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# Intestinal epithelial cell signalling and chronic inflammation: From the proteome to specific molecular mechanisms

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**Abstract**

Advancing knowledge regarding the cellular mechanisms of intestinal inflammation has led to a better understanding of the disease pathology in patients with inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis. It has become clear from numerous studies that enteric bacteria are a critical component in the development and prevention/treatment of chronic intestinal inflammation. An emerging new paradigm suggests that changes in the homeostasis of bacteria- and host-derived signal transduction at the intestinal epithelial cell (IEC) level may lead to a break in barrier function and the development of adaptive immune disturbances. The functional loss of anti-inflammatory host-derived signals in the gut including the immunosuppressive cytokines Interleukin 10 (IL-10) and transforming growth factor (TGF)-β are of high relevance to the pathogenesis of IBD. The development of analytical tools including two-dimensional (2D) high-resolution protein separation techniques and peptide mass fingerprinting via high-sensitivity mass-spectrometers (MS) allows the quantitative assessment of protein expression changes in disease-relevant cell types. By using these advanced methods, the characterization of the epithelial cell proteome from murine models of experimental colitis and human IBD patients identified novel disease-related mechanisms with respect to the regulation of the glucose-regulated endoplasmic reticulum stress response protein 78 (grp-78). In conclusion, the identification and functional analysis of differentially expressed proteins in purified intestinal target cell types will help to add important insights to the understanding of the molecular pathogenesis of these immune-mediated chronic intestinal disorders.


*Keywords:* Commensal bacteria; Endoplasmic reticulum (ER) stress; Intestinal epithelium; Chronic inflammation

## 1. Introduction

Gastrointestinal infections, the genetic predisposition to dysregulated mucosal immune responses and the concurrent prevalence of certain environmental triggers in developed countries are strong etiologic factors for the development of inflammatory bowel disease (IBD) [1–3]. Homeostasis (hyporesponsiveness) versus chronic intestinal inflammation is determined by the presence or absence of appropriate control mechanisms that terminate mucosal immune responses to the constant antigenic drive of luminal enteric bacteria. Ulcerative colitis and Crohn's disease, the two distinct idiopathic pathologies of IBD, are spontaneously relapsing, immunologically mediated disorders of the gastrointestinal tract. Both disorders affect people in approximately equal female/male proportion with a combined mean frequency of 5–200 cases per 100,000 European and North American inhabitants [4]. Of note, the incidence of Crohn's disease is still increasing in Western societies, demonstrating the importance to add mechanistic insights to the yet unknown etiology of the disease pathogenesis.

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The hypothesis that enteric bacteria accelerate and aggravate the disease pathologies of IBD patients was initially supported by the observation that inflammation in bypassed distal ileal or colonic segments of Crohn's disease patients is absent after proximal diversion of the fecal stream [5,6], but immune responsiveness and inflammation is reactivated within 1 week of perfusing ileostomy effluent into the bypassed ileum [7]. Additional clinical studies suggested that luminal enteric bacteria penetrate the intestinal mucus layer and associate with the intestinal epithelium under conditions of chronic inflammation [8,9]. Consistent with the observation that IBD patients fail to maintain immunologic tolerance towards bacterial antigens [10–13], the barrier function of the intestinal epithelium is abrogated under conditions of chronic intestinal inflammation [14–16].

In addition to these clinical findings, the selective colonization of germ-free rodent models for experimental colitis including IL-10 deficient mice (IL-10−/−), IL-2 deficient mice (IL-2−/−) and HLA-B27 transgenic rats (HLA-B27tg) implicate *Enterococcus faecalis, Escherichia coli* and *Bacteroides vulgatus* as particularly important to the induction of colitis in these models [17–19]. Interestingly, the colonization of germ-free IL-10−/− mice with *E. faecalis* and *E. coli* but not with *B. vulgatus* triggered chronic experimental inflammation. Vice versa, the reconstitution studies of gnotobiotic HLA-B27 transgenic rats [19] revealed colitogenic effects of *B. vulgatus*, with no pathological response to *E. coli* and *E. faecalis*. These studies suggest that all enteric bacteria are not equal in their capacities to induce chronic intestinal inflammation but rather specifically interact with the genetically susceptible host. It seems important to understand that the absence of colitis and pathologic immune responses in colonized wild-type mice demonstrates the non-pathogenic nature of these enteric bacterial species and most importantly suggests that normal hosts develop immunosuppressive mechanisms that control mucosal immune responses to the constant challenge of these commensal enteric bacteria.

In conclusion, the studies with IBD patients and animal models of experimental colitis demonstrate that enteric bacteria are a critical component in the development of chronic intestinal inflammation in the genetically susceptible host. In addition, accumulating evidence suggests that enteric bacteria not only have the ability to induce intestinal inflammation, but also mediate beneficial activities [20]. Probiotics are commensal micro-organisms with beneficial effects to the host, including stimulation of protective immune responses, enhancement of mucosal barrier function, suppression of pathogenic organisms and treatment of chronic intestinal inflammation [21]. Although the specific properties of probiotic micro-organisms are not yet characterized and validated in well-designed, multicenter clinical trials, studies in several animal models and human trials show considerable therapeutic relevance for VSL#3 [22,23] and *E. coli* strain Nissle [24,25]. Although advancing knowledge regarding the cellular mechanisms of bacteria-induced inflammation in the gut has led to a better understanding of the disease pathologies, still little is known about the molecular mechanisms of enteric bacteria in targeting protective and detrimental cell type-specific signal transduction pathways in the genetically susceptible host. New technologies such as genomics, proteomics (expression, functional and structural) and bioinformatics will likely contribute to the discovery of more components involved in the complex interaction of bacteria with the host under normal and pathological conditions of chronic intestinal inflammation. In this review, we summarize the novel concept that host-derived mediators including Interleukin 10 (IL-10) and transforming growth factor (TGF)-β target the intestinal epithelium to inhibit enteric bacteria-derived pro-inflammatory signal transduction mechanisms. In addition, we will discuss the contribution of proteome technologies in epithelial cell lines, animal models of experimental colitis and IBD patients to the identification and functional characterization of disease-relevant proteins and novel mechanisms for the development of chronic inflammation.

## 2. Innate and adaptive immune signals target the epithelial cell interface in the gut: a double edged sword

The surface epithelium represents a highly selective barrier between the luminal gut environment and underlying lamina propria immune cells. Intestinal epithelial cells (IEC) constitutively express, or can be induced to express, costimulatory molecules [26,27] and components of the human major histocompatibility complex (MHC) including class II, classical I and non-classical class Ib MHC molecules [28,29], Toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD2) protein receptors [30,31], inflammatory and chemoattractive cytokines [32] as well as anti-microbial peptides [33,34]. Of considerable importance, most of these molecules are at least in part transcriptionally regulated by the transcription factor NF-κB [35]. Moreover, there is accumulating evidence that IEC contribute to the

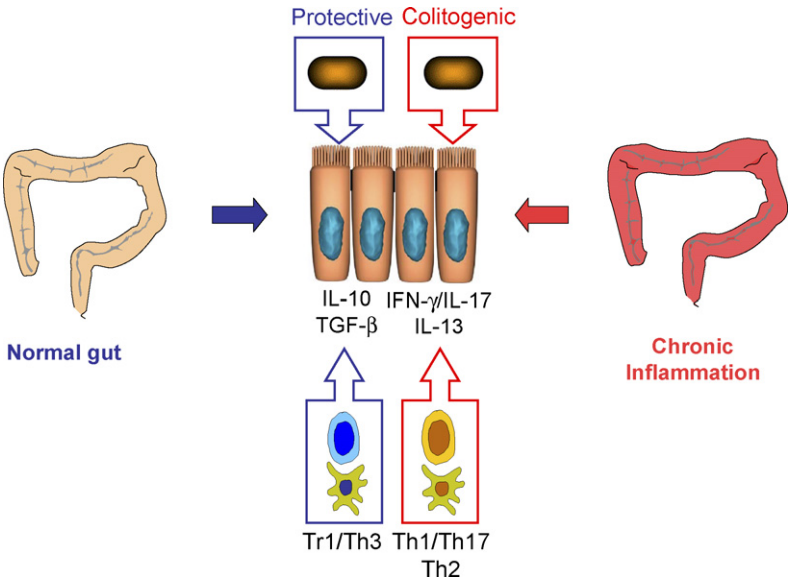


Fig. 1. Bacteria- and host-derived protective and colitogenic signals target the intestinal epithelium. The lack of protective immune-mediated signals (IL-10 and TGF-β from immunoregulatory T cells) in the gut and/or the accessive production of colitogenic mediators (IFN-γ, IL-17 and IL-13 from T helper cells) triggers chronic intestinal inflammation in the genetically susceptible host.

initiation and regulation of innate and adaptive defense mechanisms by directly interacting with lamina propria dendritic cells (DC), lamina propria lymphocytes (LPL) and intraepithelial lymphocytes (IEL) [12,36–38], and are therefore considered to be a constitutive component of the mucosal immune system.

To maintain gut homeostasis, the intestinal epithelium must adapt to a constant changing environment by processing the combined biological information of the intestinal luminal content including enteric bacteria as well as host-derived immune signals (Fig. 1). Interestingly, the break in intestinal epithelial barrier function precedes the onset of chronic immune-mediated histopathology in the intestinal tract of IBD patients as well as animal models, supporting the hypothesis that the loss of epithelial cell homeostasis is critically important for the development of chronic intestinal inflammation [14–16].

### *2.1. Innate mechanisms of chronic intestinal inflammation*

The cornerstone of innate signalling is initiated by a set of well-conserved pattern recognition receptors including TLR and NOD receptors [30,39,40]. The combined action of both sets of receptors plays a pivotal role in the recognition of extracellular and intracellular microbial patterns associated with both non-pathogenic (commensal) and pathogenic bacteria (Fig. 2). TLRs are transmembrane proteins characterized by an extra cellular domain containing leucine-rich repeats (LRR) and an intracellular domain homologous to the IL-1R or Toll/IL-1R (TIR). It is important to understand that ligand-specific binding to TLR promotes signal divergence by the differential interaction of the cytoplasmic TIR domain with various adaptor proteins (MyD88, MAL/TIRAP, TRIF/TICAM-1, TRAM/TIRP/TICAM-2) targeting down-stream effector systems such as the mitogen-activated kinases (MAPK) and the IκB/NF-κB transcriptional system [41,42]. The purpose of pattern recognition receptor signalling is to alert and protect the host, but changes in these innate signalling pathways due to host genetic predispositions may turn a physiological response into a pathological situation including failure of bacterial clearance and development of chronic inflammation [43]. This is supported by the identification of variant alleles of TLR4 [44,45] and NOD2 genes [46,47] in some IBD patient cohorts, suggesting that changes in the pattern recognition receptor signalling may indeed affect chronic intestinal inflammation.

Interestingly, Rakoff-Nahoum et al. recently demonstrated that the development of experimental colitis in IL-10-/mice was abrogated in the absence of TLR/MyD88-derived signals using IL-10−/− X MyD88−/− mice [48]. The authors demonstrated that the lack of this innate signalling pathway prevented the development of colitis at the level of

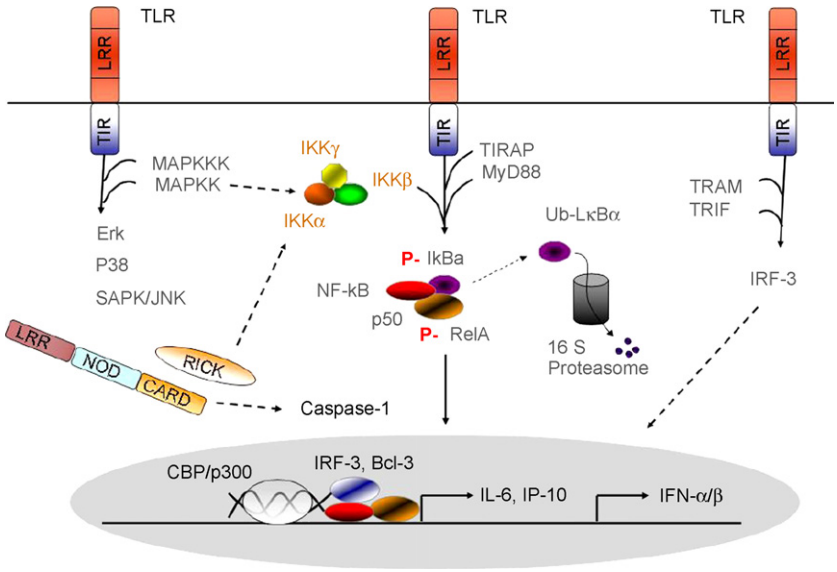


Fig. 2. Pattern recognition receptors including TLR and NOD proteins recognize extracellular and intracellular bacterial structures to trigger various signal transduction cascades including the mitogen-activated proteine kinases (MAPK) and IκB/NF-κB system. TLR and NOD proteins recognize bacterial products through interaction with their leucine-rich repeat (LRR) domain. Pattern recognition receptors (TLR, NOD); adaptor proteins (TIRAP, MyD88, TRAM, TRIF); kinases (IKK, Erk, p38, SAPK/JNK, RICK); transcription factors and co-activatiors (RelA, p50, CBP/p300, IRF, Bcl-3).

T cell-mediated adaptive immune responses. In contrast, TLR/MyD88 deficient mice developed markedly increased histopathology in the dextran sodium sulfate (DSS)-induced model of colitis, suggesting protective effects of this innate signalling cascade at the epithelial cell level [49]. The latter findings are consistent with observations that TLR4 mutant C3H/HeJ mice are more sensitive to DSS-induced colitis than wild-type mice [50,51]. In addition, the presence of TLR4-mediated signals linked enteric bacteria to the prevention of allergic responses to food antigens [52], supporting the hypothesis that the loss of pattern recognition receptor signalling may incapacitate the host to mount an appropriate innate response leading to dysregulated adaptive immune responses [53]. Further evidence for protective TLR-mediated effects on experimental colitis was recently shown by Katakura et al. [54]. The authors demonstrated that the induction of TLR9 signalling resulted in the activation of interferon regulated factors (IRF1 and 8) and triggered protective type I IFN (IFN-α/β) production through MyD88- and DNA-dependent protein kinase (DNA-PK)-dependent mechanisms. It appears to be an important question to what extend IEC as the most prominent cell type at the interface between the intestinal luminal environment and the host directly interact with bacteria and most importantly, to what extend these signals contribute to the development of chronic intestinal inflammation.

### 2.2. *Bacteria-epithelial cell cross-talk: NF-κB signal transduction at the crossroad between host defense and chronic inflammation*

Previous studies in human and experimental IBD showed increased TLR expression and nuclear factor κB (NF-κB) activity in lamina propria macrophages and the epithelium under conditions of chronic intestinal inflammation [55–59]. Interestingly, the local administration of anti-sense NF-κB RelA oligonucleotides abrogated clinical and histological signs of inflammation in trinitrobenzene sulfonic acid (TNBS)-treated mice [60], suggesting that the NF-κB transcription factor system plays an important role in conferring the colitogenic bacteria- and host-derived signals into the deleterious pro-inflammatory gene program that fuels the chronic pro-inflammatory processes. On the other hand, but equally important, the inhibition of NF-κB activity with pharmacological inhibitors during the resolution phase of carrageenan-induced acute inflammation had adverse effects on the host [61], suggesting dual functions of activated NF-κB signalling during the course of inflammation. In addition, the selective ablation of NF-κB signalling in IKKβ-deficient IEC sensitizes these animals to acute ischemia-reperfusion-induced epithelial cell apoptosis and the loss of mucosal integrity under these adverse conditions [62]. This local intestinal tissue injury under these inflammatory stress conditions

is likely due to the failure of IKK to activate a protective NF-κB-dependent gene program in IEC. These results support the hypothesis that the acute and transient activation of NF-κB may be protective for the host, while sustained and uncontrolled NF-κB signalling in the intestinal epithelium may indeed contribute to the immunopathology of experimental colitis.

It seems reasonable to assume that commensal enteric bacteria may target the TLR effector system in the gut leading to the activation of the transcription factor NF-κB [55–59]. Interestingly, we showed that Gram-negative non-pathogenic *B. vulgatus* triggered TLR4 signalling to induce NF-κB activation and pro-inflammatory gene expression in epithelial cell lines and the native intestinal epithelium [63–65]. Immunostaining of tissue sections confined the induction of RelA phosphorylation to the epithelium with no induction in underlying lamina propria immune cells of *B. vulgatus*-monoassociated wild-type rats, suggesting a transient and compartimentalized activation of NF-κB in the gut mucosa of the normal host. Consistent with these findings, Lotz et al. elegantly demonstrated that the intestinal epithelium of the normal murine host acquired postnatal endotoxin tolerance in response to TLR-induced signals by mechanisms that involve the selective ubiquitin-mediated degradation of IRAK-1 [66]. These data support the concept that non-pathogenic bacteria can transiently activate pro-inflammatory signalling processes in IEC, suggesting the hypothesis that the intestinal epithelium from the normal host is trained to be silent but not blind towards enteric bacteria.

In addition and consistent with the latter observations in the normal non-diseased rat, we showed that *E. faecalis* monoassociation of germfree wild-type mice induced a transient TLR2-mediated NF-κB activation (RelA Ser536 phosphorylation) and pro-inflammatory gene expression in the "naïve" epithelium at early stages of bacterial colonization (3 days). Consistent with this "self-limiting" induction of NF-κB signalling preceding any histological evidence of colitis, the level of TLR2 protein expression in IEC dramatically decreased after the initial colonization of the germfree host. Interestingly and in contrast to wild-type controls, the presence of persistently active TLR/NF-κB signalling in IL-10−/− IEC was associated with the development of clinical and histological signs of intestinal inflammation at late stages of bacterial colonization (14 weeks) [67]. These results suggest that pattern recognition receptor signalling in IEC may indeed contribute to the immune surveillance of enteric bacteria during the early stages of host colonization [68]. However, and most importantly for the understanding of the disease progression, the lack of host-derived feed-back mechanisms may contribute to the development of chronic intestinal inflammation in the genetically susceptible host.

### 2.3. Host-derived immune signals in the regulation of pro-inflammatory mechanisms at the epithelial cell level

Since the intestinal epithelium is renewed every 3–5 days, the biological information for the immunosuppressive effects in the colonized host should be imprinted in the gene program of pluripotent epithelial stem cells and/or mediated by the recruited professional immune cells in the lamina propria. Interestingly, several TLR pathway intrinsic mechanisms have been proposed for the development of hypo-responsiveness of the intestinal epithelium towards bacterial signals under normal conditions [66,69–72]. In addition, we provide evidence for the fact that host-derived immune signals are critical in maintaining epithelial cell homeostasis, suggesting that the lack of adequate control signals at the epithelial cell level may contribute to the development of chronic intestinal inflammation. Although many pathways are likely involved in the regulation of innate and/or adaptive immunity in the intestine, IL-10 and TGF-β signalling cascades are of high relevance to IBD.

Most elegantly, Powrie and colleagues provided experimental evidence for the importance of the immunosuppressive mediators TGF-β and IL-10 using severe combined immunodeficient (SCID) and recombination activating gene deficient (RAG−/−) mice. The adoptive transfer of CD4+ $CD45RB^{high}$ T cells from congenic donor mice into T and B cell deficient SCID and/or RAG−/− mice triggered experimental colitis. The development of chronic inflammation was associated with the production of high amounts of the pro-inflammatory Th1 mediator Interferon γ (IFN-γ) [73]. In contrast, the adoptive transfer of CD4+ $CD45RB^{low}$ T cells revealed protective mechanisms in the recipient SCID and/or RAG−/− host, depending on the presence of the immunosuppressive mediators TGF-β and IL-10 [74–76]. In accordance with these observations, $IL\text{-}10^{-/-}$ mice develop immune-mediated colitis in a specific pathogen free (SPF) environment but remain healthy and disease free when mice are born and raised under germ-free conditions. These results strongly support the observation that in the absence of host-derived immune regulators, bacterial antigens that are present in the intestinal microbiota drive the inflammatory process. In addition, the protective mechanisms of IL-10 in TNBS-induced experimental colitis were indirectly mediated through its inductive effect on TGF-β secre-

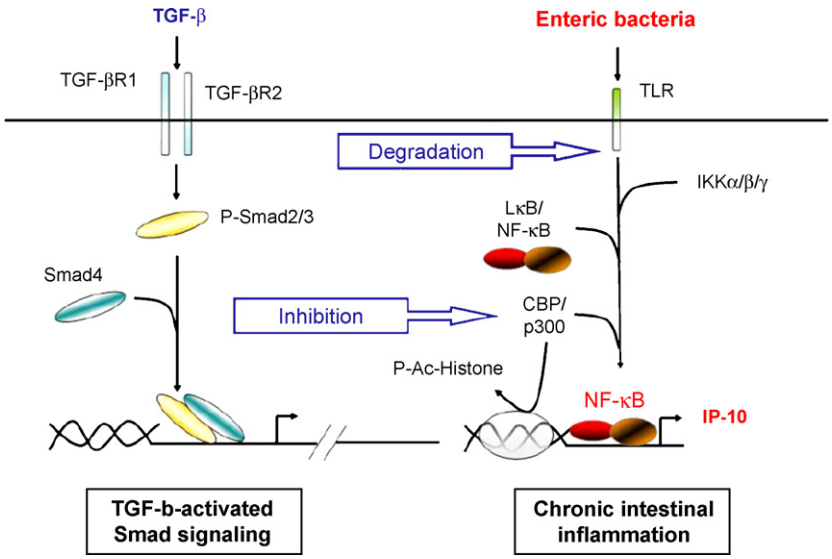


Fig. 3. Host-derived mechanisms to terminate pro-inflammatory signals through the immunoregulatory cytokine TGF-β. The TGF-β-activated Smad signal transduction cascade inhibits the TLR-induced NF-kB-dependent pro-inflammatory gene expression program at the receptor and nuclear level.

tion in lamina propria T cells, suggesting an interrelated role for these protective cytokines [77,78]. Moreover, TGF-β1 deficient mice spontaneously develop colitis [79] and the over-expression of TGF-β1 in lamina propria immune cells inhibited Th1-mediated experimental TNBS-induced colitis [80]. Consistent with these findings and most important to understand the biological function of TGF-β at the epithelial cell level, the molecular blockade of TGF-β signalling, using tissue specific transgenic mice expressing a dominant-negative TGF-β receptors in native epithelium [81], triggers colitis under conventional conditions and manifest increased susceptibility to DSS-induced disease. TGF-β1 mediates its biological effect through activation of various signalling cascades including the Smad and MAPK pathways [82]. The lack of TGF-β-activated Smad signalling in lamina propria T cells of IBD patients due to over-expression of the pathway specific inhibitor Smad7 was associated with disease progression [83,84]. These results suggest that although immunosuppressive mediators are present in the diseased tissue, the intracellular blockade of these protective signals may lead to development of chronic intestinal inflammation.

We demonstrated in *B. vulgatus*-monoassociated wild-type Fisher F344 rats as well as *E. faecalis*-monoassociated wild-type SvEv129 mice that nuclear RelA phosphorylation was followed by the induction of Smad2 phosphorylation in epithelial cells from intestinal tissue sections at early stages of bacterial colonization [63–65,67], suggesting that the presence of NF-κB and TGF-β1 signals in the intestinal epithelium under normal conditions following bacterial colonization. Interestingly and mechanistically important, we showed that TGF-β-activated Smad signalling induced rapid TLR2 degradation [67] and blocked CBP/p300-mediated histone phosphorylation in epithelial cells [64], leading to the inhibition of pro-inflammatory gene expression (Fig. 3). These results support the hypothesis that host-derived feed back mechanisms specifically inhibit bacterial-driven pro-inflammatory mechanisms in the intestinal epithelium. Additional evidence for the importance of proteasome-mediated degradation of TLRs as a strategy of the host to control pattern recognition receptor signalling was recently shown by Chuang and Ulevitch [85]. The authors demonstrated that the intrinsic RING finger protein TRID3 enhanced ubiquitination and proteolytic degradation of TLR4 and TLR9 but not TLR2 through its E3 ubiquitin-protein ligase activity, suggesting that the various negative feed-back regulators of the TLR signalling cascade may target different levels of the TLR cascade with distinct specificities for the TLR subsets. Important for the molecular understanding of the disease pathology, TGF-β1-induced Smad2 signalling in IEC was absent in *E. faecalis*-monoassociated IL-10$^{-/-}$ mice [67], suggesting that in the absence of the activated TGF-β/Smad cascade in the intestinal epithelium, bacteria-mediated TLR signalling may lead to the development of clinical and histological signs of chronic intestinal inflammation. In conclusion, these results support the hypothesis that host-derived feed-back mechanisms control epithelial cell responses towards enteric bacteria under normal conditions, but

the lack of these protective immune signals is associated with the loss of epithelial cell homeostasis and the chronic activation of pro-inflammatory immune mechanisms [86,87].

## 3. Functional epithelial cell proteomics identified the endoplasmic reticulum stress response as potential molecular target to modulate chronic intestinal inflammation

Proteome analysis includes a conglomerate of advanced analytical methods directed to assess quantitative changes in the protein expression level of various cell types and tissues including protein identification and characterization of their function in health and disease [88]. The need for separation techniques that allow the quantitative and qualitative resolution of complex protein mixtures into reproducible patterns led to the development of two-dimensional (2D) protein separation methods with the possibility to resolve 1000–10,000 protein spots using isoelectric focussing (IEF) in the first dimension and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for molecular weight separation in the second dimension [89,90]. Although the human genome assembles approximately 20,000–25,000 genes, the estimated number of proteins/peptides that may be present during the cellular life cycle ranges between 30,000 and 100,000 proteins/peptides, depending on the cell environment in the tissue and the presence of stress mechanisms such as infections and chronic inflammation [91]. Although, 2D-gel detection methods need to improve their sensitivity and resolution limits for low-abundant and insoluble membrane proteins by pre-fractionation of the tissue and cell samples, the use of high-resolution gel-based separation methods are still imperative for the identification of differentially expressed proteins [92–94]. Alternative gel-free approaches such as liquid chromatography (LC) and stable isotope labelling complement the existing methods to improve the identification of protein variations including isoforms, allelic variants and post-transcriptional modifications [95,96]. In addition to the high-resolution 2D-gel electrophoresis, peptide mass fingerprinting via high-sensitivity mass-spectrometers (MS) and the availability of genome and protein databases are instrumental for successful proteome analysis [97,98]. The development of revolutionary techniques such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), electro-spray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry replaced less sensitive slow protein degradation methods [99]. The fundamental principle of MS analysis involves the conversion of the subject molecules to either cations or anions in the ion source, followed by their separation according to the mass/charge ($m/z$) ratio in the mass analyser and subsequent detection. There are several different types of mass-spectrometers that combine ESI or MALDI with a variety of mass analysers for the detection of ions from high-molecular-mass proteins and polypeptides with extremely low concentrations.

Since the biologic information stored in the genome may be differentially transcribed and translated in various cell types due to tissue-specific and disease-related mechanisms, the detection of protein expression changes in a set of well defined cells/tissues/organs from normal and pathologic conditions will likely add important insights to the understanding of disease pathologies. Although disease and tissue-specific proteomics has been used to study physiological and pathophysiological situations at the epithelial cell level [100,101], human and experimental studies in animal models of chronic intestinal inflammation to characterize the IBD-related changes of the protein expression profile in primary cells have not yet been performed.

### *3.1. IL-10 inhibits endoplasmic reticulum stress response: implications for chronic intestinal inflammation*

Adverse environmental and metabolic conditions trigger cellular stress responses including endoplasmic reticulum (ER)-specific mechanisms to ensure the transit of correctly folded proteins to the extracellular space, plasma membrane and the exo/endocytic compartments [102]. Various biochemical and physiologic stimuli, e.g., perpetuation in calcium homeostasis or redox status, elevated protein synthesis and expression of unfolded or misfolded proteins, glucose deprevation and altered protein glycosylation, cholesterol depletion and microbial infections can induce ER stress responses [103]. Distinct signal transduction pathways, including the unfolded protein response (UPR), the ER-overload response (EOR) as well as the sterol regulatory element binding protein pathway direct the specific ER stress signals towards the nucleus [104]. In addition, ER-associated degradation processes reduce the accumulation of mis- or unfolded proteins through the initiation of proteasomal degradation. Likely upon failure of these adaptation mechanisms, the excessive and prolonged ER stress response results in cell death through mechanisms that involve mitochondria-dependent and mitochondria-independent apoptotic pathways [105,106].

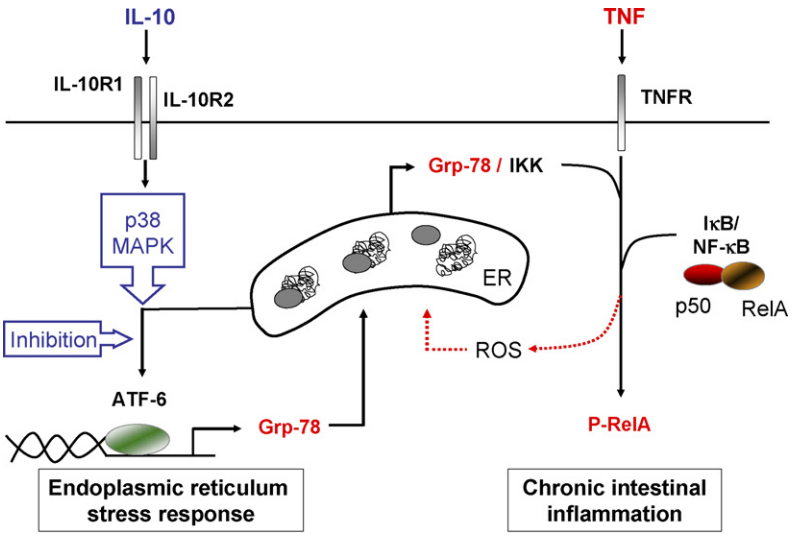


Fig. 4. Host-derived mechanisms to terminate pro-inflammatory signals through the immunoregulatory cytokine IL-10. The IL-10-activated p38 MAPK signal transduction cascade inhibits glucose-regulated endoplasmic reticulum stress protein (grp-78) response mechanisms at the level of nuclear translocation of the ATF-6 transcription factor.

The glucose-regulated protein (grp)-78 (also referred to immunoglobulin heavy chain-binding protein, BiP) was first identified as a prototypic ER stress marker and master regulator of UPR [106]. The accumulation of mis- or unfolded proteins in the ER due to environmental and/or metabolic stress conditions triggers grp-78 liberation from ER trans-membrane proteins. For example, the activating transcription factor (ATF)-6 is an ER trans-membrane protein with ER/perinuclear localization and is an important activator of the grp-78 promoter that contains one copy of the UPR response element and multiple copies of the ER stress response element (ERSE) [107,108]. At the level of ER-nuclear signal transduction, the cleaved cytoplasmic fragment of ATF-6 (p50) translocates to the nucleus and interacts in conjunction with other proteins at ER-specific grp-78 promoter sites. In addition to ATF-6, the bifunctional serine/threonine proteine kinase/endoribonuclease (IRE-1/Ern1p) and the PKR-like ER-associated kinase (PERK) represent important ER-associated stress sensors. ER stress responses were also linked to the activation of the NF-κB transcription factor system through mechanisms that involve IER-1 signalling and the induction of the TNF receptor-associated factor (TRAF) 2, $Ca^{2+}$ signalling and the production of reactive oxygen species (ROS) [104,109]. So far, ER stress response mechanisms have been associated with the development of chronic pathologies such as diabetes (Types I and II), cancer and neurodegenerative diseases [103], however, little is known about the role of ER stress response mechanisms in IBD patients.

We performed proteomic analysis in *E. faecalis*-monoassociated IL-10−/− mice, demonstrating that the expression of the ER stress response protein grp-78 was increased in primary IEC under conditions of chronic inflammation [110]. Most important for the pathologic relevance, we validated these findings in primary IEC from patients with Crohn's disease and ulcerative colitis, demonstrating increased grp-78 protein expression levels in inflamed but not in control tissues. Interestingly, we found that the ER-resident chaperone grp-78 modulates cytoplasmic TNF signal transduction through recruitment of grp-78 into the IκB kinase (IKK) complex (Fig. 4). Consistently, the small interferring (si) RNA-mediated knock-down of grp-78 prevented TNF-induced NF-κB RelA phosphorylation, supporting the hypothesis that the association of grp-78 with the IKK/NF-κB signalsome facilitates the activation of this pro-inflammatory cascade. Since TNF triggers ROS-dependent ER stress [111] independent of grp-78 re-synthesis [112], the appearance of grp-78 in the IKK complex may reflect TNF-induced ER stress response and redistribution of grp-78 from the ER lumen into the cytoplasmic space. These findings are consistent with limited published precedents in the literature, demonstrating that ER stress inducers trigger the redistribution of grp-78 from the ER lumen to the cytoplasm [113] or the cytosolic ER interface as a trans-membrane protein [114].

Interestingly, IL-10 signals through JAK1/STAT3 and p38 MAPK-dependent pathways to trigger suppressors of cytokine signalling (SOCS)-mediated [115,116] or

heme oxygenase (HO)-1-dependent anti-inflammatory mechanisms [117,118]. Although the molecular understanding for IL-10 signalling in IEC is still unclear, we showed that IL-10 receptor (IL-10R) reconstituted IEC cultures regain IL-10-mediated p38 phosphorylation. These results suggested a direct protective role for IL-10-mediated p38 signalling at the epithelial cell level. In addition and most important to understand the physiologic relevance of IL-10-derived feed-back mechanisms, we showed that the activation of the p38 MAPK signalling cascade is present in primary IEC from *E. faecalis*-monoassociated wild-type but not IL-10−/− mice. Together with the findings that IL-10-mediated p38 signalling blocked ER stress responses in the intestinal epithelium through mechanisms that inhibit nuclear recruitment of the ER transcription factor ATF-6 to the grp-78 promoter (Fig. 4), we suggest that IL-10 may directly confer protective mechanisms to the intestinal epithelium by regulating ER stress response mechanisms. Considering our previous findings that protective TGF-β-mediated Smad signalling was present at the early but not at the late phase of bacterial colonization [67], we propose that TGF-β and IL-10 may both contribute to the maintenance of epithelial cell homeostasis but differ in the timing and molecular mechanisms of their effects. To better define epithelial cell responses under the pathological conditions of chronic intestinal inflammation in the absence of IL-10, we recently characterized the protein expression profile (proteome) in primary IEC from *E. faecalis*-monoassociated WT and IL-$10^{-/-}$ mice at early (2 weeks) and late stages (14 weeks) of bacterial colonization. Additionally we characterized protein expression profile in IL-10 receptor reconstituted (IL-10R) Mode-K cells after the stimulation of the epithelial cell line with *E. faecalis* and IL-10. In total, we identified 76 target proteins with significantly altered steady state expression levels in primary and IL-10R reconstituted IEC lines using 2D-gel electrophoreses (2D SDS-PAGE) and peptide mass fingerprinting via MALDI-TOF mass spectrometry (MS) [119]. The presence of sustained ER stress response mechanisms in the intestinal epithelium may contribute to the development of epithelial cell dysfunctions and chronic intestinal inflammation [120]. An attractive hypothesis is that transient induction of NF-κB activity in epithelial cells triggers biologically active IL-10-mediated TGF-β responses in the lamina propria or the epithelium, suggesting that IL-10 and TGF-β1 have interrelated roles in maintaining epithelial cell homeostasis to commensal enteric bacteria.

### *3.2. Intestinal epithelial cell proteomics in IBD patients: translational research from model systems to the human disease*

Proteome analysis may become an important analytical tool to improve the molecular understanding of the IBD pathogenesis. The possibility to identify disease-specific biomarkers for Crohn's disease and ulcerative colitis envisions great expectations with respect to the diagnosis and treatment of these chronically relapsing intestinal disorders. In contrast to animal models of chronic experimental inflammation, the accessibility to relevant tissue sites and the availability of suitable controls are unequally more difficult to obtain. In addition and for obvious reasons, IBD patients are much harder to standardize compared to animal model systems with respect to their genetic heterogeneity and their environmental behaviour including differences between socio-economic conditions, dietary habits and medication. Since the intestinal epithelium represents an important interface between luminal- and host-derived mediators, the biological information that can be retrieved from purified ileal and colonic epithelial cells by using proteome analysis, will add important insights to the disease-mechanisms in IBD patients.

In a pilot "proof-of-principle" study, we used purified primary IEC from surgical speciments of 18 patients with active Crohn's disease (ileal tissue, $N=6$) and ulcerative colitis (colonic tissue, $N=6$) as well as non-inflamed control tissue from colorectal cancer patients (colonic tissue, $N=6$) and identified 61 different target proteins using 2D SDS-PAGE and MALDI-TOF MS [120]. It was recently demonstrated by the study of Polley et al. that the protein expression profile in the morphologically normal mucosa from cancer patients and the mucosa of healthy individuals is remarkably different [122]. This pointed to the question whether the differences in the protein expression profile found in IBD patients are exclusively due to the inflammatory condition or are influenced by the cancer status of the patients used as a control. Inflammation and cancer have been considered closely linked for many years, with colorectal cancer (CRC) being the most common malignant complication in patients with chronic intestinal inflammation (five percent of all CRCs) [123,124]. This makes difficult to say whether an aberrant protein profile expression is characteristic of cancer tissue or is a pathological factor of IBD.

To address the important question of adequate control samples, we also compared inflamed versus non-inflamed regions from the same tissue sections of two UC patients and identified additional 40 proteins with significant changes in their protein expression levels

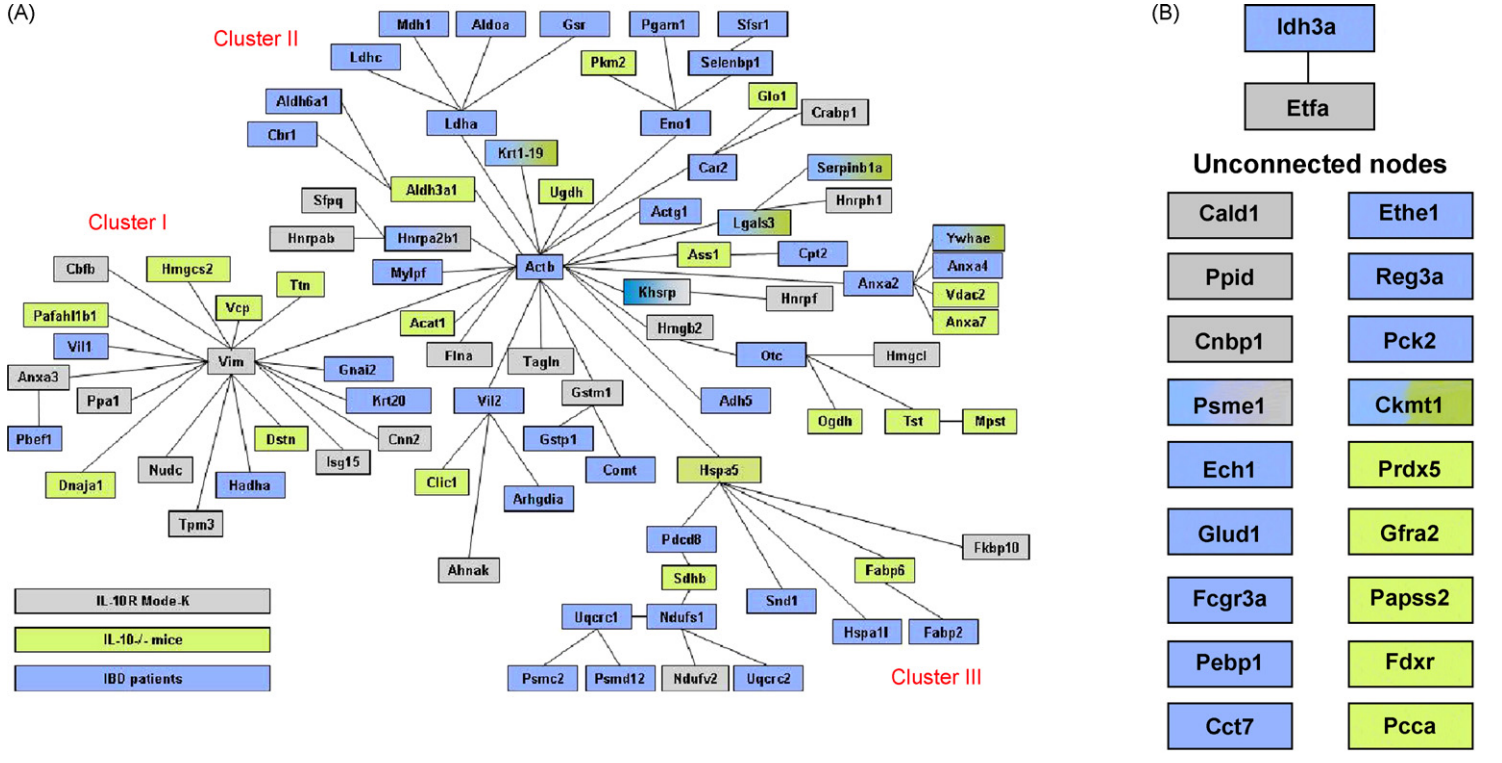


Fig. 5. (A and B) Bibliometric analysis was performed on the base of literature co-citation from NCBI Pubmed. The data-mining program Bibliosphere software (Genomatix) was used to generate protein-protein network trees from cell culture experiments (gray boxes), animal model systems (green boxes) and human IBD patient data (blue boxes). Proteins that were identified in cell culture experiments and the human data set are marked in blue/gray boxes. Proteins that were identified in the animal model and the human data set are marked in blue/green boxes. (A) One-hundred and three proteins are integrated in the highly interrelated network tree. (B) Additional 20 proteins remain unconnected to the large network tree.

[121]. Interestingly, the total number of differentially regulated proteins varied substantially between the two patients, confirming the heterogeneity of protein expression in patients with different genetic background, age and exposure to environmental factors. Nevertheless, the approach to compare inflamed and non-inflamed tissue from the same patient offers the possibility to exclude the problem of "abnormal" non-inflamed cancer considered as healthy control tissue as well as variations in the protein expression intensity due to genetic heterogeneity and medical treatment. It seems obvious from these results that follow-up studies require higher numbers of patients including IBD patient subsets with similarities in genetic polymorphisms, disease localization and disease behaviour in order to confirm and extend the number of significantly regulated proteins.

### *3.3. Bibliometric data analysis: from cell lines, animal models and human patients*

On the base of literature co-citation from NCBI Pubmed, we are able to generate protein–protein network trees from cell culture experiments, animal model systems and human patient data using the data-mining program Bibliosphere software (Bibliosphere Pathway Edition$^{TM}$, Genomatix Software, Munich, Germany). As shown in Fig. 5, we combined the proteome analysis of three different experiments. First, we stimulated IL-10 receptor (IL-10R) reconstituted Mode-K cell line with *E. faecalis* and/or recombinant murine IL-10 as previously described [110]. This experimental approach added 27 differentially expressed proteins to the bibliometric analysis. Second and in addition to the cell culture experiments, we implemented 31 additional proteins from the proteome analysis in primary IEC from *E. faecalis*-monoassociated wild-type and IL-10−/− mice after 2 and 14 weeks of bacterial colonization (Werner, Shkoda and Haller, manuscript submitted). Finally, we included 55 differentially regulated proteins from IBD patients [121]. As shown in Fig. 5A and B, the combined network tree from murine cell culture experiments (gray squares), primary murine IEC (green squares) and primary human IEC (blue squares) compiled 113 different proteins forming an highly interrelated network with 82.3% concordance rate within the total clusters of proteins (Fig. 5A). The network tree was roughly divided in three functional clusters including cytoskeletal proteins (cluster I), energy metabolism (cluster II) and protein metabolism (cluster III). We found two additional co-citated proteins outside from the big network tree and additional 18 proteins that were completely unrelated (Fig. 5 B). Interestingly, we identified five proteins that were similarly detected in primary IEC from the murine and human analysis including the lectin-galactose-binding protein, soluble three (LGALS3), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide (YWHAE), keratin complex 1, acidic, gene 19 (KRT1-19), creatine kinase, mitochondrial 1, ubiquitous (CKMT1) and the serine (or cysteine) peptidase inhibitor, clade B, member 1a (SERPINB1A). Interestingly, the ER-resident glucose-regulated chaperon grp-78 was starting point for the protein metabolism cluster (cluster III) and was directly co-citated with the human programmed cell death protein PDCD8. This protein was most dramatically upregulated (7.4-fold increase) in IEC from inflamed versus non-inflamed tissue regions of an ulcerative colitis patient [121], supporting the hypothesis that ER stress responses and epithelial cell barrier function contribute to the disease pathology. In addition, we found three overlapping proteins between the murine epithelial cell culture experiments and IBD patients including the KH-type splicing regulatory protein (KHSRP), proteasome (prosome, macropain) 28 subunit, alpha (PSME1) and the heterogeneous nuclear ribonucleoprotein A/B (HNRPA2B1).

In conclusion, these data strongly suggest that bacteria- and host-derived signals at the epithelial cell level are tightly controlled by a complex network of proteins. It seems important to define functional clusters of interrelated proteins within the complex protein network and to characterize their specific contribution in maintaining epithelial cell homeostasis. The failure to terminate pro-inflammatory mechanisms in the epithelium may lead to persistent inflammation and potentially to chronic inflammation in a susceptible host. The epithelial cell proteome together with additional technologies may help to generate novel mechanistic insights into the disease pathogenesis leading to identification of specific biomarkers according to the specific disease status, disease behaviour and individual genetic subgroup of the IBD patients.

## Appendix A

| | |
|---|---|
| Acat1 | acetyl-coenzyme A acetyltransferase 1 |
| Actb | actin beta |
| Actg1 | actin gamma |
| Adh5 | alcohol dehydrogenase 5 (class III9, chi polypeptide Ahnak AHNAK nucleoprotein (desmoyokin) |
| Aldh3a1 | aldehyde dehydrogenase family 3, subfamily A1 |

Aldh6a1 aldehyde dehydrogenase family 6, subfamily A1 Aldoa aldolase A, fructose-bisphosphate
Anxa2 annexin A2
Anxa3 annexin A3
Anxa4 annexin A4
Anxa7 annexin A7
Arhgdia Rho GDP dissociation inhibitor (GDI) alpha
Ass1 argininosuccinate synthetase 1
Cald1 caldesmon 1
Car2 carbonic anhydrase II
Cbfb core binding factor beta
Cbr1 carbonyl reductase 1
Cct7 chaperonin containing TCP1, subunit 7 (eta)
Ckmt1 creatine kinase, mitochondrial 1, ubiquitous
Clic1 chloride intracellular channel 1
Cnbp1 cellular nucleic acid binding protein 1
Cnn2 calponin 2
Comt catechol *O*-methyltransferase
Cpt2 carnitine palmitoyltransferase II
Crabp1 cellular retinoic acid binding protein I
Dnaja1 DnaJ (Hsp40) homolog, subfamily A, member 1
Dstn Destrin
Ech1 enoyl Coenzyme A hydratase 1, peroxisomal
Eno1 enolase 1 (alpha)
Etfa electron transferring flavoprotein
Ethe1 ethylmalonic encephalopathy 1
Fabp2 fatty acid binding protein 2, intestinal
Fabp6 fatty acid binding protein 6, ileal (gastrotropin)
Fcgr3a Fc fragment of IgG, low affinity IIIa
Fdrx Ferredoxin reductase
Fkbp10 FK506 binding protein 10
Flna Filamin, alpha
Gfra2 glia cell line derived neurotrophic factor family receptor alpha 2
Glo1 glyoxalase 1
Glud1 glutamate dehydrogenase 1
Gnai2 G protein, alpha inhibiting activity polypeptide 2
Gsr glutathione reductase
Gstm1 glutathione *S*-transferase, mu1
Gstp1 glutathione *S*-transferase P
Hadha trifunctional protein alpha subunit
Hmgb2 high mobility group box 2
Hmgcl 3-hydroxy-3-methylglutaryl-coenzyme A lyase
Hmgcs2 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2
Hnrpa2b1 heterogeneous nuclear ribonucleoprotein A2/B1
hnrpab heterogeneous nuclear ribonucleoprotein A/B
Hnrpf heterogeneous nuclear ribonucleoprotein F
Hnrph1 heterogeneous nuclear ribonucleoprotein H1
Hspa1l heat shock 70 kDa protein 1-like
Hspa5 heat shock 70 kDa protein 5 (glucose-regulated protein)
Idh3a isocitrate dehydrogenase 3 (NAD+) alpha
Isg15 ISG15 ubiquitin-like modifier
Khsrp KH-type splicing regulatory protein
Krt1-19 keratin complex 1, acidic, gene 19
Krt20 keratin 20
Ldha lactate dehydrogenase A
Ldhc lactate dehydrogenase C
Lgals3 lectin, galactoside-binding, soluble, 3 (galectin 3)
Mdh1 malate dehydrogenase 1, (NAD) soluble
Mpst mercaptopyruvate sulfurtransferase
Mylpf fast skeletal myosin light chain 2
Ndufs1 NADH-coenzyme Q reductase 75 kDa
Ndufv2 NADH dehydrogenase (ubiquinone) flavoprotein 2
Nudc nuclear distribution gene C homolog (Aspergillus)
Ogdh oxoglutarate dehydrogenase
Otc ornithine carbamoyltransferase
Pafahl1b1 platelet-activating factor acetylhydrolase, isoform 1b
Papss2 3′-phosphoadenosine 5′-phosphosulfate synthase 2
Pbef1 pre-B-cell colony enhancing factor
Pcca propionyl-coenzyme A carboxylase
Pck2 phosphoenolpyruvate carboxykinase 2, mitochondrial
Pdcd8 programmed cell death 8 (apoptosis-inducing factor)
Pebp1 phosphatidylethanolamine binding protein 1
Pgam1 phosphoglycerate mutase 1 (brain)
Pkm2 pyruvate kinase, muscle
Ppa1 pyrophosphatase (inorganic) 1
Ppid peptidylprolyl isomerase D (cyclophilin D)
Prdx5 peroxiredoxin 5
Psmc2 poteasome 26S subunit, ATPase, 2
Psmd12 poteasome 26S subunit, non-ATPase, 12
Psme1 proteasome activator subunit 1 (PA28 alpha)
Reg3a regenerating islet-derived 3 alpha
Sdhb succinate dehydrogenase complex, subunit B
Selenbp1 selenium binding protein 1
Serpinb1a serpin (or cysteine) peptidase inhibitor, clade B, member 1a
Sfpq splicing factor proline/glutamine rich
Sfrs1 splicing factor arginine/serine rich 1
Snd1 staphylococcal nuclease domain containing 1
Tagln transgelin
Tpm3 tropomyosin 3, gamma

| | |
|---|---|
| Tst | thiosulfate sulfurtransferase, mitochondrial |
| Ttn | titin |
| Ugdh | UDP-glucosedehydrogenase |
| Uqcrc1 | ubiquinol-cytochrome *c* reductase core protein I |
| Uqcrc2 | ubiquinol-cytochrome *c* reductase core protein II |
| Vcp | valosin containing protein |
| Vdac2 | voltage-dependent anion channel 2 |
| Vil1 | villin 1 |
| Vil2 | villin 2 (ezrin) |
| Vim | vimentin |
| Ywhae | tyrosine 3-/tryptophan 5-monooxygenase activation protein |

## References


[1] E.V. Loftus Jr., Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences, Gastroenterology 126 (2004) 1504–1517.

[2] T. Ahmad, C.P. Tamboli, D. Jewell, J.F. Colombel, Clinical relevance of advances in genetics and pharmacogenetics of IBD, Gastroenterology 126 (2004) 1533–1549.

[3] G. Bouma, W. Strober, The immunological and genetic basis of inflammatory bowel disease, Nat. Rev. Immunol. 3 (2003) 521–533.

[4] P.L. Lakatos, Recent trends in the epidemiology of inflammatory bowel diseases: up or down? World J. Gastroenterol. 12 (2006) 6102–6108.

[5] P.H. Harper, E.C. Lee, M.G. Kettlewell, M.K. Bennett, D.P. Jewell, Role of the faecal stream in the maintenance of Crohn's colitis, Gut 26 (1985) 279–284.

[6] P. Rutgeerts, K. Goboes, M. Peeters, M. Hiele, F. Penninckx, R. Aerts, R. Kerremans, G. Vantrappen, Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum, Lancet 338 (1991) 771–774.

[7] G.R. D'Haens, K. Geboes, M. Peeters, F. Baert, F. Penninckx, P. Rutgeerts, Early, lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum, Gastroenterology 114 (1998) 262–267.

[8] A. Swidsinski, A. Ladhoff, A. Pernthaler, S. Swidsinski, V. Loening-Baucke, M. Ortner, J. Weber, U. Hoffmann, S. Schreiber, M. Dietel, H. Lochs, Mucosal flora in inflammatory bowel disease, Gastroenterology 122 (2002) 44–54.

[9] A. Darfeuille-Michaud, J. Boudeau, P. Bulois, C. Neut, A.L. Glasser, N. Barnich, M.A. Bringer, A. Swidsinski, L. Beaugerie, J.F. Colombel, High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease, Gastroenterology 127 (2004) 412–421.

[10] R. Duchmann, E. May, M. Heike, P. Knolle, M. Neurath, K.H. Meyer zum Buschenfelde, T cell specificity and cross reactivity towards enterobacteria, bacteroides, bifidobacterium, and antigens from resident intestinal flora in humans, Gut 44 (1999) 812–818.

[11] T.A. Kraus, L. Toy, L. Chan, J. Childs, L. Mayer, Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease, Gastroenterology 126 (2004) 1771–1778.

[12] M. Allez, L. Mayer, Regulatory T cells: peace keepers in the gut, Inflamm. Bowel Dis. 10 (2004) 666–676.

[13] M.F. Neurath, S. Finotto, L.H. Glimcher, The role of Th1/Th2 polarization in mucosal immunity, Nat. Med. 8 (2002) 567–573.

[14] E.J. Irvine, J.K. Marshall, Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk, Gastroenterology 119 (2000) 1740–1744.

[15] K.D. Katz, D. Hollander, C.M. Vadheim, C. McElree, T. Delahunty, V.D. Dadufalza, P. Krugliak, J.I. Rotter, Intestinal permeability in patients with Crohn's disease and their healthy relatives, Gastroenterology 97 (1989) 927–931.

[16] T.S. Olson, B.K. Reuter, K.G. Scott, M.A. Morris, X.M. Wang, L.N. Hancock, T.L. Burcin, S.M. Cohn, P.B. Ernst, F. Cominelli, J.B. Meddings, K. Ley, T.T. Pizarro, The primary defect in experimental ileitis originates from a nonhematopoietic source, J. Exp. Med. 203 (2006) 541–552.

[17] S.C. Kim, S.L. Tonkonogy, C.A. Albright, J. Tsang, E.J. Balish, J. Braun, M.M. Huycke, R.B. Sartor, Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria, Gastroenterology 128 (2005) 891–906.

[18] M. Waidmann, O. Bechtold, J.S. Frick, H.A. Lehr, S. Schubert, U. Dobrindt, J. Loeffler, E. Bohn, I.B. Autenrieth, Bacteroides vulgatus protects against *Escherichia coli*-induced colitis in gnotobiotic interleukin-2-deficient mice, Gastroenterology 125 (2003) 162–177.

[19] H.C. Rath, K.H. Wilson, R.B. Sartor, Differential induction of colitis and gastritis in HLA-B27 transgenic rats selectively colonized with *Bacteroides vulgatus* or *Escherichia coli*, Infect. Immun. 67 (1999) 2969–2974.

[20] R.B. Sartor, Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics, Gastroenterology 126 (2004) 1620–1633.

[21] G. Reid, J. Jass, M.T. Sebulsky, J.K. McCormick, Potential uses of probiotics in clinical practice, Clin. Microbiol. Rev. 16 (2003) 658–672.

[22] P. Gionchetti, F. Rizzello, A. Venturi, P. Brigidi, D. Matteuzzi, G. Bazzocchi, G. Poggioli, M. Miglioli, M. Campieri, Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial, Gastroenterology 119 (2000) 305–309.

[23] P. Gionchetti, F. Rizzello, U. Helwig, A. Venturi, K.M. Lammers, P. Brigidi, B. Vitali, G. Poggioli, M. Miglioli, M. Campieri, Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial, Gastroenterology 124 (2003) 1202–1209.

[24] B.J. Rembacken, A.M. Snelling, P.M. Hawkey, D.M. Chalmers, A.T. Axon, Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial, Lancet 354 (1999) 635–639.

[25] W. Kruis, P. Fric, J. Pokrotnieks, M. Lukas, B. Fixa, M. Kascak, M.A. Kamm, J. Weismueller, C. Beglinger, M. Stolte, C. Wolff, J. Schulze, Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine, Gut 53 (2004) 1617–1623.

[26] P.E. Framson, D.H. Cho, L.Y. Lee, R.M. Hershberg, Polarized expression and function of the costimulatory molecule CD58 on human intestinal epithelial cells, Gastroenterology 116 (1999) 1054–1062.

[27] L.S. Toy, X.Y. Yio, A. Lin, S. Honig, L. Mayer, Defective expression of gp180, a novel CD8 ligand on intestinal epithelial cells, in inflammatory bowel disease, J. Clin. Invest. 100 (1997) 2062–2071.

[28] R.M. Hershberg, P.E. Framson, D.H. Cho, L.Y. Lee, S. Kovats, J. Beitz, J.S. Blum, G.T. Nepom, Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing, J. Clin. Invest. 100 (1997) 204–215.
[29] N.A. Campbell, H.S. Kim, R.S. Blumberg, L. Mayer, The non-classical class I molecule CD1d associates with the novel CD8 ligand gp180 on intestinal epithelial cells, J. Biol. Chem. 274 (1999) 26259–26265.
[30] M.T. Abreu, M. Fukata, M. Arditi, TLR signaling in the gut in health and disease, J. Immunol. 174 (2005) 4453–4460.
[31] T. Hisamatsu, M. Suzuki, H.C. Reinecker, W.J. Nadeau, B.A. McCormick, D.K. Podolsky, CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells, Gastroenterology 124 (2003) 993–1000.
[32] S.K. Yang, L. Eckmann, A. Panja, M.F. Kagnoff, Differential and regulated expression of C-X-C, C-C, and C-chemokines by human colon epithelial cells, Gastroenterology 113 (1997) 1214–1223.
[33] J. Wehkamp, M. Schmid, K. Fellermann, E.F. Stange, Defensin deficiency, intestinal microbes, and the clinical phenotypes of Crohn's disease, J. Leukoc. Biol. 77 (2005) 460–465 (Epub 2004 Dec 2023).
[34] G. Canny, S.P. Colgan, Events at the host-microbial interface of the gastrointestinal tract. I. Adaptation to a microbial world: role of epithelial bactericidal/permeability-increasing protein, Am. J. Physiol. Gastrointest. Liver Physiol. 288 (2005) G593–G597.
[35] H.L. Pahl, Activators and target genes of Rel/NF-kB transcription factors, Oncogene 18 (1999) 5853–6866.
[36] J.H. Niess, S. Brand, X. Gu, L. Landsman, S. Jung, B.A. McCormick, J.M. Vyas, M. Boes, H.L. Ploegh, J.G. Fox, D.R. Littman, H.C. Reinecker, CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance, Science 307 (2005) 254–258.
[37] M.R. Neutra, N.J. Mantis, J.P. Kraehenbuhl, Collaboration of epithelial cells with organized mucosal lymphoid tissues, Nat. Immunol. 2 (2001) 1004–1009.
[38] T.A. Kraus, L. Toy, L. Chan, J. Childs, A. Cheifetz, L. Mayer, Failure to induce oral tolerance in Crohn's and ulcerative colitis patients: possible genetic risk, Ann. N.Y. Acad. Sci. 1029 (2004) 225–238.
[39] L.A. Carneiro, L.H. Travassos, D.J. Philpott, Innate immune recognition of microbes through Nod1 and Nod2: implications for disease, Microbes Infect. 6 (2004) 609–616.
[40] E. Cario, Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2, Gut 54 (2005) 1182–1193 (Epub 2005 Apr 1119).
[41] K. Takeda, T. Kaisho, S. Akira, Toll-like receptors, Annu. Rev. Immunol. 21 (2003) 335–376.
[42] S. Akira, K. Takeda, Toll-like receptor signalling, Nat. Rev. Immunol. 4 (2004) 499–511.
[43] E. Kopp, R. Medzhitov, Recognition of microbial infection by Toll-like receptors, Curr. Opin. Immunol. 15 (2003) 396–401.
[44] F.L. Rock, G. Hardiman, J.C. Timans, R.A. Kastelein, J.F. Bazan, A family of human receptors structurally related to Drosophila Toll, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 588–593.
[45] D. Franchimont, S. Vermeire, H. El Housni, M. Pierik, K. Van Steen, T. Gustot, E. Quertinmont, M. Abramowicz, A. Van Gossum, J. Deviere, P. Rutgeerts, Deficient host-bacteria interactions in inflammatory bowel disease? The Toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis, Gut 53 (2004) 987–992.
[46] J.P. Hugot, M. Chamaillard, H. Zouali, S. Lesage, J.P. Cezard, J. Belaiche, S. Almer, C. Tysk, C.A. O'Morain, M. Gassull, V. Binder, Y. Finkel, A. Cortot, R. Modigliani, P. Laurent-Puig, C. Gower-Rousseau, J. Macry, J.F. Colombel, M. Sahbatou, G. Thomas, Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease, Nature 411 (2001) 599–603.
[47] Y. Ogura, D.K. Bonen, N. Inohara, D.L. Nicolae, F.F. Chen, R. Ramos, H. Britton, T. Moran, R. Karaliuskas, R.H. Duerr, J.P. Achkar, S.R. Brant, T.M. Bayless, B.S. Kirschner, S.B. Hanauer, G. Nunez, J.H. Cho, A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease, Nature 411 (2001) 603–606.
[48] S. Rakoff-Nahoum, L. Hao, R. Medzhitov, Role of Toll-like receptors in spontaneous commensal-dependent colitis, Immunity 25 (2006) 319–329.
[49] S. Rakoff-Nahoum, J. Paglino, F. Eslami-Varzaneh, S. Edberg, R. Medzhitov, Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis, Cell 118 (2004) 229–241.
[50] J.P. Sundberg, C.O. Elson, H. Bedigian, E.H. Birkenmeier, Spontaneous, heritable colitis in a new substrain of C3H/HeJ mice, Gastroenterology 107 (1994) 1726–1735.
[51] M. Mahler, I.J. Bristol, E.H. Leiter, A.E. Workman, E.H. Birkenmeier, C.O. Elson, J.P. Sundberg, Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis, Am. J. Physiol. 274 (1998) G544–G551.
[52] M.E. Bashir, S. Louie, H.N. Shi, C. Nagler-Anderson, Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy, J. Immunol. 172 (2004) 6978–6987.
[53] M. Schnare, G.M. Barton, A.C. Holt, K. Takeda, S. Akira, R. Medzhitov, Toll-like receptors control activation of adaptive immune responses, Nat. Immunol. 2 (2001) 947–950.
[54] K. Katakura, J. Lee, D. Rachmilewitz, G. Li, L. Eckmann, E. Raz, Toll-like receptor 9-induced type I IFN protects mice from experimental colitis, J. Clin. Invest. 115 (2005) 695–702.
[55] M. Hausmann, S. Kiessling, S. Mestermann, G. Webb, T. Spottl, T. Andus, J. Scholmerich, H. Herfarth, K. Ray, W. Falk, G. Rogler, Toll-like receptors 2 and 4 are up-regulated during intestinal inflammation, Gastroenterology 122 (2002) 1987–2000.
[56] E. Cario, D.K. Podolsky, Differential alteration in intestinal epithelial cell expression of Toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease, Infect. Immun. 68 (2000) 7010–7017.
[57] S. Schreiber, S. Nikolaus, J. Hampe, Activation of nuclear factor kappa B inflammatory bowel disease, Gut 42 (1998) 477–484.
[58] G. Rogler, K. Brand, D. Vogl, S. Page, R. Hofmeister, T. Andus, R. Knuechel, P.A. Baeuerle, J. Scholrerich, V. Gross, Nuclear Factor kB is activated in macrophages and epithelial cells of inflamed intestinal mucosa, Gastroenterology 115 (1998) 357–369.
[59] L. Andresen, V.L. Jorgensen, A. Perner, A. Hansen, J. Eugen-Olsen, J. Rask-Madsen, Activation of nuclear factor kappaB in colonic mucosa from patients with collagenous and ulcerative colitis, Gut 54 (2005) 503–509.
[60] M. Neurath, S. Pettersson, K.-H. Meyer, Z. Buschenfelde, W. Strober, Local administration of antisense phosphothioate oligonucleotides to the p65 subunit of NF-kB abrogates established experimental colitis in mice, Nat. Med. 2 (1996) 998–1004.

[61] T. Lawrence, D.W. Gilroy, P.R. Colville-Nash, D.A. Willoughby, Possible new role for NF-kappaB in the resolution of inflammation, Nat. Med. 7 (2001) 1291–1297.
[62] L.W. Chen, L. Egan, Z.W. Li, F.R. Greten, M.F. Kagnoff, M. Karin, The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion, Nat. Med. 9 (2003) 575–581.
[63] D. Haller, M.P. Russo, R.B. Sartor, C. Jobin, IKK beta and phosphatidylinositol 3-kinase/Akt participate in non-pathogenic Gram-negative enteric bacteria-induced RelA phosphorylation and NF-kappa B activation in both primary and intestinal epithelial cell lines, J. Biol. Chem. 277 (2002) 38168–38178.
[64] D. Haller, L. Holt, S.C. Kim, R.F. Schwabe, R.B. Sartor, C. Jobin, Transforming growth factor-{beta}1 inhibits non-pathogenic gramnegative bacteria-induced NF-{kappa}B recruitment to the Interleukin-6 gene promoter in intestinal epithelial cells through modulation of histone acetylation, J. Biol. Chem. 278 (2003) 23851–23860.
[65] P.A. Ruiz, S.C. Kim, R.B. Sartor, D. Haller, 15-Deoxy-delta12,14-prostaglandin J2-mediated ERK signaling inhibits gram-negative bacteria-induced RelA phosphorylation and interleukin-6 gene expression in intestinal epithelial cells through modulation of protein phosphatase 2A activity, J. Biol. Chem. 279 (2004) 36103–36111 (Epub 32004 Jun 36115).
[66] M. Lotz, D. Gutle, S. Walther, S. Menard, C. Bogdan, M.W. Hornef, Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells, J. Exp. Med. 203 (2006) 973–984.
[67] P.A. Ruiz, A. Shkoda, S.C. Kim, R.B. Sartor, D. Haller, IL-10 gene-deficient mice lack TGF-beta/Smad signaling and fail to inhibit proinflammatory gene expression in intestinal epithelial cells after the colonization with colitogenic *Enterococcus faecalis*, J. Immunol. 174 (2005) 2990–2999.
[68] R.D. Fusunyan, N.N. Nanthakumar, M.E. Baldeon, W.A. Walker, Evidence for an innate immune response in the immature human intestine: Toll-like receptors on fetal enterocytes, Pediatr. Res. 49 (2001) 589–593.
[69] K. Kobayashi, L.D. Hernandez, J.E. Galan, C.A. Janeway Jr., R. Medzhitov, R.A. Flavell, IRAK-M is a negative regulator of Toll-like receptor signaling, Cell 110 (2002) 191–202.
[70] G. Zhang, S. Ghosh, Negative regulation of Toll-like receptor-mediated signaling by Tollip, J. Biol. Chem. 277 (2002) 7059–7065.
[71] D.L. Boone, E.E. Turer, E.G. Lee, R.C. Ahmad, M.T. Wheeler, C. Tsui, P. Hurley, M. Chien, S. Chai, O. Hitotsumatsu, E. McNally, C. Pickart, A. Ma, The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses, Nat. Immunol. 5 (2004) 1052–1060 (Epub 2004 Aug 1029).
[72] D. Kelly, J.I. Campbell, T.P. King, G. Grant, E.A. Jansson, A.G. Coutts, S. Pettersson, S. Conway, Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA, Nat. Immunol. 5 (2004) 104–112 (Epub 2003 Dec 2021).
[73] F. Powrie, R. Correa-Oliveira, S. Mauze, R.L. Coffman, Regulatory interactions between CD45RBhigh and CD45RBlow CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity, J. Exp. Med. 179 (1994) 589–600.
[74] C. Mottet, H.H. Uhlig, F. Powrie, Cutting edge: cure of colitis by CD4 + CD25+ regulatory T cells, J. Immunol. 170 (2003) 3939–3943.
[75] K.J. Maloy, L. Salaun, R. Cahill, G. Dougan, N.J. Saunders, F. Powrie, CD4 + CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms, J. Exp. Med. 197 (2003) 111–119.
[76] M. Hara, C.I. Kingsley, M. Niimi, S. Read, S.E. Turvey, A.R. Bushell, P.J. Morris, F. Powrie, K.J. Wood, IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo, J. Immunol. 166 (2001) 3789–3796.
[77] I.J. Fuss, M. Boirivant, B. Lacy, W. Strober, The interrelated roles of TGF-beta and IL-10 in the regulation of experimental colitis, J. Immunol. 168 (2002) 900–908.
[78] M.F. Neurath, I. Fuss, B.L. Kelsall, D.H. Presky, W. Waegell, W. Strober, Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance, J. Exp. Med. 183 (1996) 2605–2616.
[79] A.B. Kulkarni, J.M. Ward, L. Yaswen, C.L. Mackall, S.R. Bauer, C.G. Huh, R.E. Gress, S. Karlsson, Transforming growth factor-beta 1 null mice. An animal model for inflammatory disorders, Am. J. Pathol. 146 (1995) 264–275.
[80] A. Kitani, I.J. Fuss, K. Nakamura, O.M. Schwartz, T. Usui, W. Strober, Treatment of experimental (Trinitrobenzene sulfonic acid) colitis by intranasal administration of transforming growth factor (TGF)-beta1 plasmid: TGF-beta1-mediated suppression of T helper cell type 1 response occurs by interleukin (IL)-10 induction and IL-12 receptor beta2 chain downregulation, J. Exp. Med. 192 (2000) 41–52.
[81] K.B. Hahm, Y.H. Im, T.W. Parks, S.H. Park, S. Markowitz, H.Y. Jung, J. Green, S.J. Kim, Loss of transforming growth factor beta signalling in the intestine contributes to tissue injury in inflammatory bowel disease, Gut 49 (2001) 190–198.
[82] Y. Shi, J. Massague, Mechanisms of TGF-beta signaling from cell membrane to the nucleus, Cell 113 (2003) 685–700.
[83] G. Monteleone, G. Del Vecchio Blanco, I. Monteleone, D. Fina, R. Caruso, V. Gioia, S. Ballerini, G. Federici, S. Bernardini, F. Pallone, T.T. MacDonald, Post-transcriptional regulation of Smad7 in the gut of patients with inflammatory bowel disease, Gastroenterology 129 (2005) 1420–1429.
[84] M. Boirivant, F. Pallone, C. Di Giacinto, D. Fina, I. Monteleone, M. Marinaro, R. Caruso, A. Colantoni, G. Palmieri, M. Sanchez, W. Strober, T.T. Macdonald, G. Monteleone, Inhibition of Smad7 with a specific antisense oligonucleotide facilitates TGF-beta1-mediated suppression of colitis, Gastroenterology 6 (2006) 1786–1798.
[85] T.H. Chuang, R.J. Ulevitch, Triad3A, an E3 ubiquitin-protein ligase regulating Toll-like receptors, Nat. Immunol. 5 (2004) 495–502 (Epub 2004 Apr 2025).
[86] D. Haller, Intestinal epithelial cell signalling and host-derived negative regulators under chronic inflammation: to be or not to be activated determines the balance towards commensal bacteria, Neurogastroenterol. Motil. 18 (2006) 184–199.
[87] D. Haller, C. Jobin, Interaction between resident luminal bacteria and the host: can a healthy relationship turn sour? J. Pediatr. Gastroenterol. Nutr. 38 (2004) 123–136.
[88] S. Hanash, Disease proteomics, Nature 422 (2003) 226–232.
[89] A. Gorg, W. Weiss, M.J. Dunn, Current two-dimensional electrophoresis technology for proteomics, Proteomics 4 (2004) 3665–3685.
[90] T.J. Griffin, D.R. Goodlett, R. Aebersold, Advances in proteome analysis by mass spectrometry, Curr. Opin. Biotechnol. 12 (2001) 607–612.
[91] M. Tyers, M. Mann, From genomics to proteomics, Nature 422 (2003) 193–197.

[92] G.L. Corthals, V.C. Wasinger, D.F. Hochstrasser, J.C. Sanchez, The dynamic range of protein expression: a challenge for proteomic research, Electrophoresis 21 (2000) 1104–1115.
[93] P.A. Binz, D.F. Hochstrasser, R.D. Appel, Mass spectrometry-based proteomics: current status and potential use in clinical chemistry, Clin. Chem. Lab. Med. 41 (2003) 1540–1551.
[94] E. Jung, M. Heller, J.C. Sanchez, D.F. Hochstrasser, Proteomics meets cell biology: the establishment of subcellular proteomes, Electrophoresis 21 (2000) 3369–3377.
[95] M.R. Roe, T.J. Griffin, Gel-free mass spectrometry-based high throughput proteomics: tools for studying biological response of proteins and proteomes, Proteomics 6 (2006) 4678–4687.
[96] S. Hanash, The emerging field of protein microarrays, Proteomics 3 (2003) 2075.
[97] M. Mann, P. Hojrup, P. Roepstorff, Use of mass spectrometric molecular weight information to identify proteins in sequence databases, Biol. Mass Spectrom. 22 (1993) 338–345.
[98] R.L. Caldwell, R.M. Caprioli, Tissue profiling by mass spectrometry: a review of methodology and applications, Mol. Cell. Proteomics 4 (2005) 394–401.
[99] R. Aebersold, M. Mann, Mass spectrometry-based proteomics, Nature 422 (2003) 198–207.
[100] H. Zhao, K.B. Adler, C. Bai, F. Tang, X. Wang, Epithelial proteomics in multiple organs and tissues: similarities and variations between cells, organs, and diseases, J. Proteome Res. 5 (2006) 743–755.
[101] S. Barcelo-Batllori, M. Andre, C. Servis, N. Levy, O. Takikawa, P. Michetti, M. Reymond, E. Felley-Bosco, Proteomic analysis of cytokine induced proteins in human intestinal epithelial cells: implications for inflammatory bowel diseases, Proteomics 2 (2002) 551–560.
[102] C. Xu, B. Bailly-Maitre, J.C. Reed, Endoplasmic reticulum stress: cell life and death decisions, J. Clin. Invest. 115 (2005) 2656–2664.
[103] L. Zhao, S.L. Ackerman, Endoplasmic reticulum stress in health and disease, Curr. Opin. Cell Biol. 18 (2006) 444–452.
[104] H.L. Pahl, Signal transduction from the endoplasmic reticulum to the cell nucleus, Physiol. Rev. 79 (1999) 683–701.
[105] J. Wu, R.J. Kaufman, From acute ER stress to physiological roles of the Unfolded Protein Response, Cell Death Differ. 13 (2006) 374–384.
[106] K. Zhang, R.J. Kaufman, The unfolded protein response: a stress signaling pathway critical for health and disease, Neurology 66 (2006) S102–S109.
[107] U.K. Rapp, S.H. Kaufmann, Glucose-regulated stress proteins and antibacterial immunity, Trends Microbiol. 11 (2003) 519–526.
[108] A.S. Lee, The glucose-regulated proteins: stress induction and clinical applications, Trends Biochem. Sci. 26 (2001) 504–510.
[109] P. Hu, Z. Han, A.D. Couvillon, R.J. Kaufman, J.H. Exton, Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression, Mol. Cell Biol. 26 (2006) 3071–3084.
[110] A. Shkoda, P.A. Ruiz, H. Daniel, S.C. Kim, G. Rogler, R.B. Sartor, D. Haller, Interleukin 10 blocked endoplasmic reticulum stress responses in intestinal epithelial cells: implications for chronic inflammation, Gastroenterology 132 (2007) 190–207.
[111] X. Xue, J.H. Piao, A. Nakajima, S. Sakon-Komazawa, Y. Kojima, K. Mori, H. Yagita, K. Okumura, H. Harding, H. Nakano, Tumor necrosis factor alpha (TNFalpha) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNFalpha, J. Biol. Chem. 280 (2005) 33917–33925 (Epub 32005 Aug 33917).
[112] H.L. Pahl, P.A. Baeuerle, A novel signal transduction pathway from the endoplasmic reticulum to the nucleus is mediated by transcription factor NF-kappa B, EMBO J. 14 (1995) 2580–2588.
[113] L.M. Hendershot, J.Y. Wei, J.R. Gaut, B. Lawson, P.J. Freiden, K.G. Murti, In vivo expression of mammalian BiP ATPase mutants causes disruption of the endoplasmic reticulum, Mol. Biol. Cell. 6 (1995) 283–296.
[114] R.V. Rao, A. Peel, A. Logvinova, G. del Rio, E. Hermel, T. Yokota, P.C. Goldsmith, L.M. Ellerby, H.M. Ellerby, D.E. Bredesen, Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78, FEBS Lett. 514 (2002) 122–128.
[115] W.S. Alexander, D.J. Hilton, The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response, Annu. Rev. Immunol. 22 (2004) 503–529.
[116] K.W. Moore, R. de Waal Malefyt, R.L. Coffman, A. O'Garra, Interleukin-10 and the interleukin-10 receptor, Annu. Rev. Immunol. 19 (2001) 683–765.
[117] T.S. Lee, L.Y. Chau, Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice, Nat. Med. 8 (2002) 240–246.
[118] G.A. Ricchetti, L.M. Williams, B.M. Foxwell, Heme oxygenase 1 expression induced by IL-10 requires STAT-3 and phosphoinositol-3 kinase and is inhibited by lipopolysaccharide, J. Leukoc. Biol. 7 (2004) 7.
[119] T. Werner, A. Shkoda, D. Haller, Intestinal epithelial cell proteome in IL-10 deficient mice and IL-10 receptor reconstituted epithelial cells: impact on chronic inflammation, J. Proteome Res. (2007), in press.
[120] T. Clavel, D. Haller, Bacteria-and host-derived mechanisms to control intestinal epithelial cell homeostasis: implications for chronic inflammation, Inflamm. Bowel Dis. (2007) [Epub ahead of print].
[121] A. Shkoda, T. Werner, H. Daniel, M. Gunckel, G. Rogler, D. Haller, Differential protein expression profile in the intestinal epithelium of patients with inflammatory bowel disease, J. Proteome Res. 6 (2007) 1114–1125.
[122] A.C. Polley, F. Mulholland, C. Pin, E.A. Williams, D.M. Bradburn, S.J. Mills, J.C. Mathers, I.T. Johnson, Proteomic analysis reveals field-wide changes in protein expression in the morphologically normal mucosa of patients with colorectal neoplasia, Cancer Res. 66 (2006) 6553–6562.
[123] A.J. Schottelius, H. Dinter, Cytokines, NF-kappaB, microenvironment, intestinal inflammation and cancer, Cancer Treat. Res. 130 (2006) 67–87.
[124] M. Karin, F.R. Greten, NF-kappaB: linking inflammation and immunity to cancer development and progression, Nat. Rev. Immunol. 5 (2005) 749–759.