Intestinal barrier function: regulation of epithelial permeability and mucin expression
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General introduction
1 The gastrointestinal tract

1.1 Gastrointestinal barrier function

The intestinal tract is essential in the regulation of oral tolerance since this is where functional uptake and processing of nutrients takes place and where leakage of potential antigenic proteins needs to be limited. In many pathological conditions the barrier function of the gut is affected. Patients with food allergy, food hypersensitivity (gluten, cow milk) or inflammatory bowel disease have unbalanced mucosal immune reactivity which results in tissue damage, villous atrophy and intestinal permeability. Intestinal permeability is a potential pathological event in systemic diseases like atopic dermatitis, sepsis, pancreatitis, liver disease and rheumatic disorders. In this thesis we focused on mediators improving barrier integrity of the gut and mechanisms of immune mediated barrier disruption as seen in inflammatory bowel disease.

1.2 Anatomy of the gastrointestinal tract

After passage of the esophagus and the stomach partly digested food enters the small intestine, which can be divided in the duodenum, jejunum, and the ileum respectively. The ileum connects to the cecum of the large intestine which is further divided in the ascending, transverse, descending and sigmoid colon. After passage of the colon the faeces finally reaches the rectum (figure 1). The intestinal wall is composed of 4 layers: mucosa, submucosa, muscularis propria, and serosa. The mucosa is composed of a monolayer of epithelial cells that covers the lamina propria immune cells imbedded in loose connective tissue and further down the muscularis mucosa (thin layer of smooth muscle) (figure 2). Lamina propria immune cells and (mesenteric) lymph nodes together with the Peyer's patches that are located directly underneath epithelial M-cells form the GALT (gut associated lymphoid tissue). The submucosa contains dense connective tissue with autonomic nerves and small blood vessels. In between the muscularis propria, that regulates peristalsis and the serosa, which contains large blood vessels, the Auerbach nerve plexus is situated. The mucosa contains circular folds with villi and crypts (of Lieberkühn) that greatly augment the absorptive surface area. The epithelial cells of the small intestine have microvilli at the luminal surface,
composing the brushborder. The colonic mucosa has a similar organization to that of the small intestine however only contains crypts.

**Figure 2** The intestinal mucosa contains the immunological compartment of the intestine, the GALT. Which controls oral tolerance induction and immunosurveillance. The submucosa contains blood vessels and nerves and the muscularis propria regulates intestinal motility.

### 1.3 Inflammatory Bowel Disease

There are two idiopathic inflammatory bowel diseases (IBD): Ulcerative colitis (UC) and Crohn's disease (CD). UC is characterized by superficial inflammation located in the colonic (sub)mucosa. CD however may involve the entire gastrointestinal tract and the inflammation is transmural also affecting the serosa and mesenterial fat. IBD patients have abdominal pain (CD in particular) and diarrhea (CD and UC) which sometimes contains blood (UC), however constipation is not uncommon. Systemic symptoms like fever, weight loss and fatigue are usually seen when a larger part of the intestine is involved and more common in CD. In CD, loss of appetite or pain after eating causes diminished oral intake or unbalanced eating, resulting in malnutrition. Furthermore damage to the intestinal wall decreases the absorptive area. Arthritis is the most common extra-intestinal manifestation of IBD and effective therapy of IBD results in improvement of the arthritis. UC patients have an enhanced risk of developing colon cancer. In families with increased incidence of Crohn's disease also the risk for ulcerative colitis is enlarged and vice versa. Fifteen percent of IBD patients have first-degree relatives that develop IBD and monozygotic twins have high rates of concordance, furthermore supporting a role for genetic predisposition. Specific haplotypes of HLA II and MHC genes are associated with IBD, a locus known as IBD2 on chromosome 12q enhances the risk to develop UC and loci on chromosome 16q (IBD1, that encloses the NOD2/CARD15 gene), 1p, 5q, and 14q are risk factors for CD. Although the exact etiology of IBD is unknown, environmental factors like dietary antigens and resident bacterial flora are thought to drive the increased immune responsiveness in a genetically susceptible person. This is supported by the fact that mice with gene disruptions for IL-2,
IL-10, TGF-β₁, or the T-cell receptor all develop colitis exclusively in the presence of bacterial flora. Intestinal permeability precedes relapses of chronic inflammation suggesting that increased exposure to luminal components may also contribute to the pathogenesis of IBD.

1.4 Intestinal mucosa: interplay between several cell types

The epithelial cells, subepithelial myofibroblasts, GALT, intraepithelial lymphocytes (subset of cytotoxic T cells that reside within the epithelium) and the intestinal nervous system form a well integrated network sustaining immunologic homeostasis, barrier integrity and mucoprotection (figure 3). Besides the lamina propria immune cells, epithelial cells and subepithelial myofibroblasts are potent players in regulating immune processes since they also produce inflammatory cytokines (e.g. TNF-α, IL-1β, IL-6), chemokines (e.g. IL-8, IP-10, GRO, MIP, MCP, RANTES), immunoregulatory mediators (e.g. IL-10, IL-7, PG) and growth factors (e.g. KGF, TGF-β₁).

**Figure 3** The intestinal mucosa is composed of several well integrated cell types. Under physiological conditions mucosal exposure to antigenic, microbial or toxic components is limited and mucosal immune reactivity is carefully regulated, creating conditions for optimal developed intestinal barrier integrity. In IBD uncontrolled T-cell activation results in chronic inflammation causing tissue damage, barrier disruption and ulceration. On the other hand primary barrier defects, resulting in enhanced permeability may also trigger mucosal inflammation.

1.4.1 The epithelium

The epithelium is a monolayer of cells which are continuously renewed (3-6 days). By separating the exterior compartment (gut lumen) from the interior (lamina propria and bloodstream) it not only functions as barrier but also is essential for the uptake of nutrients and water. Epithelial stem cells are located in the crypts. Along the crypt-villus axis differentiation of the absorptive columnar cells (small intestinal enterocytes or large intestine colonocytes), mucus secreting goblet cells and a sub-set of entero-endocrine cells occurs.
while in opposite direction (towards the crypt base) differentiation of other entero-endocrine (secreting substance P, serotonin, secretin) and Paneth cells (secreting bacteriocidal proteins like lysozyme and defensins) takes place. The differentiated enterocytes are specialized to digest and absorb nutrients (lipids, sugars, amino acids) and water. For this purpose the villi have brushborders (apical microvilli) expressing enzymes (such as disaccharidases, peptidases, alkaline phosphatases), transporter proteins (apolipoprotein, fatty acid binding protein), receptors and membrane transporters (glucose and amino acid transporter). The basolateral membrane of the epithelial cells of both the small intestine and the colon express ion transporters (Na⁺/K⁺-exchanger, Na⁺/K⁺-ATPase) which regulate in water and electrolyte transport. The colonocytes, have small microvilli to absorb water and electrolytes (Na⁺/Cl⁻) and are unable to absorb glucose and amino acids. Besides the absorptive function the epithelium forms an essential paracellular barrier against molecules bigger than 11 Å to avoid unspecific leakage of antigenic molecules into the mucosa. Tight junctions regulate the paracellular barrier integrity. However selective transcellular antigen uptake and processing is a specific function of M-cells, and recently enterocytes are also thought to present antigen/MHC complexes basolaterally. To protect their surface the epithelial cells are covered with a glycocalix (glycoproteins) which is part of the cell membrane and further with a gel-forming layer of viscous mucus, both are continuously renewed. Goblet cells are specialized mucus producing cells but columnar epithelial cells contain mucus granules in their apical compartment as well. Defense mechanisms that protect against invading pathogens include secretion of chloride and fluid, sIgA and anti-microbial peptides by enterocytes and colonocytes. The mucus layer contains the secreted mediators and prevents microbial adherence contributing to the elimination potentially harmful microbes. Intestinal epithelial cells constitutively express cyclooxygenase(COX)-1 and COX-2 in case of mucosal inflammation. Both isoforms are involved in processes of cytoprotection and mucosal healing, but COX-2 induction is incriminated to enhance inflammatory processes and induce pain sensation. COX enzymes convert poly-unsaturated fatty-acids to prostaglandins (PG) and thromboxanes. PG in particular have been studied for their role in mucoprotection and mucosal inflammation. Besides epithelial cells subepithelial myofibroblasts prominently contribute to mucosal prostaglandin production.

1.4.2 Subepithelial myofibroblasts

Epithelial cells rest on a basement membrane with directly underneath a thin layer of specialized subepithelial myofibroblasts that control epithelial differentiation and function. The myofibroblasts are known to induce basement membrane formation, regulate crypt-villus maturation, epithelial proliferation and restitution/differentiation by cell-cell contact and the production of extracellular matrix molecules (fibronectin, laminin and collagen IV) and growth factors like KGF and TGF-β. In case of injury, myofibroblasts can decrease the villus surface by contraction. Primary myofibroblasts
(α-smooth muscle actin and vimentin positive) isolated from the colon constitutively express COX-1 and are secreting mucoprotective PG. In inflammatory conditions, PG stimulate epithelial chloride and mucus secretion. Myofibroblasts have cytokine, acetylcholine, and toll like receptors and can be activated by pro-inflammatory mediators, the gastrointestinal nervous system, and bacterial components.

1.4.3 Lamina propria immune cells
Embedded in the connective tissue of the lamina propria reside lymphocytes (T-helper, T-suppressor/cytotoxic, B/ plasma cells), mononuclear cells (monocytes), phagocytes (dendritic cells, macrophages), eosinophils, mast cells and NK cells. Lymphoid follicles (B- and T-cells) are present in the mucosa and submucosa. Microbes or antigens that enter the mucosa are taken up by naïve dendritic cells (DC) which get activated and migrate to the lymph nodes were they mature and present processed antigen on the surface together with MHC I or MHC II. This complex is recognized by naïve T-cells which, depending on the type of DC they encounter, are triggered to mature towards Th1-, Th2- or regulatory Tr1- or Th3-cells. Selective recruitment results in accumulation of these regulatory or effector T-cells in the mucosa and antigen presentation by epithelial cells may maintain these cells. Th1-cells produce IFN-γ, IL-2 and TNF-α, activate B-cells (IgA, IgM, IgG2) and macrophages and are necessary for elimination of intracellular microbes and viruses by phagocytes. Th2-cells produce IL-4,-5,-10,-13 and activate B-cells (IgE, IgG1 (-human IgG4)), mast cells/basophils, and eosinophils to remove nematodes and parasites phagocyte independent. Th1 and Th2 homeostasis is tightly regulated since immunological reactivity can be dangerous for the host as tissue damage may occur. Regulatory Tr1- and Th3-cells which produce respectively IL-10 and TGF-β1 are essential to downregulate immune reactivity of T-cells and macrophages or induce antigen-specific tolerance. Consequently, depletion of IL-10 and TGF-β1 may result in loss of tolerance to the commensal microflora. Unbalanced immune reactivity skewing towards mucosal Th1 responses is typical in patients with Crohn's disease and Th2 skewing is typical for atopic disorders.

1.5 Immunopathology in IBD
CD is a Th1 type inflammatory response, while in UC the response, although less clearly, is skewed towards Th2. In CD, inflammation is initiated by infiltration of the lamina propria with T-cells and macrophages. Activated macrophages release high amounts of TNF-α and IL-1β. Both in CD and in UC enhanced mucosal IL-1β concentrations were found during active disease. IL-1β activates T-cells which produce IL-2 inducing clonal expansion of cytotoxic T-cells (Tc) and supporting B- and Th-cell function. IFN-γ, TNF-α produced by activated T-cells and macrophages respectively affect the epithelial barrier integrity, enhancing permeability and increasing the antigenic load in the mucosa. Scid
mice replete with CD45RB\(^{hi}\) Th-cells develop colitis while antibodies against IFN-\(\gamma\) completely and antibodies against TNF-\(\alpha\) partially abrogated the colonic inflammation\(^{13}\). Reactive oxygen species released by activated neutrophils may further deteriorate the barrier integrity\(^{58-60}\). Current medication is directed towards downregulation of pro-inflammatory cytokine production (corticosteroids (prednisone, budesonide), in anti-TNF\(\alpha\)), reducing tissue damage by reactive oxygen species with antioxidants (sulfasaline or active metabolite 5-aminosalicylate (5-ASA)) and immunomodulation (azathioprine or metabolite 6-mercaptopurine (6-MP))\(^{13,61}\).

2 Mechanisms regulating gut barrier function

2.1 Physical/mechanical barrier: mucus layer

The gastrointestinal mucosa is protected against mechanical and chemical damage by a viscous layer of gel-forming mucus which covers the epithelium. The mucus gel contains 95% water and 5% secreted glycoproteins (mucins) and is stored in apical granules. Mature secreted mucins which determine the physical properties of the mucus layer are very large, e.g. the apparent molecular weight of intestinal type secreted mucin(MUC)-2 is 550 kDa\(^{62,63}\). The most prominent mucins present in the small intestine are MUC-2 and -3 and MUC-2 can be found most prominently in the colon, however MUC-1, -4, -5A/C and -6 are expressed as well\(^{63-65}\). Secretory mucins MUC-5A/C and MUC-6 are mainly present in the stomach\(^{63,64}\). The mucin protein backbone is largely O-glycosylated with oligosaccharides containing terminal sialic acid or sulphate residues which determine intrinsic negative charge of the glycoprotein\(^{62,65}\). Extensive O-glycosylation of mucin protein backbones contributes to filament formation at the epithelial surface, one of the barrier protective features\(^{66}\). Sulphate residues are believed to protect mucins against bacterial degradation\(^{67,68}\). Disulphide bridges couple monomeric mucin molecules to large networks and determine the gel-forming and viscous properties of the mucus layer\(^{65}\). Baseline mucus secretion is enhanced by agents which stimulate chloride secretion, but mucin secretion is independent from the chloride fluid flow. Epithelial cells have G protein-coupled EP receptors which can cause increased intracellular Ca\(^{2+}\) (EP\(_1\)), increase cAMP (EP\(_2\) and EP\(_4\)) or decrease cAMP (EP\(_3\))\(^{69,70}\). Both the cAMP and Ca\(^{2+}\) pathway may induce mucin exocytosis independently\(^{69}\). Prostaglandins, primarily known to increase cAMP levels and secretion of mucins and water, were found to induce epithelial mucin exocytosis via the EP\(_4\) receptor\(^{41,69}\). PGE\(_2\) is also ligand for the DP receptor which is present in intestinal goblet cells and transduces signals via cAMP production\(^{70}\). Other mediators of intestinal mucus secretion are neurotransmitters or -peptides (e.g. VIP), gut hormones (e.g. serotonin), and inflammatory mediators (PG, NO, IL-1\(\beta\))\(^{50,69,71-74}\). Furthermore, short chain fatty acids which are bacterial fermentation products
are important luminal regulators of mucin synthesis and secretion in the large intestine \(^{75-77}\). COX inhibitors are widely used in patients with arthritis or other inflammatory mediated diseases for their anti-inflammatory and analgesic effects \(^{78}\). A major drawback of the use of NSAIDS is the loss of mucoprotection resulting in gastrointestinal ulceration, PG analogs reduce these side effects \(^{30}\). Patients with inflammatory bowel disease have high risk of these side effects and because NSAIDS also interfere with mucosal tolerance, they are contra indicated in these patients \(^{79}\). It is presently unknown whether this also holds true for COX-2 specific inhibitors.

2.1.1 IBD: mucus defects

Several defects in the molecular structure of mucin glycoproteins have been identified in IBD patients. Reduced sulphation and length of the oligosaccharide chains and increased sialylation was found in both CD and UC, implying that the mucus viscosity and gel-forming properties are affected in these patients \(^{65,67,80,81}\). Furthermore, UC patients have a very thin colonic mucus layer during active inflammation and MUC-2 levels were significantly decreased which was associated with goblet cell depletion. In remission mucus thickness and goblet cell numbers were found to be at normal levels \(^{65,82-84}\). In contrast the mucus layer of CD patients was found to be thicker than normal and the number of goblet cells was not affected \(^{65,82}\).

2.2 Epithelial barrier: tight junctions

The epithelial cells are interconnected by belt-forming adherens junctions, button-forming desmosomes and apical tight junction complexes (figure 4) \(^{25}\). Only the tight junctions seal the paracellular pathway by formation of 'kissing points', preventing passage of

Figure 4 Epithelial cells compose a monolayer of cells separating the gut lumen from the GALT. Tight junctions seal the paracellular route of permeability by formation of 'kissing points'. Adherens junctions and desmosomes interconnect cells. The tight junction complex is connected to the peri-junctional actomyosin ring. Pro-inflammatory cytokines enhance epithelial permeability by enhancing contraction of this ring and disruption of tight junction structures.
macromolecules. Tight junction are selectively permeable for cations, water and small uncharged molecules, such as sucrose. Selectivity for ion strength and size, depends upon the proteins of which the tight junctions are composed. Tight junctions are formed by a network of proteins. Transmembrane proteins consist of occludin and several members of the recently discovered claudin family (20 family members have been discovered) (figure 5). Cytosolic tight junction protein zonula occludens-1 and 2 have been identified to connect occludin and claudin-1 and 2 with the actin filaments which attach to the peri-junctional actomyosin ring (directly underneath the adherens junction). Phosphorylation of myosin light chain (MLC) induces contraction of this ring, hereby increasing paracellular permeability. Transmembrane proteins from two neighbour cells interconnect with each other forming zipperlike structures. Occludin was first to be discovered having a functional role in sealing the paracellular barrier. Phosphorylation of occludin is essential for tight junction assembly and dephosphorylation retracts occludin from the intercellular tight junction complex and decreases epithelial resistance. However other tight junction proteins are evidently of importance since occluding-deficient mice do have well-developed tight junctional strands and normal intestinal barrier integrity. Claudin-1,-2,-3 and -4 have been recently identified in the intestinal epithelium. When transfected into fibroblasts claudin-1 and -2 from homogeneous or heterogeneous paired tight junctional strands with claudin-3 and co-polymerize with occludin. Claudin-1 and -4 are involved in sealing the paracellular barrier since overexpression of these proteins resulted in increased transepithelial resistance. In general it is thought that the number of junctional strands determines the transepithelial electrical resistance however the relation is not linear but rather exponential. On the other hand, MDCK cells (kidney epithelial cells) of low and high resistance appeared to have equal amount of tight junctional strands. It became apparent that, in the low resistance cells, claudin-2 was expressed while this protein was absent in the high resistance cells. Transfection studies with claudin-2 rendered the high resistance cells towards low resistance and increased ion permeability. Therefore claudin-2 is believed to form aqueous channels. On the other hand introduction of claudin-2 did not result in increased permeability for macromolecules as measured by FITC-dextran flux. Tight junction assembly is triggered by Ca\(^{2+}\) and maintenance requires extracellular Ca\(^{2+}\) and intracellular ATP. Pathogens like enteropathogenic Escherichia coli, Entamoeba histolytica, or toxins like Bacteroides fragilis toxin 2, Rotavirus enterotoxin disrupt tight junctions and/or F-actin by a variety of mechanisms. Clostridium toxin A for example has been shown to inactivate Rho-GTPase resulting in dissociation of tight junction proteins (by internalization) from the tight junction complex and F-actin, increasing paracellular permeability. E. coli cytotoxic necrotizing factor-1 causes dramatic barrier disruption by internalizing tight junction protein occludin thus removal from the junctional complex without having profound effects on peri-junctional actomyosin. Pro-inflammatory cytokines like IFN-\(\gamma\), TNF-\(\alpha\), IL-4 and IL-13 are often studied for their
capability to affect tight junction structures inducing permeability. Reactive oxidants released by activated neutrophils also impair barrier integrity. Sub-epithelial myofibroblasts and epithelial cells themselves produce mediators like growth factors (TGFβ, KGF, FGF, HGF), prostaglandins (PGE₁, PGE₂) and trefoil factors that may support barrier function and regulate processes of epithelial migration, regeneration and differentiation.

2.2.1 IBD: intestinal permeability
In patients with IBD intestinal permeability is often increased, when assessed using with permeability markers. During periods of active inflammation mucosal tissue injury results in increased passage of antigenic molecules through paracellular pathways. In CD patients enhanced epithelial permeability was even found to precede relapse to active disease. The ratio of the non-digestible oligo-/monosaccharides lactulose/ mannitol as measured in urine is mostly used to determine intestinal permeability. Mannitol travels through the transcellular pathway while lactulose follows the paracellular route. Decreased absorbing surface results in decreased transcellular uptake in IBD while lactulose uptake was found to be increased presuming that most prominently the paracellular route of permeability is enhanced. Ex vivo intestinal mucosal biopsies of inflamed IBD tissue even without visible defects were found to be more permeable as measured electronically or with para- and transcellular tracers. Furthermore, mucosal biopsies from UC and CD patients with active disease were found to have reduced expression levels of ZO-1 and especially occludin and disrupted tight junction strands. Thus increased paracellular permeability was found to correlate with disruption of tight junction structures during the inflammatory process.

2.3 Signaling pathways regulating epithelial barrier function
In this thesis we worked with the in vitro model of IFN-γ mediated barrier disruption. The most prominent known pathway of IFN-γ signaling is activation of STAT1. However, ERK1/2 MAPK, PKC and PI3K activity may also be regulated after receptor phosphorylation.
IFN-γ Signaling
General Signaling Pathway: JAK/STAT1

extracellular

IFN

JAK1

JAK2

STAT-1

STAT-1

nucleus

GAF

STAT-1

GAS

IFN-γ signal transduction is regulated via STAT1 and other cascades. IFN-γ activation of the receptor activates Jak tyrosine kinases inducing receptor dimerization and Jak phosphorylation. STAT1 molecules are attracted and phosphorylated by Jak5. They dimerize and translocate into the nucleus where they organize gene transcription. Besides STAT1 other routes are activated by IFN-γ, like PI3K-PKB, PKC and Ras-ERK MAPK. G-proteins (Ras, Raf, Rho, Rac, Cdc42) are upstream activators of MKK which control MLC function and MAPK signaling cascades. At least three subfamilies of MAPK are known: ERK, p38 and JNK, all activated by specific MKKs. Upon activation MAPKs translocate into the nucleus where they target specific transcription factors (Elk-1, c-fos/myc/jun, ATF-2) which induce gene transcription. Gene transcription may result in proliferation, apoptosis or production of pro-inflammatory cytokines.

Figure 6 IFN-γ signal transduction is regulated via STAT1 and other cascades. IFN-γ activation of the receptor activates Jak tyrosine kinases inducing receptor dimerization and Jak phosphorylation. STAT1 molecules are attracted and phosphorylated by Jak5. They dimerize and translocate into the nucleus where they organize gene transcription. Besides STAT1 other routes are activated by IFN-γ, like PI3K-PKB, PKC and Ras-ERK MAPK. G-proteins (Ras, Raf, Rho, Rac, Cdc42) are upstream activators of MKK which control MLC function and MAPK signaling cascades. At least three subfamilies of MAPK are known: ERK, p38 and JNK, all activated by specific MKKs. Upon activation MAPKs translocate into the nucleus where they target specific transcription factors (Elk-1, c-fos/myc/jun, ATF-2) which induce gene transcription. Gene transcription may result in proliferation, apoptosis or production of pro-inflammatory cytokines.
2.3.1 Signal transduction: Jak/STAT

IFN-γ like other cytokines and growth factors uses the Jak-STAT (janus kinase-signal transducer and activator of transcription) signaling pathway to transduce signals from the membrane into the cell nucleus. Six mammalian STAT and four Jak family members have been identified, of which IFN-γ uses STAT1. Upon receptor binding of IFN-γ the IFNγR(Receptor)1 and R2 subunits oligomerize, Jak1 and Jak2 are recruited to the cytoplasmatic tail of the transmembrane receptor and phosphorylate tyrosine residues. Tyrosine kinase Jak is required for STAT signaling and activation of the Ras-MAPK and PI3K-AKT pathway. STAT1 that is present in the cytoplasm docks onto the receptor complex and is cross-phosphorylated followed by dimerization and translocation into the nucleus regulating transcription of several GAS dependent genes (e.g. IP-10, IRF-1 and Fc-γ receptor) (figure 6). Other docking sites may be available for signaling molecules like PLCγ, Src-kinases and adaptor signaling molecules like Shc and Grb.

2.3.2 MAP kinases

MAPK (mitogen activated protein kinases) consist of 20 members and are divided into 5 subfamilies of which most prominently studied are ERK (extracellular signal regulated kinase), JNK (c-jun amino terminal kinase) and p38. MAPK activation results in gene transcription regulating cell proliferation and differentiation, metabolism, cytoskeletal functions and cell death but also inducing pro-inflammatory signaling cascades. MAPK activation is regulated via a cascade activation starting by activation of MKKK (MAPK kinase kinase) or MKK (MAPK kinase). Theoretically, MAPK activation can proceed through many pathways, but it seems that distinctive routes are imposed by protein scaffolds, which bind fixed combinations of proteins used in the MAPK signaling cascades. In general MKK 1 and 2 (MEK 1 and 2) activate ERK, MKK 4 and 7 activate JNK and MKK 3, 4 and 6 activate p38 resulting finally in downstream activation of the AP-1 (activator protein-1) family of transcription factors Elk-1 and c-fos (ERK), c-Jun and ATF-2 (JNK) and MEF-2 and ATF-2 (p38). In several cell types IFN-γ activates small GTPases Ras and Raf-1 controlling ERK MAPK. On the other hand the Rho family of small GTPases Rho, Rac and Cdc42 may regulate p38/JNK MAPK, MLC and actomyosin, and epithelial barrier function. These GTPases activate Pak (p21-activated kinase) or ROCK (Rho kinase) which phosphorylate MLCK resulting in dephosphorylation of MLC, enhancing barrier integrity. Activation of JNK MAPK by small GTPases may be Pak dependent or independent. Pathogens like E. coli and Salmonella typhimurium activate MAPK and enhance IL-8 secretion by intestinal epithelial cells.
2.3.3 PI3K and protein kinase C

PI3K (phosphoinositide-3 kinase) activity is involved in a wide range of biological responses, among which cytoskeletal rearrangements. PI3K is attracted to the IFN-γ receptor complex but also small protein GTPases are known regulators of PI3K activity. PI3K was shown to be involved in immune mediated barrier disruption. The regulation of barrier function by protein kinase signaling pathways is prominently studied. Phospholipase (PLC)-γ is docking in the activated IFN-γ receptor complex. PLC-γ may activate PKC (protein kinase C) via Ca\(^{2+}\) release and activation of DAG (diacylglycerol).

The PKC family consists of three subtypes: conventional α, β, γ (cPKC) which depend upon second messager DAG and Ca\(^{2+}\), novel δ, ε, η, μ, θ (nPKC) depending on DAG and atypical τ, τ, α (aPKC) independent to both. PKC isoforms regulate myosin light chain kinase (MLCK) that controls MLC phosphorylation.

2.3.4 IBD: barrier integrity and signaling

In colonic biopsies of patients with active Crohn's disease mucosal JNK and p38 MAPK were found to be activated. In these patients JNK/p38 MAPK inhibitor CNI-1493 reduced JNK phosphorylation (no obvious reduction of p38 activation was seen), which improved ulcer healing and reduced Crohn's disease activity index. However in mice, p38 inhibition did not reduce disease activity caused by TNBS. Mucosal biopsies of patients with active UC and CD were also found to have increased STAT1 expression and activation, which was decreased after induction of remission. Previous studies show STAT1 and MAPK indeed to be relevant targets since their activation is related to chronic inflammation of the mucosa. However these studies mainly focussed on activation of signaling routes in mucosal immune cells. Furthermore, PKC activity was enhanced in inflamed mucosal biopsies of UC patients. Activation of similar signaling pathways in mucosal epithelial cells may contribute to inflammatory induced barrier disruption.

Aim and outline of the thesis

Regulation of intestinal barrier integrity is essential for gut health under physiological and inflammatory conditions. Mucosal barrier defects are thought to play an important role in the immunopathogenesis of many diseases. Barrier preservation is controlled by several mucosal cell types, but the mechanisms of intercellular communication and signal transduction cascades that are involved in barrier regulation are poorly understood. Because the regulation of epithelial cell function by subepithelial myofibroblasts in particular is interesting in this regard, we developed a coculture model in which intestinal epithelial cells were cultured directly on subepithelial myofibroblasts, mimicking the in situ situation.
In this thesis we addressed the following issues:

1. What is the role of subepithelial myofibroblasts in regulation of epithelial cell function? In chapter 2 the barrier properties of the coculture model were compared with monocultures epithelial cells when incubated with stimulated lamina propria immune cells in order to simulate mucosal inflammation. In chapter 3 both models were compared with regard to epithelial mucin expression after incubation with bacterial fermentation products (short chain fatty acids). Prostaglandin secretion by subepithelial myofibroblasts was determined since PG are known stimulators of mucin secretion.

2. Soluble mediators derived from several mucosal cell types effectively regulate epithelial cell function. KGF is such a factor, known to regulate epithelial mucoprotection. KGF is secreted by subepithelial myofibroblasts but not by the epithelial cells themselves. However, the myofibroblasts are also prominent producers of prostaglandins. In chapter 4 we determined whether KGF also might have autocrine functions by regulating myofibroblast derived prostaglandin secretion.

3. What mechanisms regulate barrier homeostasis and barrier disruption during inflammation? In chapter 5 the effects of IFN-γ on tight junction protein expression was determined with special interest in newly discovered claudin tight junction proteins. Mechanistic studies were performed using inhibitors of proteases and protein synthesis. In chapter 6 several signal transduction cascades were inhibited in order to determine whether these routes are involved in regulation of basal barrier properties and/or IFN-γ mediated barrier disruption. Natural food flavonol quercetin was tested as candidate molecule potentially ameliorating IFN-γ affected barrier integrity.

References
Chapter 1


General introduction

64. van Klinken, B. J. et al. The human intestinal cell lines Caco-2 and LS174T as models to study cell-type specific mucin expression. *Glycoconj J* 13, 757-68 (1996).
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Chapter 1


General introduction

Chapter 1


