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Protein Kinases and Ulcerative Colitis

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1. Introduction

Ulcerative colitis (UC), together with Crohn's disease (CD), collectively called inflammatory bowel disease (IBD), is a chronic, spontaneously remitting, and relapsing disorder of large intestine, characterized by abdominal pain and diarrhea. UC differs dramatically from CD with the respects of disease distribution, morphology, and histopathology; meantime, they share a lot of inflammatory similarities, such as epithelial barrier dysfunction. UC may result in significant morbidity and mortality, with compromised quality of life and life expectancy. While there is no cure for UC, the last two decades have seen tremendous advances in our understanding of the pathophysiology of this intestinal inflammation. Even though the precise etiology of IBD remains elusive, it is accepted that UC arises from abnormal host–microbe interactions, including qualitative and quantitative changes in the composition of the microbiota, host genetic susceptibility, barrier function, as well as innate and adaptive immunity.

Intracellular signaling cascades mediated by protein kinases are the main route of communication between the plasma membrane and regulatory targets in various intracellular compartments. The signaling pathway mediated by protein kinase plays an important role in transducing signals from diverse extra-cellular stimuli (including growth factors, cytokines and environmental stresses) to the nucleus in order to affect a wide range of cellular processes, such as proliferation, differentiation, development and apoptosis, and more importantly, also involved in intestinal inflammation.

In this chapter, we are going to focus on the involvement protein kinases in the pathogenesis of UC, try to shed some light on the clues of intervention of UC.

2. Genetic factor

Population-based studies provided compelling evidence that genetic susceptibility plays an essential role in the pathogenesis of UC, evidence including an 8- to 10-fold greater risk among relatives of UC and greater rates of concordance between twins in UC patients (15.4% in monozygotic vs 3.9% in dizygotic twins) (Cho & Brant, 2011). Some of genes encoding protein kinase like ERK1 (Hugot et al. 1996) and p38α (Hampe et al. 1999) are located in major IBD susceptibility regions on chromosome 16 and 6. Recently, substantial advances have been achieved in defining the genetic architecture of UC since the genome-wide association study (GWAS) analysis heralded a new era of complex disease gene discovery with notable success in CD initially and latterly also in UC. To date, over 60 published IBD susceptibility loci have been discovered and replicated, of which

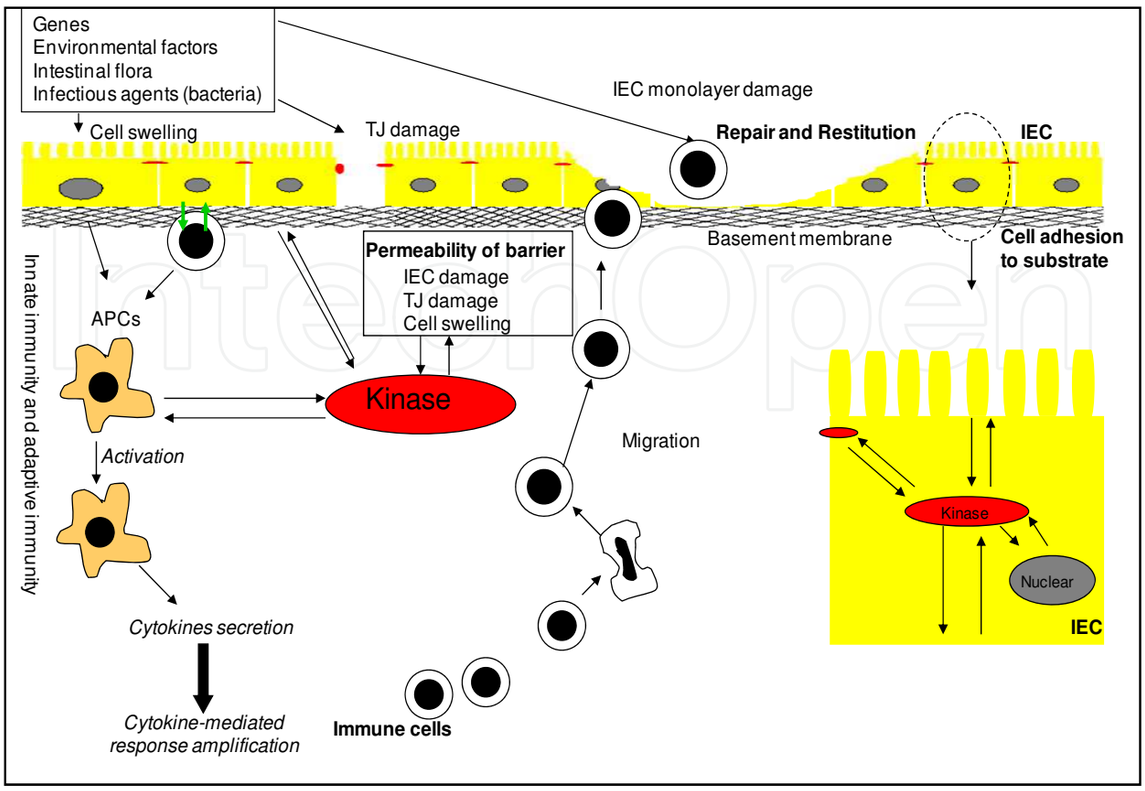

Fig. 1. Pathogenesis of UC. Many different factors, such as genetic factors, environmental factors, and intestinal non-pathogenic or pathogenic bacteria can damage the mucus, epithelium, or the tight junction, to initiate the inappropriate regulation or deregulation of the immune response, leading to the secretion of pro-inflammatory cytokines, decrease in epithelial barrier function and initiation of the inflammation-related signaling pathways. IEC: Intestinal epithelial cell; APC: Antigen presenting cell; TJ: Tightjunction. This model adapted from the model presented previously (Yan 2008)

approximately one third are associated with both UC and CD, although 21 are specific to UC and 23 to CD (Thompson & Lees, 2011). Importantly, most of the genes have been linked to defects in innate and adaptive immunity and epithelial barrier function. Notably, extracellular matrix *gene* 1 (ECM1) (Festen et al. 2010), E-cadherin gene (CDH1), Hepatocyte nuclear factor 4 alpha gene (HNF4a), and laminin B1 (Barrett et al. 2009) are genes implicated in mucosal barrier function, conferring risk of UC; ECM1 interacts with the basement membrane, inhibits matrix metalloproteinase 9 (MMP9), and strongly activate NFκB (Chan et al. 2007; Matsuda et al. 2003). The Wnt/beta-catenin signal transduction pathway has been shown to influence ECM1 expression (Kenny et al. 2005). E-cadherin is the first genetic correlation between colorectal cancer and UC, Chimeric mice with impaired E-cadherin function due to expression of dominant–negative N-cadherin developed colitis despite possessing an intact immune system (Hermiston & Gordon 1995a, 1995b). Notably, all of these 4 genes are regulated or related to protein kinases, for example, HNF4alpha-DNA binding activity is dependent on its phosphorylation by protein kinase A (PKA) (Viollet et al. 1997), while its transcription activity was dependent on AMP-activated protein kinase(AMPK) (Hong et al. 2003).

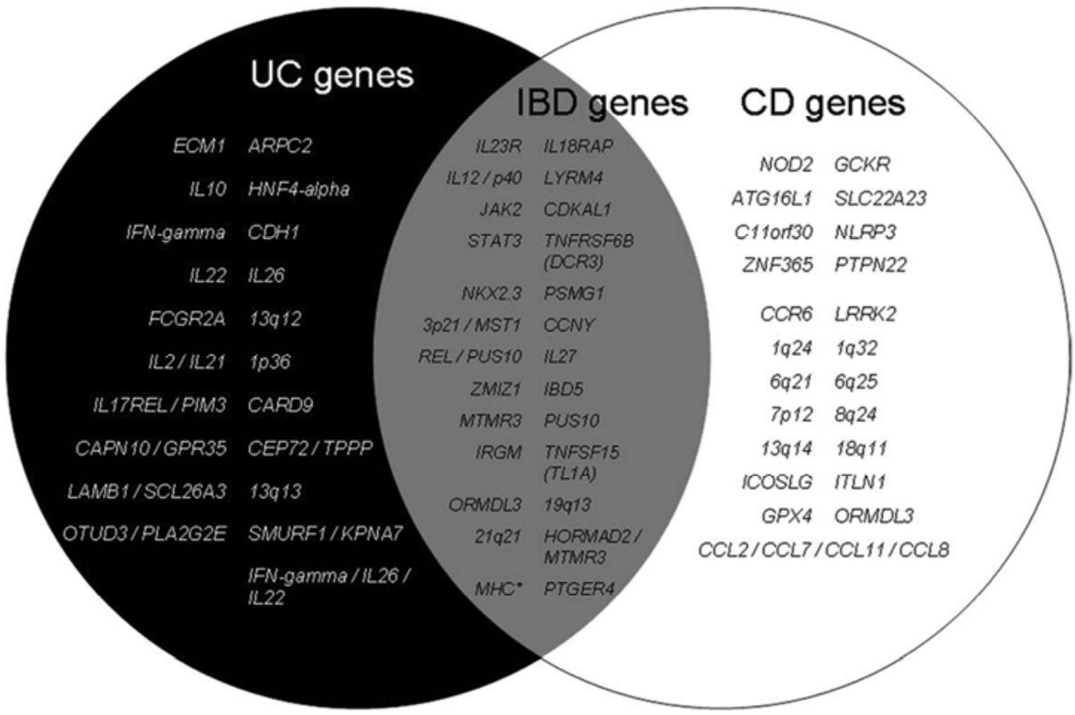

Fig. 2. Susceptible loci for UC. This model is adapted from the model presented previously (Thompson & Lees 2001)

3. Microbiota and immune responses

The human gastrointestinal (GI) tract contains as many as 10^{14} individual bacteria, comprising over 500 different species. These commensal bacteria serves as a primary barrier between the intestinal epithelial cells and the external environment, which is critical to the healthy host, as it modulates intestinal development, maintains a healthy intestinal pH, promotes immune homeostasis, and enhances metabolism of drugs, hormones and carcinogens. Evidence from immunologic, microbiologic, and genetic studies implicates abnormal host-microbial interactions in the pathogenesis of UC. But the mechanisms underlying the involvement of microbiota are elusive, and the effects of microbiota are due to their interaction with other factors, such as immunologic factors, genetic factor or epithelial junction proteins. The postulated mechanisms (Packey & Sartor, 2008) are as followed with little modification: (a) Pathogenic bacteria. A traditional pathogen or functional alterations in commensal bacteria, including enhanced epithelial adherence, invasion, and resistance to killing by phagocytes or acquisition of virulence factors, can result in increased stimulation of innate and adaptive immune responses. (b) Abnormal microbial composition. Decreased concentrations of bacteria that produce butyrate and other short-chain fatty acids (SCFA) compromise epithelial barrier integrity. (c) Defective host containment of commensal bacteria. Increased mucosal permeability can result in overwhelming exposure of bacterial toll like receptor (TLR) ligands and antigens that activate pathogenic innate and T cell immune responses. (d) Defective host immunoregulation. Antigen-presenting cells and epithelial cells overproduce cytokines due to ineffective downregulation, which results in TH1 and TH17 differentiation and

inflammation. Dysfunction of regulatory T cells (T-reg) leads to decreased secretion of IL-10 and TGF-β, and loss of immunological tolerance to microbial antigens (an overly aggressive T cell response).

UC is commonly regarded as the consequences of an enhanced inflammatory response or the lack of a down regulatory response to bacteria abnormality. The dysregulated immune response involving the innate (for example, TLR, DC, etc) and the adaptive immune system (e.g. effector T-cells, regulatory T-cells, eosinophils, neutrophils, etc) may follow or precede the macroscopic lesions. Crohn's disease is a predominantly TH1- and TH17-mediated process, while the immunopathogenesis of UC has been a more difficult disease to ascertain, neither IFN-γ (a major Th1 cytokine) nor IL-4 (the major Th2 cytokines) was increased (Fuss et al, 2008). In fact, IL-4 production was found to be decreased in cells extracted from UC tissue and only the fact that an additional Th2 cytokine IL-5 secretion by these cells was somewhat increased hinted that the disease may have a Th2 character. Further, enhanced level of IL-13 was noticed in lamina propria from UC specimens, whereas those from Crohn's disease specimens were producing IFN-γ (Fuss et al, 1996). Fuss (Fuss et al, 2004) found that antigen-presenting cells bearing a CD1d construct (and thus expressing CD1d on its surface, which presents lipid rather than protein antigens to T cells.) could only induce lamina propria mononuclear cells from UC patients but not that of Crohn's disease to produce IL-13. Thereby, the cytokine secretion profile seen in UC was produced from a non-classical CD1 dependent NK T cell whereas the cytokines produced in Crohn's disease were from that of an activated classical Th1 CD4 + T cell. In addition, Lamina propria cells enriched for NK T cells from the patients could be shown to be cytotoxic for epithelial cells and such cytotoxicity was further enhanced by IL-13. Antigens in the mucosal microflora activate NK T cells because of barrier dysfunction that, in turn, cause cytolysis of epithelial cells and the characteristic ulcerations associated with the disease. As suggested, enhancement of cytolytic activity was observed *in vitro* in the presence of IL-13. Further, IL-13 was shown to have direct effects on activation of cytokine transcription. These studies demonstrated that TGF-β transcription was dependent upon IL-13. In short, UC is associated with an atypical TH2 response mediated by a distinct subset of NK T cells that produce IL-13 and are cytotoxic for epithelial cells (Fuss et al. 2008). Further, UC is characterized by the presence of various types of autoantibodies against goblet cells and the isoforms 1 and 5 of human tropomyosin.

The intestinal mucosa must rapidly recognize detrimental pathogenic threats to the lumen to initiate controlled immune responses but maintain hyporesponsiveness to omnipresent harmless commensals. Pattern recognition receptors (PRRs) may play an essential role in allowing innate immune cells to discriminate between ''self'' and microbial ''non-self'' based on the recognition of broadly conserved molecular patterns. Toll-like receptors (TLRs), a class of transmembrane PRRs, play a key role in microbial recognition, induction of antimicrobial genes, and the control of adaptive immune responses. Individual TLRs differentially activate distinct signaling events via diverse cofactors and adaptors. To date, at least five different adaptor proteins have been identified in humans: MyD88, Mal/TIRAP, TRIF/TICAM-1, TRAM/Tirp/TICAM-2, and SARM (O'Neill et al. 2003). The first identified so-called ''classical'' pathway (Cario 2005) involves recruitment of the adaptor molecule MyD88, activation of the serine/threonine kinases of the interleukin 1 receptor associated kinase (IRAK) family, subsequently leading to degradation of inhibitor kB (IkB) and translocation of nuclear factor kB (NFkB) to the nucleus, then result in activation of specific transcription factors, including NFkB, AP-1, Elk-1, CREB, STATs, and the subsequent

transcriptional activation of genes encoding pro- and anti-inflammatory cytokines and chemokines as well as induction of costimulatory molecules. All of these various downstream effects are critically involved in the control of pathogen elimination, commensal homeostasis, and linkage to the adaptive immunity. Signaling through different TLRs can result in considerable qualitative differences in TH dependent immune responses by differential modulation of MAPKs and the transcription factor c-FOS (Agrawal et al. 2003). So TLR signalling protects intestinal epithelial barrier and maintains tolerance, but aberrant TLR signalling may stimulate diverse inflammatory responses leading to UC.

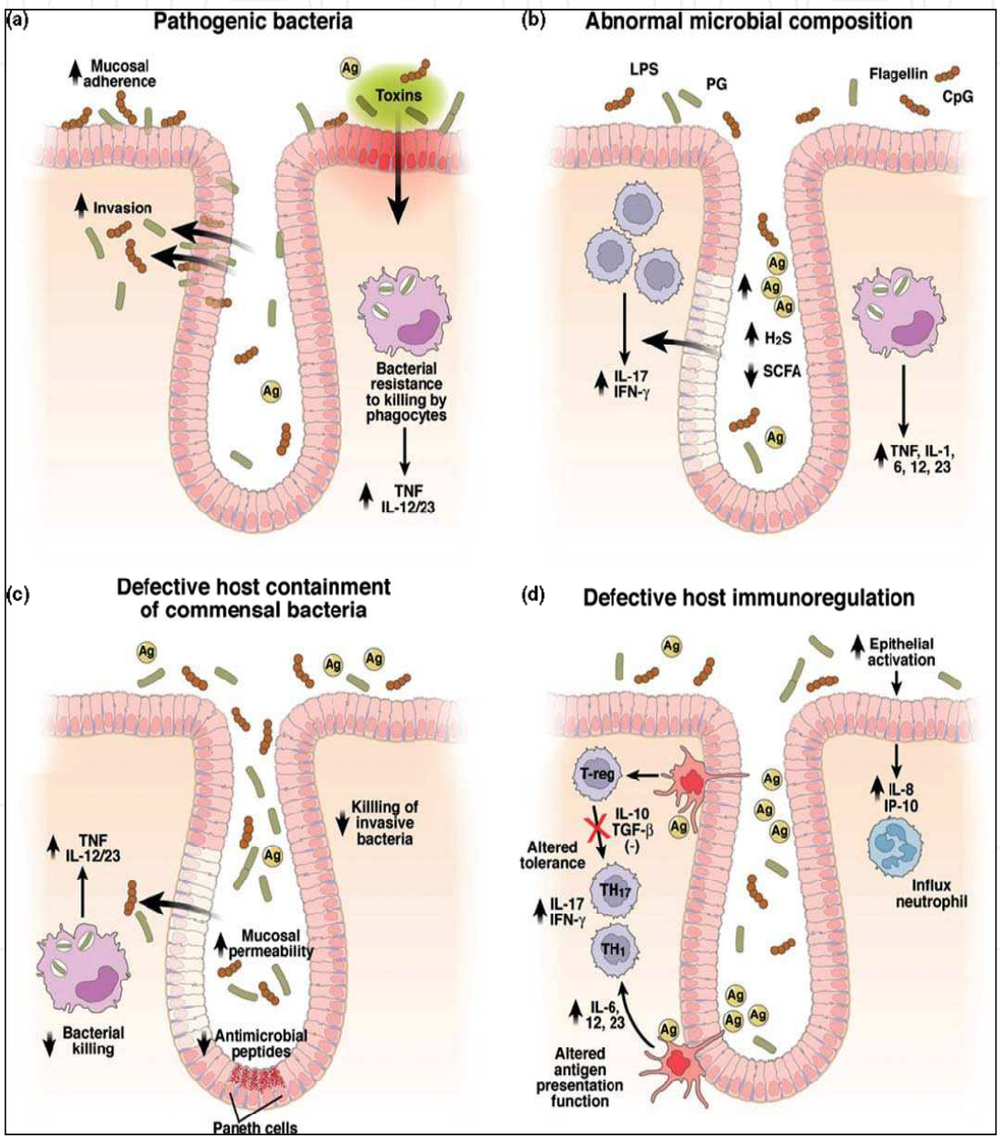

Fig. 3. Proposed mechanisms by which bacteria and fungi induce chronic immune-mediated inflammation and injury of the intestines. This model adapted from the model presented in the work by Dr Sartor (Packey & Sartor 2008) (a) Pathogenic bacteria. (b) Abnormal microbial composition. (c) Defective host containment of commensal bacteria. (d) Defective host immunoregulation

TLR comprise a family of (so far) 11 type-I transmembrane receptors. Different pathogen associated molecular patterns selectively activate different TLRs: (Lipoptroteins) TLR1, 2 and 6; (dsRNA) TLR3; (LPS) TLR4; (Flagellin) TLR5; (ssRNA) TLR7 and 8; (CpG DNA) TLR9. These signals all converge on a single pathway via myeloid differentiation primary response protein MyD88, which activates NFκB. the NFκB pathway was thought to have predominantly pro inflammatory activities and NFκB is activated in the tissues of UC patients and its inhibition can attenuate experimental colitis (Neurath et al 1996).

In intestine, tolerance is an essential mucosal defence mechanism maintaining hyporesponsiveness to harmless lumenal commensals and their products. Several molecular immune mechanisms that ensure tolerance via TLRs in intestinal epithelial cells (IEC) have recently been described, for example, low expression of TLRs at resting conditions in IEC can maintain hyporesponsiveness to microbiota; high expression levels of the downstream signaling suppressor Tollip which inhibits IRAK activation (Otte et al. 2004), ligand induced activation of peroxisome proliferator activated receptor c (PPARc) which uncouples NFkB dependent target genes in a negative feedback loop (Dubuquoy et al. 2003. Kelly et al. 2004), and external regulators which may suppress TLR mediated signalling pathways. Commensal bacteria may assist the host in maintaining mucosal homeostasis by suppressing inflammatory responses and inhibiting specific intracellular signal transduction pathways (Neish et al. 2000), uncoupling NFkB dependent target genes in a negative feedback loop (Dubuquoy et al. 2003) which may lead to attenuation of colonic inflammation (Kelly et al. 2004).

In addition, NF-κB is normally grouped into one of the pro-inflammatory mediators, a protective role for epithelial NF-κB signaling by either bacteria, IL-1, or TNF stimulation of TLRs, or cytokine receptors is demonstrated by conditional ablation of NEMO (IκB kinase) in intestinal epithelial cells causing spontaneous severe colitis (Nenci et al. 2007). Blockade of epithelial NF-κB signaling led to increased bacterial translocation across the injured epithelium, similar to toll like receptor (TLR)4-deficient mice treated with dextran sulphate sodium (DSS) (Fukata et al. 2006).

4. Barrier dysfunction

Generally, intestinal barrier function consists of different level of defense lines, the mucus layer, commensal microbiota, epithelial cells themselves, the junction between lateral epithelial cells, innate and adaptive immune systems and enteric nerve system. Any stresses which interfere with any level of this defense lines could potentially lead to intestinal barrier dysfunction and result in intestinal inflammation.

Epithelial cells form a continuous, polarized monolayer that is linked together by a series of dynamic junctional complexes. Except function as a physical barrier, epithelial cells maintain a mucosal defense system through the expression of a wide range of PRRs, such as TLRs. These PRRs form the backbone of the innate immune system through the rapid response and recognition of the unique and conserved microbial components, (Medzhitov & Janeway. 2002; Akira et al. 2006). Tight junctions are composed of transmembrane proteins (claudins, occludins, and junctional adhesion molecule [JAM]), peripheral membrane or scaffolding proteins (zonula occludens [ZO]), and intracellular regulatory molecules that include kinases and actin. An anatomically and immunologically compromised intestinal epithelial barrier allows direct contact of the intestinal mucosa with the luminal bacteria and

plays a crucial role in the development and maintenance of UC by initiating chronic inflammatory responses, although it is unclear whether this is a primary pathogenic process or secondary to inflammation. Since the contribution of genetic factors, microbiota and immune responses to the pathogenesis to UC, we high light the involvement of mucus layer, tight junction itself in the pathogenesis of UC.

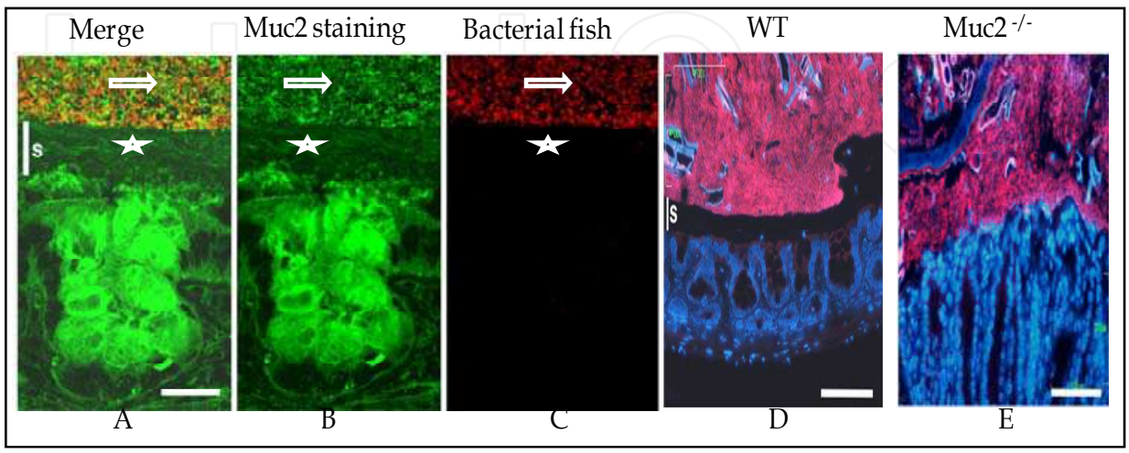

Fig. 4. Merged figure (A) of Muc2immunostaining (green, B) *and FISH analysis using the general bacterial probe EUB338-Alexa Fluor* 555 (red, C) of distal colon, it was shown muc2-positive goblet cells and the outer mucus layer (Arrow) and inner mucus layer (Star) on the epithelium. The inner layer (Star) is devoid of bacteria, which can only be detected in the outer mucus layer. The inner mucus generates a spatial separation between the cells and microflora. (Scale bar: 20 μm). (D) FISH using the EUB338-Alexa Fluor 555 probe staining bacteria and DAPIDNA staining in colon show a clear separation of the bacterial DNA and epithelial surface in WT mice, but not in Muc2$^{-/-}$ mice. This separation corresponds to the inner mucus layer (s). (Scale bar: 100m). These models adapted from the models presented previously (Johansson et al. 2008)

4.1 Mucus layer

As mentioned in previous part of this chapter, the digestive tract is home to 10^{14} bacteria and bacteria genome is as many 10 times as human genome, which has evolved to ensure homeostasis. How to manage this enormous bacterial load without overt immune responses from the adaptive and innate systems is not well understood. When the equilibrium is altered, as in the disease ulcerative colitis, inflammatory responses are initiated against the commensal bacteria. An important component, often neglected due to lack of understanding, is the mucus layer that overlies the entire intestinal epithelium as a protective gel-like layer (Johansson et al. 2008). This thick and hyperviscous mucus layer secreted by goblet cells overlies the entire intestinal epithelium as a protective gel-like layer that can extend up to as much as 150 μm thick in mouse colon (and 800 μm thick in rat colon). There exist two different kinds of mucus layer-out layer and inner layer. The majority of microorganisms in the lumen can be found in the outer mucus layer, there is an inner, protected, and unstirred layer that is directly adjacent to the epithelial surface and is relatively sterile. The sterility of this layer contributes to the retention of a high concentration of antimicrobial proteins (such as cathelicidiens, defensins, and cryptidens)

produced by various intestinal epithelial lineages, including enterocytes and Paneth cells. The inner firmly attached mucus layer forms a specialized physical barrier that excludes the resident bacteria from a direct contact with the underlining epithelium. This organization of the colon mucus, as based on the properties of the Muc2 mucin, should be ideal for excluding bacteria from contacting the epithelial cells and thus also the immune system. Alterations or the absence of these protective layers, as in the Muc2$^{-/-}$ mouse colon, allow bacteria to have a direct contact with epithelial cells, to penetrate lower into the crypts and also translocate into epithelial cells. That such a close contact between bacteria and epithelia can trigger an inflammatory response (Johansson et al. 2008; Shen et al. 2009). The surface mucus layer also impacts mucosal permeability, as demonstrated by spontaneous colitis in Muc-2- deficient mice (Bergstrom et al. 2010), and increased DSS-induced colitis in intestinal trefoil factor deficient mice (Mashimo et al. 1996) and in human UC. The importance of the Muc2 mucin in organizing the colon mucus protection is further strengthen by the report that two mouse strains with diarrhea and colon inflammation were shown to have two separate spontaneous mutations in the Muc2 mucin (Heazlewood et al. 2008). Importantly, the production of mucin is regulated by protein kinases, for example, resistin and resistin-like molecule (RELM) beta upregulated mucin expression which dependent on the kinase activities of protein kinase C (PKC), tyrosine kinases, and extracellular-regulated protein kinase (Krimi et al. 2008); Cathelicidin stimulates colonic mucus synthesis by up-regulating MUC1 and MUC2 expression through a mitogen-activated protein kinase pathway (Tai et al. 2008).

4.2 Epithelial cell and its tight junction

The cellular components of the intestinal barrier consist of the complete array of columnar epithelial cell types (enterocyte, paneth cells, enteroendorine cells, and goblet cells) present within the intestine. These cells are polarized with an apical membrane and a basolateral membrane, and apical membrane composition is distinct from the basolateral membrane, for example, the nutrient transporters are located on the apical membrane; they use Na^+ ions cotransport to provide the energy and directionality of transport. In contrast, the Na^+K^+-ATPase, which establishes the Na^+ electrochemical gradient, is present on basolateral, but not apical membranes. In addition, the lipid composition of the membrane differs; the apical membrane is enriched in sphingolipids and cholesterol relative to the basolateral membrane. One result of this cellular polarization is that the apical membranes of intestinal epithelial cells are generally impermeable to hydrophilic solutes in the absence of specific transporters. Thus, the presence of epithelial cells, particularly the apical membranes, contributes significantly to the mucosal barrier (Shen et al. 2009). Among the most important structures of the intestinal barrier are the epithelial tight junctions (TJs) that connect adjacent enterocytes together to determine paracellular permeability. The tight junction is composed of multiple proteins including transmembrane proteins such as occludin, tricellulin, claudins and junctional adhesion molecule (JAM). The intracellular portions of these transmembrane proteins interact with cytoplasmic peripheral membrane proteins, including zona occludens (ZO)-1,-2,-3 and cingulin (Mitic & Anderson. 1998). These tight junction and cytoplasmic proteins then interact with F-actin and myosin II, thereby anchoring the tight junction complex to the cytoskeleton. Once thought to be static, the association of these proteins with the tight junction is highly dynamic (Shen et al. 2009) and may play a role in epithelial barrier regulation. Occludin was the first tight junction-associated integral

membrane protein identified (Furuse et al. 1993). Although occludin knockout mice exhibit intact intestinal epithelial tight junctions and display no observable barrier defect (Schulzke et al. 2005, Saitou et al. 2000). But *in vitro* studies demonstrate crucial roles in tight junction assembly and maintenance (Yu et al. 2005; Suzuki et al. 2009; Elias et al. 2009). This suggests that further analysis of occludin knockout mice under stressed condition may reveal *in vivo* functions of occludin and provide new insight into mechanisms of tight regulation (Turner 2006). Given the phylogenetic and structural similarities between occludin and tricellulin (Ikenouchi et al. 2005), it may be that the tricellulin accounts for normal intestinal barrier function in occludin knockout mice. This hypothesis could also be applied to inflammatory bowel disease, where intestinal epithelial occludin expression is reduced (Heller et al. 2005). The fact that occludin knockout mice exhibit intact intestinal epithelial barrier function led to the search for additional tight junctional components and ultimately to the discovery of the claudins (Furuse et al. 1998). The claudins are a large family of proteins that also interact with partners on neighboring cells to affect junctional adhesions via extracellular loops. At least 24 different claudin proteins are present in mammals (Van Itallie et al. 2003, 2004, 2006), and these proteins are the primary component of tight junction strands (Furuse et al. 2006). Claudins are expressed in a tissue-specific manner, studies on human intestine confirm the expression of claudins-1, -2, -3, -4, -5, -7, and -8 in the colon, expression of claudins-1, -2, -3, and -4 in the duodenum, and expression of claudins-2 and -4 in the jejunum (Burgel et al 2002; Escaffit et al 2005, Szakal et al. 2010; Wang et al. 2010; Zeissing et al. 2007).

The molecular anatomy of transport through tight junction is not yet clear, at least two routes allow transport across the tight junction, and the relative contributions of different paracellular transport are regulated independently (Fihn et al. 2000; Van Itallie 2008; Watson et al. 2005). One route, the size-dependent pathway, allows paracellular transport of large solutes, including limited flux of proteins and bacterial lipopolysaccharides (Van Itallie 2008; Watson et al. 2005). Although at what size particles are excluded from the leak pathway has not been precisely defined, it is clear that materials as large as whole bacteria cannot pass. Flux across the leak pathway may be increased by cytokines and protein kinases, including IFNγ, TNF *(*Watson et al. 2005; Wang et al. 2005; Clayburgh et al. 2006), MAPKs, myosin II light chain kinase (MLCK) (Turner 2006) and SPAK (Yan 2011). A second pathway is charge-dependent pathway, characterized by small pores that are defined by tight junction-associated pore-forming claudin proteins (Amasheh et al. 2002; Colegio et al. 2003; Simon et al. 1999). These pores have a radius that excludes molecules larger than 4 A (Van Itallie 2008; Watson et al.2005). Thus, tight junctions show both size selectivity and charge selectivity, and these properties may be regulated individually or jointly by physiological or pathophysiological stimuli. It need to point out that barrier dysfunction may be caused by increased paracellular permeability, but mainly by epithelial damage, including erosion, and ulceration (Zeissig et al. 2004; Schulzke et al.2006). In addition, in epithelial cells, the site of claudin protein polymerization to form strands depends on ZO family protein expression (Furuse & Tsukita 2006), and cells lacking ZO-1 and ZO-2 fail to form tight junctions at all.

Generally, TJ proteins can be subdivided into ‘‘tightening’’ TJ proteins that strengthen epithelial barrier properties (such as occluding and claudin-1 and -4 etc) and ‘‘leaky’’ TJ proteins (like claudin-2) that selectively mediate paracellular permeability. Dysfunctional intestinal barrier is a feature of gut inflammation in humans and has been implicated as a

pathogenic factor in IBD for the last 30 years. The factors responsible for barrier dysfunction in UC are similar to those in CD, including an increase in epithelial antigen transcytosis and a change in TJ structure with a reduction in TJ strand count and in the depth of the TJ main meshwork; although, in contrast to CD, strand breaks are not as frequent as in UC (Schmitz et al. 1999; Schurmann et al. 1999). Again, the downregulation of occludin and downregulation of several ''tightening'' TJ proteins like claudin-1 and -4, together with an upregulation of the pore-forming TJ protein claudin-2 contribute to the barrier defect observed in UC (Heller et al.2005; Oshima et al. 2008). These disruptions of tight junction proteins could lead to a breakdown in the protective barrier and can be used as a portal of entry by the luminal bacteria. This breach in intestinal barrier can result in inflammatory infiltrate and enhanced production of cytokines and other mediators (such as neutrophil) that can further contribute to the altered barrier function.

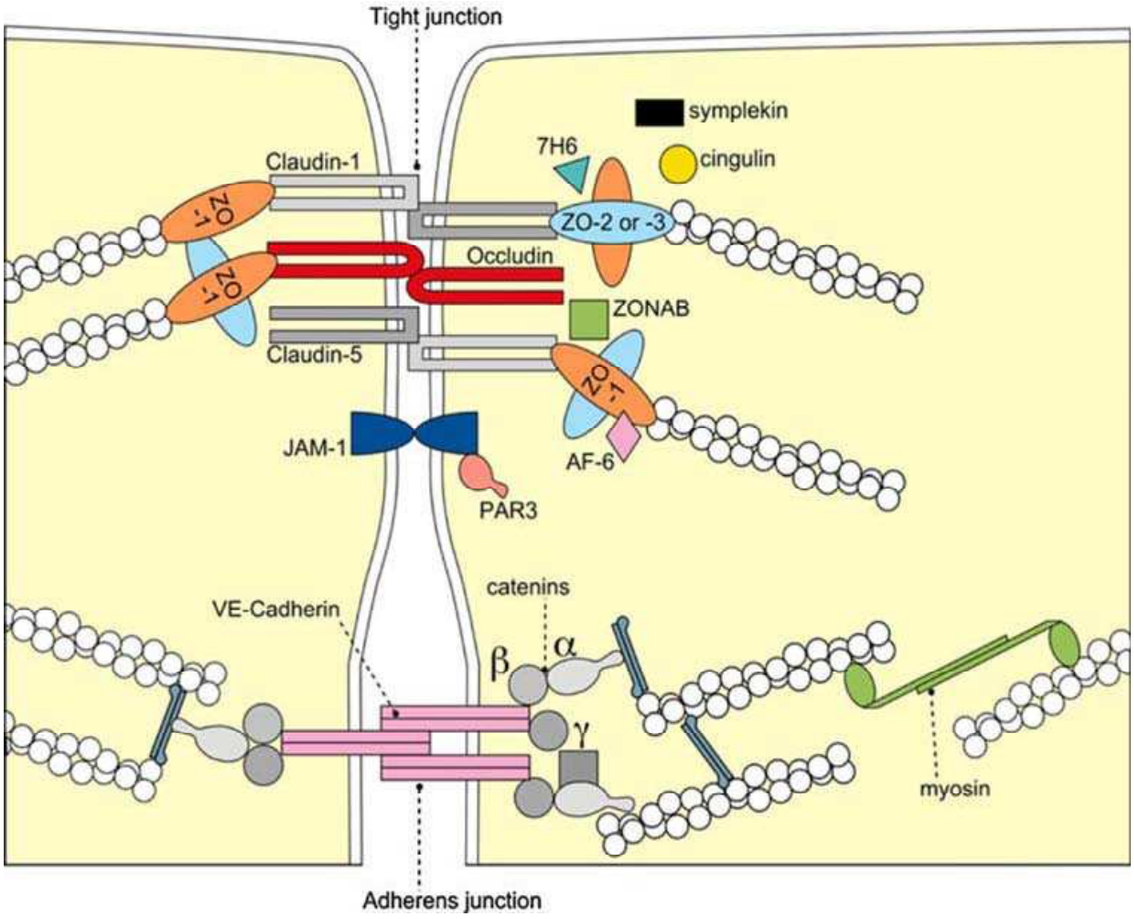

Fig. 5. Molecular composition of tight junctions. This model adapted from the model presented previously:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2413111/?tool=pubmed

Mucosal permeability is influenced by several factors. The surface mucus layer also impacts mucosal permeability, as demonstrated by spontaneous colitis in Muc-2- deficient mice (Van

der Sluis et al. 2006), and increased DSS-induced colitis in intestinal trefoil factordeficient mice (Mashimo et al. 1996). Luminal microbiota can also compromise the intestinal barrier function (Packey & Sartor, 2008). The third is the integrity of the epithelial cell layer and the basement membrane. Molecularly this can be compromised by downregulating tight junction components Claudins 5 and 6, upregulating pore-forming Claudin 2 (Zessig et al. 2007), which can be accomplished by TNF and IL-13, or increasing epithelial apoptosis, which has been achieved in mice by blocking nuclear factor kappa-B (NFκB) signalling. Genetic factors are involved in the loss of intestinal barrier function (Cho & Brant 2011). Dysregulated innate and adaptive immune system can lead to the enhanced epithelial permeability (Fuss 2008). Finally, autonomic nerve system function affects epithelial permeability, as demonstrated by mice that develop fulminant jejunoileitis following ablation of enteric glial cells (Bush et al. 1998).

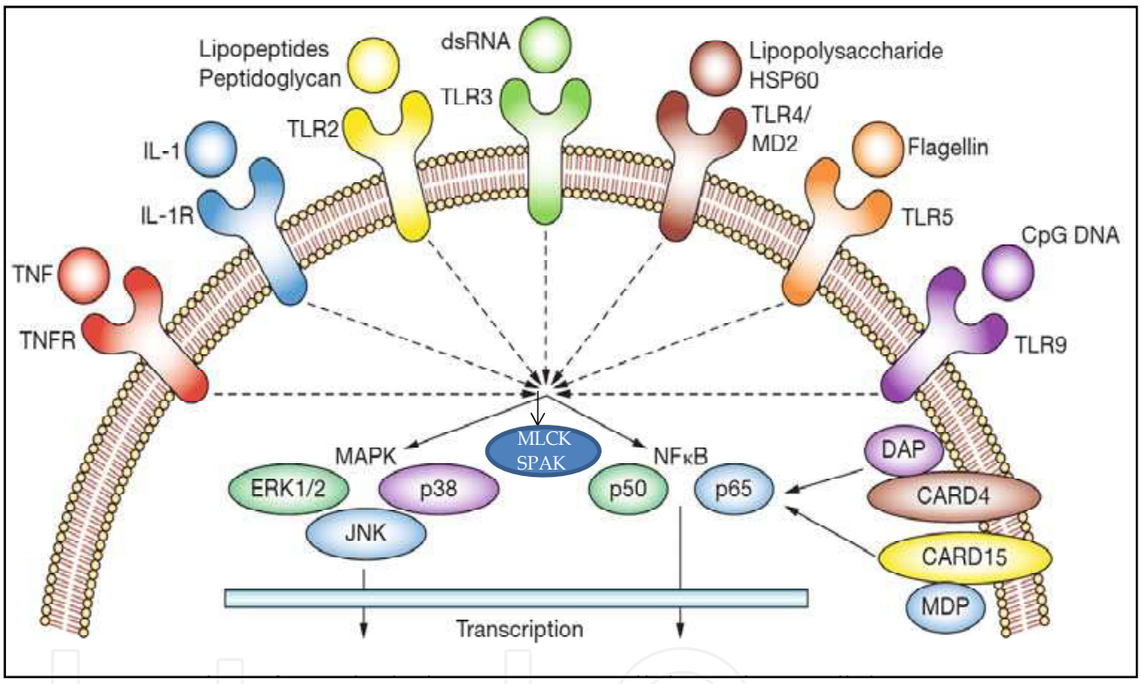

Fig. 6. Binding of microbial adjuvants to extracellular and intracellular pattern-recognition receptors and initiate their function by activating protein kinases. Toll-like receptors on the cell membrane selectively bind to various bacterial, viral or fungal components. This ligation activates conserved signaling pathways that activate NFκB and mitogen-activated protein kinases. These transcription factors stimulate the expression of a number of proinflammatory and antiinflammatory genes. This model adapted and modified from the model presented previously.
http://www.nature.com/nrgastro/journal/v3/n7/full/ncpgasthep0528.html

The increased uptake of antigens and macromolecules from the intestinal lumen mediated through this epithelial barrier dysfunction can further exacerbate the inflammatory process, ending up in a vicious circle. In this manner, barrier dysfunction is a perpetuating principle during gastrointestinal inflammation. Since epithelial TJs are important in the maintenance of barrier function, regulatory changes in their function that are commonly found during

intestinal inflammation can have severe consequences. For example, the resulting passive loss of solutes into the intestinal lumen and the subsequent osmotically driven water flow results in ''leak flux diarrhea'', one of the main consequences of UC. The tight junction is, therefore, the rate-limiting step in transepithelial transport and the principal determinant of mucosal permeability. But it has to be pointed out that barrier dysfunction itself is not sufficient to cause intestinal diseases, such as in MLCK (Turner 2006) and SPAK (Yan 2011) transgenic mice, these two different transgenic mice revealed increased transepithelial permeability, but neither of them demonstrated any UC characterization, for example, these mice develop normal, no significant weight loss, histologically normal crypts were found, no abscesses was noticed.

Recent molecular advances as well as studies of cellular physiology in model epithelia have instead revealed that both the permeability and selectivity of tight junctions can be modulated dynamically by a variety of signals (Mitic et al., 2000). Much of the progress in this field has rested on a significantly enhanced understanding of the proteins that make up the junction itself, as well as those components of the junction on its cytoplasmic face that link the junctional region both to the cellular cytoskeleton and to signal transduction modules (González-Mariscal et al. 2003).

5. Protein kinase and pathogenesis of UC

5.1 Mitogen activated protein kinases (MAPK)

Interestingly, protein kinases are associated with all different level of aspects, demonstrated promising potential as intervention targets against UC. Intracellular signaling cascades are the main route of communication between the plasma membrane and regulatory targets in various intracellular compartments. The evolutionarily conserved mitogen activated protein kinases (MAPK) signaling pathway plays an important role in transducing signals from diverse extra-cellular stimuli (including growth factors, cytokines and environmental stresses) to the nucleus in order to affect a wide range of cellular processes, such as proliferation, differentiation, development, stress responses and apoptosis. MAPK (Coskun et al,2011) signaling cascades, which comprise up to seven levels of protein kinases, are sequentially activated by phosphorylation and also involved in intestinal inflammation. These families can be divided into two groups: the classical MAPKs, consisting of ERK1/2, p38, JNK and ERK5, and the atypical MAPKs, consisting of ERK3, ERK4, ERK7 and NLK (Coulombe & Meloche, 2007). The signalling pathways which the members of these families influence can be independent of each other or overlapping. The classical pathway leading to activation of ERK1/2 is through the upstream activation of the Raf MAPKKKs, which activate sequentially the MAPKKs, MEK1/2, which can specifically bind and phosphorylate ERK1/2. At this stage, and depending upon the signal being propagated, the ERK1/2 proteins commonly then phosphorylate the downstream MAPK activated proteins (MAPKAP) 1/2. However, other proinflammatory proteins such as cytosolic phospholipase A_2 can be activated, as well as several transcription factors including Ets-1, Elk and c-myc. These transcription factors aid the inflammatory process by inducing other related cellular processes such as cell migration and proliferation. Interestingly, a role for ERK1/2, using an ERK1/2 inhibitor, was found in cells of the immune system and colonocytes in the development and progression of IBD, through its mediation in the signalling pathways induced by various cytokines, for example IL-21, and IL-1 (Caruso et al.2007; Kwon et

al.2007). Indeed, several studies, cell line cultures and isolated crypts from human biopsies, have shown that it is not only over-expressed in IBD tissue (both colonocytes and cells in the underlying lamina propria), but that its phosphorylation state and therefore activation state is increased significantly during the active stages of IBD (Waetzig et al.2002; Dahan et al.2008). Study also found that Erk activation is involved in claudin-4 protein expression and claudin-4 is involved in the maintenance of the intestinal epithelial cell barrier function (Pinton et al. 2010) as a "tightening" junction protein. Activation of p38/MAPK and Akt signal transduction pathways in the epithelial cells have also been implicated as key mediators of these protective effects (Resta-Lenert and Barrett. 2006). For example, *Lactobacillus GG* (LGG) prevents cytokine-induced apoptosis in both human and mouse intestinal epithelial cells through activating antiapoptotic Akt in a phosphatidylinositol-3-kinase (PI3K)-dependent manner and inhibiting proapoptotic p38/MAPK activation (Yan and Polk. 2002). The p38 family is composed of four members: α, β, γ and δ. Expression of the isoforms varies between tissues. Different ligands, via their respective receptors, are able to activate one or several of p38 targets TAK1, ASK1, MLK3, MEKK1-4 and TAO1-3 (Thalhamer et al.2008). Several studies using the p38 inhibitor, SB203580, have indicated that p38 phosphorylation is increased significantly in IBD tissue (Waetzig et al.2002; Dahan et al.2008). This finding is substantiated further by an *in vitro* study, indicating that inhibition of p38 using the natural IL-1 receptor antagonist, in a colonocyte cell line, leads to reduced IL-6 and -8 production, and an *in vivo* study using a murine model of IBD, where inhibition of p38 reduced significantly cytokine mRNA and NFκB activation (Garat et al.2003; Hollenbach et al.2004). However, Heat-killed *L. brevis* SBC8803 induced Hsps, phosphorylated p38 MAPK, regulated the expression of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-12, and improved the barrier function of intestinal epithelia under oxidant stress (Ueno et al. 2011).

There are three JNK isoforms, JNK1, 2 and 3, of which there are 10 splice forms in total. Studies using a specific inhibitor against JNK1/2 in induced IBD in rodent models or with isolated colonic tissue found that proinflammatory cytokine production was reduced in conjunction with reduced inflammatory cell infiltration. Similarly, increased phosphorylation of JNK1/2 was seen in inflamed tissue from IBD patients (Dahan et al.2008; Assi K et al.2006; Mitsuyama et al.2008). RDP58 (Loftberg et al.2002) is a peptide consisting of 9 D-amino acids blocking p38 and JNK, further attenuate UC.

5.2 Serine and threonine kinase

5.2.1 Ste20 related proline/alanine rich kinase (SPAK)

SPAK is defined as a ste20-like proline-/alanine rich kinase that contains an N-terminal series of proline and alanine repeats (PAPA box) followed by a kinase domain, a nuclear localization signal, a consensus caspase cleavage motif, and a C-terminal regulatory region (Johnston et al.2000). Colonic SPAK exists as a unique isoform that lacks the PAPA box and F-α helix loop in the N-terminus (Yan et al.2007). The diversity of domains present in SPAK protein might be associated with a variety of biological roles. For example, SPAK has been shown to play roles in cell differentiation, cell transformation and proliferation, and regulation of chloride transport (Piechotta et al.2002; Gagnon et al.2006). More importantly, a linkage has been established between SPAK and inflammation, SPAK, as an upstream kinase to Na^+-K^+-$2Cl^-$ co-transporter 1 (NKCC1), can phosphorylate Thr203, Thr207, and

Thr212 amino acids on NKCC1, which play an important role in inflammation (Topper et al.1997). Furthermore, we have demonstrated that SPAK can activate p38 pathway (Yan et al. 2007) that is well known involving inflammation. SPAK caused an increase in intestinal permeability, and SPAK transgenic (TG) mice were more susceptible to experimental colitis. Additionally, increased cytokine production and bacterial translocation were associated with the increased colitis susceptibility (Yan Y et al 2011).

5.2.2 Myosin II light chain kinase (MLCK)

MLCK is a specific Serine and threonine kinase which can phosphorylate MLC. It has been found that MLCK activity is required for TNF-induced acute diarrhea. Further, TNF treatment resulted in increased myosin light chain kinase expression (Wang et al. 2005), as a result of transcriptional activation (Graham et al. 2006) in vitro and in vivo. Constitutive MLCK activation accelerates onset and increases severity of experimental UC. MLCK inhibition, either pharmacologically or by genetic knockout, prevented both intestinal epithelial MLC phosphorylation and barrier dysfunction. More remarkably, MLCK inhibition also restored net water absorption, and therefore corrected the TNF-dependent diarrhea (Clayburgh DR).

6. Conclusions

Protein kinase have been implicated in the pathogenesis of a variety of human disorders including UC, continuing progress in the understanding of the roles of protein kinases in intestinal barrier dysfunction, further in IBD pathogeneses offers hope for a new generation of therapeutic strategies targeted at the modulation of protein kinase activity.

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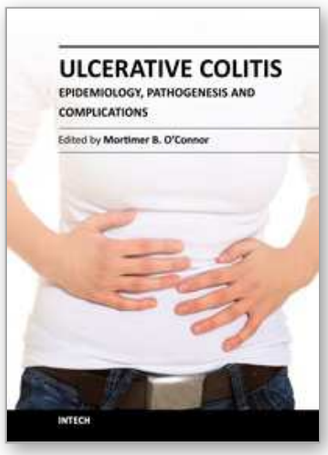

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This book is intended to act as an up-to-date reference point and knowledge developer for all readers interested in the area of gastroenterology and in particular, Ulcerative Colitis. All authors of the chapters are experts in their fields of publication, and deserve individual credit and praise for their contributions to the world of Ulcerative Colitis. We hope that you will find this publication informative, stimulating, and a reference point for the area of Ulcerative colitis as we move forward in our understanding of the field of medicine.

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