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Manning the Barricades: Role of the Gut Epithelium in Crohn's Disease

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1. Introduction

Crohn's disease is a chronic inflammation of the gut that affects an estimated 800,000 people in North-America alone. Crohn's disease most commonly affects the ileum, and to a lesser extent, the colon, however can be found throughout the entire gastro-intestinal tract (Shanahan, 2002). The cause of this disease is as yet largely unknown, despite tremendous progress in research efforts over the last decade. It is increasingly clear that inflammation and disease progression involves a complex interplay between the environment, host genes and microbes (Baumgart and Carding, 2007). Increasingly, and predominantly based on genomic analyses, involvement of components of the innate immune system have been recognized in inflammatory bowel disease (Baumgart and Sandborn, 2007). The first major susceptibilty locus that was identified for Crohn's disease was the IBD1 locus, encoding nucleotide oligomerization domain 2 or NOD2 (Hugot et al., 2001; Ogura et al., 2001). Various variations in genotypes and single nucleotide polymorphisms have been identifed in NOD2 that are strongly associated with Crohn's disease development (Economou et al., 2004; Lesage et al., 2002). In a recent genome-wide study, a total of 71 loci were identified to be associated with Crohn's disease, with the potential involvement of more genes (Franke et al., 2010). Among the genes identified were the autophagy-related 16-1 or *ATG16L1* gene and the interleukin-23 (IL-23) receptor gene. Autophagy is a mechanism that regulates protein degradation and is essential for immune balance. Disturbance of this mechanism may lead to inflammation or disease and therapeutic applications of manipulating this mechanism are under investigation (Fleming et al., 2011). The IL-23 receptor is a key feature of the Th17 subset of T helper cells which are a critical component of the antibacterial defense (Abraham and Cho, 2009). Both IL-17 and the IL-23 receptor ligand are currently targeted for therapy (De Nitto et al., 2010).

The epithelium of the gut is an important component of innate immunity. Epithelial cells perform an essential, yet selective barrier function, physically separating the gut lumen from underlying cells and tissues (Peyrin-Biroulet et al., 2008). This physical barrier limits the exposure of microbes and infectious agents to the underlying mucosal immune system, while at the same time allowing exchange and uptake of fluids and nutrients. More than a physical barrier, the gut epithelium actively participates in host defense. Epithelial cells

form a critical link between mucosal immunity and the microbial intestinal flora via germ-line encoded receptors and specific signaling pathways (Abreu, 2010; Koch and Nusrat, 2009; Wells et al., 2010). For example, epithelial cells from distinct lineages express NOD2 or ATG16L1, critical for recognition and clearance of intracellular microbes and linked to Crohn's disease as mentioned (Bevins, 2004, 2005; Kaser and Blumberg, 2011). Barrier functions of the gastro-intestinal tract is regulated by chemokines and cytokines released in underlying compartments as well (Zimmerman et al., 2008). The exact sites and mechanisms of how cytokines affect epithelial permeability is not known, however it involves mainly the Th1 cytokines tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). Therapy directed against these cytokines is currently widely applied in the clinic (Ford et al., 2011). Additionally, specialized epithelial cells have evolved in the gut that are critical in three areas: **1**) Goblet cells secrete mucins and are a source of trefoil peptides, important for mucosal repair (McGuckin et al., 2011); **2**) Paneth cells secrete antimicrobial peptides (Bevins, 2006; Ouellette, 2011); and **3**) M cells transport antigen and micro-organisms, thus sampling the gut lumen (Miller et al., 2007). In this chapter, we will discuss in detail the active barrier function of the indivdual cellular components of the intestinal epithelium in context of immune homeostasis and Crohn's disease.

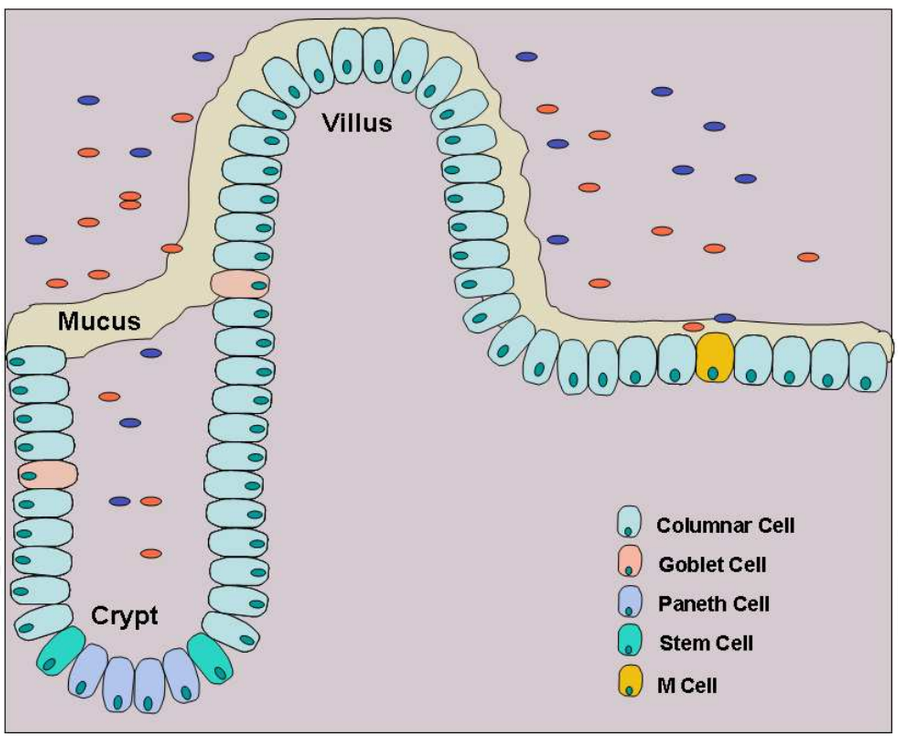


Fig. 1. Epithelial cells of the ileum.

The ileum is predominantly populated by columnar enterocytes or columnar cells which provide essential barrier function to the gut, separating the lumen from underlying tissue. Specialized goblet cells produce mucus, the first line of defence against microorganisms, but also microhabitat for bacteria. Paneth cells produce a host of antimicrobial factors resulting in a relatively sterile environment in the crypt base. Poliplurent stem cells continually self-replicate and differentiate to ensure high turnover rate of epithelial cells. M cells are

optimized for antigen sampling and transport and are in close proximity with underlying components of adaptive immunity.

2. Goblet cells

Goblet cells are glandular simple columnar epithelial cells that are found scattered among the epithelia of the intestinal and respiratory tracts, as well as the urogenital, visual, and auditory systems. The primary function of goblet cells is to secrete mucin into the lumen of the gut and airways. The majority of the cytoplasm of goblet cells is occupied by secretory granules containing a variety of proteins that form the mucus layer upon granule exocytosis. Rough endoplasmic reticulum, mitochondria, nucleus, and other organelles are located in the basal portion of the cell. The apical plasma membrane of goblet cells contains microvilli to increase the surface area for secretion.

2.1 Mucus

Mucus comprises a visoelastic layer of fluid that plays an important defensive role against foreign environmental substances. Mucus covers all exposed epithelia of mammals, as well as the epidermis of amphibians and the gills of fish. In addition to trapping and removing foreign substances, mucus serves to lubricate the some epithelial surfaces, principally those of the gastro-intestinal tract. A layer of mucus along the inner walls of the stomach is vital to protect gastric epithelial cells from the highly acidic environment. The average human body produces about one liter of mucus per day (Thorton, 2008). Mucus consists of water, salts and various macromolecules, including mucins, proteinases, proteinase inhibitors, proteoglycans, and defensive proteins. In the latter category are proteins such as lysozyme, lactoferrin, and immunoglobulins. Proper concentrations of these components are required for the optimum function of mucus, and an alteration in the quality or quantity of the individual constituents of mucus may lead to pathological conditions.

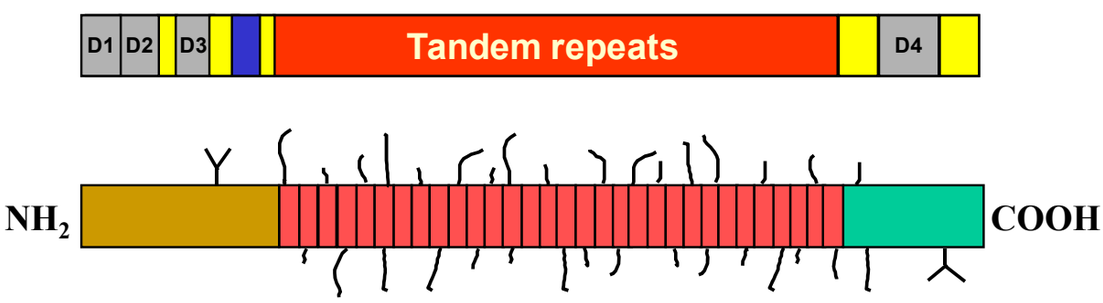
2.2 Mucins

Mucins are the primary protein constituents of mucus (Lillehoj and Kim, 2002). These high molecular weight glycoproteins contain variable numbers of tandem repeats (VNTRs) in which serine, threonine, and/or proline residues are highly enriched. Serines and threonines are responsible for extensive mucin glycosylation that contributes to size and charge heterogeneity of the molecules. Glycosylation within the VNTR takes place between the serine/threonine moieties of the peptide backbone and N-acetylgalactosamine of the oligosaccharides, characteristic of O-linked glycoproteins. In addition, a limited amount of N-linked glycosylation between asparagines residues of the protein backbone and N-acetylglucosamine of the oligosaccharides also are present. Mucins can be broadly classified as either gel-forming/secreted mucins or membrane mucins. Gel-forming mucins are produced by goblet cells and account for the visoelastic property of the mucus layer as a result of protein cross-linking between mucin monomers. Cross-linking occurs following disulfide bonding between cysteine-rich D domains in the NH_2- and COOH-termini of the proteins. Membrane mucins are expressed in a polarized fashion on the apical surface of all epithelial cells. Eighteen mucin (MUC) genes have been cloned and the particular distribution of mucin gene expression varies by epithelial type. In the gastro-intestinal tract, 15 mucin glycoproteins are present (McGuckin et al., 2011). These include both gel-forming

(MUC2, MUC5AC, MUC5B, MUC7, and MUC19) and membrane (MUC1, MUC3, MUC4, MUC12, MUC13, MUC15, MUC16, and MUC17) mucins.

2.2.1 MUC2, the major gel-forming mucin of the intestinal tract

MUC2 is the major component of the secreted mucus barrier in the small and large intestines (Figure 2). MUC2 knockout mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection (Van der Sluis et al., 2006). The MUC2 gene product is a very large, greater than 5,100 amino acids in length, and contains two VNTRs with different amino acid sequences (Gum et al., 1994). The VNTR domain contains 50 -100 threonine/proline-rich 23 amino acid continuous repeats, while the second is composed of a 347 residue irregular and discontinuous serine/threonine/proline-rich repeat. MUC2 contains four cysteine-rich D domains, three located at the NH_2-terminus and the fourth at the COOH-terminus of the protein. This D domain organization is similar to that seen in von Willebrand factor, a glycoprotein involved in hemostasis. The MUC2 D domains contain a characteristic -cysteine-X-X-cysteine- sequence (where X is any amino acid) that mediates mucin oligomerization through disulfide bonding. The glycan moieties of MUC2 contain an equal fraction of neutral (40%) and sialylated (40%) residues with the remainder being sulphated (Karlsson et al., 1996). Mass spectrometry identified the sulfate group attached to C-6 of the N-acetylglucosamine moiety.



Upper, MUC2 cDNA. D1, D2, D3 = dimerization domains; yellow = cysteine-rich regions; blue = cysteine knot. Lower, MUC2 protein. Tan = non-repeat NH_2–terminal region; red = VNTRs; green = non-repeat COOH-terminal region.

Fig. 2. Schematic structure of MUC2.

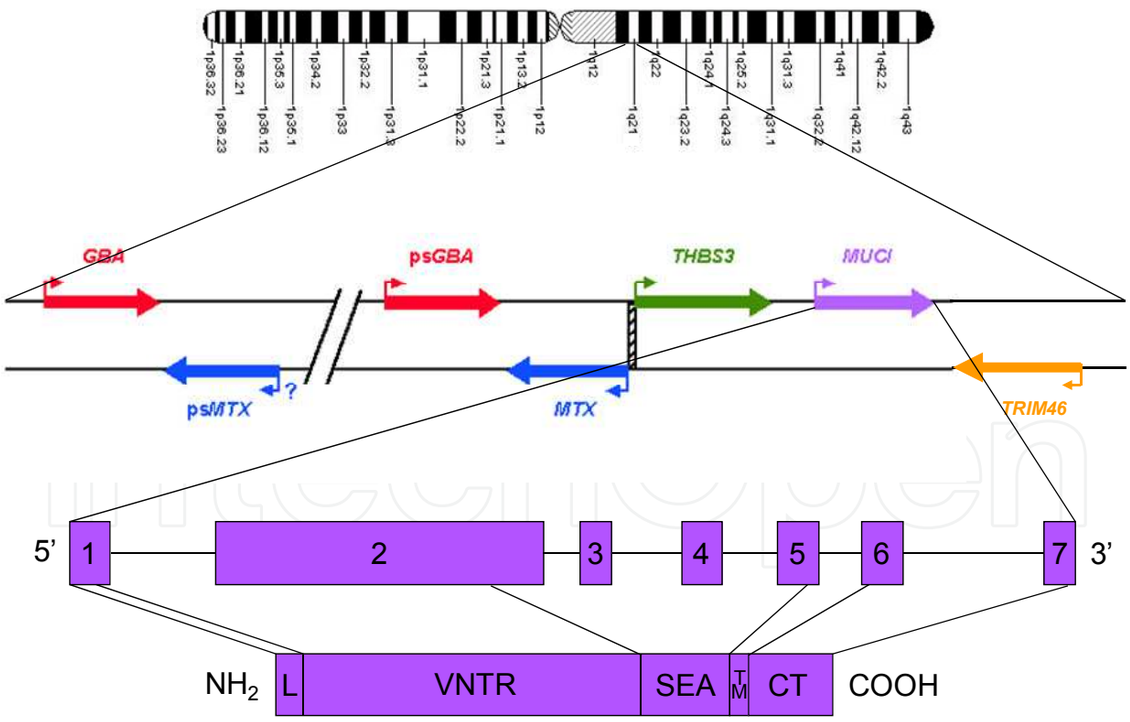
2.2.2 MUC3, the major membrane mucin of the intestinal tract

MUC3 is the most abundantly expressed membrane mucin in the small intestine (Kim and Ho). Here, MUC3 expression on epithelial cells shows a maturational gradient with increasing expression from the crypt to villus. The MUC3 protein consists of two subunits, an extracellular region containing heavily O-glycosylated VNTR domains and two epidermal growth factor (EGF)-like domains. The EGF-like regions are separated by a SEA (sperm protein, enterokinase, and agrin) module, containing a proteolytic cleavage site during biosynthesis. A membrane-spanning, hydrophilic region that is responsible for incorporation of MUC3 into the lipid bilayer and an intracellular cytoplasmic tail (CT) with potential phosphorylation sites involved in signalling, lie distal to the SEA domain. The MUC3 ectodomain may be shed from the cell surface by the activation of membrane-associated metalloproteinases, by the separation of two subunits in the SEA domain, or by alternative splicing of its mRNA. Despite the mechanism involved, shed MUC3 contributes

to the mucus gel overlying the intestinal epithelium. In mice, the cysteine-rich EGF-like domains inhibit apoptosis and stimulate cell migration, implying a regulatory role in maintaining the structure and function of the intestinal epithelial layer.

2.2.3 MUC1, a membrane mucin with signaling potential

MUC1 was the first mucin gene to be cloned (Gendler et al., 1990; Lan et al., 1990). Several studies have provided evidence that MUC1 plays a critical role in the intestinal tract. First, mice deficient in MUC1 expression have reduced amounts of intestinal mucus (Parmley and Gendler, 1998). Second, lack of intestinal MUC1 mucin in knockout mice impairs cholesterol uptake and absorption (Wang et al., 2004). Similar to MUC3, MUC1 consists of a large extracellular domain which is heavily glycosylated through N-acetylgalactosamine O-linkages, a single-pass transmembrane region, and a cytoplasmic CT (Figure 3). The MUC1 ectodomain serves as a binding site for pathogenic microorganisms, including *Pseudomonas aeruginosa* (Kato et al.; Lillehoj et al., 2001), *Helicobacter pyori* (Linden et al., 2004; Linden et al., 2009), *Campylobacter jejuni* (McAuley et al., 2007), *Escherichia coli* (Parker et al., 2010; Sando et al., 2009), and *Salmonella enterica* (Parker et al., 2010). During intracellular biosynthesis, the MUC1 ectodomain is autoproteolytically cleaved in its SEA domain to yield two noncovalently associated protein chains. The 72-amino acid CT domain of MUC1 contains 7 evolutionally conserved tyrosine residues. Many of these tyrosines are phosphorylated, leading to MUC1 interaction with receptor and cytosolic kinases as well as various adapter



Upper, the MUC1 gene is located on chromosome 1q21 between the genes for thrombospondin 3 (THBS3) and tripartite motif containing 46 (TRIM46). Lower, the seven exon structure of the MUC1 gene and corresponding protein regions. L, leader peptide; VNTR, variable number of tandem repeats; SEA, sperm protein, enterokinase, and agrin; TM, transmembrane; CT, cytoplasmic tail.

Fig. 3. Genomic organization of MUC1.

proteins, including phosphoinositide 3-kinase (PI3K), Shc, phospholipase C-γ (PLC-γ), c-Src, and Grb-2 (Hattrup and Gendler, 2008; Theodoropoulos and Carraway, 2007). Binding of PI3K, c-Src, and Grb-2 to the CT have been experimentally verified, while Shc and PLC-γ are only inferred based upon the presence of the predicted amino acid sequence motifs. Other proteins bind to non-tyrosine sites, including glycogen synthase kinase 3β (GSK3β), protein kinase C-δ (PKC-δ), and β-catenin. Consensus sequences resembling an ITAM (immunoreceptor tyrosine-based activation motif) and ITIM (immunoreceptor tyrosine-based inhibitory motif) are also present in the MUC1 CT region. Estrogen receptor α (ERα), p53, p120ctn, ErbB1-4, adenomatous polyposis coli (APC), heat shock protein 70 (Hsp70), and Hsp90 also have been reported as binding partners of the CT, but specific amino acid residues have not been identified. Analysis of downstream signaling events indicated that the MUC1 CT activated a Ras → MEK1/2 → ERK1/2 pathway, but the mechanism is unclear.

2.3 Mucus proteoglycans

Proteoglycans are large molecular weight glycoconjugates characterized by variable numbers of glycan repeats (Meisenberg, 2006). The basic proteoglycan unit consists of a core protein with one or more covalently attached glycosaminoglycan chain(s) to a serine residue. The serine residue is generally in the sequence -serine-glycine-X-glycine-, although not every protein with this sequence has an attached glycan moiety. The chains are long, linear carbohydrate polymers that are negatively charged under physiological conditions, due to the occurrence of sulfate and uronic acid groups. As a result of the later modifications, proteoglycans are highly acidic in physiologic conditions allowing them to bind to cations, such as Na^+, K^+, and Ca^{2+}. Three types of proteoglycans were shown to be secreted into mucus by epithelial cells cultured *in vitro*, hyaluronic acid containing proteoglycans, chondroitin sulfate containing proteoglycans and heparan sulfate containing proteoglycans (Kim, 1985; Paul et al., 1988; Wu et al., 1985). While the physiologic roles of proteoglycans in mucus remain largely unknown, suggested functions include epithelial development, remodeling, inflammation, and host defense (Forteza et al., 2001; Huang et al., 1999; Ohkawara et al., 2000; Zhao et al., 1999).

2.4 Mucus proteinases and proteinase inhibitors

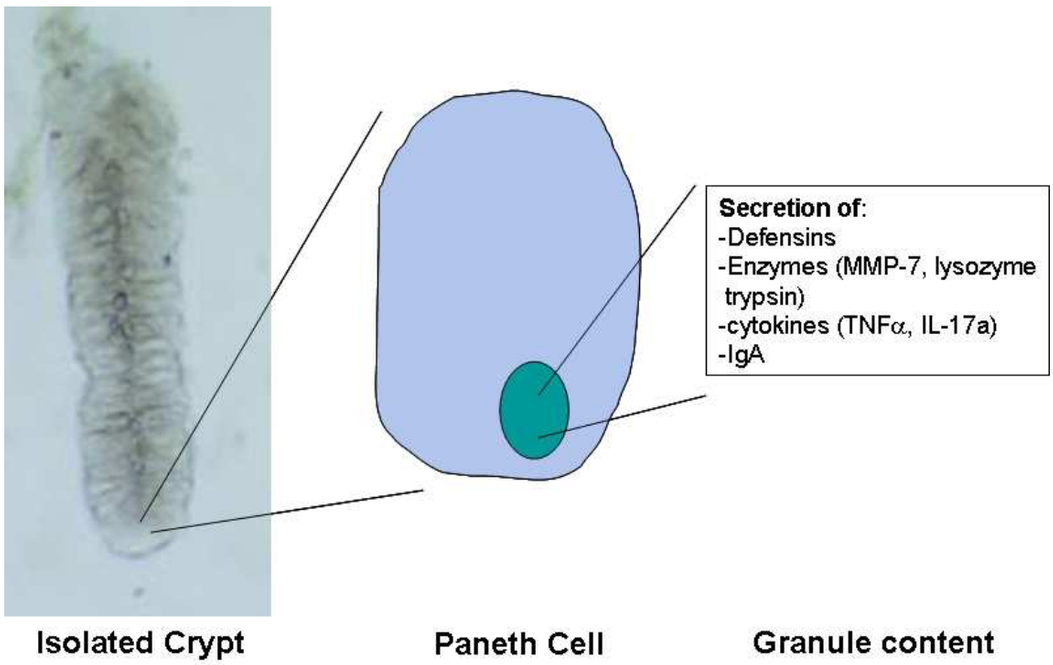
A number of proteinases are present in mucus, all of which known to be associated with inflammation and derived from inflammatory cells. Among these are elastase and various cathepsins from neutrophils and chymase and tryptase from mast cells. Neutrophil elastase has been shown to cause destruction of elastin (Snider et al., 1984), stimulate mucin release from goblet cells (Kim et al., 1987), and induce chemotaxis via production of IL-8 by the underlying epithelial cells ((Nakamura et al., 1992). Excess elastase released from neutrophils during injury and inflammation is balanced by several proteinase inhibitors, including α1-anti-trypsin, soluble leukocyte protease inhibitor (sLPI), and elafin ((Perlmutter and Pierce, 1989; Sallenave et al., 1993; Thompson and Ohlsson, 1986). Attenuated induction of sLPI and elafin has been reported in Crohn's disease (Schmid et al., 2007). Chymase and trypase are proteinases produced by mast cells, the former being responsible for disruption of the epithelial cell barrier allowing antigens and inflammatory mediators to enter the intestinal mucosa, while the latter is responsible for stimulating mucus secretion as well as TGF-β release from the extracellular matrix (Sommerhoff et al., 1990; Taipale et al., 1995).

2.5 Trefoil peptides

Trefoil peptides, or trefoil factors (TFFs), are a group of molecules that are characterized by having at least one copy of the trefoil motif, a 40-amino acid domain that contains three conserved disulfide bonds (Wong et al., 1999). Trefiol peptides are stable secretory proteins expressed in the gastro-intestinal tract. Their functions are not well defined, but they may protect the mucosa from insults, stabilize the mucus layer, and regulate healing of the epithelium. The close physical association between trefoil peptides and mucins supports these possible roles. The trefoil domain is found in a variety of extracellular eukaryotic proteins, including TFF1 (or protein pS), a protein secreted by the stomach mucosa, TFF2 (or spasmolytic polypeptide), a protein of about 115 residues that inhibits gastro-intestinal motility, and TFF3 (or intestinal trefoil factor, ITF). Other proteins with trefoil domains are *Xenopus laevis* stomach proteins xP1 and xP4, *Xenopus* integumentary mucins A.1 and C.1, *Xenopus* skin protein xp2, zona pellucida sperm-binding protein B (ZP-B), and intestinal sucrase-isomaltase. TFF1 and TFF3 contain one trefoil domain, TFF2 contains two domains, and the *Xenopus* proteins contain multiple copies. All three human proteins are clustered on chromosome 21q22.3. Overexpression of human TFF1 in mice was reported to reduce their susceptibility to dextran sodium sulfate (DSS)-induced colitis and TFF-deficient mice exhibited increased disease susceptibility (Mashimo et al., 1996; Playford et al., 1996). Unfortunately, however, these animal studies have not been translated into an effective clinical therapy (Mahmood et al., 2005).

3. Paneth cell

Paneth cells are specialized intestinal epithelial cells located at the base of ileal crypts in healthy individuals (Bevins, 2004; Ouellette, 2011). These cells are pivotal in maintaining the balance between the host and the microbiome. These cells act as sentinels for the detection of microbial molecules which are recognized by Toll-like receptors (TLRs), germ-line encoded receptors specific for bacterial and viral antigens. Genetic polymorphisms in these receptors and their signaling pathways affect Paneth cell function and have been associated with Crohn's disease (Inohara et al., 2005; Kobayashi et al., 2005). Paneth cell function is regulated by two additional mechanisms, the so-called unfolded protein response or UPR and autophagy, a process involved in clearance of intracellular microbes. The process of autophagy is induced by stress in the endoplasmatic reticulum (ER), which in turn is activated by UPR. Genetic mutations in proteins involved in both of these mechanisms have been linked to Crohn's disease as well. Variations in the autophagy protein ATG16L1 were identified in genome-wide studies and found to be associated with increased risk of disease development (Hampe et al., 2007; Rioux et al., 2007). Alterations in the gene encoding the UPR transcription factor protein Xbox-binding protein 1 or XBP-1 are signifiantly associated with inflammatory bowel disease in humans (Kaser et al., 2008). Further, loss of XBP-1 decreases the number of Paneth cells and thus the antimicrobial capacity of the intestine and leads to spontaneous enteritis in mice (Kaser and Blumberg, 2009; Kaser et al., 2008). Paneth cells are equipped with a vast arsenal of antimicrobial agents which are deployed following the recognition of potential microbial threats. These include enzymes, such as lysozyme , trypsin, phospholipase A2 and matrix metalloproteases, cytokines such as TNF-α and IL-17, as well as the bactericidal defensin peptides (Figure 4). In the following sections, we will discuss in detail the role of defensins as effectors of the innate immune system and their involvement in epithelial mucosal barrier function.



(Left) Light microscope image of isolated human ileal crypt. Paneth cells are localized at the base of the crypt as indicated. The box on the right lists confirmed compounds localized in dense secretory granules.

Fig. 4. Localization and cellular contents of Paneth cells.

3.1 Defensins

Defensins constitute a major family of antimicrobial peptides that play a protective role against microbial invasion of various epithelial surfaces, including the skin, respiratory tract and gastro-intestinal tract. Primarily, these small cationic peptides act as effectors of the innate immune system with the ability to kill a variety of microbial pathogens, including bacteria, fungi and viruses (Ganz, 2003; Zasloff, 2002). Based on a difference in disulfide connectivity of six conserved cysteine residues, defensins have been divided in two families, termed α and β. Both families are believed to have evolved from a common ancestral β-defensin gene (Patil et al., 2004; Schutte et al., 2002), and share similar tertiary structures despite low amino acid sequence identity (Hill et al., 1991; Pazgiera et al., 2006; Szyk et al., 2006). In humans, β-defensins are widely expressed in epithelial cells. Defensins of the α-family are expressed predominantly in neutrophils (termed human neutrophil peptides, or HNPs) or in ileal Paneth cells in the case of Human Defensin 5 and 6 (HD-5 and HD-6) (Porter et al., 2002; Selsted and Ouellette, 2005).

In addition to their antimicrobial activities, increasing evidence suggests that defensins play a significant role in innate and adaptive immunity. Such functions include chemoattraction and immune cell activation and promotion of cell proliferation, often involving interactions with cellular receptors (Aarbiou et al., 2002; Biragyn et al., 2002; Grigat et al., 2007; Yang et al., 1999). The capacity to chemoattractant monocytes was first described for HNPs (Territo et al., 1989). Subsequently, HNPs were shown to chemoattract different subsets of T lymphocytes and immature dendritic cells (Chertov et al., 1997; Yang et al., 2000). Similar functions were reported for β-defensins, which were shown to selectively chemoattract

immature dendritic cells and memory T lymphocytes (Yang et al., 1999; Yang et al., 2001). More recently, β-defensins were shown to act as endogenous ligands for TLRs on immature dendritic cells directly. This interaction mediated signaling for dendritic cell maturation and triggered a polarized immune response *in vivo* (Biragyn et al., 2002). In the case of human β-defensin-2 (HBD-2), the observed chemotaxis of immature dendritic cells and memory T cells was shown to result from directly binding the chemokine receptor CCR6 (Yang et al., 1999). Subsequently, a murine β-defensin was shown to recruit tumor-infiltrating dendritic cell precursors through CCR6 also (Conejo-Garcia et al., 2004). In contrast to these earlier studies, it was reported recently that β-defensins chemoattract mast cells and macrophages but not dendritic cells and lymphocytes and that CCR6 was not involved (Soruri et al., 2007). Specific receptors for the chemotactic activity of α-defensins have not been identified. Several studies however have shown that also for α-defensins this activity is blocked by pertussis toxin, indicating the involvement of G_{i}-coupled receptors (Chertov et al., 1996; Yang et al., 2000).

3.1.1 Alpha-Defensins and gastro-intestinal inflammation

There is increasing evidence that aberrant defensin expression is correlated to inflammation of the gastro-intestinal tract. A specific deficiency of the enteric α-defensin HD-5 was observed in patients suffering from ileal Crohn's disease (Wehkamp et al., 2005b). Interestingly, the HD-5 deficiency was more pronounced in patients carrying loss-of-function mutations in the cellular receptor NOD2, an intracellular receptor for the bacterial peptidoglycan component muramyl dipeptide (Inohara et al., 2005). NOD2 is predominantly expressed in the distal part of the ileum in a number of cell types including Paneth cells, which are the sole source of HD-5 (Bevins, 2006; Porter et al., 2002). In addition to recognition of bacterial ligands, NOD2 monitors the expression of enteric α-defensins. Genetic polymorphisms in the *NOD2/CARD15* gene have been identified to be tightly linked with susceptibility to Crohn's disease (Hugot et al., 2001; Ogura et al., 2001) and with decreased defensin expression.

A number of recent animal model studies have underscored the importance of NOD2 and defensin expression in relation to infection. Compared with wild-type mice, NOD2 deficient mice showed reduced expression of certain α-defensins, resulting in increased susceptibility to oral infection by *Listeria monocytogenes* (Kobayashi et al., 2005). Similarly, mice that lack mature cryptdins (the murine orthologue for α-defensins) are more susceptible to ileal colonization by non-invasive *Escherichia coli* (Wilson et al., 1999). Paneth cell expression of HD-5 rendered mice markedly resistant to oral, but not peritoneal, challenge with a virulent strain of *Salmonella typhymurium* (Salzman et al., 2003). Interestingly, HD-5 transgenic mice showed a striking loss of segmented filamentous bacteria and had fewer IL-17-producing lamina propria T cells (Salzman et al., 2010). These findings are in support of the notion that defensin deficiency may alter the microbiome, which in turn affects the adaptive immune response of the host. IL-17-producing T cells, however, were also observed in wild-type mice with functional defensins, in the specific absence of this class of bacteria. Additionally, HD-5 was shown to slightly improve mortality in lethal DSS-induced colitis in mice by intraperitoneal injection; however no effect on disease was noted when the defensin was administered orally (Ishikawa et al., 2009). This may suggest that HD-5 directly affects components of adaptive immunity in addition to affecting the microbiome.

A number of recent studies report on the role of ileal defensins in mucosal immunity and inflammation in humans. Single nucleotide polymorphisms in the gene encoding HD-5 have also been described recently in a New Zealand Caucasian population that may confer susceptibility to inflammatory bowel disease (Ferguson et al., 2008). Luminal processing of pro-HD-5 to its mature form was found to be impaired in Crohn's patients specifically (Elphick et al., 2008). As in mice, human enteric defensins HD-5 and HD-6 are synthesized as pro-peptides in Paneth cells and processed after secretion by trypsin in humans (Ghosh et al., 2002). In the majority of Crohn's disease patients, HD-5 appeared in a complex with its processing enzyme trypsin or chymotrypsin, thus rendering the peptide inactive (Elphick et al., 2008). Additionally, expression of HD-5 was markedly decreased in transplanted human small intestinal allografts (Fishbein et al., 2008). Rejection of allografts resembles Crohn's disease clinically and pathologically (Podolsky, 2002; Shanahan, 2002). Notably, decrease in the expression of HD-5 preceded visible damage to the intestinal epithelium. Finally, expression of both HD-5 and HD-6 was reported to be non-significantly decreased in active ileal Crohn's disease and decreased expression correlated positively with decreased *Vil1* expression, a marker for epithelial integrity (Arijs et al., 2009).

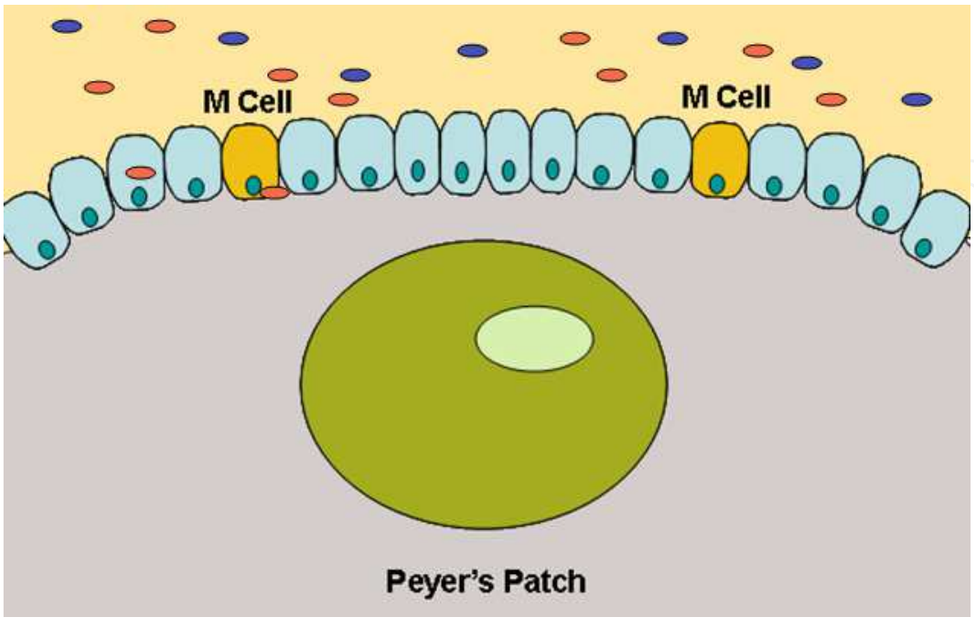
3.1.2 Beta-Defensins and gastro-intestinal inflammation

Impaired induction of β-defensins in the mucosal epithelium has been predominantly linked to colonic Crohn's disease (Fellermann and Stange, 2001; Wehkamp et al., 2002; Wehkamp et al., 2005a; Wehkamp et al., 2005c). The most widely studied β-defensin in the context of gut inflammation is human β-defensin-2 or HBD-2. Genetic polymorphisms (Fellermann et al., 2006), and especially gene copy number of HBD-2 (Fellermann et al., 2006; Hollox, 2008; Hollox et al., 2003), have been identified as risk factors in colonic Crohn's. More recently, expression of HBD-2 at both RNA and protein levels was found to be dysregulated in biopsies from colonic Crohn's patients (Aldhous et al., 2009). Interestingly, in this study, HBD-2 expression correlated with IL-10 production, irrespective of variations in HBD-2 gene copy number or variations in the HBD-2 promoter region. Additional studies on other members of the human β-defensin family emphasize their involvement in mucosal defense. Expression of HBD-1 was found to be protective in colonic Crohn's disease (Peyrin-Biroulet et al., 2010). Protective expression of HBD-1 occured via activation of the peroxisome proliferator-activated receptor (PPAR)-γ with rosiglitazone (Peyrin-Biroulet et al., 2010) or independently via a single nucleotide polymorphism in the HBD-1 gene promoter region (Kocsis et al., 2008). Two studies have reported on colonic Crohn's association of gene copy number of the gene encoding HBD-2, however with contrasting results (Bentley et al., 2010; Fellermann et al., 2006).

4. M cells

M cells, or microfold cells, are specialized epithelial cells of the ileum that have evolved to sample the gut lumen and relay this information to the underlying tissues. They are located in a region of the epithelium that is commonly referred to as the follicle-associated epithelium, or FAE, comprising the Peyer's patches and underlying lymphoid follicles (Figure 5). M cells exhibit microfolds, but not microvilli, and display a thin glycocalyx compare with absorptive enterocytes, making them more accessible to microbes (Gebert, 1996; Kyd and Cripps, 2008). Sensing and transport of microbes by M cells is facilitated by

the expression of TLRs, integrins and microbial adhesion molecules such as galectin-9 (Kyd and Cripps, 2008; Pielage et al., 2007).



Microfold (M) cells specialize in antigen sampling of the gut lumen and act as a selective conduit to underlying components of adaptive immunity without compromising epithelial barrier function.

Fig. 5. Structure of the follicle-associated epithelium.

M cells do not harbor many lysosomes and do not express major histocompatibility (MHC) class II molecules, suggesting that most antigens that are transported are not degraded (Owen et al., 1986; Pickard and Chervonsky, 2010). Because of their relatively weak defenses compared with other sites of the ileal mucosa, M cells are exploited by pathogens as a potential entry site for infection. Such pathogens include EHEC and EPEC strains of *Escherichia coli* (Fitzhenry et al., 2002; Phillips et al., 2000), as well as *Shigella flexneri* and *Salmonella typhymurium* (Jensen et al., 1998). Viruses may also use M cells as a point of entry and specific receptors for HIV (Fotopoulos et al., 2002) and reovirus (Helander et al., 2003) on M cells have been identified.

4.1 M cells and gastro-intestinal inflammation

It is technically challenging to study human M cells *in vitro*, mainly because of the absence of clear cellular markers. Differentiation of enterocytes into M cells likely requires epithelial cell-T lymphocyte cross-talk as indicated by a co-culture model of these two types of cells (Kerneis et al., 1997). Most of our current knowledge on M cells and their role in gastro-intestinal disease comes from animal studies. Various models of chemically induced intestinal inflammation have been used to study M cells, the FAE and interplay with the underlying Peyer's patches. In an indomethacin-induced enteritis model in rats, M cell numbers increased initially and showed increased apoptosis in inflamed tissue only (Kucharzik et al., 2000; Lugering et al., 2004). In the DSS-induced model of colitis in mice, increased severity of disease was associated with lack of both Peyer's patches and lymph

nodes, but not with mice lacking Peyer's patches only (Spahn et al., 2002). Three further studies emphasize the role of epithelial cross-talk with the underlying mucosal tissue at the FAE. The SAMP1/Yit mouse strain develops spontaneous ileal inflammation (Matsumoto et al., 1998). In this model, as well as in a water avoidance stress-induced rat model, early inflammatory lesions were observed in the FAE (Kosiewicz et al., 2001; Velin et al., 2004). Very recently, the FAE and M cells were shown to be targeted specifically by adhesive-invasive *E. coli* bacteria associated with Crohn's disease (Chassaing et al., 2011). The interaction between these bacteria and Peyer's patches of mouse and human was shown to depend on bacterial production of long, polar fimbriae. Such interactions may trigger the recruitment of subsets of dendritic cells or Th1 cells with increased potential for the production of TNF-α, as observed in mucosa of Crohn's disease patients (de Baey et al., 2003; Koboziev et al., 2010; Kudo et al., 2004).

5. Conclusion

It is becoming increasingly evident that intestinal health requires a controlled and balanced interplay between microbes and the host. The host provides microbes with a unique environment of constant nutrition and temperture, whereas microbes aid in food degradation and shape host immunity. In maintaining this balance, the epithelium stands guard, constantly sampling and relaying messages to elicit a rapid immune response if neccesary. At the same time, epithelial cells are continually self-renewing and differentiating to cope with the dynamics of this balance and have evolved into specialized, recognizable subsets. Together, these subsets form a selective barrier consisting of physical, chemical and biological components. In spite of harboring a tremendous arsenal of defensive agents, this barrier does have weaknesses which can be exploited by potentially harmful organisms. Some of these weaknesses have become apparent in an environment where the host is genetically predisposed. The inability of the host to timely recognize or eliminate microbes provides a window of opportunity for penetration of the epithlium, which may eventually lead to inflammation.

In addition to chemical drug treatment, biological therapy has proven its efficacy in treatment of active Crohn's disease. In particular, treatment to eliminate excess tumor necrosis factor alpha or decrease cell trafficking and adhesion by administration of monoclonal antibodies is clinically used in mild to severe cases. Both excess of tumor necrosis factor alpha as well as increased cell adhesion negatively affect the barrier function of the epithelium. Whether an epithelial imbalance is primarily caused by changes in innate or adaptive immunty is currently unclear and will likely vary between individuals. It is clear that disturbance of this delicate balance by environmental factors, pathogens or underlying genetic predispositions of the host may lead to inflammation. Clinically, an imbalance caused by one of these factors is often indistuinguishable from the other. For these reasons, having an understanding of the patients genetic background may help to determine the preferential clinical therapy. Restoration of epithelial barrier function will be an important goal of any therapy, either by strenthening the antibacterial capacity of the gut or by restoring the underlying inflammatory cascade. Additionally, as more and more is revealed about the "black box" which we refer to as the microbiome in the human intestinal tract, alternative approaches to restoration of immune balance may become apparent.

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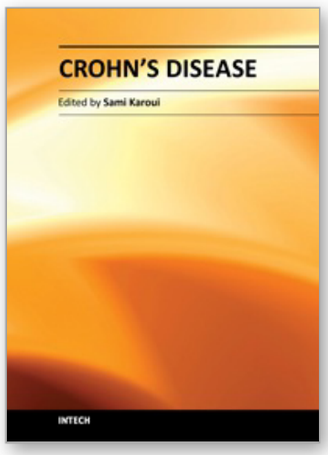

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In this book, several important points regarding Crohn's disease are discussed. In the first section, we focus on etiopathogeny of Crohn's disease and the recent advances in our overall understanding of the disease - specifically, the role of the gut epithelium, alterations of the epithelial crypts, and the roles of the different cytokines in the pathophysiology of Crohn's disease. In the second section, a diagnosis of Crohn's disease is discussed. Another particular area of focus is in the diagnosis of intestinal tuberculosis, and the role of mycobacterium avium in Crohn's disease. In the third and final section, the management of Crohn's disease is discussed, with a focus on recent evidence-based medicine recommendations.

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