COMPARATIVE LEVELS OF MCT1 AND SMCT1 IN ULCERATIVE COLITIS, CROHN’S DISEASE AND NORMAL PATIENTS

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Inflammatory Bowel Disease: Pathogenetic Mechanisms

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Abstract
Significant progress has been made in the understanding of ulcerative colitis and Crohn’s Disease over the last decades. Despite this, the pathogenesis of inflammatory bowel disease (IBD) remains obscure, especially at molecular level. Various contributing factors have been identified so far, but their respective contributions are not entirely clear cut. In this review, we focus on the genetic and environmental factors linked with IBD pathogenesis. We also explore the role of pro-inflammatory cytokines on the molecular pathophysiology of IBD.

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Introduction
Ulcerative colitis (UC) and Crohn’s Disease (CD) are the two commonest forms of inflammatory bowel disease (IBD). UC was first described in a case report by Sir Samuel Wilks in 1859.1 CD, on the other hand, was identified as a separate disease entity by Crohn and colleagues in 1932.2 Despite the many decades following initial reports, the pathogenesis of IBD remains largely unknown and therefore, the pathophysiology poorly understood.

Both UC and CD have a similar clinicopathological presentation with a small proportion of patients showing features of both diseases. Typical presenting complaints in IBD include: diarrhoea, abdominal cramping, blood in the stool and weight loss. A small percentage of patients also display extra-intestinal manifestations which include skin lesions, arthritis/arthritis and iritis/uveitis amongst others. Inflammation in UC is typically superficial and confined to the colon and rectum. CD involves the entire gastrointestinal tract (GIT) and the inflammation affects deeper mucosal layers in a discontiguous fashion giving rise to ‘skip’ lesions. Bowel inflammation in UC and CD are generally distinct with respect to: the anatomical site; depth of bowel wall involvement and disease-specific complications. Differentiating between the two conditions are especially challenging when inflammation is confined to the colon.3

IBD is a relapsing and remitting condition and in most instances, patients will experience intermittent bouts of acute flares after a varying period of remission. A small fraction of patients displays continuous disease activity with no remission, while others experience just a single acute attack with no subsequent symptoms.

The incidence of IBD is rising globally. Although the disease appears more prevalent in the developed world, its incidence is steadily rising in developing countries and South Africa is no exception. Current treatment strategies in IBD include the induction and maintenance of remission. Currently, there is no cure for IBD and patients remain on long-term drug treatment. Drug therapy, however, is costly and has considerable side-effects. Without understanding the pathogenetic mechanisms in IBD, curative treatments will remain a dream for many patients.

Aetiology
Currently, no precise pathogenetic mechanism exists for IBD. However, genetic, environmental, and immunologic factors have been linked with its development (Figure 1). The consensus view is that the disease results from an aberrant mucosal immune response to environmental triggers in genetically susceptible individuals.4 Studies showing discordant rates of IBD in monozygotic twins, with identical inherited genetic material, suggest that environmental factors may have a strong role in IBD development.5,6 Monozygotic twins have been shown to have differences in their gut microbiota.7 El Aidy et al. showed in a mice model that genetic factors may have a
role in determining the composition of the gut microbiota and the maintenance of homeostasis in the intestines. Environmental triggers implicated in IBD include: diet, gut microbiota, stress and mood, medications, lifestyle, and influence of free radicals.

Genetic susceptibility

Explosion of genome-wide association studies (GWAS) in recent years have increased our knowledge into the genetic basis of IBD. A recent meta-analysis of GWAS on UC and CD identified a total of 163 IBD susceptibility loci that meet genome-wide significance threshold. Of the 163 IBD loci, 110 were associated with both diseases whiles 30 and 23 loci were more specific to UC and CD, respectively. These findings suggest that UC and CD have common genetic characteristics and hence mechanistic features. Although UC and CD share similar genetic susceptibilities, the signalling pathways invoked by these genetic variants and how they affect intestinal homeostasis are different in both diseases. For example, CD susceptibility genes have been associated with innate immunity, autophagy, and phagocytosis, while UC genetic variants are linked to intestinal barrier function.

Mutation in the Nucleotide binding oligomerization domain-containing 2 (NOD2) gene, also known as Caspase recruitment domain (CARD)-containing 15 or IBD1 has been identified as the most important and frequent genetic alterations in IBD, particularly in CD. NOD2 gene or CARD15 is located on chromosome 16 and encodes the cytoplasmic protein, NOD2, which recognises bacterial peptidoglycans, such as muramyl dipeptide (MDP), leading to downstream activation of NF-κB and mitogen-activated protein kinases (MAPK) signalling in dendritic cells. Additionally, NOD2 has recently been identified to function in autophagy induction, cells that present antigen as part of their major histocompatibility class II (MCH II), and intracellular bacteria resistance. Mutations in the leucine-rich repeats (LRR) of NOD2 have been reported and the resulting defect leads to loss of function in the NOD2 pathway, and hence the development of IBD. Genetic studies have also abetted the identification of two important autophagy genes (Autophagy related 16-like 1, ATG16L1 and Immunity-related GTPase family M, IRGM), whose different variants have been implicated in IBD. Autophagy is an intracellular event that involves catabolic lysosomal degradation of ingested bacteria as well as self-digested cellular components. Although NOD2 can directly initiate autophagy pathways, it has been observed that for an effective elimination of intracellular bacteria, an intact NOD2 and proteins encoded by ATG16L1 are required. Activation of NOD2 by bacterial ligands such as MDP leads to the formation of ATG16L1-dependent autophagy vacuoles in dendritic cells and subsequent initiation of intracellular bacteria degradation. Alterations in either NOD2 or ATG16L1 genes have been shown to compromise this activity, resulting in reduced bacterial clearance and a shift in gut microbiota. Furthermore, Cadwell et al. demonstrated that a specific virus infection plus a mutation in ATG16L1 increases the risk of colon inflammation, similar to CD. This observation further supports the role of genetic-environmental interactions in IBD development.

Tight junctions in the intestinal mucosa protect the epithelium from luminal microorganisms and their products. A disturbance in this functionality often leads to increased gut permeability, which has been implicated in inflammatory bowel disease. Various gene candidates, whose alterations affect mucosal integrity have been identified in IBD. Examples of such genes include Cadherin 1 (CDH1), Guanine nucleotide binding protein alpha 12 (GNA12), and Protein tyrosine phosphatase non-receptor type 2 (PTPN2). Muise et al. confirmed that polymorphisms of CDH1 resulted in a truncated expression of E-cadherin, a main component of adheren junctions and a key mediator of epithelial intercellular communication, leading to abnormal cytoplasmic accumulation and impaired plasma membrane localisation. Such mis-localisation of E-cadherin disrupts intestinal permeability and may result in uncontrolled entry of bacteria into the submucosal layers. A missense mutation of Mucin 2 (MUC2) gene has also been associated with chronic intestinal inflammation in mice arising from mucosal epithelial defects. Alterations in this gene have been shown to compromise MUC2 biosynthesis leading to an inappropriate regulation of the innate and adaptive immune systems through endoplasmic reticulum stress, reduced goblet cell numbers, decreased mucous production, and spontaneous intestinal inflammation. The association of Interleukin 23 receptor (IL23R) gene with IBD has also been described. This gene encodes a receptor subunit protein for IL-23, an important pro-inflammatory cytokine, which regulates the generation of T lymphocytes (T cells) in IBD. IL-23 inhibits the production of regulatory T cells (Tregs) and, therefore, counteracts the inhibitory effects of Tregs on T-helper type 1 (Th-1) and Th-17 cells. Finally, micro ribonucleic acids (miRNAs) have been identified as causal factors to IBD susceptibility. miRNAs are minute non-coding RNAs that inhibit protein synthesis either by suppressing translation processes or degradation of target
messenger RNA (mRNA). In quiescent IBD, Fasseu et al. showed that the expression of miRNA in non-inflamed mucosa was dysregulated in both UC and CD cohorts.27 This over-expression of miRNA in non-inflamed mucosa suggests that miRNA can possibly initiate IBD or cause a relapse in quiescent disease by targeting genes that are associated with the risk of IBD.

**Immunologic factors**

Even under normal physiologic conditions, the GIT is in a constant state of low grade inflammation and the intestinal immune system maintains its energy. Imbalance of this homeostasis has been implicated in the development of IBD. Both the innate and adaptive intestinal immune systems have been implicated IBD development.28 The intestinal epithelium, which forms a protective barrier against luminal bacteria, together with other immune cells form the backbone of the innate immune system of the GIT. The intestinal epithelium is a single layer of cells that is in direct contact with luminal contents of the bowel. It consists of four different cell types: columnar (absorptive) cells, Paneth cells, goblet (mucosecreting) cells and neuroendocrine (enteroendocrine) cells, all of which are derived from multipotent intestinal stem cells.29 Columnar cells are more abundant in the small intestines and they contain microvilli which are covered by glycopexy forming a sticky barrier that prevents pathogens from invading the mucosa.30 These cells also produce antimicrobial peptides such as defensins, which are cationic broad spectrum microcidal peptides, that forms micropores in microbial membranes and cause their death.31 Human defensins are classified into two groups: alpha-defensin (HD) and beta-defensin (HBD), based on three characteristic intracellular disulphide bonds. While HBD have been shown to be produced by columnar cells, Paneth cells which are located at the base of the crypts of Lieberkühn secrets HD5 and HD6 to keep the crypts pathogen free.32,33 Goblets cells on the other hand secrets a negatively charged viscous mucous, which forms a thick layer of about 150µm on top of the epithelium to provide external mechanical protection.30 Mucous contains glycosylated mucins, trefoil factors, and defensins, which together modulates the defensive properties of the mucous layer by preventing microbial invasion into the epithelium. In addition, the intestinal epithelial cells regulate intestinal permeability by forming tight connections with adjacent cells to deny entry of luminal microorganism into submucosal tissues. These connections comprise of transmembrane proteins of adherent junctions, tight junctions, and desmosomes; which are important for controlling cellular adhesion and paracellular transport.34

In a nutshell, the entire intestinal epithelium with functional intercellular junctions covered with mucous layer and a shield of defensins constitutes the mucosal innate barrier (figure 2). In health, this barrier provides effective protection against luminal antigens. Defects in the mucosal innate barrier, on the other hand, are thought to perpetuate the development of IBD. The development of CD, for example, has been associated with decreased expression of human defensins independent of the degree of inflammation. While patients with ileal CD have reduced levels of human alpha defensins, HB5 and HB6, patients with colonic CD have reduced human beta-defensin, HBD1 levels.35,36 One study suggested an association between reduced human alpha-defensins and NOD2 mutation in ileal CD, but this was refuted by another study.37,38 In UC, on the other hand, expression of human defensins were relatively preserved during inflammation compared to CD.39,40 Although the defensin release is appropriate in UC, the physical and chemical barrier provided by the mucous layer is deficient in patients with UC.41,42,43 Therefore, in CD there is deficiency in the production of defensins, while in UC there is abnormality in the mucous layer formation. This allows luminal microorganisms and their products easier access through the intestinal epithelium to activate secondary cells of the intestinal innate immune system such as macrophages and dendritic cells (figure 2).

Activation of innate cells leads to downstream induction of the adaptive immune system, which is highly specific and confers long lasting immunity.28 The adaptive immune system together with molecules and cells of the innate system work together to effectively eliminate pathogens as well as foreign antigens from attacking host tissues. The principal components of the adaptive immune system are T cells, which upon stimulation differentiate into other specialised T cell forms, with specific pro-inflammatory cytokine and chemokine production. In IBD, three T cell types have been described: Th1, Th2, and Th17.44 Dysregulation of T cell response secondary to disruption of the innate immune system leads to abnormal development of a subset of T cells resulting in excessive amounts of cytokine and chemokine production, which orchestrate the inflammatory damage observed in IBD. The development of CD has been observed to be mediated by a Th1 and Th17 response with IFN-γ, IL-17, and IL-22 production. UC, on the other hand, is characterised by Th2-like response with the development of natural killer (NK) cells producing IL-13 as well as IL-5,28,44,45

**Figure 2:** An illustration of an intact healthy intestinal epithelium and the epithelia of CD and UC. A healthy individual’s epithelium produces sufficient amount of human defensins fixed in a thick layer of mucous preventing luminal microbes from reaching the epithelium. The production of defensins and mucous is impaired in CD and UC, respectively. This biochemical alteration in IBD epithelium allows luminal microbes and antigens to invade the epithelium to cause the activation of secondary immune structures.
Gut microbiota

Literature suggests that the human gut microbiota play a crucial role in IBD pathogenesis. The gut microbiome consists of approximately 1,000 bacterial species, with at least 160 species in every individual host. The proximal colon contains approximately 1012 microorganisms and the terminal ileum roughly 108 microorganisms. Microbiota colonise the gut a few weeks after birth and thereafter, it changes under the influence of social and environmental factors as well as immune maturation. Functions of the gut microbiota include: digestion of dietary compounds and xenobiotics as well as fermentation of complex carbohydrates with the production of short chain fatty acids (SCFAs); maintenance of the mucosal barrier by inhibiting pathogen invasion and strengthening of the epithelial barrier integrity; and regulation of the mucosal immune system. However, they have been shown to drive mucosal inflammation in their host, especially in dysbiosis. The high concentration of bacteria in the colon and distal ileum, commonest sites of UC and CD, led to the suggestion that the gut microbiota could be responsible for the development of IBD. The fact that mice raised under germ-free conditions do not develop colitis supported this notion. Moreover, the fact that microorganisms are fertile has revealed that IBD development is strongly associated with environmental factors (Figure 3). The involvement of environmental triggers in IBD development has been documented by various epidemiological studies. A wide range of environmental factors including cigarette smoking, diet, ROS, medication as well as mood and depression have been described.

Environmental factors

In addition to genetic susceptibility, studies from monzygotic twins have revealed that IBD development is strongly associated with environmental factors (Figure 3). The involvement of environmental triggers in IBD development has been documented by various epidemiological studies. A wide range of environmental factors including cigarette smoking, diet, ROS, medication as well as mood and depression have been described.

Smoking

Tobacco smoking appears to have a divergent effect in the development of IBD. Smoking confers a higher risk for initiation and exacerbation of CD, with the risk lasting for years after cessation. Smoking appears to be protective in UC, with patients’ often experiencing relapse after cessation. This paradoxical effect of smoking in IBD can be explained by the differential activities of nicotine on the small and large intestines, where it increases and decreases the generation of free radical-mediated oxidative stress, respectively. In UC, nicotine may be protective by inducing mucin production and decreasing the expression of IL-8 and TNF-α, through the activation of nicotinic acetylcholine receptors. There have also been interesting reports that marijuana smoking appears to improve the symptoms of IBD. A Canadian study found that 33% of UC patients and 50% of CD patients who used marijuana experienced relieve from the following IBD-related symptoms: abdominal pain, diarrhea and reduced appetite. The long-term effect of marijuana on the natural history of IBD is still unclear at present.
Oxidative stress

Oxidative stress has been described as a contributing factor to the development and progression of IBD. Oxidative stress is defined as a disturbance of the normal redox state of body cells, where there is an imbalance between the formation of ROS (superoxide anion radical, hydroxyl radical, hydroperoxyl radical, nitric oxide, and oxygen) and antioxidant activity. In addition to IBD, oxidative stress underlies disease process in the cardiovascular system, neurodegenerative disorders, cancer and chronic inflammatory disease. In IBD, infiltrated leukocytes including polymorphonuclear neutrophils, mononuclear cells as well as B and T lymphocytes produce large amount of ROS, which is accumulated into the affected area of the intestine. Enzymes such as COX, lipoxygenase, and xanthine oxidase are also able to produce ROS when activated. It has been demonstrated that the ROS-antioxidant balance is disturbed in IBD and that patients with the disease have an increased production of ROS by activated phagocytic cells. These free radicals are generated primarily to target offending pathogens but may also affect the host cellular systems. Molecular targets of ROS include unsaturated fatty acids of lipid membranes, proteins, and nucleic acids. Free radicals can damage lipid membranes by forming lipid peroxides, which destroys the structural integrity of the lipid membrane leading to leakages, and eventually causing a complete membrane breakdown. ROS, together with lipid peroxidation products can cause structural disturbance of protein molecules leading to cross-linked reaction products and fragmentation products with other cellular components. Oxidative damage to proteins can render them dysfunctional, for instance, oxidative damage to tight junction proteins may lead to an increased penetration of bacteria into the lamina propria to stimulate T cell production of pro-inflammatory cytokines and aggravate IBD. Accumulation of hydroxyl radicals in colonic epithelial cells can cause depolymerisation of mucous and destroy their protective functions.

Diet

Diet is believed to play a dual role in the development of IBD. Although not identified as a primary aetiological factor, diet may influence metabolic activities and contribute to the progression of the disease. Diet may affect IBD by exposing dietary antigens to the mucosa, alter gut permeability and influence the composition of the gut microbiota. The typical “Western” style diet with an imbalance in the consumption of fatty acids, vegetables, and fruits may possibly confer risk for IBD. High intake of protein from animal source has been associated with the risk of IBD. Proteins derived from fish gave conflicting risk of IBD with more than two courses of antibiotic use. Data suggest that tetracyclines and cephalosporins have a strong correlation with IBD development. Non-steroidal anti-inflammatory drugs (NSAIDs) have multiple gastrointestinal adverse effects and their disruption of the gastrointestinal barrier may influence IBD development. Prostaglandins synthesised from arachidonic acid by the action of COX enzymes, and NSAIDs primarily target COX-1 and COX-2 to reduce the production of prostaglandins in the mucosa. Prostaglandins play a vital role in mucosal defence, maintenance of microcirculation and modulation of the immune system in the intestinal epithelium. In one study, the risk of IBD development was linked to reduced prostaglandin production by NSAIDs, particularly early and frequent relapse, with non-selective NSAIDs associated with 17-28% relapse rate in quiescent disease. Although other studies have reported similar findings, two studies by Bonner et al. did not find any association between non-selective NSAIDs and IBD onset or relapse. COX-2 selective NSAIDs appear safe with no increased risk reported in a recent meta-analysis. Clearly, more robust data are needed to settle the NSAID and IBD relapse debate.
Psychological factors
There is positive correlation between anxiety and depression and IBD. While we appreciate that mood disorders may predispose to IBD, the development of anxiety and depression is likely a consequence of IBD. Mood disorders like anxiety are usually high among IBD patients with knowledge about the disease-specific complications and the prospect of surgical intervention. Mawdsley et al. demonstrated that acute psychological stress increased systemic and mucosal production of pro-inflammatory cytokines (TNF-α and IL-6) in patients with inactive UC, which could have been responsible for the disease exacerbation in such patients. Psychological stress has been proposed to directly affect pro-inflammatory cytokine production, increased gut permeability, activation of mucosal mast cells, and stimulation of both central and peripheral release of corticotrophin-releasing factor. Poor medication adherence and smoking, indirectly associated with stress, can potentially trigger exacerbation of the disease. Psychological stressors have the potential to initiate IBD or triggering a relapse in a patient with quiescent disease as described above.

Molecular pathogenesis
The precise molecular mechanism of IBD remains unclear despite significant progress made in understanding the underlying pathogenesis of the disease. The consensus view of IBD pathogenesis has mainly focused on the gut microbiota and the mucosal immune system, involving two broad hypotheses. One theory proposes an abnormal immune response to mucosal antigens from normal gut microbiota – defective mucosal immune system theory. An opposing theory proposes dysbiosis causing abnormal immune regulation in the presence of normal functioning mucosal immune system – abnormal gut microbiota theory. In both scenarios, an abnormal and excessive immune response is raised leading to the inflammatory cascades observed in IBD.

Mucosal immune defect theory
Able to distinguish self from non-self, the gut immune system maintains tolerance to luminal commensals, but is able to mount appropriate responses to pathogens. An abnormal adaptive immune response to luminal antigens was previously thought central to the IBD pathogenesis theory. However, recent evidence suggests a defective innate immune system to be the primary underlying cause. The innate immune cells including macrophages are able to sense microorganism-associated molecular patterns (MAMPs) through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), to initiate the production of chemical mediators in defence against offending triggers. NLRs are important in the induction of gut mucosal inflammation and indeed, polymorphism within the NOD2 gene confers greater IBD risk, specifically CD development. NOD2 is a PRR and a member of NLR protein family expressed by monocyte derived intestinal epithelial cells and Paneth cells. It contains a NOD domain that is linked on its carboxylic-terminal side to LRR and on its amino-terminal side to two tandem CARD domains. The LRR domain is the site for MAMPs recognition, whereas the CARD domain interacts with downstream signalling molecules. On sensing MAMPs, such as muramyl dipeptide (MDP) derived from bacterial peptidoglycans, NOD2 undergoes an auto-conformational change in structure, which results in an unfolding of the molecule, followed by oligomerisation and exposure of the CARD domain. This specific change in conformation allows NOD2 to bind to and activate a receptor interacting protein–like interacting caspase-like apoptosis regulatory protein kinase, RICK (also known as receptor interacting protein 2, RIP2) through CARD-CARD interactions involving the respective domains of the two molecules. This interaction has been identified to be a crucial step in NOD2 signalling. Activated RICK or RIP2 undergoes polyubiquitination resulting in the recruitment of transforming growth factor beta-activated kinase-1 (TAK1) complex, which drives IKK induction with subsequent activation of NF-kβ.

NOD2-NF-kβ signalling induces the expression of cytokines, chemokines and antimicrobial factors by deferent cells in the gut epithelium (Figure 4). MDP-dependent NOD2 induction resulted in expression of defensins and the anti-inflammatory cytokine, IL-10. NOD2 activation, as part of the Myd88 pathway, has an inhibitory effect on the NF- kβ stimulated release of the pro-inflammatory cytokines, TNF-α, IL-8, and IL-6. Therefore, it makes perfect sense that mutation in NOD2 will allow unchecked production of inflammatory cytokines that will propagate the inflammatory cascade in IBD. As described above, NOD2 signalling plays an important functional role in down-regulating the expression of pro-inflammatory factors via the TLR signalling. TLRs comprise a class of transmembrane PRRs, which are either induced or constitutively expressed in intestinal epithelial cells as well as various professional immune cells subset (for example macrophages or monocytes, dendritic cells, and T cells) within the intestinal lamina propria. TLRs are a family of glycoproteins, which contain a multiple LRR motif, divergent ectodomain and a highly conserved intracellular tail domain called toll-interleukin-1 receptor (TIR) domain. A variety of antigens ranging from lipopolysaccharide, lipopeptide and flagellin from bacteria to viral derived double-stranded RNA have an affinity for TLRs. Ligand binding elicits receptor activation through conformational changes which then lead to the activation of adaptor proteins, such as myeloid differentiation primary response 88 (Myd88). It has been found that all TLRs signal through Myd88 adaptor protein except for TLR-3. Induction of Myd88 activates a series of intermediate signalling molecules including TAK1, which then lead to the activation of transcriptional factors, including NF-kβ, MAPK, and STAT.
Like NOD2, signalling stimulation through TLRs is important in maintaining gut homeostasis in health. Thus, both NOD2 and TLRs are important innate immune factors necessary for maintaining tolerance to luminal antigens in the gut. In addition to its role in innate immune responses, NOD2 also exerts a regulatory inhibitory function to innate responses, particularly in TLR signalling. NOD2 signalling inhibited TLR-2-driven activation of NF-κB while mutated NOD2 exerted stimulatory effect. Furthermore, chronic NOD2 stimulation of macrophages from healthy individuals led to a down-regulation of pro-inflammatory cytokines, TNF-α, IL-8, and IL-1 following TLR-4, TLR2, and IL-1 receptor (IL-1R) receptor activation. The mechanism behind NOD2 regulation of TLR signalling is poorly understood, but it has been proposed that NOD2-dependent mediators, including interferon regulatory factor 4 (IRF4) and IL-1R-associated kinase-M (IRAK-M) may be the primary molecular signals for this regulation. In summary, functional defects in NOD2 lead to a primary disturbance of the mucosal innate immune homeostasis, which is primarily caused by decreased production of defensins and anti-inflammatory cytokines; loss of NOD2-dependent autophagy function; as well as impaired regulation of TLR responses. This results in luminal microorganisms invading the intestinal epithelium and stimulating other innate factors such as the TLRs to increase the production of pro-inflammatory cytokines to drive the adaptive immune responses leading to the inflammatory cascades observed in IBD (Figure 4).

Pro-inflammatory cytokines in inflammatory bowel disease
Pro-inflammatory cytokines are small non-structural proteins secreted by nearly all nucleated cells. Their function is to promote inflammation. Immune activation in IBD primarily involves the innate immune cells including macrophages and dendritic cells within the mucosal epithelium. The primary innate immune response activates the adaptive immune system which is regulated by B lymphocytes (B cells) and T cells in the lamina propria. Macrophages and dendritic cells, acting as antigen presenting cells (APC), stimulate adaptive cells through antigen presentation as part of the MHC class II together with the cytokines they release upon activation. Upon stimulation, naïve T helper cells differentiate into specific T helper subtypes producing different sets of cytokines, depending on the inflammatory milieu. Differentiation of naïve T helper cells into Th1 is induced by IL-12 and it is associated with high amount of IFN-γ, IL-2 and TNF-α release. In an IL-4 microenvironment, naïve T helper cells differentiate into Th2 with increased production of IL-5, IL-13, and IL-4. Another subset of T helper cells, Th17, produces large amount of IL-17, IL-21, and IL-22 when induced by transforming growth factor-β (TGF-β) and IL-6.

CD is commonly associated an abnormal Th1 response with Th1 cytokine profile and UC with Th2 response with characteristic Th2 cytokine profile. Matsuoke et al. showed that CD patients exhibited increased IL-12 expression in their lamina propria compared with healthy controls. Further, mucosal T cells from patients with CD produce large amount of IFN-γ and IL-2 compared to patients with UC. Lamina propria T cells from inflamed CD mucosa showed increased IFN-γ secretion compared to those from UC or control, but T cells from inflamed UC lamina propria produced increased amount of IL-5 compared to inflamed CD. The association of UC with Th2 response has been based on studies showing increased production of IL-13 by NK T cells, rather than conventional T cells. CD is therefore considered as exhibiting a Th1 response with excessive IFN-γ and IL-2 release, whereas UC is classified a Th2 disease characterised by increased production of IL-5 and IL-13 (Figure 5). The Th1/Th2 concept in CD and UC has been contested by different observations about mucosal Th1 and Th2 cytokines in IBD. Rovedatti et al. showed that ex vivo mucosal biopsy cultures from both UC and CD patients released high and comparable amounts of IFN-γ. In addition, lower levels of IL-13 have been reported in UC patients compared with CD patients or control. Moreover, IL-13 is suggested to be an anti-inflammatory rather than a pro-inflammatory cytokine in the gut.

More recently, Th17 or inflammatory Th, has been implicated in IBD pathogenesis. IL-17 gene and protein expression was increased in the mucosa of IBD patients. Similarly, increased IL-23 transcript and protein expression was shown in CD mucosa compared to UC or control. IL-23 is a p19 cytokine that forms a heterodimer with p40 of IL-12 to form a biologically active
cytokine. Its main function is the stabilisation and expansion of differentiated Th17 cells that express IL-23 receptors. Adding to the confusion of the Th1/Th2 debate in IBD, a new subset labelled Th1/Th17 cells have recently been identified in patients with CD. They are derived from naive T cells and produce both IFN-γ and IL-17. Some studies have confirmed that Th1 mediated IFN-γ is driving the inflammation in cell-transfer colitis models, while others showed Th-17 the key mediator of such colitis models.

Supporting the Th17 concept, studies showed that recombination activating gene-deficient mice deficient in IL-23p19, developed no colitis upon the transfer of naive T cells, but the same mice deficient additionally in IL-12p35, did develop colitis. TNF-α and IFN-γ, were greatly reduced in IL-23p19 deficient mice, whereas only moderately reduced in IL-12p35 deficient mice. This and other findings led to the suggestion that Th17 could be the main effector response in experimental colitis and hence IBD. However, impact studies of the Th17 response on regulatory T cells or Tregs have led to the notion that Th17 responses may be crucial, but not necessarily an effecter response in IBD. Tregs are a subset of T cells that inhibit the proliferation of naive T cells and thereby suppress inflammation. They exert their function by producing the anti-inflammatory cytokines IL-10 and TGF-β and by suppressing the activities of effector T cells that escape other regulatory mechanisms. IL-23, a facilitator of the Th17 response, has been shown to suppress the development of Tregs. Additionally, IL-17 exhibited a protective response in experimental colitis by directly acting on IL-17 receptors on Th1 cells to suppress the production of IFN-γ. It appears therefore, that although Th17 may have a pro-inflammatory response in IBD, it may also have a regulatory function.

The activation of the above disease-specific cytokines, stimulate the production of TNF-α, IL-6, and IL-1β, among other secondary cytokines, which are generally non-specific in function and are associated with both CD and UC. These secondary cytokines activate NF-κB or MAPK which promote inflammation by upregulating various downstream pro-inflammatory responses or by acting as upstream facilitators of inflammation. The final result of this pro-inflammatory cytokine activation is the mucosal pathology observed in IBD. Important functions of TNF-α include: (i) promotes the differentiation of lamina propria stromal cells into fibroblast and (ii) the production matrix metalloproteinases (MMP) from mononuclear cells. MMPs are a class of tissue-degrading enzymes that induce enterocyte apoptosis by digesting extracellular matrix and basement membrane of the intestinal epithelium resulting in ulcer formation. TNF-α promotes mucosal inflammation through enhancement of IL-12 production and inhibiting the suppressive functions of Tregs. The relative success with anti-TNF treatment in IBD corroborate the importance of TNF-α in IBD pathogenesis. IL-4 and IL-13 was shown to cause increased intestinal permeability and induction of enterocyte differentiation and apoptosis in IBD. IL-17 stimulation on the other hand, produced pro-inflammatory cytokines, such as IL-1β and IL-21. In IBD, IL-1β promoted inflammation by increasing intestinal tight junction permeability and allowing microorganisms through the epithelium. IL-21 was shown to stimulate the production of MMPs and the secretion of macrophage inflammatory protein-3α (MIP-3α), a chemokine which promotes the migration of inflammatory cells to the site of inflammation.

**Conclusion**

The molecular interplay in IBD is complex as discussed above. It appears many factors are involved in the pathogenesis of IBD and no single factor has an overriding role in its establishment. The consensus view in the initiation of IBD is the effect of environmental factors on a genetically susceptible host. At present, the treatment of IBD revolves around suppressing inflammation, but offers no cures or permanent resolution of the disease process. This will only be feasible when we have a greater understanding of the triggering events in IBD. We have come very far in understanding the pathogenetic mechanisms in IBD, yet we still have a long way to go.

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