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1.1. What is Inflammatory Bowel Disease?

Inflammatory bowel disease (IBD) is a name given to two different diseases that cause inflammation in the intestine *Crohn's disease* (CD) and *ulcerative colitis* (UC). In both the diseases, the lining of the digestive tract becomes inflamed and sores develop, which may cause diarrhea, abdominal pain, cramping, bloody stools, fatigue, weight loss, and fever. IBD can lead to malnutrition because of a loss in appetite due to pain associated with poor absorption by the diseased intestines. Both diseases are chronic with periods of relapse and remission.

The etiology of IBD is largely unknown. However, most researchers have dismissed the traditional belief that it is caused by high levels of stress and anxiety (Lerebours et al. 2007). Although these factors may worsen the progression of the disease, many researchers believe IBD is largely an autoimmune disease a disease characterized by the immune system's attack on its own tissues (Braun et al. 2007). Mucosal inflammation is regulated by the interplay of resident microbiota, intestinal epithelium, and the mucosal immune system, which comprise the native intestinal community. Genetic allelism and environmental factors modify this interplay, driving the mucosal inflammatory state toward or away from clinically significant intestinal inflammation. IBD Figure 1.

![Figure 1. Factors responsible for IBD (Braun et al. 2007).](image-url)
1.2. History of IBD

In 850 AD King Alfred, "England's Darling" had a GI illness that began at age 20. At the time the illness was thought to be due to witchcraft, or a punishment for the King's infidelities. It is now thought to be Crohn's disease.

As early as, 1612 a Doctor performed an autopsy on a young boy, who had complained of abdominal pain, and noted ulcerations in the intestine, similar to those found in IBD. Similar reports by others during the 19th century identified the intestinal pathology of what we know today as IBD. An article was published in the British Medical Journal of 1913 by T. Kennedy Dalziel, who reported treating 13 patients who had suffered from intestinal obstruction. On autopsy it was found that all 13 patients had inflamed gut, especially in the jejunal, ileal and colonic areas. On examining the inflamed bowel more closely, the transmural inflammation that is characteristic of the disease was clearly seen.

Between the 1920s and the 1930s increasingly more patients, particularly young adults, were being seen for symptoms that resembled appendicitis- abdominal cramps, fevers, diarrhoea, and weight loss.

In 1923, Drs. Berg, Oppenheimer, and Ginzberg, surgeons at the Mt Sinai Hospital in New York, collected 12 patients with similar symptoms, and showed that these symptoms were not the result of any other known disease or organism.

Burril Crohn, in 1930, showed similar findings in two patients that he was treating. On the suggestion of Paul Klemperer, the two groups of doctors combined their information, and published their findings in the 1932 JAMA. At the time the disease was called "terminal ileitis", in view of the predilection of the disease for the end portion of the
small bowel. However, the term "terminal" has rather unfortunate implications, and the disease is not solely confined to the small bowel. The term "Crohn's Disease" is now generally accepted.

UC was once again reported by Aretaeus of Cappadocia in 300AD. "Bloody Flux" was a term given to a diarrhoeal disease in the late 1600's by Thomas Sydenham, and this description aptly describes Ulcerative Colitis.

Sir Samuel Wilks described the first case of Ulcerative Colitis, and hence the discovery has been linked to his name, the true article that was written was in fact a letter to The Medical Times and Gazette in 1859.

1.3. Genetics of Inflammatory Bowel Disease

The strongest evidence supporting the contribution of inherited factors in the pathogenesis of Crohn's disease and ulcerative colitis comes from concordance rates in twin pairs. Both types of IBD occur in genetically susceptible individuals through interplay with poorly understood environmental factors. IBD, considered a polygenic disorder, is familial in 5-10% of individuals and sporadic in the remainder (Kaser et al. 2009). Monozygotic twins exhibit phenotypic concordance in 50-75% of CD patients, and the relative risk of developing CD is 800-fold greater compared to the general population (Kaser et al. 2009). In three large studies from Sweden, Denmark, and the UK, the combined concordance rate was 36% in monozygotic twins (Tysk et al. 1988; Orholm et al 2000; Thompson et al. 1996) and only 4% in dizygotic twins (Russell et al. 2004). IBD tends to run in families. About 20 to 25 percent of people with Crohn's disease have a blood relative with some form of IBD. The disease is most prevalent in North American and northern European populations (Bousvaros et al., 2007; Katsanos et al., 2007). Specifically, IBD is more likely to occur in people of European decent, with the risk being higher for individuals of European Jewish descent (Katsanos et al., 2007). The epidemiology of IBD suggests role of genetic factors in disease development.

In 2001, researchers identified a specific gene that, if mutated, may play a role in the development of Crohn's disease. The gene, called Nod2, is believed to have a role in preventing harmful bacteria from invading the intestine. Researchers studied Crohn's patients with a family history of the disease and found that in some of the cases, the Nod2
gene was mutated (Figueroa et al, 2006). Researchers have also identified 170 other genes that may be associated with IBD (O'Callaghan et al, 2003).

1.4. Types of Inflammatory Bowel Disease

1.4.1. Crohn's Disease

Crohn's disease was first described by Scottish physician Dr. Kenny Dalziel in 1913. However, it is named after Dr. Burrill B. Crohn who published a highly publicized paper, with other colleagues, in the Journal of American Medical Association (JAMA) in 1932. This disease can affect the entire length of the digestive tract from mouth to anus. Most often, it affects the lower part of the small intestine, extending through every layer of bowel tissue. The inflammation occurs in patches, with normal healthy bowel in between diseased bowel Figure 2. Complications of Crohn's disease include scarring and narrowing of the small intestine, which may cause food to get stuck in the intestine. Ulcers, pus pockets (abscesses) or abnormal passage ways between organs (fistulas) can also develop. There is no cure for Crohn's but surgery and drug treatments are available to help control the disease and improve the quality of life (Figure 3.).

1.4.2. Ulcerative Colitis

This disease affects the innermost lining of the colon and rectum. The affected areas are continuous, rather than in patches Figure 2. It always starts at the rectum and moves to the rest of the colon. Complications of ulcerative colitis include toxic megacolon, a condition in which the colon becomes paralyzed by the inflammation. As a result, gases can accumulate and the colon can explode, allowing the stool and gas into the abdominal cavity. Toxic megacolon can be fatal unless treated promptly (Molina-Infante et al, 2005; Sriram et al, 2004). Ulcerative colitis can be cured with surgery and can be managed with medication (Pal et al, 2005) (Figure 3.). Table 1. showing the comparison between UC and CD.
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Figure 2. The affected are in UC and CD.

Figure 3. Colonoscopy. A, showing normal colon; B, showing CD; C, showing UC.

Table 1. Comparison between UC and CD

<table>
<thead>
<tr>
<th></th>
<th>Crohn’s Disease</th>
<th>Ulcerative Colitis</th>
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<tbody>
<tr>
<td>Most common site</td>
<td>Terminal ileum</td>
<td>Rectum</td>
</tr>
<tr>
<td>Distribution</td>
<td>Mouth to anus</td>
<td>Rectum to colon</td>
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<tr>
<td></td>
<td></td>
<td>“backwash” ileitis</td>
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<tr>
<td>Spread</td>
<td>Discontinuity “skip” lesions</td>
<td>Continuous</td>
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<tr>
<td>Gross features</td>
<td>o Focal aphthous ulcers with intervening normal mucosa</td>
<td>o Extensive ulceration</td>
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<td></td>
<td>o Linear fissures</td>
<td>o Pseudo-polyps</td>
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<td></td>
<td>o Cobblestone appearance</td>
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<td>o Thickened bowel wall “linitis plastic”</td>
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<td>o Creeping fat</td>
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<tr>
<th>Micro</th>
<th>Noncaseating granulomas</th>
<th>Crypt abscess</th>
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<tbody>
<tr>
<td>Inflammation</td>
<td>Transmural</td>
<td>Limited to mucosa and submucosa</td>
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| Complication | - Strictures  
- String sign on barium study  
- Obstruction  
- Abscess  
- Fistula  
- Sinus tract | o Toxic megacolon |

| Genetic Association | HLA-B27 |
| Extraintestinal manifestation | Uncommon | Common |
| Cancer risk | Slight 1-3% | 5-25% |
| Presentation | Variable: Pain, diarrhea, weight loss | Bloody diarrhea |

*Kaplan USMLE Lecture Notes* (http://medchrome.com/basic-science/pathology/crohn's-disease-vs-ulcerative-colitis-ibd/)

### 1.5. Resident Microflora of Intestine

Designing of the intestinal tract has a bearing on the microbial flora (Savage 1977). The structure of the gastrointestinal tract dictates the localization of the localization of the microbiota and, to some extent, the composition of the microbiota as well. Recent studies have shown that the biota of the rumen, cecum, and large bowels of man and other animals have striking similarities. In all three types of tracts, microbial habitats may exist in any area from the esophagus to the anus. Some of these habitats may cover visible areas of the mucosal epithelium, others may be of microscopic dimensions, *i.e.* microhabitats (Savage 1977). They may occur in any major areas of the tract but may be colonized normally in areas of relative stasis, such as the rumen, cecum, and large intestine, where the flow rate of the contents does not exceed the doubling rate of the microbial population levels. Each of the general habitats apparently provided for its microbial colonizers a different type of environmental or nutritional challenge (Savage 1977).
For pretty long period, *Escherichia coli* was thought to be the chief inhabitant of the animal bowel. But now it is well known that *E. coli*, is actually a minority inhabitant of most gastrointestinal ecosystems. This confusion arose primarily because culturing methods for oxygen-intolerant anaerobic bacteria were not available earlier. After development of such methods (especially for ruminant bacteria) studies revealed that in most systems in adults the strict anaerobes outnumber the facultative microbes such as *E.coli* as much as 1000 to 1 (Savage 1977).

1.6. Composition of Commensal Microbiota in Intestine

The concentration and population of microorganisms that constitute the normal intestinal flora vary with the location along the intestine. The mammalian small intestine can be divided into the upper, mid, and lower areas, called the duodenum, jejunum, and the ileum, respectively. Flora in the stomach, duodenum, jejunum and proximal ileum are sparse, usually less than $10^5$/mL. The distal ileum represents a transitional zone between the sparse flora of the proximal small intestine and the luxuriant flora of the lower bowel, where microorganism concentrations reach $10^{11}$/mL. The predominant species are strict anaerobes, including *Bacteroides*, anaerobic *Streptococci*, *Bifidobacteria* and *Clostridium*. The commonest aerobic organisms are *E.coli*; however, their concentration ($10^8$/mL) is only 1/1,000 of the usual concentration of anaerobes in the colon (Savage 1977).

Few mechanisms control the normal small intestinal flora. First, bile added in the duodenum has additional antibacterial properties. Second, small intestinal motility mechanically sweeps bacteria downstream, helping to maintain a low concentration of organisms in the proximal small intestine. Third, the ileocecal valve plays an important role in preventing reflux of large amounts of colonic organisms. Additionally, mucus secreted by goblet cells and immunoglobulins has antibacterial properties.

Microorganisms are isolated frequently from the contents taken from all regions of the small intestine of nonruminant mammals of several types. In the lower part of the ileum, they could be microbes from the rich biota of the cecum finding their way by the ileocecal valve into the small intestine. In either case, the organisms would be only transients in the lumen of the small intestine and not indigenous inhabitants (Drasar & Hill 1974). The contents of the small intestine normally flow rapidly, possibly becoming
static for an appreciable period only, in the distal ileum. The microbes involved in lower small bowel are segmented; filamentous prokaryotes (Davis & Savage 1976) are unique in that they attach to intestinal epithelial cells via a segment on one of their ends that inserts into an invagination in the membrane of the epithelial cell. Intestinal biopsies taken with capsules from living persons may not yield satisfactory results because the sampled area is relatively smaller to the total surface area. Even biopsies taken at surgery are unsatisfactory because the patients usually have had their diets manipulated in some ways or may have been treated with antimicrobial drugs.

In all adults humans, the large bowel, including the cecum and colon, harbors much complex microbiota composed undoubtedly of both autochthonous and allochthonous microorganisms. Microbes in food are known to pass down into human feces. Microbes from habitats above the large bowel certainly pass down into the lumen of that region and their population is much lower than those of the chief inhabitants of the large bowel. Enormous microbial populations can develop in the lumen of the large bowel, and especially in that of the cecum, because these are areas of relative stagnation in the flowing stream that is the gastrointestinal tract. In these areas, the passage rate of luminal content does not exceed the doubling time of the bacteria. In most mammals, including humans, these luminal populations are composed in the main of oxygen-intolerant anaerobic bacteria of various types. Many genera, and in some cases hundreds of bacterial species can be isolated (Moore & Holdeman 1975). The luminal community therefore, is extremely complex. Luminal community in mammals is very similar to rumen luminal community in terms of complexity, types of microbial species involved, their nutritional, fermentative and symbiotic activities (Bryant 1974). Figure.4 showing list of organisms and number of organisms colonizing different parts of human gastrointestinal tract.
1.7. Factors Influencing Composition of the Microbiota

Many factors affect the composition of the large-intestinal microbiota in humans. These include the age, susceptibility to infections, nutritional requirements, and immunologic status of the host and the pH, transit time, interactions between flora components, and presence and availability of fermentable material in the gut (Collins et al 1999). Bacteria have been estimated to constitute 35-50% of the volume of the contents in the human colon. Figure 5. Showing that microbial flora profoundly influence nutritional, physiologic and protective processes (Cortot et al 2009). Both direct and indirect defensive functions are provided by the normal microbiota. Specifically, gut bacteria directly prevent colonization by pathogenic organisms by competing for essential nutrients or for epithelial attachment sites. By producing antimicrobial compounds, volatile fatty acids, and chemically modified bile acids, indigenous gut bacteria also create a local environment that is generally unfavorable for the growth of enteric pathogens. This phenomenon is called ‘Colonization Resistance’, which can be defined as the ability of microorganisms belonging to the normal gut microflora to impede the implantation of pathogens. This function of the microflora is also known as the barrier effect. The population levels and types of microbes in the many climax communities in
the gastrointestinal ecosystem, and the successions of these communities are regulated by multifactorial processes. Some of the regulatory forces in these processes are exerted by the host; some are exerted by the microbes themselves. Some microbial communities can exert direct influences to exclude other microbes from their habitats and niches. Some can effect changes in the functions of the host that regulate the biota and thus exert indirect influences on its composition and geographic distribution (Savage 1972).

Figure 5. Intestinal microflora ecology and its functions. The human intestine harbours roughly $10^{14}$ microorganisms, comprising bacteria, fungi and protozoa. Bacteria represent the majority of the gut flora, accounting for 60% of the total. The gut microflora has multiple functions, as represented here (Custom probiotics inc.).

1.8. Forces Exerted By the Host and Its Diet and Environment
Diet may exert a major influence on gut bacterial populations and their development. The main fermentable dietary substrates in the adult gut are carbohydrate-based materials such as dietary fibers, resistant starches, oligosaccharides, food sweeteners, and other nonabsorbed sugars. There is a lesser contribution from nitrogen-based materials like proteins and amino acids and some dietary lipids may also reach the colon in a metabolizable form. In the infant gut, the form of the milk substrate can have important effects on the composition of gut flora (Collins et al 1999). IBD encompasses a group of diseases triggered and perpetuated by a variety of diverse genetic, environmental, and immunologic factors that share similar clinical manifestations and primary affect the...
small intestine and colon (Figure 2). Antigenic similarities between intestinal microorganisms and their animal hosts suggest that such microbes have evolved to a close immunological relationship with the host (Keita et al, 2006). At least the surfaces of the microbes that contact the animal’s cells must be sufficiently related to the host’s antigens so as to render them recognizable as “self” by the animal’s immune system (Foo & Lee 1974). Being recognized as self would give indigenous microbe’s enormous advantage in colonizing their habitats. In this context, then, it would be rational to think if bacteria from the feces of human secretors of blood group specific glycoproteins differed in antigenic type depending upon the secretor types of the individual (Hoskins & Boulding 1976). If an animal’s immunological system influences at all the composition of the microbiota in the gastrointestinal canal, the main effect of that influence may be to prevent allochthonous species from colonizing habitats in the system. Interestingly, some cells in the epithelium itself function as phagocytes. Paneth cells in the epithelium at the bases of the crypts of Lieberkuhn in the small intestines of rats have been identified both functionally and structurally as active phagocytes. (Savage 1977) These cells may function to clear the crypts of microbes (through the secretion of lysozyme and a family of antimicrobial peptides known as cryptidins (Falk et al 1998) that progress too deeply into those areas where the epithelial cells are actively dividing (Savage 1977). Thereby, paneth cells play important role in limiting where microbes can localize in that region. Some evidences suggest that certain influences in the environment of an animal may alter the composition of the microbiota. Air pressure as altered during changes in altitude may change the composition of the fecal biota in man and mice (Gillmore & Gordon 1975). Such factors undoubtedly alter animal biota, which in turn alters the composition of the microbial communities. The precise physiological mechanisms that might be working behind this are unknown. Any physiological change that would decrease or increase peristaltic rate, the amount of HCl secreted in the stomach or perhaps even mucus secreted anywhere in the tract, could conceivably alter the microbial communities in local habitats. Microbes in the gastrointestinal ecosystem contribute to the regulation of the composition and localization of their communities not only directly but also by altering physiological responses of the host that may be involved in the regulatory processes. (Savage 1972)
Intestinal microbes deconjugate and otherwise alter the bile acids (Drasar & Hill 1974) and induce immunological responses in the host (Freter & Abrams 1972) that may be factors regulating the composition of the biota. The biota also stimulates peristalsis, which influences colonization by the microbes in all areas of the tract especially in small intestine. For any given community, the factors must balance delicately to maintain the structure of the community and this delicate balance can be perturbed by forces such as antimicrobial drugs (Savage 1972).

1.9. Probiotics, prebiotics and synbiotics: as an alternative to antibiotic therapy

The microbiota of the human large intestine influences health and well-being. Whereas it has long been accepted that gut bacteria play a role in host pathogenesis, current opinion is that certain microflora components can have beneficial effects on gastroenteritis resistance, blood lipids, antitumor properties, lactose tolerance, and gastrointestinal immunity. Probiotics, prebiotics, and synbiotics may contribute toward nutritional modulation of the gut microecology, with emphasis on the neonatal intestine where appropriate (Collins et al 1999). The term probiotic is often used for orally ingested (usually with food) live microorganisms that provide health benefits for the host. The definition of probiotic has changed over the years and was recently proposed as: “A preparation of or a product containing viable, defined micro-organisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host” (Schrezenmeir et al 2001). Bacterial species that have been used as probiotics include *Lactobacillus* (e.g. *L. acidophilus*, *L. plantarum*), *Bifidobacterium* (e.g. *B. longum*) and *Streptococcus* (e.g. *S. lactis*) (Gibson et al 1995; Bezirtzoglou et al 2011).

A prebiotic is defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson et al 1995). Prebiotics are generally non-digestible oligosaccharides such as inulin and lactulose. Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism, hence synbiotics. The live microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for growth (e.g. a fructooligosaccharide in conjunction with a bifidobacterial
strain or lactitol in conjunction with a lactobacillus organism) (Collins et al 1999). This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation, and result in advantages to the host that the live microorganism and prebiotic offer.

1.10. Interactions between Gut Bacteria and Host Immunity

Resident bacterial flora have been suggested to be an essential factor in driving the inflammatory process in human inflammatory bowel diseases. Intestinal mucosa is the main interface between the immune system and the external environment. Thus, that gut-associated lymphoid tissues (GALT) contain the largest pool of immunocompetent cells in the human body (Brandtzaeg et al 1989). The dialogue between host and bacteria at the mucosal interface seems to play a part in development of a competent immune system. Many and diverse interactions between microbes, epithelium and gut-associated lymphoid tissue are involved in modeling the memory mechanisms of systemic immunity. For instance, flora has been implicated in oral tolerance. The systemic response to a specific antigen can be abrogated after ingesting the same antigen. The interaction between gut-associated lymphoid tissue and flora early in life seems to be crucial for appropriate development of complex mucosal and systemic immunoregulatory circuits. In adults, immunity may be constantly reshaped by persistent interactions between the host and its bacteria that take place in the gut. Commensal organisms try to circumvent the immune response. For instance, *B. fragilis*, a predominant species in the human colon, can change its surface antigenicity by producing distinct capsular polysaccharides (Krinos et al 2001). Surface diversity seems to allow the organism to escape immunosurveillance and maintain an ecological niche of predominance in the intestinal tract. However, host defenses adapt and keep an active control of bacterial growth. The immune response to microbes relies on innate and adaptive components, such as immunoglobulin secretion. In addition, patients with Crohn’s disease or ulcerative colitis have increased intestinal mucosal secretion of IgG type antibodies against a broad spectrum of commensal bacteria. Immunoinflammatory responses mediated by IgG can damage the intestinal mucosa since, unlike normal IgA responses,
they activate the complement and the cascade of inflammatory mediators (Guarner et al 2003).

In general, the mucosal immune system is homeostatic despite the considerable antigenic load in the intestine. When an imbalance does occur in the regulation of this response, gut barrier dysfunction and inflammatory bowel disease are observed. Gut bacteria that gain access to the host through the mucosal tissue of the alimentary tract may influence the development of such intestinal inflammatory disorders. Gut inflammatory diseases are associated with the production of various inflammatory cytokines including interleukin-1 (IL-1), IL-8, or tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) that may be produced by mucosal epithelial cells and/or by neighboring cells from the immune system (Wang et al, 2006). These immune products may act as chemoattractants (chemokines) for specific inflammatory cells, including macrophages, monocytes, neutrophils, and lymphocytes, that contribute to the mucosal inflammation. Elevated levels of nitric oxide (NO)-derived metabolites have been associated with these Th1-mediated inflammatory disorders. Although this proinflammatory response may be necessary to clear the infection, it may invoke pathologic and potentially destructive changes in the tissue. In normal physiological conditions, a homeostatic balance is maintained and the inflammatory disorders are prevented by downregulation of the immune response in the intestine (Kasper and Buzoni-Gatel, 2001).

Intestinal immune homeostasis is dependent upon the successful interaction of several compartments within the intestinal tract. These include organized secondary lymphoid organs, such as mesenteric lymph nodes, Peyer’s patches, and leukocytes that are dispersed throughout the intestinal wall and within the mucosa, the intraepithelial lymphocytes (IEL). Epithelial cells or enterocytes lining the alimentary tract serve both as a physiological barrier separating the lumen from underlying tissues and as a source of immune inflammatory products. These enterocytes or immunocytes play a critical role in mucosal immunophysiology that in part consists of a paracrine network between enterocytes and the underlying immune and inflammatory cells (Kasper and Buzoni-Gatel, 2001).

Also an immuno suppressive state has been observed on delayed cutaneous hypersensitivity against amebic antigens. On the other hand, high levels of Interferon-
gamma (IFN-γ) (profile Th1) are associated with a higher cytotoxic effect of macrophages against trophozoites of *Entamoeba histolytica* (Ghadirian & Denis, 1992). Additionally, studies in a murine model suggest that the induction of the Th1/Th2 profile is dependent on specific peptides of *E. histolytica* (Talamas et al, 1995).

### 1.11. Mucosal responses to luminal bacteria

Moreover, patients with inflammatory bowel diseases have higher amounts of bacteria attached to their epithelial surfaces than than do healthy people (Swidsinski et al 2002). These bacteria are from diverse genera and some of them, especially bacteroides, were identified within the epithelial layer, in some instances, intracellularly (Swidsinski et al 2002). Thus, unrestrained activation of the intestinal immune system by elements of the flora could be a key event in the pathophysiology of inflammatory bowel disease. Host mucosa is exposed to vast numbers of metabolically active microbial cells and cell wall components, such as LPS (lipopolysaccharide) and peptidoglycan. A unique feature of host microbial interactions in the intestine is the lack of pro-inflammatory responses in the mucosa exposed to the resident luminal microflora, whilst retaining the capability to respond to luminal pathogenic bacteria via the recruitment of acute inflammatory cells from the systemic circulation. In general, host responses to pathogenic microorganisms are mediated by innate and adaptive immune responses. In the intestine (Figure 6), components of innate immunity are either preexisting or are rapidly activated and, in addition to mediating antimicrobial effects, also regulate the highly specific adaptive immune responses.

### 1.12. Innate and adaptive immune connections in inflammatory bowel diseases

The study of inflammatory, innate and adaptive responses has led to the discovery of sensors {TLRs, nucleotide-binding oligomerization-domain receptors [nucleotide-binding domain leucine-rich repeat containing families (NLRs); including nucleotide oligomerization domains (NODs) and NALPs], C-type lectin receptors (CLRs) and retinoic acid-inducible gene (RIG-I)-like receptors (RLRs)} (Figure 7.) (Jarchum et al 2011). These sensors recognize microbial (bacteria, viruses and fungi) and nonmicrobial (potassium ion efflux, products of cell death and tissue injury such as heat shock proteins...
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and components of the extracellular matrix) triggers. These sensors, located on human macrophages, dendritic cells, and other cells that recognize bacteria, initiate cascades that result in effector responses such as inflammation, tissue repair and, in the case of microbial sensing pathways, adaptive immune responses (Medzhitov et al 2010). Importantly, the specific arm of the effector response [whether isotype-specific B-cell responses, CD8-mediated and CD4 T helper (Th) 1, Th2, Th17, Tr1 and inducible regulatory T cells (Tregs)] is appropriate for the initiating trigger (Takeuchi et al 2010; Gong et al 2010; Jarchum et al 2011).

Figure 6. Control of intestinal microbiota and the host response at mucosal interface. Innate and adaptive microbial-sensing systems in epithelial cells and immune cell types adjust the levels of local microbial clearance activity. Limits to mucosal inflammation are set by levels and activity of local immunoregulatory cell types (e.g., macrophages and neutrophils).
regulatory CD4$^+$ and CD8$^+$ T cells, plasmacytoid dendritic cells). TGF, transforming growth factor; IL-10, interleukin-10; PGE$_2$, prostaglandin E2; IFN, interferon; IDO, indoleamine oxidase; Treg, regulatory CD4$^+$ T cells; B, B cell; M cell, mucosal follicle-associated epithelial cell; sIgA, secretory immunoglobulin A; CCR, CC-type chemokine receptor; IEC, intestinal epithelial cell.

**Figure 7. Structure and cellular location of TLRs and NOD1 and NOD2.**

The classic pathway of innate control of adaptive immune responses active in IBD pathogenesis (as reviewed above), innate–adaptive connections in IBD also involve the adaptive control of innate immune responses. Regulatory T cells have been shown to regulate innate-mediated colitis in various animal models (Maloy et al 2003). In addition, products of the adaptive immune system [such as secretory immunoglobulin A (IgA)] may indirectly impact on the adaptive immune system by binding microbial peptides and other antigens, thus limiting the amount of antigen that contacts cells on the intestinal surface, and regulatory T cells may control this IgA response Figure 8 (Cong et al 2009). Although it has been shown that luminal IgA specific either to commensal antigen or molecular patterns, such as lipopolysaccharide or flagellin, appears important in limiting activation of the innate immune response, the significance of this phenomenon to the
pathogenesis of animal and human IBD is unknown (Cong et al 2009; Peterson et al 2007).

**Figure 8. Innate and acquired immunity.**

sm, Smooth muscle; ens, enteric nervous system; g, goblet cell; p, Paneth cell; eb, epithelial barrier; ap, antimicrobial peptides; m, mucus layer; pp, Peyer’s patch.

### 1.13. Role of NOD (nucleotide-binding oligomerization domain) as intracellular sensors

Highly conserved structures of pathogenic microorganisms, designated PAMPs (pathogen-associated molecular patterns), are recognized by pattern-recognition receptors (Janeway et al, 2001). Recent studies have shown that, some transmembrane receptors for PAMPs, and there are also intracellular sensors of bacterial products, which have been designated as Nod1 or Card4 and Nod2 or Card15 (Girardin et al 2003, Inohara et al 2003; Fujimoto et al 2011). The **NOD1/CARD4** gene has been considered as a candidate gene not only because of the structural and functional similarities of the gene product with NOD2/CARD15 protein but also because of its location on the 7p14-p15 chromosome band, which has been previously reported to contain an IBD susceptibility locus in few British families (Satsangi et al 1996). Both Nod1 and Nod2 have a C-terminal leucine-rich repeat domain, N-terminal CARD(s) [caspase activation and recruitment domain(s)] and a central NBS (nucleotide-binding site), Nod1 contains a single CARD but Nod2 contains two CARDS (Figure. 5). Nod1 and Nod2 have been shown to detect peptidoglycan (which is a constituent of the cell wall of Gram-positive
and Gram-negative bacteria), but with specificity for distinct muropeptides, with subsequent activation of the NF-κB pathway [Girardin et al 2003, Inohara et al 2003, Girardin et al 2003; Tang et al 2011]. Nod1 has been shown to detect a muropeptide found mostly in Gram-negative bacterial peptidoglycan. By contrast, Nod2 senses a muropeptide found in most bacteria (both Gram-negative and Gram-positive) as shown in figure 9 [Girardin et al 2003]. They are mainly expressed by two cell types’ (that are exposed to this component under physiological conditions) antigen-presenting cells (APCs) and epithelial cells (Inohara et al 2003). A more compelling interest in the function of these proteins arises from the recent finding that they have a role in the pathogenesis of human gastrointestinal disease: CARD15 is a susceptibility gene for Crohn’s disease (Hugot et al 2001; Ogura et al, 2001); and polymorphisms in CARD4 are associated with inflammatory bowel disease (McGovern et al, 2005) and asthma (Hysi et al, 2005). Moreover, CARD4 is involved in host defence against Helicobacter pylori infection of the gastric mucosa, a chronic infection that can lead to peptic ulcers and gastric cancer (Viala et al, 2004). Signaling function of NOD1 and NOD2 contributes to inflammation and host defence as depicted in (Figure 9).

Figure 9. Nod1 and Nod2 both sense peptidoglycan through the detection of distinct muropeptides. The structure detected by Nod1, GM-TridAP (GlcNAc-MurNAc-L-Ala-D-Glu-mesoDAP), is a muropeptide found mostly in Gram-negative bacterial peptidoglycan. Nod2 detects two types of muropeptides: GM-Di (GlcNAc-MurNAc-L-Ala-
D-Glu, also known as GMDP) present in peptidoglycans from Gram-negative and Gram-positive bacteria, and GM-TriLys (GlcNAc-MurNAc-L-Ala-D-Glu-L-Lys), a muropeptide found only in peptidoglycans from Gram-positive bacteria. Abbreviations: LTA, lipoteichoic acid; LPS, lipopolysaccharide; TA, teichoic acid.

NOD1 and NOD2 are members of the phylogenetically conserved NLR protein family (Martinon et al, 2005; Inohara et al, 2003; Ting et al, 2005; Chamaillard et al, 2003), the mammalian NLR family is composed of more than 20 members, which encompasses proteins that were previously identified as members of the CATERPILLER (CARD, transcription enhancer, R (purine)-binding, pyrin, lots of LRRs), NOD, NOD-LRR and NALP groups of proteins (Figure 5.). In general, members of this family share a tripartite domain structure that consists of the following: a carboxy (C)-terminal LRR domain, which is involved in ligand recognition; a central NOD (also known as a NACHT domain), which facilitates self-oligomerization and has ATPase activity; and an amino (N)-terminal domain that is composed of protein–protein interaction cassettes, such as CARDs or pyrin domains (Inohara and Nunez, 2003). Although NLR-family members that contain CARDs interact with different downstream adaptor molecules that contain pyrin domains. They are functionally related and both types of molecules activate nuclear factor-κB (NF-κB) and/or caspases. In addition, mutations in both CARD-containing and pyrin-domain-containing NLRs have been linked to inflammatory diseases (Ting et al 2005, Hoffman et al 2001). Although members of the NOD protein family have similar structures, this does not necessarily imply that they have similar functions.

1.14. Expression of NOD1 and NOD2
Both NOD1 and NOD2 are mainly expressed by two cell types that are exposed to and/or deal with microorganisms that express PGN: APCs and epithelial cells. In both humans and mice, APCs such as macrophages and dendritic cells (DCs) express NOD1 and NOD2, whereas other haematopoietic cells (such as T cells and B cells) do not express these proteins (Inohara et al, 2003; Gutierrez et al, 2002; Ogura et al, 2001). Most (but not all) intestinal epithelial cell (IEC) lines and, more importantly, most primary epithelial cells express NOD1. However, whereas most IECs express NOD2 at the mRNA level, expression at the protein level is low or undetectable (Gutierrez et al, 2002; Ogura et al, 2001; Hisamatsu et al, 2003; Lala et al, 2003; Hisamatsu et al, 2003; Kim et
**Figure 8a.** NLRs, homologs and adaptors. The NLRs are characterized by three distinct domains: an N-terminal effector domain, which can be a pyrin domain (PYD), a CARD or a Bir domain; a central NACHT domain (NACHT stands for domain present in Naip, CIITA, HET-E (plant het product involved in vegetative incompatibility) and TP-1 (telomerase-associated protein 1)) and a C-terminal LRR domain thought to constitute the microbe-sensing portion. Bottom, adaptors involved in NLR signaling, including RIP2, ASC and CARDINAL.
al, 2004). In addition, among primary epithelial-cell populations, NOD2 expression seems to be limited to Paneth cells, which are located at the base of the intestinal crypts (Ogura et al, 2003).

Both NOD1 and NOD2 are expressed mainly in the cytosol (Inohara et al, 2003). So, whereas TLRs function as cell-surface receptors, NOD1 and NOD2 do not have this property. However, there is recent evidence that, at least in epithelial cells, NOD2 contains molecular sequences that allow association with the plasma membrane (Barnich et al, 2005; Zurek et al, 2011). This pattern of intracellular expression suggests that the ligands for these molecules are not native microbial components but, instead, products that are derived from microbial components. Indeed, NOD1 and NOD2 have been shown to recognize peptides that are derived from the degradation of PGN, which is a component of bacterial cell walls (Inohara et al, 2003; Chamaillard et al, 2003; Girardin et al, 2003; Girardin et al, 2003; Tang et al, 2003).

The expression of both NOD1 and NOD2 is regulated by pro-inflammatory cytokines, but this occurs in a slightly different way for each. In the case of NOD1, constitutive (but variable) baseline expression by epithelial cell lines is upregulated by interferon-γ (IFNγ) acting through the transcription factor IFN-regulatory factor 1 (IRF1) at the CARD4 promoter; however, NOD1 expression is not upregulated by tumour-necrosis factor (TNF) (Hisamatsu et al, 2003). In the case of NOD2, baseline expression of protein by epithelial cells is low, and TNF induces upregulation of expression; furthermore, this positive effect is augmented by IFNγ (Rosenstiel et al, 2003). NF-κB-binding sites in the CARD15 promoter are involved in this response to TNF, implying that, when NOD2 activates NF-κB following activation by its ligand, NOD2 can upregulate itself. A similar regulatory system for expression of NOD1 and NOD2 has not yet been reported for APCs. Finally, consistent with the role of NOD proteins in innate immunity, type-I-IFN-mediated signaling might be involved in the expression of NOD1 and NOD2.

The CARD15 is overproduced in the CD colon, and is found in both macrophages and related cells and abnormally in mucosa epithelial cells. This dysregulated expression of the CARD15 gene may be explained by an inappropriate relationship between genetic and environmental factors.
1.15. Signaling through NOD1 and NOD2

Detection of microbes is mediated by the recognition of conserved and unique pathogen structures by specific host pattern-recognition molecules, such as the TLRs and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). The initial studies identified lipopolysaccharide (LPS) as a NOD2 ligand (Ogura et al, 2001), it is now well established that the NOD1 and NOD2 ligands are the PGN-derived peptides γ-d-glutamyl-meso-diaminopimelic acid (iE-DAP) (Chamaillard et al, 2003; Girardin et al, 2003) and muramyl dipeptide (MDP) (Inohara et al, 2003; Girardin et al, 2003), respectively (Figure 10.). Because PGN from both Gram-positive and Gram-negative bacteria contains MDP, NOD2 functions as a general sensor of most, if not all, bacteria. By contrast, because PGNs from Gram-positive bacteria do not contain iE-DAP (except for PGNs derived from specific Gram-positive bacteria such as Listeria and Bacillus spp. and from many Gram-positive bacteria in the soil) (Inohara et al, 2004), NOD1 mainly senses products from Gram-negative bacteria. Confirmation of these specificities has come from the finding that macrophages isolated from NOD1- or NOD2-deficient mice are completely unresponsive to their respective ligands (Chamaillard et al, 2003; Pauleau et al, 2003 Zurek et al 2011).

The NOD-protein ligands need to reach the LRR domains of the respective NOD protein for activation of this protein to be initiated. However, information on how this is accomplished is not well defined at present, especially in the case of APCs. One possibility that relates to phagocytic cells such as macrophages or DCs is that these cells generate the peptide ligands by ingesting whole bacteria and then digesting them in phagolysosomes (Gupta et al, 1985; Araki et al, 1971). In epithelial cells, a slightly different process might occur in that the apical peptide transporter PEPT1 seems to have a role in the delivery of MDP. This is indicated by the finding that MDP that is taken up by PEPT1 into colonic epithelial cells subsequently mediates the activation of NF-κB (Vavricka et al, 2004) (Figure 10.). In addition, it has recently been shown that H. pylori can ‘inject’ PGN into cells through a type IV secretion system, which is encoded by a pathogenicity island (Viala et al, 2004). This discovery indicates that PGN can enter cells by various mechanisms that involve bacteria-host interactions.
After small peptides derived from PGN have been released into the cytosol, they are thought to interact with NOD1 or NOD2 through the LRR domains of these molecules. However, it should be noted that, as is the case for activation of most TLRs by their respective ligands (Bell et al, 2003), there is, as yet, no direct evidence for the binding of the NOD1 and NOD2 ligands to these domains. The postulated interaction is then proposed to initiate the activation of NOD1 and NOD2 through the induction of a complex conformational change (Inohara et al, 2000; Tanabe et al, 2004).

This change comes, in part, from studies of activation of apoptotic-protease-activating factor 1 (APAF1), an NLR-family member that is involved in caspase activation and apoptosis (Saleh et al, 1999; Benedict et al, 2000). Activation of APAF1 is initiated by the interaction of its WD40 domain with its ligand (cytochrome c), as well as by the binding of dATP or ATP to an ATP-binding cassette (ABC) or oligomerization cassette in the NOD. The molecule then undergoes self-oligomerization, which enables it to bind its downstream effector molecule, caspase-9, through a CARD–CARD interaction. The large molecular complex that is formed in this way, which is known as the apoptosome, then facilitates activation of the bound caspase-9, possibly by bringing caspase molecules into juxtaposition (Saleh et al, 1999; Benedict et al, 2000). That this activation model applies to NLRs in general (and to NOD1 and NOD2 in particular) is indicated by the presence of structural similarities between NOD proteins and APAF1: the N-terminal region of the central NOD in NLRs contains both an ABC and an oligomerization module. At least in the case of NOD2, the introduction of mutations into the ABC region abolishes NOD2 signaling (Tanabe et al, 2004). In addition, it has been shown that both NOD1 and NOD2 undergo self-oligomerization following the binding of PGN-derived ligand (Inohara et al, 2000; Tanabe et al, 2004). In one model of NOD-protein activation based on the APAF1–caspase-9 pathway, Inohara et al. proposed that oligomerization of NOD1 or NOD2 also allows binding to a downstream effector molecule through a CARD–CARD interaction, in this case involving the serine/threonine kinase RICK (receptor-interacting serine/threonine kinase; also known as RIP2 or CARDIAK), and this, in turn, leads to cross activation of RICK (Figure 10.).
Figure 10. Signalling pathways of NOD1, NOD2 and TLR. Recognition of muramyl dipeptide (MDP) and γ-d-glutamyl-meso-diaminopimelic acid (iE-DAP) through leucine-rich repeat (LRR) domains activates the NOD (nucleotide-binding oligomerization domain) proteins NOD2 and NOD1, respectively, which then recruit receptor-interacting serine/threonine kinase (RICK) through caspase-recruitment domain (CARD)–CARD interactions. In addition to NF-κB activation, NOD1 and NOD2 signalling gives rise to the activation of mitogen-activated protein kinases (MAPKs) such as JUN amino-terminal kinase (JNK), extracellular-signal-regulated kinase (ERK) and p38MAPK by as-yet-unknown mechanisms (denoted by dashed arrows). TLR2 and TLR6 activate the transcription factor JNK, P38 and ERK by a signaling pathway. AP1, activator protein 1; PGN, peptidoglycan

(Strober et al, 2006)
1.16. Role of NOD proteins in disease

Nod proteins are a group of key switching proteins that are involved in recognition of upstream signaling molecules and turn on the downstream events by activating effector molecules. The importance of NOD1 and NOD2 in innate immunity has been highlighted by the findings that mutations affecting the function of these proteins are associated with the occurrence of disease (Hugot et al, 2001; Ogura et al, 2001; Miceli-Richard et al, 2001; Kanazawa et al, 2005; Holler et al, 2004) and that these proteins are important host-defence factors (Viala et al, 2004; Opitz et al, 2005) (Table 2).

Table 2. Disease related to polymorphism in NOD proteins.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutations*</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD1 Helicobacter pylori infection</td>
<td>No mutation</td>
<td>Delivery of PGN to epithelial cells through type IV secretion system</td>
<td>Viala et al 2004</td>
</tr>
<tr>
<td>Chlamyphila pneumoniae infection</td>
<td>No mutation</td>
<td>Activation of NF-KB in endothelial cells</td>
<td>Opitz et al 2005</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Deletion polymorphism in LRR domain</td>
<td>Risk factor for inflammatory bowel disease</td>
<td>McGovern et al 2005</td>
</tr>
<tr>
<td>Asthma and high IgE levels</td>
<td>Insertion polymorphism in LRR domain</td>
<td>Risk factor for asthma</td>
<td>Hysi et al 2005</td>
</tr>
<tr>
<td>Blau syndrome</td>
<td>Arg334Trp, Arg334Gln, Leu469Phe</td>
<td>Constitutive NF-KB activation</td>
<td>Miceli-Richard et al 2001</td>
</tr>
<tr>
<td>Early-onset sarcoidosis</td>
<td>Arg334Trp, His496Leu, Thr605Pro</td>
<td>Constitutive NF-KB activation</td>
<td>Kanazawa et al 2005</td>
</tr>
<tr>
<td>Graft-versus-host disease</td>
<td>Arg702Trp, Gly908Arg, Leu1007fsinsCys</td>
<td>Risk factor for graft-versus-host disease</td>
<td>Holler et al 2004</td>
</tr>
</tbody>
</table>
1.17. Bacterial induction of proinflammatory cytokines in IBD

During pathogen infection, innate immunity is initiated via the recognition of microbial products by pattern recognition receptors and the subsequent activation of transcription factors that upregulate proinflammatory genes. The transcription factor nuclear factor-kappa B (NF-κB) playing the central function in expression of cytokines, chemokines, anti-bacterial peptides and adhesion molecules. Signaling pathways of PAMP recognition converge into the activation of the pleiotropic transcription factor nuclear factor-kappa B (NF-κB) that, in the context of innate immunity, regulates the expression of proinflammatory genes encoding cytokines, chemokines, anti-bacterial peptides and adhesion molecules (Beutler et al 2009) (Figure 11).

Figure 11. Pathogenic bacteria such as Salmonella typhimurium trigger IκB kinase activation, IκBα degradation and nuclear translocation of p50/p65 NF-κB subunits. Some commensal bacteria offset these affects by promoting the nuclear export of activated p65 through associations with peroxisome proliferator-activated receptor (PPAR)γ, thereby terminating promoter activation. Other commensal bacteria inhibit IκBα degradation. (Beutler et al 2009)
IL-17A are leukocyte-derived cytokines that have a major impact on epithelial cells in various tissues. Increasing evidence suggests that IL-17 are key regulators of homeostasis and epithelial barrier function (Eyerich et al 2010). It can be protective against infections, but also turn pathological in several inflammatory diseases such as psoriasis, asthma and inflammatory bowel disease. Furthermore, IL-17 is involved in the pathogenesis of several autoimmune diseases. Although early studies have suggested that both cytokines are almost exclusively co-expressed by CD4+ T helper (Th)17 cells (Eyerich et al 2010). The demonstration of genetic associations in the interleukin 23 (IL-23) pathway in multiple chronic inflammatory disorders, including inflammatory bowel disease (IBD), has coincided with significant advances in the understanding of its key role in host defense and organ-specific autoimmunity. Evidence for the importance of the IL-23 pathway in IBD has come from mouse models of IBD, in the which IL-23 deficiency or blockade protects from disease (Elson et al 2007; Kullberg et al 2006; Yen et al 2006), as well as human IBD (Fujino et al 2003; Schmidt et al 2005; Annunziato et al 2007; Holtta et al 2008; Saruta et al 2007; Fina et al 2008). In particular, genetic polymorphisms in the IL-23 receptor, IL23R, represent one of the strongest associations in Crohn’s disease (CD) and are also associated in ulcerative colitis (UC) (Duerr et al 2006), psoriasis (Cargill et al 2007), and ankylosing spondylitis (Burton et al 2007).

1.18. Aims and Objectives

Insights into inflammatory bowel disease are advancing rapidly owing to immunologic investigations of a large number of animal models of intestinal inflammation background – breaking advances have been made in the interrogation of diseases that are inherited as complex genetic traits. The development of culture independent methods have helped to define the composition of the intestinal microbiota more comprehensively. These advances are bringing a deeper understanding to the genetically determined interplay between the commensal microbiota, intestinal epithelial cells, and the immune system and the manner in which this interplay might be modified by relevant environmental factors in the pathogenesis of IBD.

Although proposed several years ago, genetic findings to date have generally been consistent with this conceptual model. Indeed, if this model is true, it is presumed that eventually, knowledge of a patient’s genotype and altered microflora may allow the
clinician to apply a molecular classification to his/her disease and, presumably, better predict both the future disease course and appropriate therapeutic options. So present aim of the study are listed below:

1. Alteration in mucosa associated microbial flora in IBD patients.
2. Changes in expression level of Nod like receptor (NLR) family of genes.
3. Detection and analysis of polymorphism in inflammatory related genes.
4. To draw possible correlation between the altered mucosal flora and the IBD associated genes.