). These drugs, which can be given either orally or rectally, interfere with the body's ability to control inflammation. They are effective in treating mild-to-moderate episodes of ulcerative colitis and Crohn's disease, as well as preventing relapses and maintaining remission.

Oral Medications

Sulfasalazine (Azulfidine®), the first aminosalicylate to be widely used for IBD, is effective in achieving and maintaining remission in people with mild-to-moderate disease. The active portion of the drug, 5-ASA, is bonded to sulfapyridine, a compound that delivers 5-ASA to the intestine but comes with disagreeable side effects in some patients, such as headache, nausea, and rash. However, sulfasalazine is inexpensive and effective for the many patients who can tolerate it. The newer oral drugs that deliver 5-ASA without sulfapyridine include:

- mesalamine (Asacol®, Pentasa®);
- olsalazine (Dipentum®); and
- balsalazide (Colazal™).

Up to 90 percent of people who cannot tolerate sulfasalazine are able to take other 5-ASAs.

Alternative Methods of Delivery

In addition to conventional oral preparations, there are several other ways to deliver 5-ASA to the bowel. Patients with Crohn's disease or ulcerative colitis may have bowel
inflammation in different locations, hence various 5-ASAs have been designed to be released in different areas of the bowel:

- Local mesalamine preparations are effective precisely because they bypass the stomach to avoid early digestion, and then release close to the inflamed section of the bowel. There, the medication coats the inflamed bowel lining, thus decreasing the inflammation.

- Enema formulations (Rowasa®) allow mesalamine to be applied directly to the left colon. Rowasa is effective in mild-to-moderate colitis that affects only the left side of the colon. Up to 80 percent of patients with left-sided disease benefit from using this therapy once a day.

- Suppositories (Canasa®) deliver mesalamine directly from the rectum up to the sigmoid colon. A high proportion of patients with ulcerative proctitis will respond to mesalamine suppositories. These are usually given in single or twice-daily doses. A combination of mesalamine enemas and pills may be more effective than pills alone.

- Oral, delayed-release preparations (Pentasa) can release 5-ASA directly to the small intestine and colon, or to the ileum and/or the colon (Asacol), or to the colon only (Dipentum, Colazal, sulfasalazine).

### Side Effects

- **Sulfasalazine:** Side effects may include headache, nausea, loss of appetite, vomiting, rash, fever, and decreased white blood cell count. Sulfasalazine can also decrease sperm production and function in men while they are taking the medication (sperm count becomes normal after the medication is discontinued). It has been rarely associated with pancreatitis.

- **Mesalamine:** Side effects may include abdominal pain and cramps, diarrhea, gas, nausea, hair loss, headache, and dizziness. People with kidney disease should use caution when taking mesalamine, as some studies have found that the medication may be linked to kidney problems. Patients on long-term mesalamine therapy may be monitored regularly for any signs of decreased kidney function. Pancreatitis is a rare side effect of mesalamine use.

- **Olsalazine:** Diarrhea is the most common side effect. It can be reduced by taking the medication with food. Less common side effects may include headache, rash, and fatigue. Even rarer are hair loss, pancreatitis, or pericarditis.


- **Balsalazide**: The most common side effects are headache and abdominal pain. Less common are nausea, diarrhea, and vomiting.

**Drug Interactions**

- Sulfasalazine cannot be used by people who are allergic to or cannot tolerate sulfa drugs (approximately one-third of people being treated). Other sulfa-containing drugs should be used with caution while taking sulfasalazine.

**B. Corticosteroids**

Corticosteroids were introduced as therapy for IBD in the 1950s. Since that time, these anti-inflammatory drugs have been the mainstay of treatment for acute flare-ups of disease. In addition to their anti-inflammatory action, corticosteroids also are immunosuppressive. Corticosteroids are recommended only for short-term use in order to achieve remission. Long-term use is not advised because of undesirable side effects. Corticosteroids are usually given in the lowest possible dosage for the shortest amount of time.

**Oral Medications**

In people with moderate to severe active disease, corticosteroids in pill form are usually effective. These include:

- prednisone (Deltasone®)
- methylprednisolone (Medrol®)
- hydrocortisone

The drugs may be used alone or together with aminosalicylate to reduce acute inflammation.

**Budesonide**

Budesonide (Entocort® EC), is used to treat mild-to-moderate Crohn's disease. Because 90% of the drug is inactivated before it reaches the rest of the body, it causes fewer side effects than traditional corticosteroids such as prednisone. Side effects include headache, respiratory infection, and nausea, among other corticosteroid-associated side effects.
Alternative Methods of Delivery

For people who do not respond to oral forms of the drugs, it may be necessary to administer corticosteroids through other routes. These include:

- Rectally as enemas (hydrocortisone, methylprednisone, Cortenema®), foams (hydrocortisone acetate, ProctoFoam-HC®), and suppositories. These preparations are helpful for patients with mild-to-moderate ulcerative colitis that is limited to the rectum or lower part of the colon. They also may be used, together with other therapies, in people with mild-to-moderate disease.
- Intravenously (IV): methylprednisone and hydrocortisone. Patients with severe and extensive disease may require treatment with IV corticosteroids.

Side Effects

The undesirable side effects of corticosteroids are dependent on both dose and duration of treatment. For many, the side effects of steroids outweigh their anti-inflammatory benefits. Some of the most common ones include the following:

- high blood pressure
- rounding of the face ("moon face")
- increased risk of infection
- weight gain
- acne
- mood swings
- psychosis and other psychiatric symptoms
- increased facial hair
- cataracts
- stretch marks
- high blood sugar levels
- osteoporosis
- insomnia

There are number of ways to reduce the risk of developing side effects. These include rapid but careful tapering off of steroids; alternate-day dosing; rectally applied corticosteroids; and rapidly metabolized corticosteroids such as budesonide. To help prevent osteoporosis, calcium supplements as well as vitamin D can be prescribed. Another option is the use of bisphosphonates, such as risedronate (Actonel®) and...
alendronate (Fosamax®). These compounds, which have been shown to help avert bone loss, are effective in treating and preventing steroid-induced osteoporosis.

C. Immunomodulators

Immunomodulators weaken or modulate the activity of the immune system. Immunomodulators are most often used in organ transplantation to prevent rejection of the new organ, and in autoimmune diseases such as rheumatoid arthritis. These drugs are appropriate for those who:

- do not respond to aminosalicylates, antibiotics, or corticosteroids
- have steroid-dependent disease or frequently require steroids
- have experienced side effects with corticosteroid treatment
- have perineal disease that does not respond to antibiotics
- have fistulas
- need to maintain remission

An immunomodulator may be combined with a corticosteroid to speed up response during active flares of disease. Lower doses of the steroid are required in this case, producing fewer side effects. Corticosteroids also may be withdrawn more rapidly when combined with immunomodulators. For that reason, immunomodulators are sometimes referred to as "steroid-sparing" drugs.

Oral Medications

The first two immunomodulators to be used widely in IBD are azathioprine (Imuran®, Azasan®) and 6-mercaptopurine (6-MP, Purinethol®), drugs that are chemically quite similar. They are used to maintain remission in Crohn's disease and ulcerative colitis. Both have a slow onset of action (three to six months for full effect). Accordingly, they are usually given along with another faster-acting drug such as corticosteroids.

Other immunomodulators to treat IBD are cyclosporine A (Sandimmune®, Neoral®) and tacrolimus (Prograf®), both used for organ transplantation as well. Cyclosporine A has a more rapid onset of action (one to two weeks) than azathioprine and 6-MP. It is useful in people with active Crohn's disease, but only when given intravenously and at high doses. Both cyclosporine A and tacrolimus have been more effective in treating people with severe ulcerative colitis, and are generally given until one of the
slower-acting immunomodulators begins to work or until the patient undergoes curative surgery. Tacrolimus can be used in Crohn's disease when corticosteroids are not effective or when fistulas develop.

**Alternate Methods of Delivery**

Tacrolimus may be applied topically for Crohn's disease that affects the mouth or perineal area. Topical tacrolimus is also used to treat *pyoderma gangrenosum*, an ulcerating skin disorder often associated with IBD. Methotrexate (MTX®, Rheumatrex®, Mexate®) works more rapidly than azathioprine or 6-MP, and is given by weekly injections. It is an effective option for people with Crohn's disease who have not responded to other treatments and cannot tolerate other immunosuppressants.

**Side Effects**

- **Azathioprine and 6-MP**: Infrequently reported side effects may include headache, nausea, vomiting, diarrhea, and malaise. Sometimes changing from azathioprine to 6-MP or vice versa may reduce some of these reactions. Canker sores in the mouth, rash, fever, joint pain, and liver inflammation are unlikely to be affected by changing from azathioprine to 6-MP or vice versa. Less common side effects include pancreatitis and bone marrow suppression, which may increase the risk of infection or serious bleeding. A return to normal blood cell production may take several weeks after discontinuing the medication.
- **Cyclosporine and tacrolimus**: Infrequently reported side effects include decreased kidney function, hepatitis, increased risk of infections, diabetes, increased cholesterol levels, sleep problems, headache, mild tremor, high blood pressure, swollen gums, tingling of the fingers and feet, increased facial hair, and increased risk of lymphoma.
- **Methotrexate**: Infrequently reported side effects include flu-like symptoms (nausea, vomiting, headache, fatigue, and diarrhea) and low white blood cell count. Less common but more serious side effects include scarring of the liver and lung inflammation. Scarring of the liver can be made worse by diabetes, being overweight, and alcohol consumption.
Drug Interactions

- Proneness to mild infection with symptoms like fever, chills, or sore throat.
- Bone marrow suppression, renal and hepatotoxicity.
- Methotrexate use should be avoided by pregnant women and by both men and women for several months before conception because it may lead to pregnancy loss or possible birth defects.

D. Antibiotics

Antibiotics are frequently used as a primary treatment approach in IBD. They control symptoms of IBD by reducing intestinal bacteria and by directly suppressing the intestine's immune system. Antibiotics are effective as long-term therapy in some people with IBD, particularly Crohn's disease patients who have fistulas or recurrent abscesses. Patients whose active disease is successfully treated with antibiotics may be kept on these as maintenance therapy as long as the medications remain effective.

Oral Medications

The two most commonly prescribed in IBD are:

- Metronidazole (Flagyl®)
- Ciprofloxacin (Cipro®)

Metronidazole is the most extensively studied antibiotic in IBD. As a primary therapy for active Crohn's, this drug has been shown to be superior to placebo and equal to sulfasalazine—especially when the illness affects the colon. Metronidazole also has been shown to reduce the recurrence of Crohn's for the first three months after ileum resection surgery. In more than 50 percent of those treated, metronidazole can be effective in managing perineal Crohn's disease. Metronidazole also is used to suppress an overgrowth of *C. difficile*, a type of bacteria that causes inflammation. Another indication for metronidazole is in people who develop "pouchitis" after ileal-pouch anal anastomosis surgery. Ciprofloxacin is commonly used to treat active Crohn's disease and pouchitis. Both metronidazole and ciprofloxacin are also used in intravenous forms.
Side Effects

Metronidazole: Common side effects may include nausea, vomiting, loss of appetite, a metallic taste, diarrhea, dizziness, headaches, and discolored urine (dark or reddish brown). Another side effect of long-term use is tingling of the hands and feet, which may persist even after the drug is discontinued.

Ciprofloxacin: Side effects may include headache, nausea, vomiting, diarrhea, abdominal pain, rash, and restlessness, all of which are rare.

Drug Interactions

- Metronidazole affects the metabolism of alcohol, which may result in nausea and vomiting.
- Reduces effects of Ciprofloxacin is seen due to interaction with antacids, vitamin and mineral supplements that contain calcium, iron, or zinc.
- Phototoxicity.
- Antibiotics can decrease the effectiveness of oral contraceptive medications.
- Antibiotics can interfere with warfarin (Coumadin®) and increasing the risk of bleeding. Adjustments in the dose of warfarin may be required if antibiotics are started.

E. Biological therapy

Promising targets for biological therapy include drugs modulating the release or actions of TNF-α, interleukins, interferon gamma, integrin receptors, adhesion molecules, colony-stimulating factors, GM-CSF and others. They affect the body's immune response in IBD by inhibiting proinflammatory and promoting anti-inflammatory cytokines actions.

Anti-TNF agents: TNF-alpha is a cytokine, a specialized protein that promotes inflammation in the intestine in other organs and tissues. Anti-TNF has been used in ulcerative colitis which suppress tumor necrosis factor alpha. Examples include Adalimumab (Humira®), Certolizumab pegol (Cimzia®), Infliximab (Remicade®) and Thalidomide.

Infliximab (Remicade®) is the first FDA-approved biological therapy for Crohn's disease and fistulizing Crohn's disease, as well as for ulcerative colitis. It is given as a
Review of Literature

drip via intravenous infusion. It is used for people with moderately-to-severely active disease who have not responded well to other therapies.

Adalimumab (Humira®) is approved for use in Crohn's disease. It also binds to and inactivates tumor necrosis factor alpha, but it is a fully human monoclonal antibody. It is given by injection. It is used for people with moderately to severely active disease who have not responded well to other therapies, and who have lost response or are unable to tolerate infliximab.

Certolizumab pegol (Cimzia®) is also approved for use in Crohn's disease. It is the first and only PEGylated anti-TNF-alpha. The antibody portion combines with a special chemical called polyethelyene glycol, which delays its excretion from the body. It is given by injection. It is used to reduce the signs and symptoms of moderately to severely active Crohn's disease in adult patients who have not been helped enough by usual treatments.

Thalidomide was originally used for its sedative and antiemetic properties, has recently been shown to inhibit TNF-α production by monocytes and other cells. Thalidomide was found to be efficacious in patients with chronically active, steroid-dependent CD.

**Integrin Receptor Antagonist**

Integrin receptor antagonists block integrin receptors playing key role in inflammation. Example includes Natalizumab (Tysabri®). Natalizumab (Tysabri®) was approved for use in Crohn's disease. It is a recombinant humanized monoclonal antibody thought to inhibit certain types of white blood cells that are involved in the inflammatory process. It is given by IV infusion for moderate to severely active Crohn's patients, who have had an inadequate response to, or are unable to tolerate, conventional and anti-TNF disease therapies.

**Side Effects of anti-TNF agents and Integrin receptor antagonist:**

The most common side effects with the anti-TNF agents include infusion or injection site reactions (redness, swelling, itching, bruising, rash), upper respiratory infections, headaches, rash, and nausea. There have been some reports of serious infections associated with anti-TNF agent use, including tuberculosis and sepsis. Treatment may lead to development of new or worsening symptoms of heart failure—namely shortness of breath or swelling of the ankles or feet. On rare occasions, bleeding disorders may be seen. Nervous system disorders also have been reported
occasionally. Reports of lymphoma in patients taking anti-TNF agents are rare. Progressive multifocal leukoencephalopathy, a rare brain infection, has been reported with natalizumab use. Natalizumab may also cause liver damage and allergic reactions. Patients with HIV infection or AIDS, leukemia or lymphoma, or an organ transplant are not recommended to take Natalizumab.

3.10.2 Non-pharmacological Treatment

**Pro-, Pre- and Synbiotics in the treatment of IBD** (Dimitrios and George, 2008)

Microbiota modulates gut physiology and immunological function in IBD. Use of probiotics, prebiotics and synbiotics aim to restore the balance of the gastrointestinal microflora in order to reduce or prevent intestinal inflammation.

**Probiotics**

Probiotics are living microorganisms, able to survive stomach acid and bile, maintain viability throughout extended periods of storage, and safe for human consumption, inducing beneficial results in the host. Several mechanisms of action of probiotics relative to prevention and treatment of IBD have been reported (Table 3.9) (Bengmark, 2002), such as antimicrobial activity and suppression of bacterial growth, immunomodulation and initiation of an immune response, enhancement of barrier activity and suppression of human T-cell proliferation. Derived originally from cultured food, especially dairy products, this group includes *Lactobacillus* species, *Bifidobacterium* species, *E. coli* Nissle 1917, *Saccharomyces boulardii*, *C. butyricum*, VSL#3 and *Lactococcus lactis* genetically engineered to secrete IL-10. Another new approach is the use of helminthes as probiotic.

**Prebiotics**

Prebiotics are indigestible carbohydrates, which stimulate the growth of particular species of the microflora of the host, resulting in an ameliorated enteric function (Table 3.10). These nondigestible food constituents act primarily by increasing the population of certain bacteria and thus quantitatively altering the microflora (Bengmark, 2002). When reaching the colon, they are fermented by anaerobic bacteria, producing short-chain fatty acids and gas (CO2 and H2). As a result, intraluminal pH drops, favouring the increase of *Bifidobacteria*, *Lactobacilli* and nonpathogenic *E. coli* and decreasing *Bacteroidaceae*. 
The fermentation of carbohydrates also leads to the production of acetic, propionic and butyrate acids that are involved in several colon-specific and systemic pathways. Acetate is used as cell fuel and propionic acid is involved in cholesterol synthesis, amongst others. Of these, butyrate is of great importance to the metabolism of the colonocyte. It has also been proven that butyrate exerts anti-inflammatory action, by in vitro reducing the expression of TNF-α-related cytokines and upregulating IL-10 in mice, possibly by inhibition of the nuclear translocation of NF-κB (Segain et al, 2000). Butyrate enemas have been used with success in UC, but the need for continuous administration limits its use. The most commonly used prebiotics in experimental models and clinical trials are lactulose, lactosucrose, oligofructose and inulin, psyllium, germinated barley foodstuff, fructo- and milk-oligosaccharides.

**Synbiotics**

Synbiotics are combined products of pro- and prebiotics (Bengmark, 2002). Suppression of colonic carcinogenesis is observed by use of a combination of oligofructose-enriched inulin, *Lactobacillus* and *Bifidobacterium*, while the same mixture stimulated secretion of IgA and IL-10 by the caecum (Bengmark, 2008). A combination of *B. breve*, *Lactobacillus casei* and galacto-oligosaccharides substantially improved bowel function in a girl with short bowel syndrome. In UC patients, combination of *B. longum*, inulin and oligofructose reduces sigmoidoscopy and rectal biopsy inflammation scores, with a concurrent reduction of TNF-α and IL-1b levels (Furrie et al, 2005).
### Table 3.9: Mechanism of Probiotics on the intestinal pathophysiology

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Effects on intestinal pathophysiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus</td>
<td>Inhibition of NF-κB nuclear translocation, blockage of IκB degradation (<em>L. reuteri</em>)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of production of IL-6 (<em>L. casei</em>)</td>
</tr>
<tr>
<td></td>
<td>Upregulation of intestinal MUC3 and MUC3 mRNA expression</td>
</tr>
<tr>
<td></td>
<td>Inhibition of apoptosis of intestinal epithelial cells (<em>L. GG</em>)</td>
</tr>
<tr>
<td></td>
<td>Decreased translocation of commensal bacteria via the mesenteric lymph nodes and liver (<em>L. plantarum, L. GG</em>)</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>Induction of COX-2 expression (<em>L. rhamnosus</em>)</td>
</tr>
<tr>
<td></td>
<td>Suppression of the growth of <em>Bacteroides vulgatus</em> (<em>B. infantis</em>)</td>
</tr>
<tr>
<td></td>
<td>Increase in IL-10 secreted by mesenteric lymph nodes</td>
</tr>
<tr>
<td></td>
<td>(Bifidobacterium-fermented milk)</td>
</tr>
<tr>
<td></td>
<td>Reduction of MPO activity, tissue contents of immunoglobulin, TNF-α (Bifidobacterium-fermented milk)</td>
</tr>
<tr>
<td></td>
<td>Alteration of bacterial translocation and SCFA production</td>
</tr>
<tr>
<td></td>
<td>(<em>B. infantis</em>)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of discordant T-cell activation</td>
</tr>
<tr>
<td><em>Escherichia coli Nissle 1917</em></td>
<td>Downregulation of the expansion of newly recruited T cells into the mucosa</td>
</tr>
<tr>
<td></td>
<td>Intestinal inflammation regulation via TLR-2 and TLR-4</td>
</tr>
<tr>
<td></td>
<td>Restoration of disrupted epithelial barrier in the colonic epithelial cell line T84</td>
</tr>
<tr>
<td><em>Saccharomyces boulardii</em></td>
<td>Limitation of infiltration of T-helper 1 cells into the mucosa</td>
</tr>
<tr>
<td></td>
<td>NF-κB blocking and IL-8 downregulation</td>
</tr>
<tr>
<td><em>Clostridium butyricum</em></td>
<td>Production of high levels of short chain fatty acids</td>
</tr>
<tr>
<td>VSL#3</td>
<td>Reduction of secretion of TNF-α and interferon-γ</td>
</tr>
<tr>
<td></td>
<td>Improvement of the colonic barrier function</td>
</tr>
<tr>
<td></td>
<td>Inhibition of Salmonella Dublin invasion into T-84 cells</td>
</tr>
<tr>
<td></td>
<td>Conversion of linoleic acid into conjugated linoleic acid</td>
</tr>
<tr>
<td></td>
<td>Inhibition of TNF-α-induced IL-8 secretion, mitogen-activated protein kinase activation and NF-κB activation in HT-29 cells (Cpg DNA)</td>
</tr>
<tr>
<td></td>
<td>Upregulation of mucin expression</td>
</tr>
<tr>
<td><em>Helminthes</em></td>
<td>Skewing of the immune response towards Th2</td>
</tr>
</tbody>
</table>

### Table 3.10: Mechanism of Prebiotics on the intestinal pathophysiology

<table>
<thead>
<tr>
<th>Prebiotics</th>
<th>Effects on intestinal pathophysiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common effects</td>
<td>Reduction of intraluminal pH</td>
</tr>
<tr>
<td></td>
<td>Favouring of <em>Bifidobacteria</em> and <em>Lactobacilli</em> vs. <em>Bacteroidaceae</em></td>
</tr>
<tr>
<td></td>
<td>Short-chain fatty acid (SCFA) production</td>
</tr>
<tr>
<td></td>
<td>Regulate colonic mucosa physiology via the production of SCFA</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Reduction of MPO activity</td>
</tr>
<tr>
<td></td>
<td>Production of TNF-α and leukotriene B</td>
</tr>
<tr>
<td>Germinated barley foodstuff</td>
<td>Decrease in serum IL-8 and α1-acid glycoprotein</td>
</tr>
<tr>
<td>Fructo-oligosaccharides</td>
<td>Upregulation of IL-10 expression in dendritic cells</td>
</tr>
<tr>
<td>Goat’s milk oligosaccharides</td>
<td>Decrease the colonic MPO activity, increase MUC-3</td>
</tr>
</tbody>
</table>
Dietary Therapy (David and Phil, 1998)

There is no specific dietary therapy for patients with UC, although a few (< 5%) may improve with avoidance of cow’s milk, and some with proctitis and proximal constipation may benefit from fibre supplementation. Patients with stricturing small bowel CD should avoid high residue foods (eg. citrus fruit segments, nuts, uncooked vegetables) which might cause bolus obstruction. All patients with IBD, particularly if it is active or extensive, are at risk of nutritional deficiencies which need replacement as necessary. Over the last 20 years, it has become clear that in children with CD, as well as in adults with extensive small bowel disease and in those who respond poorly to, or prefer to avoid corticosteroids, an alternative therapy is a liquid formula diet. This can either be elemental (aminoacid-based), protein hydrolysate (peptide containing) or polymeric (containing whole protein and not therefore hypoallergenic), and is given for 4-6 weeks as the sole nutritional source (Griffiths et al, 1995). This approach is probably as effective as corticosteroid therapy in the short-term, about 60% patients achieving remission. Unfortunately, after the resumption of a normal diet, many patients relapse (50% at 6 months): whether this can be prevented by selective and gradual reintroduction of particular foods to which individual patients are not intolerant, or by the intermittent use of further enteral feeding for short periods, remains to be proven.

The success of enteral nutrition as a primary treatment for CD is also limited by its cost, the unpleasant taste of some of the available preparations, the need often to give the feed by nasogastric tube, and the poor compliance of many patients in adhering to it. Such therapy does, nevertheless, offer a valuable alternative in the well-motivated minority of patients for whom it is appropriate.

Correcting nutrient deficiencies in UC and CD:  (Galland, 1999; Gassull et al, 2004)

✓  **Folic Acid:** Reduced folic acid in patients with IBD is associated with hyperhomocysteinemia, a risk factor for deep vein thrombosis, an extra-intestinal complication of inflammatory bowel disease.

✓  **Vitamin B12:** Because vitamin B12 absorption may be impaired by ileal inflammation and by small bowel bacterial overgrowth, deficiency of vitamin B12 has long been described as a potential complication of CD.
**Vitamin B6:** Vitamin B6 levels are significantly lower in patients with IBD than controls; low levels are associated with active inflammation and hyperhomocysteinemia. Although some homocysteine is removed by folate-B12-dependent remethylation, the bulk of homocysteine is converted to cystathionine in a reaction catalyzed by vitamin B6.

**Vitamins E and C:** Blood levels of vitamins E and C are often reduced in patients with IBD. Administration of alpha-tocopherol 800 IU per day and vitamin C 1000 milligrams per day to patients with stable, active CD decreased markers of oxidative stress.

**Vitamin A:** Although levels of carotenoids and retinol are diminished in patients with active CD, low levels appear to be related not to malabsorption but to inflammation and a reduction in circulating retinol binding protein.

**Vitamin D:** Reduced blood levels of 25-OH cholecalciferol, the major vitamin D metabolite, are common in patients with CD, and are related to malnutrition and lack of sun exposure. Administration of vitamin D, 1000 IU per day for one year, prevented bone loss in patients with active disease. The major causes of bone loss in IBD, however, are the effects of inflammatory cytokines and glucocorticoid therapy which should be monitored in patients with IBD receiving vitamin D supplements.

**Vitamin K:** Biochemical evidence of vitamin K deficiency has been found in patients with ileitis and in patients with colitis treated with sulfasalazine or antibiotics. Serum vitamin K levels in CD are significantly decreased compared with normal controls and are associated with increased levels of undercarboxylated osteocalcin, indicating a low vitamin K status in bone. In patients with CD, undercarboxylated osteocalcin is inversely related to lumbar spine bone density. Optimal dose of vitamin K for correction of deficiency is not known. Patients with active disease may not absorb oral vitamin K, even at high dosage.

**Calcium:** Calcium supplementation is recommended for maintaining bone density in patients with IBD, especially those receiving glucocorticoids.

**Zinc:** Low plasma zinc is common in patients with CD and may be associated with clinical manifestations such as acrodermatitis, decreased activity of zinc-
dependent enzymes like thymulin and metallothionein, reduction in muscle zinc concentration and poor taste acuity. Zinc absorption is impaired and fecal zinc losses are inappropriately high. In patients with active disease, zinc sulfate, 200 milligrams per day significantly increases plasma zinc and thymulin activity.

- **Selenium**: Low selenium levels in patients with CD are associated with increased levels of TNF-alpha and decreased levels of the antioxidant enzyme, glutathione peroxidase. Patients with small bowel resection are at risk for severe selenium deficiency; monitoring of selenium status and selenium supplementation has been recommended for this group.

- **Magnesium**: Magnesium deficiency is a potential complication of IBD, a result of decreased oral intake, malabsorption and increased intestinal losses due to diarrhoea.

- **Chromium**: Glucocorticoid therapy increases urinary chromium excretion and chromium picolinate, 600 micrograms per day, can reverse steroid-induced diabetes in humans, with a decrease in mean blood glucose from 250 milligrams per dL to 150 milligrams per dL. Chromium supplementation may be of benefit for patients receiving glucocorticoids who manifest impaired glucose tolerance.

- **Iron**: Anemia occurs in about 30 percent of patients with IBD. Its causes include iron deficiency due to blood loss, cytokine-induced suppression of erythropoiesis and side effects of medication. It is also speculated that iron deficiency actually increases the IFN-gamma response in T_{H-1} driven inflammation and may contribute to aggravation of CD.

**Fish oils**: Biochemical studies indicate that 25 percent of patients with IBD show evidence of essential fatty acid deficiency. In experimental animals, fish oil feeding ameliorates the intestinal mucosal injury produced by methotrexate. In tissue culture, omega-3 fatty acids stimulate wound healing of intestinal epithelial cells. For patients with UC, a fish oil preparation supplying 3200 milligrams of eicosapentaneoic acid (EPA) and 2400 mg of docosahexaenoic acid (DHA) per day decreased symptoms and lowered the levels of leukotriene B4 (LTB4) in rectal dialysates, with improvement therapy of UC (Uritski et al, 2004). EPA, the active ingredient of fish oil capsules, decreases synthesis of leucotriene B4, thromboxane A2, prostaglandin
E2, platelet activating factor and interleukin-1 (David and Phil, 1998). An enteric-coated fish oil preparation, which is better tolerated than standard formulations, has been reported to reduce substantially the relapse rate in patients with inactive CD. Table 3.11 describes the recommended daily allowances for various nutrients as supplements.

**Table 3.11:** Correcting Nutrient Deficiencies in IBD (Kathleen and Julie, 2003)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Suggested Supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>10,000-25,000 IU daily*</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>25,000-100,000 IU daily</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>400-800 IU daily</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>500-1,000 mg daily</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>500 mcg-1 mg daily</td>
</tr>
<tr>
<td>Folic acid</td>
<td>400 mcg-1 mg daily</td>
</tr>
<tr>
<td>Calcium</td>
<td>500-1,000 mg daily**</td>
</tr>
<tr>
<td>Iron</td>
<td>30-60 mg daily***</td>
</tr>
<tr>
<td>Magnesium</td>
<td>300-500 mg daily</td>
</tr>
<tr>
<td>Selenium</td>
<td>200-400 mcg daily</td>
</tr>
<tr>
<td>Zinc</td>
<td>15-45 mg daily</td>
</tr>
<tr>
<td>Copper</td>
<td>1-3 mg daily</td>
</tr>
</tbody>
</table>

* Do not exceed 7,500 IU if pregnant
** Citrate or citrate malate forms
*** Supplement only if anemic

3.10.3 New therapies aimed at specific pathophysiology targets (David and Phil, 1998):

Elucidation of the pathogenesis of IBD has led to the evaluation in experimental animal models for several different therapeutic approaches aimed at specific pathophysiological targets.

**Non-pathogenic Escherichia coli** (David and Phil, 1998)

There are evidences that patients with UC have increased proportions of adhesive and enterohaemorrhagic *E.coli* in their large bowel. Two preliminary reports suggest that oral administration of capsules containing nonpathogenic *E.coli* may have a role in maintaining remission in patients with inactive UC (Rembacken *et al.*, 1997), but further work is required to confirm the efficacy of this or other (e.g., lactobacillus) probiotic approaches.
**Short chain fatty acids (SCFA)** (David and Phil, 1998)
Normal colonic epithelial cells depend for their energy metabolism on a luminal supply of SCFA, derived from bacterial flora. In UC, colonocytes inadequately utilise SCFA; low luminal SCFA levels in UC exacerbate this metabolic defect (Roediger et al., 1980). Efforts to remedy the defect by treatment of patients with distal UC with enemas containing SCFA, principally butyrate, have unfortunately not proved uniformly successful; furthermore, the appeal of this very safe therapy is restricted by the unpleasant smell of the enemas.

**Modifying leucocyte numbers and function** (David and Phil, 1998)
Depleting leucocyte numbers, by use of leucocyte apheresis, antiCD4 antibodies or bone marrow transplantation has been shown in uncontrolled reports to suppress activity of CD. Furthermore, trials are in progress to assess the clinical efficacy in IBD of inhibiting leucocyte migration into the gut mucosa using antibodies or antisense oligonucleotides to adhesion molecules such as ICAM-1.

**Modulation of cytokine activity** (David and Phil, 1998)
Recognition of altered cytokine expression in IBD has prompted therapeutic trials using interleukin-1 receptor antagonist, interferon-alpha and gamma, anti-TNF-alpha antibody and interleukin-10.

**Interleukin-10** (David and Phil, 1998)
IL-10 is an anti-inflammatory and immunosuppressive cytokine. A recent placebo controlled trial of recombinant human IL-10 gave promising results in steroid-refractory CD.

**Antisense oligonucleotide to NFkB.**
The upregulation of NFkB in IBD tissue may play a central role in its pathogenesis as a result of stimulation of the synthesis of proinflammatory cytokines such as TNF, IL-1 and IL-6. It remains to be seen whether trials of antisense oligonucleotides to NFkB will prove as effective and safe in human IBD as they appear to be in experimental colitis in mice (Neurath et al., 1996).

**Modifying the effects of lipid mediators** (David and Phil, 1998)
Reducing synthesis of proinflammatory prostaglandins with non selective NSAIDs shows adverse rather than beneficial effect in IBD, perhaps because of the concomitant suppression of cytoprotective prostaglandins. The efficacy and safety of selective cyclooxygenase-2 (COX2) inhibitors have not yet been formally assessed in
IBD. Trials with inhibitors of the synthesis of the extremely potent inflammatory mediator, leucotriene B4, in UC have shown modest benefit (Laursen et al, 1992). Ridogrel, a dual thromboxane synthesis inhibitor and receptor antagonist, has been shown to induce remission in over 40% patients with moderately active UC, and is under trial in active Crohn’s. Antagonists to platelet activating factor (PAF) have been ineffective in active UC.

**Antioxidants** (David and Phil, 1998)

UC is characterized by signs of increased oxidative stress in the intestinal mucosa that may be secondary to inflammation. Superoxide dismutase (SOD) is a scavenger of free radicals, and as such may have therapeutic application in UC by scavenging free oxygen radicals. Published trials of antioxidant therapy in human IBD are limited to one open study of patients with steroid resistant CD who appeared to benefit from intramuscular injections of superoxide dismutase. Many patients with IBD in the West use over the counter antioxidant drugs in an effort to ameliorate their disease.

**Heparin** (David and Phil, 1998)

Active IBD is characterised by a procoagulant diathesis which may contribute not only to the increased risk of systemic thromboembolism characteristic of the disease, but also to the intramucosal inflammatory process (Wakefield et al, 1991). Patients with UC have a greater risk of developing coagulation problems such as deep vein thrombosis (DVT). In treating patients for DVT with heparin, an unexpected improvement in UC was noted. Several pilot studies suggest that intravenous heparin may have a beneficial effect on disease activity in both UC and CD. Mechanisms of action of heparin in IBD are likely to include interference with leucocyte endothelial cell adhesion and of platelet activation, its anticoagulant effects and potential anti-inflammatory effects.

**Modulation of enteric nerve function** (David and Phil, 1998)

Neuronal hyperplasia, hypertrophy and degeneration, together with abnormalities of neurotransmitter content, have been described in the gut mucosa of patients with IBD. In open studies, it is reported to have clinical and sigmoidoscopic improvement in 90% of UC patients treated with lidocaine enemas for up to 12 weeks; similar uncontrolled results using ropivacaine gel rectally. The beneficial effect of lidocaine or other local anaesthetics is due to modulation of enteric nerve function or to inhibition of production by mucosal leucocytes of inflammatory mediators.
Smoking: Nicotine (David and Phil, 1998)
Two controlled studies have confirmed that nicotine patches can induce remission in active UC. The mechanism of the therapeutic effect of nicotine in UC, possibilities include increased colonic mucus secretion, alterations of cell-mediated immunity, and reductions in gut permeability, prostaglandin E2 production and rectal mucosal blood flow. Smoking has an adverse effect on the natural history of CD, including the recuperation rate.

NK-1 Receptor Antagonists (Kara et al, 2007)
The ability of proinflammatory cytokines to modulate expression of NK-1 receptors on colonic mucosa cells, the increased expression of SP seen in some studies of IBD patients, suggests that these receptors represent a potential therapeutic target for the treatment of IBD. NK-1 receptor antagonists are expected to decrease SP-mediated activation and mediator release from several immune and inflammatory cell types, as well as reduce ion secretion, intestinal permeability, and the increased colonic motility frequently seen in IBD patients.

Melatonin (Helen Burgess et al, 2013)
Melatonin may exert benefit in IBD by interacting with inflammatory mediators. Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is induced by TNF-α and believed to be involved in inflammation associated with IBD. Furthermore, oxidative stress is considered to be important in the pathogenesis of UC and melatonin is a significant free radical scavenger.

Phosphatidylcholine/Phosphatidylinositol
Oral supplementation with phosphatidylcholine prevents collagen deposition and subsequent stricture formation in inflamed colonic tissue. Both phospholipids resulted in significant mucosal recovery and decreased permeability.

Estrogen
Data on the relationship between estrogen and ulcerative colitis has been conflicting, with pregnancy increasing risk of UC flare-ups in severe, uncontrolled colitis, but not in milder cases or those in remission. In animals 17α-estradiol has been found to decrease inflammation in some experimental models of colitis.
Sphingosine kinase

Sphingolipid metabolism has emerged as a potential new therapeutic target for many diseases. Sphingosine kinases (SK1 and SK2) phosphorylate sphingosine, producing sphingosine-1-phosphate (S1P) which regulates a variety of important cell processes. SK1 appears to be a highly regulated enzyme that is activated by many growth factors and cytokines; prominent among them is tumor necrosis factor alpha. TNF activates SK1, leading to S1P generation, which, in turn, appears to mediate many cellular TNF responses. TNF activation of SK1 in endothelial cells generates S1P and leads to endothelial NOS (eNOS) activation. Similarly, SK1 activation by TNF- and consequent increased S1P production induce cyclooxygenase-2 (COX-2) expression and production of prostaglandin E2 (PGE2) (Snider et al, 2009).

Tryptophan hydroxylase

Serotonin (5-hydroxytryptamine (5-HT)) has an important role in gastrointestinal function. The biosynthesis of 5-HT occurs in 2 steps: a rate-limiting enzyme, tryptophan hydroxylase (TPH), first converts L-tryptophan to 5-hydroxytryptophan, which then is converted to 5-HT by aromatic L-amino acid decarboxylase. There are 2 isoforms of TPH, which are the products of separate genes, TPH1 and TPH2. TPH1 is found in EC cells, mast cells, and pinealocytes, whereas TPH2 is restricted to central and enteric neurons. The TPH1-dependent 5-HT of EC cells is released in response to a variety of signals, including increased intraluminal pressure and acid, which initiate peristaltic and secretory reflexes. Reports of gene polymorphisms abnormalities in 5-HT secretion, TPH1, serotonin transporter, and 5-HT receptors in subsets of patients with IBD suggest that abnormalities of 5-HT production, secretion, or action. Preclinical studies carried out using different TPH inhibitors from which LX1031 showed positive results in colitis model (Brown et al, 2011).

Heme oxygenase

Heme oxygenase (HO) catalyzes the first and rate-limiting enzymatic step of heme degradation and produces carbon monoxide (CO), iron and biliverdin, which are converted into bilirubin (BR) via biliverdin reductase which act as anti-inflammatory compound. Two genetically distinct HO ribozymes, HO-1 and HO-2, are known. HO-2 represents the constitutive non-inducible isoform and is primarily expressed in brain
and testis. The inducible isoform HO-1, which exhibits low basal expression levels in
most cells and tissues, is highly up-regulated by a wide variety of oxidative stress
stimuli. More recently, HO-1 has been recognized to have major immunomodulatory
and anti-inflammatory properties, which have been demonstrated in HO-1 knockout
mice and a human case of genetic HO-1 deficiency.

The immunomodulatory role of HO-1 is associated with its cell type-specific
functions in myeloid cells (eg. macrophages and monocytes) and in endothelial cells,
as both cell types are crucially involved in the regulation of inflammatory responses.A
potential link between HO-1 and inflammation has initially has been demonstrated in
a HO-1 knockout mouse model that these animals develop a chronic inflammatory
bowel disease. CO and BR enhance production of anti-inflammatory cytokine, through
JAK/STAT3 pathway and inhibit transcription of proinflammatory
molecules. Statins, COX-2 inhibitors, 5-ASA agents, Proton pump inhibitors are proven
as HO-1 inducers and showed protective effect in animal models of colitis (Ananta et
al., 2010).

Peroxisome proliferator-activated receptor

PPARγ also inhibits tissue injury associated with immune activation which points to
PPARγ as a novel anti-inflammatory mediator with broad therapeutic potential.
Desreumaux and colleagues in 2001 reported the effects of both PPARγ and RXR
ligands in the treatment of experimental colitis using the 2, 4, 6-trinitrobenzene
sulfonic acid (TNBS)-induced colitis model. They showed important therapeutic
contributions of ligands for both molecules. Notably, PPARγ forms heterodimers with
RXR and inhibit NFκB signaling and JAK/STAT pathway. The PPARγ-deficient
(+/−) mice exhibited an aggravation in inflammation in the TNBS model in
comparison with wild-type mice. It is reported that PPARγ ligands are effective in
three different models of colitis: DSS-, ischemia-, and TNBS-induced colitis. Based
upon these reports, endogenous PPARγ pathways play a central role in anti-
inflammatory responses in the small intestine and colon (Wahli, 2008).

Calprotectin

Calprotectin is heterodimer complex of two inflammatory proteins S100A8 and
S100A9. S100A8 and S100A9 proteins became the focus of intensive current research
due to their association with numerous human disorders, including acute and chronic inflammatory conditions, autoimmune diseases, cancer, atherosclerosis, cardiomyopathies and neurodegenerative diseases, as well as due to their crucial roles in normal physiological processes within cells. Apparently these proteins are able to perform a wide plethora of intra- and extracellular functions, including cytokine-like and chemokine-like activities via activation of the receptor for advanced glycation end products and Toll-like receptor 4 dependent signalling cascades and potentially other signalling pathways, promotion of calcification in the blood vessels and prostate, regulation of cytoskeleton via tubulin polymerization and others (Foell et al, 2008). Calprotectin complexes have emerged as very potent biomarkers of a wide range of inflammatory processes, including rheumatoid arthritis, juvenile idiopathic arthritis, inflammatory bowel disease, acute lung inflammation and vasculitis (Foell et al, 2009)

**Botanicals and Flavonoids** (Kathleen and Julie, 2003)

Over 20 different botanicals have been used alone or in combination in both animal models and clinical trials of UC. Clinical trials have been conducted on a *Ginkgo biloba* extract (Cedemin), *Boswellia serrata*, and a botanical combination. Animal studies or case reports offer preliminary information on the potential efficacy of flavonoids, bromelain, and other plant extracts.

**Ginkgo biloba** (Kathleen and Julie, 2003)

In a small open trial, 10 patients with mild to moderate UC were given Cedemin enemas nightly for three weeks. Three of 10 patients achieved remission and two experienced some improvement, but these results were not statistically better than placebo. It was hypothesized that the mechanism responsible for Cedemin’s effect in patients who improved or attained remission was due to the extract’s inhibition of platelet-activating factor, which mediates mucosal inflammation.

**Boswellia serrata** (Kathleen and Julie, 2003)

An open, non-randomized clinical trial of 30 patients with chronic colitis investigated the effect of *Boswellia serrata* gum resin (BWGR) for six weeks on various UC disease parameters. Stool properties, histopathology of colonic mucosa, sigmoidoscopic scores and various laboratory markers of anemia and inflammation were assessed at the beginning and end of the trial. Twenty of 30 patients received 900 mg daily of BWGR in three divided doses. Ten control patients received 3 mg
daily sulfasalazine in divided doses. Eighteen of 20 patients receiving BWGR showed an improvement in one or more of the parameters assessed, particularly in sigmoidoscopic scores, and 14 achieved remission. This was compared to four of 10 patients in the control group receiving sulfasalazine. After statistical analysis of results for all parameters measured, the degree of improvement was not statistically significantly better for BWGR-treated patients than for patients receiving sulfasalazine (Gupta et al, 2001).

**Combination Herbal Treatment** (Kathleen and Julie, 2003)

Another study examined the effectiveness of an herbal combination, containing *Taraxacum officinale, Hypericum perforatum, Melissa officinalis, Calendula officinalis*, and *Foeniculum vulgare*, on 24 patients with chronic non-specific colitis. By day 15 of the study, 23 patients had a complete resolution of pain in the large intestine. In addition, diarrhea resolved and fecal content normalized (Chakurski *et al*, 1981).

**Peumus boldus** (Kathleen and Julie, 2003)

A study compared the efficacy of boldine – an alkaloid from *Peumus boldus*, an evergreen tree found in Chile – to 5-ASA. The study investigated boldine’s cytoprotective and anti-inflammatory properties in mucosal tissue of rats with induced colitis. Boldine was found to have an anti-inflammatory effect in the colon as evidenced by reduced colonic neutrophil infiltration, protection against edema and cell death, and enhanced fluid absorption in the colon (Gotteland *et al*, 1997).

**Plant Sterols and Sterolins** (Kathleen and Julie, 2003)

Sterols and sterolins (phytosterols) are fats present in all plants, including fruits and vegetables. β- sitosterol (BSS) is the major phytosterol in higher plants, along with its glycoside β- sitosterolin (BSSG). In *in vitro*, animal and human studies a proprietary BSS: BSSG mixture had shown promise in normalizing T-cell function and dampening overactive antibody responses.

### 3.10.4 Surgical Treatment

Like medication and nutritional approaches, it represents another important therapeutic option. For many people with IBD, surgery can result in a healthier, more active lifestyle. Indeed, for those with ulcerative colitis, surgery to remove diseased intestinal tissue actually represents a "cure." Crohn's disease, on the other hand, cannot be eradicated by an operation. However, surgery can relieve symptoms and
thereby vastly improve the quality of life. Medical treatment is definitely the preferred first line of treatment. But when it no longer keeps the disease under control, then surgery is another option for long-term relief of symptoms. Surgery may also reduce or completely eliminate the need for ongoing use of medication.

A. Surgery for Ulcerative Colitis

Some people elect to have surgery if they experience chronic severe symptoms or if medical therapy fails to adequately control symptoms. Surgery may also become necessary if complications arise. Complications of ulcerative colitis which can require emergency surgical intervention include:

- Perforation of the colon
- Massive bleeding in the colon
- Sudden, severe ulcerative colitis
- Toxic megacolon (in which the muscle wall of the colon dilates and bacteria and gases build up inside the colon)

The standard surgical procedure for ulcerative colitis is proctocolectomy. Unlike Crohn's disease, which can recur after surgery, ulcerative colitis is "cured" once the colon is removed.

**Proctocolectomy with Ileostomy:** Proctocolectomy is performed along with a procedure called ileostomy. An ileostomy—performed after the colon, rectum and anus have been removed—involve bringing the end of the ileum through a hole in the abdominal wall, allowing drainage of intestinal waste out of the body. After the procedure, an external bag is worn over the opening at all times to collect waste which is emptied several times a day. The usual site for an ileostomy is the right lower abdomen just below the belt line, to the right of the navel.

**Restorative Proctocolectomy:** The newer procedure, called an ileoanal pouch anal anastomosis or restorative proctocolectomy, allows the patient to continue to pass stool through the anus. This procedure has become the most commonly performed surgical procedure for ulcerative colitis.
B. Surgery for Crohn's Disease

Surgery may be needed for serious complications; for disease that doesn't respond to medication; or as a last resort to relieve symptoms that cannot be brought under control. Complications of Crohn's disease that may require surgery include:

- Intestinal obstruction or blockage
- Excessive bleeding in the intestine
- Perforation of the bowel
- Formation of a fistula or abscess
- Toxic megacolon

Crohn's cannot be cured with surgery. Even if the diseased portion of the intestine is removed, the inflammation can reappear in a previously unaffected portion of the intestine.

**Strictureplasty**

When Crohn's disease affects the small intestine, areas of diseased bowel may alternate with areas of normal bowel. The areas of active disease may narrow, forming strictures, which can block the passage of digested food. The sections of normal bowel compensate by pushing against this strictured area, causing severe crampy pain. When this occurs, a procedure called strictureplasty may be performed. This procedure widens the strictured area without removing any portion of the small intestine.

**Resection**

If a stricture is long, or there are multiple strictures close to one another, it may be necessary to remove the affected section of the intestine. This is called a resection. The two ends of healthy intestine are then joined together in a procedure called anastomosis. A bowel resection may offer patients many years of symptom relief. However, the disease can recur at or near the site of the anastomosis.

**Colectomy or Proctocolectomy**

This allows the person to continue to pass stool in a bowel movement.

**Surgery for Abscesses and Fistulas**

About one in four adults with Crohn's disease develops fistula or abscess during their lifetime. An abscess is a tender mass filled with pus from an infection. A fistula is an abnormal tunnel that may lead from an abscess to a hollow organ like the intestines. A
fistula can also connect two adjacent loops of intestine or may connect the intestine to the bladder, vagina, skin, or other organ. Fistulas are usually initially treated with medication, but surgery may be required if the fistula is causing symptoms that don't respond to drugs. The surgical procedure is resection of the involved bowel and anastomosis. An abscess must be drained. This may be done with a needle inserted through the skin, which is guided to the correct location with the help of a CT scanner. In many cases, surgery is required to drain the abscess or to perform a resection.

**Disease Recurrence after Surgery**

- About 50 percent of adult patients have a recurrence of symptomatic Crohn's disease within five years after having a resection. The disease usually recurs at the site of the anastomosis or ileostomy.
- The chances of a recurrence can be reduced by taking medication, such as 5-ASA agents and immunomodulators.
- Recurrent Crohn's disease often can be successfully treated with medications. However, about half of people with recurrent symptoms will need a second surgery.

### 3.11 Animal Models of IBD

Animal models of intestinal inflammation have provided useful insights into the pathogenesis of the intestinal inflammatory response. Animal models of IBD have been referred to as spontaneously occurring or induced. Induced models of IBD include

(i) animals which have been treated with agents that promotes intestinal inflammation, (ii) rodents that have been genetically manipulated through gene targeting or the introduction of transgenes, and (iii) immunodeficient animals into which cell populations that mediate intestinal inflammation have been transferred (Barbara *et al.*, 2002). IBD features in humans can be reproduced in animals models of IBD as shown in Figure 3.7.
Animal models of IBD can be divided into 5 different categories (Jorg et al, 2002):
1. Antigen-specific forms of colitis
2. Inducible forms of IBD animal models
3. Genetic forms of IBD animal models
4. Adoptive transfer models
5. Spontaneous forms of IBD animal models

1. **Antigen-specific forms of colitis**

Three CD animal models fall into this category, the granulomatous colitis induced by intramural injection of mycobacterial cord factor into mice or rats, the granulomatous colitis induced by intramural injection of purified bacterial cell wall fragments (also known as peptidoglycan-polysaccharide complex, PG-PS) and ovalbumin-specific colitis. In addition to the typical histology seen in CD, PG-PS rats develop arthritis and hepatitis as extraintestinal manifestations, as in CD.

2. **Inducible forms of IBD animal models**

- Induction by Chemicals
- Trinitrobenzene Sulphonic acid / Dinitrobenzene Sulphonic acid (TNBS/DNBS)
- Dextran Sodium Sulphate
Luminal agents capable of damaging the intestinal mucosa are able to produce an inflammatory response in genetically susceptible hosts and may mimic some aspects of human IBD. For example, colitis can be induced by administering 5% dextran sulfate sodium in the drinking water, treating the host with indomethacin, or giving an enema containing trinitrobenzene sulfonic acid in ethanol. However, the absence of chronicity is a significant shortcoming of acute nonspecific injury models of IBD. Nonetheless, regeneration of the colonic mucosa after repeated cycles of 5% dextran sulfate sodium administration takes several weeks and is a frequent model used for studying mucosal repair mechanisms (Barbara et al., 2002).

DNBS is a nitroaryl oxidizing acid with extreme oxidizing properties. DNBS dissolved in 50% alcohol is used to induce colitis. After disruption of mucosa integrity by ethanol, hapten DNBS gets bound to colon tissue proteins and changes into a modified protein compound, which is recognized by macrophages as an abnormal antigen and presented quickly to the sensitizer T-lymphocytes. So a series of immune responsiveness and severe colon inflammation are initiated subsequently. The colon injury correlates with a significant rise of apoptosis which is associated with a significant increased expression of proapoptotic Bax and decreased colon content of antiapoptotic Bcl-2. This inflammatory response is also related to activation of NF-κB and phosphorylation of c-Jun as well as FAS ligand expression in the colon (Emanuela et al., 2006).

3. Genetic forms of IBD animal models

- Alteration of cytokine function
  - IL-10 knock-out mouse
  - IL-2 knock-out mouse
  - TNF ΔARE mice
  - STAT-4 transgenic mice
- Alteration of T-cell function
  - T-cell receptor α-knock-out mouse
T-cell receptor β-knock-out mouse
HLA-B27 transgenic rat
Impairment of epithelial barrier function
Mutated multidrug-resistant gene mice
Intestinal trefoil factor knock-out mice

A number of mouse strains generated by gene-targeting methods exhibit intestinal inflammation when housed under conventional conditions. Several of these mouse models have targeted mutations that affect cytokine secretion or the CD4\(^+\) T-cell population.

Cytokines are local mediators of inflammatory and immune responses. CD4\(^+\) lymphocytes can be classified as T\(_{H1}\)-type cells or T\(_{H2}\)-type cells on the basis of their cytokine secretion profiles. T\(_{H1}\)-type cells are stimulated by IL-12 and produce IL-2, gamma interferon (IFN-γ), and TNF. T\(_{H2}\)-type cells secrete IL-4, IL-5, IL-10, and IL-13. In general, T\(_{H1}\) cells are effective inducers of cellular immune responses and T\(_{H2}\) cells support humoral immune responses. The cytokine IL-2 stimulates the growth and expansion of T lymphocytes and the activation of other cells, including macrophages.

IL-2 knockout mice develop a symptomatic pancolitis with intermittent gastrointestinal bleeding, diarrhea, and often rectal prolapse. The colon is grossly thickened and contains mucosal ulcerations. On histopathologic testing, epithelial hyperplasia, crypt distortion and abscesses, and acute and chronic inflammatory cells infiltrating into the lamina propria are noted. Various studies have suggested that CD4\(^+\) T cells, rather than B cells, are critical in the pathogenesis of the gut inflammation in IL-2-deficient mice.

IL-10 is a cytokine secreted by T\(_{H2}\) cells as well as other cells that inhibits the synthesis of IL-12 and proinflammatory cytokines such as IFN-γ and TNF. IL-10-deficient mice develop a chronic enterocolitis mediated by T\(_{H1}\) cells under conventional housing conditions. Histologically, the disease is characterized by excessive regenerative hyperplasia of the mucosa, leading to a marked thickening of the intestinal wall, abnormal crypt and villous architecture, and extensive lymphoplasmocytic and histiocytic infiltration of the lamina propria and submucosa.

AU-rich elements (ARE) in the TNF gene regulate TNF biosynthesis. Mice lacking the ARE produce a more stable TNF mRNA and fail to appropriately repress TNF.
expression. These mice develop CD-like IBD that is presumably related to overexpression of TNF.

Mice with targeted mutations of either T cell receptor α or T-cell receptor β genes develop colitis with crypt distortion and an inflammatory infiltrate in the lamina propria. The pathogenesis of disease in these T-cell receptor knockout mice is unclear, but the extent of disease markedly varies among different inbred strains of mice, suggesting that other susceptibility loci influence disease expression.

HLA-B27 transgenic rats with susceptible genetic backgrounds develop chronic colitis and joint inflammation. Inflammation is T cell mediated, particularly by CD4+ T cells, and the presence of commensal bacteria in the intestine appears necessary for disease. Studies suggest that the colitis may result from a loss of tolerance to enteric bacteria.

Investigations in other rodent models suggest that a primary defect in macrophage function also can lead to intestinal inflammation. For instance, mice which are lacking the transcription factor STAT-3 have macrophages that fail to be inhibited by IL-10, which generally down-regulates inflammatory responses. When STAT-3-deficient mice are treated with lipopolysaccharide, which activates the macrophages to secrete proinflammatory cytokines such as TNF, the mice develop mucosal inflammation.

Intestinal epithelial cells are crucial in maintaining the barrier to luminal antigens and bacteria. Investigations using mice generated by gene targeting have shown that epithelial defects in cellular transport, repair and barrier function also may lead to mucosal inflammation. For instance, mice carrying a mutated multidrug resistance gene lack the ability to pump out small amphiphilic and hydrophobic molecules from within the cell and have an increased sensitivity to certain drugs. These mice develop intestinal inflammation that resembles UC, and repletion with normal lymphocytes does not abrogate the colitis. Although both intestinal epithelial cells and lymphocytes express multidrug resistance genes, this observation suggests that the defect in epithelial cell function may be of primary importance. Prophylactic treatment with oral antibiotics prevented colitis in these mice. Cadherins are important mediators of epithelial cell adhesion and migration. Mice with altered N-cadherin expression in the intestine develop IBD resembling CD. Another example involves the trefoil peptides, which are protease-resistant molecules secreted by mucin cells in the intestine and which have been implicated in mucosal healing. Mice lacking intestinal trefoil factor
died of extensive colitis after oral administration of dextran sulfate sodium (Barbara et al, 2002).

4. Adoptive transfer models
The adoptive transfer of CD4\(^+\) T cells expressing high levels of CD45RB into severe combined immunodeficiency (SCID) mice, which lack B and T cells, results in intestinal inflammation accompanied by diarrhea and weight loss. In this model, simultaneous transfer of the CD45RB low CD4\(^+\) T cells prevents the development of inflammation, possibly through the secretion of transforming growth factor β (Barbara et al, 2002).

5. Spontaneous forms of IBD animal models
Spontaneous colitis developed in cotton-top tamarins resembles IBD. In addition, selective breeding of C3H/HeJ mice at Jackson Laboratories (Bar Harbor, Maine) produced a strain of mice referred to as the C3H/HeJ Bir strain. These mice spontaneously develop inflammation of the cecum and right colon that generally peaks at 3 to 6 weeks of age and resolves by 10 to 12 weeks of age. Small lesions at the anorectal junction, however, are common throughout life. The etiology of the colitis remains uncertain, but the C3H/HeJ parenteral strain is particularly sensitive to mucosa-injuring agents such as trinitrobenzene sulfonic acid and dextran sulfate sodium.

In addition, a subline of the senescence-accelerated mouse (SAM) P1/Yit strain has been established from AKR/J mice. This strain of mice exhibits spontaneous enteric inflammation and skin ulcerations under specific-pathogen-free conditions. The intestinal disease involves the distal part of the small intestine and cecum and histologically appears similar to human CD. Germ free (SAM) P1/Yit strain mice does not develop intestinal inflammation, but reintroduction of fecal bacteria into the gut induces disease (Barbara et al, 2002).
3.12 Plant Profile:

3.12.1 Description of *Holarrhena antidysenterica* (Sharma *et al.*, 2005):

**Botanical name:** *Holarrhena antidysenterica* (*Roxb. Ex Flem.*) *Wall.*

**Family:** Apocynaceae

**Classical name:** Kutaja

**Vernacular names:**

- **English:** Connessi bark
- **Hindi:** Kuda, Kura, Kora, Karchi, kurchi
- **Gujarati:** Indrajavanu, Dhowda
- **Marathi:** Pandhara kuda, Kodaga, Dola kuda
- **Sanskrit:** Kutaja

![Figure 3.8: Holarrhena antidysenterica plant and dried bark](image)

**Parts used:** Stem bark, leaf, seed.

**Distribution:** Plant is found throughout drier or deciduous forest areas of India in the tropical Himalaya from Chenab eastwards, common in Sal forests, Aravalli hills, Bihar, Central India, South Konkan and Kerala.

**Botanical description:** It is a glabrous or pubescent tree or large shrub. Leaves are nearly sessile, ovate-oblong, acute. Flowers are white, in terminal, corymbose cymes. Follicles are glabrous, slender, terete, 20-37 cm long, distinct from the base, usually
curved and touching at the tips. Seeds are numerous, crowned with a tuft of long, silky hairs.

**Pharmacognosy:** Stem bark- Dried bark occurs in form of small recurved pieces of varying sizes and thickness, outer surface buff to brownish, longitudinally wrinkled and bearing horizontal lenticels, inner surface brownish, rough and scaly. Fracture is hort and granular, taste acrid and bitter. Transverse section of dried stem bark shows cork consisting of 4-12 rows of tangentially elongated cells, followed by cork cambium of a single row of thin walled tangentially elongated cells. Secondary cortex is usually wide, parenchymatous, interspersed with strands of stone cells. Stone calls are rectangular to oval, with numerous pits often containing prismatic crystals of calcium oxalate. Pericyclic fibres are non-lignified. Secondary phloem is wide, consisting of sieve tubes, companion cells, phloem parenchyma and stone cells. Stone cells are arranged in tangential rows in concentric manner associated with crystal sheath containing prisms of calcium oxalate. Medullary rays are mostly bi or triseriate, rarely uniseriate, becoming wide towards outer part and consist of thin walled, radially elongated, parenchymatous cells. Medullary ray cells near stone cells become sclerosed.

**Active constituents:** Steroidal Alkaloids conessine and holacetine (root bark); conessine, holarrhenine and holarrhimine, alkaloids- kurchine, kurchicine, conessine , nor conessine, holarrhimine, hollarhine, conarrhimine, conamine, conimine, conessimine, isoconessimine, conessidine, conkurchine, holarrhenine lettocine and alkaloid holarricine (bark); a steroidal alkaloid- antidysentericine, crystalline glucoalkaloid, other alkaloids viz. kurchiline, kurchiphyllamine, kurchiphylline, holarrhesmine, kurchessine, holarrhidine, holonarmine, holantosine E and trimethyl conkirchine (seeds); 9 – D- hydroxy-cis-12-octadecenoic acid and holarricine ( seeds oil ); two amino-glyco-steroids-holantesine A,B,C and D, holaresine E, holaresine F, three aminoglycosteroids- N- acetyl holantesine C,N- acetylholtantesine D and N- acetyl holarosine A, N- acetyl-L-holontosamine and its α- and β- methyl derivatives (leaves); a steroidal alkaloid- holacetine (20 S-acetamido- 5- α pregnan, 3β ol), aspartic acid and arginine, the major free amino acids ( root bark); a triterpene characterized as 5, 20 (29)- lupadien - 3β- ol-together with known steroid sitosta- 5, 23- dien- 3 β- ol, two alkaloids named holacine and holacimine (bark extract).
**Actions and uses:** Bark and seeds are bitter, constipating, stomachic, astringent, powerful antidysenteric, refrigerant, anthelmintic, aphrodisiac, carminative, digestive, expectorant, febrifuge, and tonic. Both are useful in amoebic dysentery, diarrhoea, asthma, bronchopneumonia, internal hemorrhages, bleeding piles, rheumatism, fever, malaria, vomiting, worm infestation, calculus, wounds, leprosy and skin diseases. Bark is rubbed over body in dropsy. Seeds are carminative, astringent, lithotriptic, and aphrodisiac. Leaves are used in chronic bronchitis, dysentery, locally for boils and ulcers. Several Indian tribes have used the plants in ailments like anemia, epilepsy, stomach pain and cholera. It is also indicated in flatulence and colic.

**Pharmacological activities:** Antitubercular, hypotensive, antiprotozoal, hypoglycemic, antispasmodic, anti-jaundice, antifungal, antiamoebicidal, anti-diarrhoeal, antitumour, antispirochetal, astringent, anthelmintic, stomachic, febrifuge and tonic, and antibacterial. Various fractions of *H. antidysenterica* showed promising activity against experimental amoebiasis in rats and hamsters (Dutta and Iyer, 1968). The fruit extract (50% ethanolic) showed antiprotozoal effect against *Ent. histolytica* strain STA. *Trypanosoma evansi* (Dhar *et al.*, 1968). Clinical tests with connessine on patients with intestinal and hepatic amebiasis have been found to give results, comparable to those obtained with emetine (Signier *et al.*, 1949).

**Formulations and preparations:** Kutajarishtha, New Diarex (Himalaya Drugs), katajavaleha, vrindhamangadhara churna, laghugangadhara churna, jirakadya churna, brihanmarichadya taila, panchanimba churna, palashabijadi churna.

**Dosage:** Bark powder 1-4 gm, Dry extract(10% alkaloids) - 200-600mg, Alkaloids concentrate 50% - 30-75 mg, Kutajarishtha – 15 ml, Kurchi liquid extract IP 66- 8-16 ml, Kurchi- bismuth iodide IP 66- 0.3- 0.6 g.
3.12.2 Description of *Cyperus rotundus* Linn (Sharma et al, 2005):

**Botanical name:** *Cyperus rotundus* Linn.

**Family:** Cyperaceae

**Classical name:** Musta, Mustaka, Varida, Varidanamaka

**Vernacular names:**

English: Nutgrass

Hindi: Nagarmotha, Mustak

Gujarati: Motha

Marathi: Bimbal, Motha

**Parts used:** Tuber

**Distribution:** It is a perennial herb found throughout India up to elevation of 1800 km, from Kashmir to Simla, Garhwal, Khasia, Mountabu, Pune to Nilgiri hills. Mustak is mostly found as weed in rice fields ie in wet marshy lands.

![Figure 3.9: *Cyperus rotundus* plant and dried tuber](image)

**Botanical description:** The plant is 10-75 cm high, stolons 10-20 cm long, bearing hard, black, fragrant tubers. Leaves are 10-18 cm long, narrowly linear. Spikelets are 0.8-1.0 x 0.1 cm, linear, brown. Nuts are about 15 mm long, broadly obovoid, greenish-black.
Pharmacognosy: Stolon is slender, 10-20 cm long, tubers hard, ovoid, tunicate, black from outside, fragrant, 0.8 to 2.5 cm in diameter, inner surface white, fracture mealy; root fibres clothed with flexous hairs. In transverse section, the rhizome is characterized by a thick walled endodermis dividing a cortical portion and central ground tissue. Epidermis consists of typical parenchymatous cells with brownish pigments. Hypodermis consists of 2-3 layers of thick walled cells. Cortex is composed of parenchymatous cells, outer part compact, inner part parenchymatous with large intercellular spaces. Some cells in cortex region contain brownish oleoresinous matter and others starch grains. Vascular bundles are closely scattered in the pith. Pith is composed of parenchymatous cells containing starch grains and a few filled with oleoresinous contents. Each vascular bundle is enclosed by a lignified fibrous sheath of 1-3 layers. Vessels have blunt or tapering ends.

Root- Transverse section of root shows a broad zone of cortex, outer cortex two layered, inner cortex rapidly breaking down, only 3-4 innermost layers persistent, cells tangentially flattened, thick walled. Endodermis consists of uniformly thickened roundish cells. Xylem consists of 12 small vessels adjacent to pericycle and 4 large vessels of unequal size near centre. Central ground tissue is thick walled, fibrous.

Active constituents: Fat, sugar, gum, carbohydrate, essential oil, albuminous matter, starch, fibre and ash, traces of alkaloid. β- sitosterol, 4α 5α oxidoeudesm-11-en-3α-ol from (rhizome); pinene, cineol, alcohol- isocyperol (essential oil from the tubers); linolenic, linolic, oleic, myristic and stearic acids and glycerol, (fatty oil); a sesquiterpeneketone- mustokone and copaene, cyperotundone; sesquiterpenes-(+)-copadiene, (+)- epoxyguaïne, (-)-rotundone and cyperolone; cyperenone designated as isopatchoul- 4(5)-en-3-one and aureusidin , eugenol, cyperol (essential oil); two sesquiterpenic ketoalcohols, α- rotunol, β- rotunol, kobusone and isokobusone; oleanolic acid and its glycoside, oleanolicacid- 3-O- neohesperidoside alongwith sitosterol, sesquiterpenes- α cyperone, cyperene-1 , cyperene-2 (sesquiterpene hydrocarbon), β- selinine and cyperenone(tubers); luteolin and aureusidin (leaves). The essential oil from Cyperus rotundus contains at least 27 components comprising sesquiterpene hydrocarbons, epoxides, ketones, monoterpenes and aliphatic alcohols and triterpenes like β- sitosterol and linoleic acid. Flavonoids, sugars and minerals have also been isolated from the tuber.
Actions and uses: Tubers are acrid, bitter, astringent, cooling, anti-inflammatory, galactogogue, intellect promoting, nerve tonic, digestive, carminative, stomachic, anthelmintic, diuretic, expectorant, diaphoretic, demulcent, febrifuge, stimulant, demulcent, emmenagogue, vermifuge. They are useful in hyperdipsia, anorexia, dyspepsia, flatulence, colic, vomiting, intestinal worms, chronic diarrhea with mucus, dysentery, vomiting, inflammation, fevers, skin diseases, leprosy, scabies, pruritus, wounds, ulcers, epilepsy, cough, bronchitis, amenorrhoea, dysmenorrhea, renal and vesical calculi, ophthalmic disorders and general debility.

Pharmacological activities: Tranquillizing, anti-inflammatory (petroleum ether extract), antipyretic, diuretic, estrogenic, anti-emetic, anthelmintic, antimalarial, antibacterial, fungicidal, smooth muscle relaxant, inhibitory activity against $^{3}$H flunitrazepam binding to benzodiazepine receptor, antimicrobial and juvenile hormone mimicking activity. Alcoholic extract showed hypotensive, antihistaminic, antiemetic, smooth muscle relaxant and antipyretic effects. It has been reported to have anticonvulsant activity (Porwal et al, 2011), antidysmenorrhea effect (Yuan Hao, Wan-Sheng Ji et al, 2006) along with antispasmodic effect (Shamkuwar et al, 2012).

Formulations and preparations: New Diarex (Himalaya Drugs), Mustakadi kvatha, mustakarishta, mustadi churna, mustadi leha, shadangapaniya, ardrakakhanawaleha, kutajashtaka kvatha, darvyadi kvatha, dhanyapanchaka kvatha, vatsakadi churna, stanyashodhana kashaya.

Dose: powder 3-6 gm, decoction- 50 –100 ml