Intestinal barrier function: regulation of epithelial permeability and mucin expression
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Summarizing discussion
1 The intestinal epithelial barrier function

1.1 In vitro models to study intestinal barrier function

Adequate intestinal epithelial barrier function is crucial for the maintenance of physiological function and for the prevention of uncontrolled immune activation in the gut. The viscous mucus layer covering the intestinal epithelium not only provides a physical-chemical barrier preventing exposure of the mucosal cells to dietary and microbial antigens but also protects against mechanical and chemical damage. Tight junction complexes positioned between epithelial cells control the paracellular route of permeability for potentially harmful agents. The epithelial cell lines T84, Caco-2 and HT29/B6 cells are often used to study the role for the tight junction complex and its individual proteins in regulation of intestinal barrier integrity. These cell lines are suitable since they are capable of forming differentiated monolayers containing functional tight junction strands. To determine tight junction barrier integrity, transepithelial resistance (TER) and permeability for macromolecules were measured (chapter 2, 5 and 6). The transepithelial resistance is determined with an epithelial voltohmeter which measures the current pulse induced voltage gradient between the apical and basolateral compartment of transwell cultures. This resistance is an almost complete reflection (95%) of the paracellular tight junction permeability. Moreover, high resistance correlates well with the development of tight junctional complexes and epithelial barrier integrity. Functional permeability for macromolecules was explored by adding marker molecules FITC-dextran (4kDa) or HRP (40 kDa) to the apical compartment followed by recovery of the molecules form the basolateral compartment (flux). FITC-dextran exclusively passes via the paracellular route, while HRP may also be transported via the transcellular pathway. We chose to perform our epithelial barrier studies with T84 cells because these are often used to study epithelial barrier function and immune mediated barrier disruption, and develop higher transepithelial resistance in comparison with Caco-2 or HT29/B6 cells. T84, Caco-2 and HT29 (absorptive cell type), and LS174T and HT29-MTX (goblet cell type) human intestinal epithelial cell lines are commonly used to study in vitro mucin synthesis and secretion. Of these, T84, HT29 and LS174T are known to synthesize human colon type mucin (MUC-2), the gel-forming secreted mucin that is found in the intestine. HT29-MTX less actively produces MUC-2 whereas synthesis is absent in Caco-2 cells. We selected the T84 cell line to study mucin expression since these cells could also be used for evaluation of barrier integrity. Besides T84 cells, the MUC-2 assays were also performed with LS174T cells since these have a goblet cell phenotype. MUC-2 molecules are of extremely high molecular weight, making them particularly difficult to handle in conventional western blotting experiments. Therefore cellular MUC-2 expression was evaluated using the dot blot method (chapter 3).
In vivo, the epithelial cell function is closely regulated by subepithelial myofibroblasts which are located directly underneath the epithelial basal membrane. Myofibroblasts, together with the epithelial cells and mucosal immune cells form a well integrated network and produce various mediators, e.g. cytokines, growth factors and matrix molecules which affect epithelial and immune functions. Unfortunately, in vitro studies of intestinal barrier function only use epithelial cells and do not take into account the important regulatory functions of other mucosal cell types such as the mesenchymal cell types. Hence, instead of using only epithelial cells, we studied epithelial mucin expression and barrier function in an in vitro coculture model integrating epithelial cells, myofibroblasts, and lamina propria mononuclear cells (chapter 2, and 3, figure 1).

1.2 Introduction of an in vitro coculture model to study barrier function

A coculture model was set up in which myofibroblasts and epithelial cells were grown in direct contact resembling their in situ spatial organization (chapter 2, and 3). Subepithelial myofibroblasts are important regulators of epithelial cell function. These cells play a key role in the organization of epithelial proliferation, restitution, secretion and differentiation. Previous studies have used a similar setting to focus more on the regulation of physiologic functions of epithelial cells by myofibroblasts. However, different from our model, these studies did not address the potential regulatory role of myofibroblasts in immune mediated barrier disruption and epithelial mucin expression. The coculture model is described in chapter 2. Human intestinal epithelial cells (T84) were cultured on top of a confluent layer of myofibroblasts (CCD-18Co, primary cells derived from the human colon). The latter were grown on transwell inserts. After reaching confluency of the epithelial toplayer the cocultures were examined histologically. This revealed T84 cells to form a monolayer of well developed polarized cells on a layer of CCD-18Co, resembling
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the in situ spatial organization of these cell types in the intestinal mucosa. CCD-18Co cells underneath the epithelium were functionally active since they produced TGF-β1 and KGF. The model was also validated to perform barrier studies since transepithelial resistance was found to be high and basal HRP fluxes low. Under basal conditions there were no differences in development of tight junction barrier integrity (transepithelial resistance) as compared to T84 monocultures (chapter 2). Subepithelial myofibroblasts are known to produce mediators that contribute to epithelial differentiation and barrier integrity. Since we did not observe differences in barrier formation between mono- and cocultures, the growth factors that were already present in the culture medium may have overruled the contribution of the myofibroblasts to the development of functional barrier properties. In contrast, under basal, non stimulated conditions it became apparent that MUC-2 expression was dramatically enhanced in cocultures as compared to monolayers of T84 cells alone (chapter 3). This further supported the regulatory role of the subepithelial myofibroblasts. Indeed these cells may have enhanced epithelial differentiation and regulated mucin expression. Myofibroblast derived growth factors, like KGF which is known to enhance the goblet cell number and hereby stimulating mucin production, may have contributed to this effect. Despite T84 cells being described as the absorptive type, these cells were found to have intracellular mucin granules and to contain a subpopulation of cells with goblet cell like features. Recently, it was found that also in vivo columnar cells secrete mucins. Myofibroblasts therefore may have an important role in the regulation of mucin synthesis and secretion by intestinal epithelial cells in general. Different from T84 cells, MUC-2 expression was not enhanced when goblet cell type LS174T cells were cultured on top of the CCD-18Co. The differences in regulation of mucin expression in LS174T or T84 cells by myofibroblasts therefore deserves further exploration. These differences may involve the production of regulatory factors by myofibroblasts as well as specific responsiveness of different epithelial cell lines and differences in growth factor receptor expression (e.g. KGFR) on epithelial cells. To study immune mediated barrier disruption we introduced intestinal mucosal immune cells in the coculture model (chapter 2, figure 1). Lamina propria mononuclear cells (LPMC) that were stimulated with the mitogen PMA or αCD2/αCD28 were also introduced in the coculture system. The latter stimulation route represents T-cell receptor activation through mimicking antigenic cross linking; it is known that intestinal mucosal T-lymphocytes are preferentially stimulated through CD2 rather than CD3. Both stimulation routes enhanced the LPMC derived secretion of pro-inflammatory cytokines like IFN-γ and TNF-α, that are known to affect epithelial barrier integrity. Presence of the myofibroblasts did not alter IFN-γ, TNF-α and IL-10 secretion by LPMC. For further study on anti-inflammatory properties of myofibroblast derived mediators, it may be more adequate to incubate stimulated LPMC directly upon a layer of CCD-18Co cells, bringing the cell types in intimate contact or to use concentrated conditioned CCD-18Co culture supernatants. This might provide useful information since myofibroblasts may undergo direct stimulation by dietary or
bacterial antigens and may also be exposed to nutritional components or products of bacterial fermentation (chapter 3). This then may give rise to the local production of important immunoregulatory substances, e.g. PG, TGF-β and IL-10 29,30,40,41.

1.3 Myofibroblast derived factors regulating mucosal barrier function and epithelial mucin expression

Subepithelial myofibroblasts secrete growth factors, prostaglandins and matrix molecules all known to contribute to epithelial barrier integrity. Typically, αCD2/αCD28-stimulated LPMC only marginally affected barrier integrity in the coculture, while barrier integrity of the monolayer was dramatically reduced. This finding points to an important regulatory role for the myofibroblasts in immune mediated barrier disruption (chapter 2). Indeed, in culture supernatants of the coculture model, the TGF-β1 concentrations were increased in comparison with monocultures of epithelial cells alone. TGF-β1 induces epithelial organization, and differentiation (microvilli, alkaline phosphatase) and was found to enhance or maintain epithelial barrier function during exposure to inflammatory mediators 8,9,15,26,32. We initially postulated that myofibroblast derived TGF-β1 could have contributed to barrier preservation during LPMC co-incubations. However, cocultures incubated with PMA- or αCD2/αCD28-stimulated LPMC did not differ in TGF-β1 concentration, while only the αCD2/αCD28 route of stimulation resulted in barrier protection. Typically during these stimulations myofibroblast derived KGF concentrations were increased. It has been shown that KGF enhances epithelial woundhealing (restitution, proliferation and differentiation) and reduces ulceration in animal models of IBD 36,42-44. Moreover, KGF suppresses transcription of IFN-α and -γ genes by preventing STAT1 trafficking into the nucleus, in human airway epithelium cells45. Hence, KGF may downregulate mucosal Th1-type inflammatory reactions and therefore is an interesting therapeutic candidate especially since KGF, unlike other growth factors such as EGF, IGF and HGF, KGF, does not enhance proliferation of colon cancer cells 26,46,47. Furthermore, different from TGF-β1, there are no reports implicating KGF to have detrimental effects on collagen deposition and pathogenic fibrosis despite the known effects of KGF-2 to enhance collagen expression in myofibroblasts 43,46,48-50. Recently, repifermin (truncated KGF-2) was found not to be effective in UC patients, however dosages were low and only given for a short period of time 51. The question remains whether additional KGF in IBD patients may further support mucosal protection since mucosal KGF expression is already high in IBD patients 52-54.

In chapter 2 we observed a role for myofibroblasts in the regulation of epithelial barrier integrity. Previous investigations have demonstrated myofibroblasts to regulate epithelial Cl- secretion via production of prostaglandins (PG) 30,32,33,41. In a coculture setting COX derived products were not found to be essential for the regulation of the tight junction barrier,
however in a porcine ischemia model, COX-1 or COX-2 derived PG were found to enhance barrier recovery \(32,55,56\). Also in vitro barrier disruption by mucosal T-cells and IFN-\(\gamma\), and NSAID induced colitis in DSS rats could be prevented or ameliorated by administration of PGE\(_2\) \(57,60\). Besides their role in epithelial Cl\(^-\) responses and possibly in regulation of barrier integrity, prostaglandins can regulate epithelial mucus secretion \(20,39,61,62\). During basal as well as pro-inflammatory conditions epithelial cells have low COX enzyme expression and prostaglandin secretion when compared with the subepithelial myofibroblasts \(32-34,40,41,63,64\). Mucosal PG secretion is enhanced by local production of bacterial fermentation products in the intestinal lumen (SCFA), which are also known to stimulate intestinal mucin secretion \(65-68\). Both PGE\(_1\) and PGE\(_2\) (converted from specific substrates by COX enzymes) stimulate mucin exocytosis via the EP4 receptor which resulted in similar amounts of mucin secretion in rat colon loops \(62,69\). The EP4 receptor was found to be essential in mucoprotection since EP4-deficient mice developed severe colitis with low dose DSS treatment \(60\). Furthermore, PGE\(_1\) analogue misoprostol is particularly effective in preventing NSAID induced gastric and duodenal lesions in arthritis patients \(70-72\). We compared the efficacy of these PGs in our in vitro culture models and found PGE\(_1\) to enhance epithelial MUC-2 expression more efficiently than PGE\(_2\). Subepithelial myofibroblasts may play an important role in regulation of epithelial mucin expression. To further explore this we determined the effects of SCFA on myofibroblast derived PG secretion and mucin expression in the coculture of epithelial cells and myofibroblasts (chapter 2). Typically the SCFA acetate, propionate, and butyrate, enhanced the PGE\(_1\)/PGE\(_2\) ratio secreted by the myofibroblasts, due to enhancing PGE\(_1\) and reducing PGE\(_2\) secretion. SCFA enhanced MUC-2 expression when incubated with epithelial cells alone, but the mucus stimulating effect was dramatically pronounced in the coculture model where myofibroblasts were present. These effects were also seen when epithelial cells were incubated with supernatants of myofibroblasts stimulated with SCFA. We found these effects to be mediated by prostaglandin synthesis (by both epithelial cells and myofibroblasts), since indomethacin treatment could totally abrogate the SCFA effect on MUC-2 expression. Hence, myofibroblasts were found to increase epithelial mucin expression during culture and further enhanced expression after SCFA stimulation.

Similar to SCFA it was found that KGF enhances PGE\(_1\) secretion by subepithelial myofibroblasts (chapter 4). Where IL-1\(\beta\) enhances both PGE\(_1\) and PGE\(_2\) secretion derived from pro-inflammatory COX-2, KGF was found in particular to enhance COX-1 expression. In addition, experiments with specific COX-inhibitors revealed KGF derived PGE\(_1\) secretion to predominantly depend on COX-1 activity. The general viewpoint regarding the role of COX enzymes in intestinal immunoregulation is that constitutive COX-1 expression is involved in mucosal protection whereas COX-2 induction and PGE\(_2\) secretion is associated with inflammation, hyperalgesia and fever \(73\). For this purpose selective COX-2 inhibitors were developed in order to block inflammation while preserving mucoprotection \(74,75\).
Although this concept has generated selective inhibitors which indeed have anti-inflammatory properties, recent studies have shown that both COX-1 and COX-2 derived PG can be mucoprotective during NSAID or ischaemic induced intestinal damage. Our data showed PGE₁ to enhance mucin expression more potently than PGE₂, and SCFA and KGF stimulate a mucoprotective PG profile. Polyunsaturated fatty acids, arachidonic acid (AA) and dihomo-γ-linolenic acid (DGLA), are COX substrates for respectively PGE₂ (e.o) and PGE₁ and can be liberated from the phospholipid pool in the cell membrane by PLA₂ (phospholipase A₂). Dihomo-γ-linolenic acid (DGLA) is derived from essential fatty acid linolenic acid which has to be obtained by nutritional intake. DGLA can also be converted to arachidonic acid by Δ5-desaturase. In our hands, SCFA and KGF differentially regulated PG secretion by subepithelial myofibroblasts under comparable experimental conditions (culture medium) while substrate availability was not limited since PGE₁ and PGE₂ levels could be easily enhanced after IL-1β stimulation (via COX-2). SCFA and KGF may therefore interfere with cellular processes that regulate COX enzyme substrate selectivity or deliberation of substrates from the phospholipid pool. Selective stimulation of mucosal PGE₁ release may be an interesting target for nutritional intervention to support barrier maintenance. Nutritional supplementation of PGE₁ substrate DGLA might be considered, however DGLA can easily be converted to AA, the substrate for PGE₂. Development of nutritional strategies targeting these mucoprotective pathways, might have important beneficial effects on barrier integrity and thus for the treatment or prevention of inflammatory and allergic disease.

2 Immune mediated barrier disruption

2.1 Pro-inflammatory mediators affecting tight junction structures

Intestinal barrier integrity is a dynamic process involving regulation of tight junction protein expression. For example, tight junctions open up to enhance nutrient uptake since activation of sodium-glucose transporter SGLT-1 induces contraction of the peri-junctional actomyosin ring. Furthermore dendritic cells and neutrophils use tight junction proteins to migrate into the intestinal lumen in order to sample or eradicate potentially harmful bacteria or antigens. However, increased intestinal permeability in IBD is associated with disruption of tight junction structures. Pro-inflammatory cytokines, e.g. TNF-α and IFN-γ, reduce epithelial barrier integrity and mucosal levels of these cytokines are found to be increased during chronic inflammation. In chapter 2, activated LPMC and recombinant cytokines induced barrier disruption in monocultures and less dramatically in cocultures. To simplify our working model we determined the effects of pro-inflammatory cytokine IFN-γ on epithelial tight junction structures in the absence of myofibroblasts (chapter 5). We chose to
study IFN-\(\gamma\) mediated barrier disruption since it is far more potent than TNF-\(\alpha\), at least in T84 cells\(^1\). The mechanisms and signaling pathways leading to IFN-\(\gamma\) mediated barrier disruption are largely unknown, and targets to prevent this process are yet to be identified\(^{1,10-14}\). We therefore studied the effect of IFN-\(\gamma\) on the expression of the newly discovered claudin family of transmembrane tight junction proteins and occludin (chapter 5). Previously, occludin, claudin-1 and claudin-4 were found to be functionally involved in maintenance of barrier integrity\(^{43,90-92}\), while claudin-2 has been associated with leaky tight junctions since claudin-2 transfection reduced transepithelial resistance of MDCK cells\(^93\). In contrast, our studies implicate claudin-2 to play a functional role in barrier maintenance. IFN-\(\gamma\) mediated barrier disruption was associated with reduced protein expression and enhanced fragmentation of claudin-2. This effect was specific because no such effect of IFN-\(\gamma\) on claudin-1,-3, and -4 protein expression was observed. We identified IFN-\(\gamma\) to affect barrier disruption via two separate mechanisms. IFN-\(\gamma\) mediated reduction of occludin expression involved de novo protein synthesis whereas inhibition of de novo protein synthesis could not prevent claudin-2 fragmentation. Preservation of occludin expression abrogated barrier disruption. On the other hand serine protease inhibition prevented IFN-\(\gamma\) mediated barrier disruption and was associated with preservation of claudin-2 expression while occludin expression remained reduced. Typically, also basal claudin-2 expression was enhanced by serine protease inhibition. This suggests that serine proteases regulate claudin-2 turnover under normal conditions and that IFN-\(\gamma\) increases serine protease activity, enhancing claudin-2 degradation. Identification of the protease involved in cleavage of the claudin-2 in our view may provide a new target for therapeutic intervention. Matrix metalloproteinases comprise another group of proteases which were not addressed in this thesis but also are of interest since they are activated during inflammation and known to degrade occludin\(^94\). IFN-\(\gamma\) has also been reported to diminish expression of ZO-1 by increasing protein turnover, furthermore IFN-\(\gamma\) decreased ZO-1 RNA synthesis and occludin promotor activity, and perturbed tight junctional actin organization\(^7,14\). How these effects relate to our observations deserves further exploration. However, in our experiments IFN-\(\gamma\) induced barrier disruption was much less severe compared with the studies in which diminished ZO-1 expression and actin disorganization were found and the effects on occludin promotor activity were obtained in a transfection model. Apart from affecting tight junction protein expression, IFN-\(\gamma\) also phosphorylates MLC\(^95,96\). Although MLC is a physiological regulator of tight junction permeability, MLC phosphorylation may decrease transepithelial resistance over 90% by contraction of the peri-junctional actomyosin ring\(^81,97\). Similar to IFN-\(\gamma\) other barrier disruptive agents are capable of enhancing MLC phosphorylation\(^98,99\). Thus, together with prevention of tight junction disruption, inhibition of MLC phosphorylation may provide an interesting target for intervention. Rho controls paracellular permeability via downstream effectors ROCK-MLCK-MLC. This can take place independent of disruption or altered
distribution of tight junction proteins, but also Rho dependent tight junction protein redistribution has been shown $^{97,100-105}$. Recently, Rho activation was found to be increased in the intestinal mucosa of patients with active Crohn's disease and rats with TNBS colitis. Inhibition of Rho in TNBS rats reduced colonic inflammation $^{106}$. Authors linked the effect to in vitro inhibition of NFκB activation and consequently reduced TNF-α production by LPMC, however inhibition of Rho activation may have led to reduced intestinal permeability. Hence, enhanced barrier permeability in IBD has been shown to involve disruption of tight junction structures and may relate to activation of Rho GTP-ase. It remains to be determined whether enhanced MLC phosphorylation is a causative factor in the development of Crohn's disease by increasing intestinal permeability and could therefore be a mechanistic target for therapeutic intervention.

### 2.2 IFN-γ signaling pathways during epithelial barrier disruption

In *chapter 5*, we describe IFN-γ to specifically target tight junction proteins via distinct mechanisms, thereby increasing epithelial permeability. Figure 2 represents current status of exploration of the mechanisms and signaling pathways that regulate barrier maintenance and IFN-γ mediated barrier disruption, including observations from our own lab. In *chapter 6*, the signaling pathways that are involved in regulation of barrier maintenance and IFN-γ mediated barrier defects were explored. The most startling observation, studying the effects of signal transduction inhibitors, was that JNK MAPK inhibition resulted in profound increase in basal resistance and ameliorated IFN-γ induced permeability; p38 and ERK MAPK were less or non effective respectively. JNK is known to be activated in the mucosa of IBD patients and mucosal healing in Crohn's patients treated with a JNK/p38 inhibitor improved $^{107,108}$. These effects involved reduced activation of mucosal immune cells but could also have caused decreased intestinal permeability, thereby reducing mucosal antigenic exposure. Studies exploring JNK activation in the mucosal epithelium of IBD patients may provide further insight in the feasibility of the use of JNK inhibitors for diseases involving intestinal barrier disfunction. Indeed, JNK activation was found to enhance vascular permeability this process was mediated via redistribution of tight junction proteins $^{109}$. In our *in vitro* model serum derived growth factors may have stimulated JNK activation under basal culture conditions, explaining the enhancement of basal resistance by JNK inhibition $^{110}$. Since JNK inhibition enhances barrier integrity without affecting claudin-2 and occludin expression (data not shown), and JNK is under regulation of Rho GTP-ase, we speculated that JNK inhibition may reduce basal and IFN-γ induced MLC phosphorylation, resulting in relaxation of the perijunctional actomyosin ring thus tightening the junctional complex. Alternatively, JNK inhibition may have resorted in recovery of altered tight junction protein redistribution (like in the vascular endothelium). In a porcine ischaemic-reperfusion model, JNK inhibition also enhanced epithelial barrier integrity which was associated with induction of COX-2 in
Figure 2 IFN-γ mediated barrier disruption correlated with reduced claudin-2 and occludin expression. Serine proteases were involved in claudin-2 turnover under basal conditions and IFN-γ enhances the protease activity resulting in increased cleavage and diminished claudin-2 expression. Serine proteases did not reduce occludin expression, de novo protein synthesis is required for IFN-γ to affect occludin. Recent studies have provided evidence for a role of MLC in the regulation of barrier maintenance, physiological opening of tight junctions and IFN-γ mediated barrier disruption. PKC and small protein GTP-ases (e.g. Rho) are known to regulate MLC phosphorylation. We observed JNK MAPK inhibition to enhance basal TER, revealing a role for JNK in barrier maintenance, whereas PI3K, PKC and p38/ERK MAPK were not effective or less involved. In contrast, PI3K was identified to regulate IFN-γ mediated barrier disruption. Of the other pathways ERK MAPK was not effective and whether p38/JNK MAPK and PKC interfered with IFN-γ signaling remains unclear since these inhibitors also affected basal TER. STAT1 was activated after IFN-γ exposure but because STAT1 inhibitors are unavailable up to date the role for STAT1 in IFN-γ mediated barrier disruption remains to be elucidated.
the epithelium. Hence, another explanation might be that COX-2 derived PG contributed to enhanced barrier integrity.

The PI3K signaling pathway is known to be involved in immune mediated mediated barrier disruption. Interestingly, natural occurring food flavonols exert their effects via inhibition of several signal transduction cascades and may be potential therapeutic candidates to ameliorate intestinal barrier disruption. PI3K indeed partially inhibited IFN-γ mediated barrier disruption and similar results were obtained using quercetin which is known to have PI3K inhibitory capacities. Structure equivalents of the flavonol quercetin were found to ameliorate macroscopic damage and inflammation in TNBS colitis. However, these effects were marginal. Although quercetin was only partially effective it still is an interesting candidate for nutritional intervention when used in combination with compounds that inhibit JNK and MLC activation. Typically PKC inhibition aggravated IFN-γ mediated barrier disruption. Activation of PKC was shown to decrease MLC phosphorylation. Thus, inhibition of PKC in synergism with IFN-γ exposure may have induced MLC phosphorylation. This proposed mechanism remains speculative so far because PKC is also known to regulate redistribution of tight junction proteins. None of the inhibitors of PI3K, MAPK and PKC used in this study could totally abrogate IFN-γ signaling pathways resulting in barrier disruption. The intriguing question remains whether total blockade of STAT1 activation by IFN-γ can prevent barrier disruption. This can only be solved when specific pharmacological STAT1 inhibitors become available. McKay et al. did use STAT1 transcription factor decoys, however these were found to be only partially effective in transcription blockade.

**Conclusions**

Application of the coculture model to study immune mediated barrier disruption and epithelial mucin expression revealed that myofibroblasts protect barrier integrity under pro-inflammatory conditions and contribute to epithelial mucin expression. These effects involve myofibroblast derived soluble factors including growth factors and prostaglandins. Myofibroblasts provide an interesting target for nutritional intervention since they support epithelial function and should not be neglected when studying intestinal barrier integrity and epithelial mucin expression. Myofibroblasts enhanced epithelial mucin expression via the secretion of prostaglandins. We observed PGE1 to be more effective in enhancing epithelial mucin expression than PGE2. Growth factors (KGF) and bacterial fermentation products were found to selectively enhance myofibroblast derived PGE1 secretion, hereby supporting a mucoprotective PG profile. In general, nutrients that stimulate bacterial fermentation, or enhance mucosal KGF or PGE1 secretion may provide optimal conditions for intestinal barrier maintenance, in particular regarding mucin expression.
Our studies on IFN-γ mediated barrier disruption have shown that besides decreased occludin expression, claudin-2 expression is reduced most probably due to enhanced protease activity resulting in cleavage of claudin-2. Both selective preservation of claudin-2 (by serine protease inhibition) or occludin (by inhibition of de novo protein synthesis) abrogated IFN-γ mediated barrier disruption. Hence, functional involvement of claudin-2 and occludin in barrier maintenance was revealed. Identification of the protease enhancing claudin-2 turnover under pro-inflammatory conditions may provide an essential target for intervention since serine protease inhibition could totally overcome IFN-γ mediated barrier disruption. Another finding of particular scientific interest was that JNK MAPK inhibition resulted in profound increase in basal barrier resistance and reduced IFN-γ mediated permeability, implying that JNK is involved in homeostatic regulation of epithelial barrier function. Whether JNK activation is enhanced in the epithelium of patients with disturbed barrier function and by what mechanism JNK exerts its effect remains topic for further investigation. We therefore would like to encourage the exploration of signaling pathways regulating epithelial barrier function since this may result in the identification of new targets for nutritional intervention in diseases involving increased intestinal permeability.

References

Chapter 7


