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REVIEW

Defects in the adherens junction complex (E-cadherin/ β-catenin) in inflammatory bowel disease

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Abstract The epithelial monolayer of the intestine is a selective barrier permitting nutrient and electrolyte absorption yet acting to protect the underlying tissue compartments and cellular components from attack and infiltration by antigens, bacteria and bacterial products present in the lumen. Disruption of this barrier has been associated with inflammatory bowel disease (IBD). The adherens junction (AJ), together with tight junctions (TJ) and desmosomes, form an apical junction complex that controls epithelial cell-to-cell adherence and barrier function as well as regulation of the actin cytoskeleton, intracellular signalling pathways and transcriptional regulation. Numerous studies and reviews highlight the responses of TJs to physiological and pathological stimuli. By comparison, the response of AJ proteins, and the subsequent consequences for barrier function, when exposed to the IBD inflammatory milieu, is less well studied. In this review, we will highlight the roles and responses of the AJ proteins in IBD and provide suggestions for future studies. We will also consider recently proposed therapeutic strategies to preserve or restore epithelial barrier functions to prevent and treat IBD.

Keywords Adherens junction · E-Cadherin · Beta-catenin · Inflammatory bowel disease

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Introduction

The epithelial monolayer of the intestine functions as a vital barrier protecting the underlying tissue compartments and cellular components from exposure to antigens, bacteria and bacterial products in the lumen. The adherens junction (AJ), together with tight junctions (TJ) and desmosomes, form an apical junction complex (AJC) that controls epithelial cell-to-cell adherence and barrier function as well as regulation of the actin cytoskeleton, intracellular signalling pathways and transcriptional regulation. Numerous studies and reviews highlight the responses of AJC components to physiological and pathological stimuli. However, most have focused on the role of the TJ proteins in epithelial cells; for example, the induction of endocytosis by proinflammatory cytokines such as interferon-gamma (IFN-γ) (reviewed in Utech et al. 2010). Chronic inflammatory bowel diseases [IBD; Crohn's disease (CD) and ulcerative colitis (UC)] are characterised by prolonged cytokine stimulation in the gut, which is known to induce endocytosis of a number of TJ proteins (Bruewer et al. 2003; Prasad et al. 2005; Weber and Turner 2007; Zeissig et al. 2007). By comparison, the response of AJ proteins to the inflammatory milieu to which they are exposed is less well studied. In this review, we will highlight the roles and responses of the AJ proteins in epithelial cells when exposed to the inflammatory conditions associated with IBD. We will provide perspectives for future studies and consider recently proposed therapeutic strategies to preserve or restore epithelial barrier functions to prevent and treat IBD.

The structure and function of adherens junction

AJs are located in the apical part of the lateral plasma membrane of a cell immediately inferior to the TJ. The structure of the AJ is summarised in Fig. 1. The transmembrane component

of AJs, epithelial cadherin (E-cadherin), is a single-pass glycoprotein that belongs to the classical cadherin family of Ca^{2+}-dependent adhesion proteins (Takeichi 1988). E-cadherin has characteristic extracellular cadherin repeat domains, which create homophilic trans-cadherin interaction with the cadherin molecules of the neighbouring cell (Nose et al. 1990). The exact mechanism of this cell–cell trans-cadherin interaction has been a topic of debate for some time and has yet to be fully resolved.

Intracellularly, the cytoplasmic component of E-cadherin binds to p120-catenin, also known as γ-catenin, and β-catenin (Nagafuchi and Takeichi 1989; Ozawa et al. 1989). These proteins comprise characteristic amino acids repeat folded to form a rigid elongated, armadillo repeat domain. P120-catenin binds to the juxtamembrane domain (JMD) of the E-cadherin adjacent to the cell membrane whereas β-catenin binds at a catenin-binding domain (CBD) located further down along the tail of E-cadherin (Aberle et al. 1994; Yap et al. 1998). Both JMD and CBD are thought to be involved in the regulation of the adhesive property of the E-cadherin. P120-catenin is believed to prevent internalisation and proteolytic degradation of cadherin as well as being responsible for stabilisation of cadherin within the cell membrane (Davis et al. 2003; Xiao et al. 2003). On the other hand, β-catenin was believed to provide a link to α-catenin forming the cadherin–β-catenin–α-catenin complex (Aberle et al. 1994; Pokutta and Weis 2000) which in turn interacts with actin and actin-associated proteins such as α-actinin, vinculin, and formin-1. This hypothesis has been questioned, and there is evidence that α-catenin does not bind to the cadherin–β-catenin complex and actin filaments (Drees et al. 2005). A recent study suggests that the cadherin–catenin complex may act as a mechanical adhesion stress sensor that sends signals to actin and actin-associated proteins regulating the structural integrity of the cell (Michael and Yap 2013).

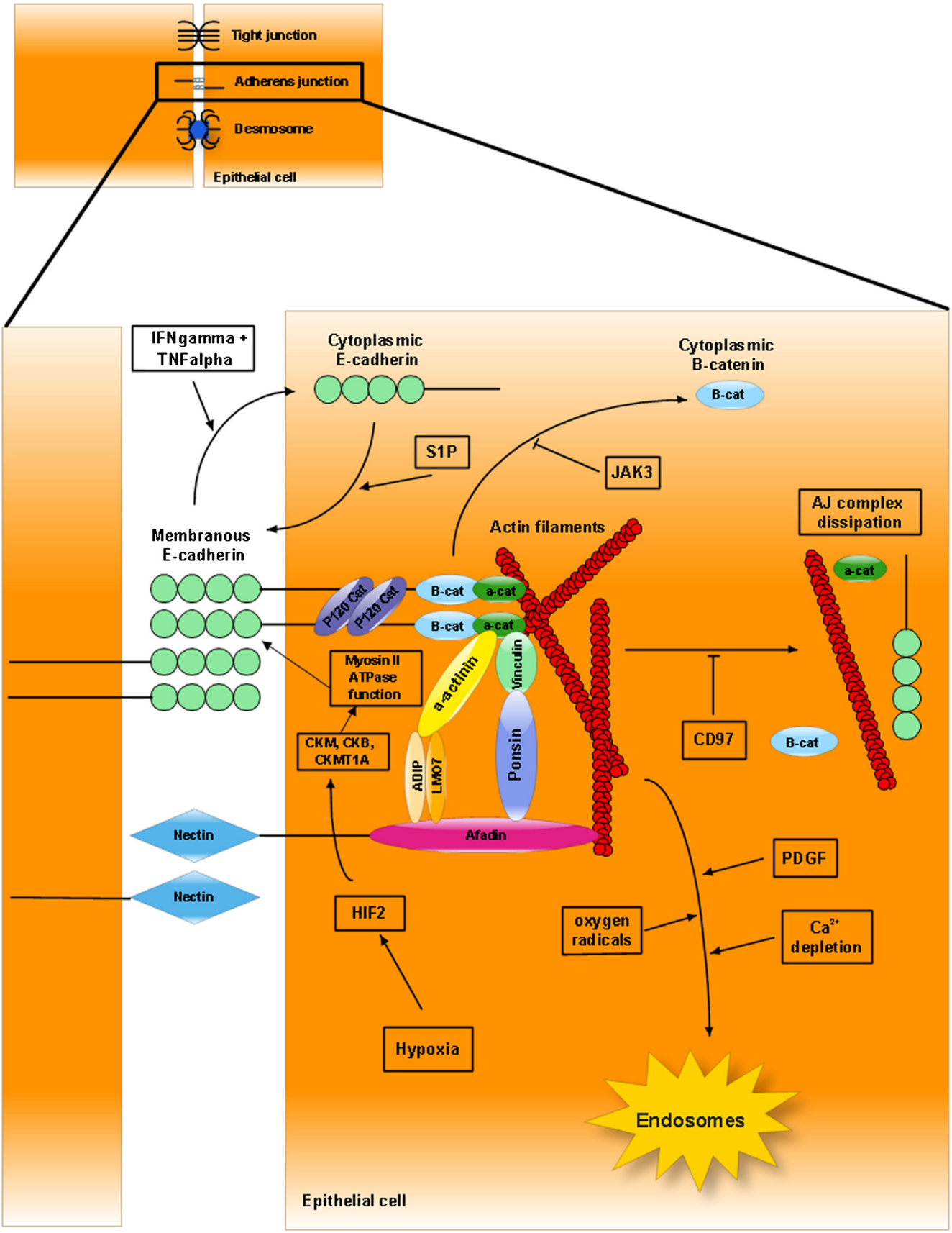
One of the main functions of AJs is to provide the dynamic adhesive connection between epithelial cells. Its interaction with actin and actin-associated proteins through bidirectional signalling pathways is believed to create a dynamic protein system that facilitates and regulates cell-to-cell integrity as well as the overall tissue structure which allows migration and dispersal of cells (Han and Yap 2012). Another function of AJs is an involvement in gene regulation: catenins such as p120-catenin and β-catenin are known to interact with transcriptional machinery in the nucleus acting as transcriptional co-factors (Perez-Moreno and Fuchs 2006). A phosphorylation-dependent cadherin–catenin complex could recruit or release catenins, in turn altering the free cytoplasmic availability of the catenins and affecting the transcription of genes (Daugherty and Gottardi 2007). One of the best examples is β-catenin and the transcription factor T cell factor/lymphoid enhancer factor (TCF/LEF) in the Wnt pathway, which promotes trancription of numerous genes involved in cell proliferation and differentiation (Nelson and Nusse 2004).

Fig. 1 The membrane-bound AJ complex in health and intestinal inflammation. The membrane-bound AJ is shown in the *centre*. The extracellular domain of E-cadherin binds to E-cadherin molecules on adjacent cells. The intracellular domain binds to both p120-catenin and β-catenin at separate sites forming an armadillo repeat domain. The cadherin–β-catenin–α-catenin complex interacts with actin and actin-associated proteins as shown. In addition, afadin links transmembranous nectin to actin and actin-associated proteins via the AJ complex. During inflammation, a number of stress factors affect the AJ complex. The combination of pro-inflammatory cytokines IFNγ and TNFα causes a greater proportion of cellular E-cadherin to be cytoplasmic rather than membranous, whereas S1P has the opposite effect. JAK3 inhibits a similar process involving β-catenin. CD97 has been shown to exert a protective effect against the dissipation of the entire AJ complex. Oxygen radicals, PDGF and calcium depletion all increase internalisation of AJ proteins for degradation via endocytosis. Tissue hypoxia, a common consequence of inflammation, causes induction of CK isoenzymes in a HIF2-dependent manner, which co-localise with AJ proteins and enhance E-cadherin function through myosin II ATPase function. *AJ* adherens junction, *IFNγ* interferon gamma, *TNFα* tumour necrosis factor alpha, *S1P* sphingosine-1-phosphate, *JAK3* janus kinase 3, *HIF2* hypoxia-inducible factor 2, *PDGF* platelet-derived growth factor, *CK* creatine kinase, *ADIP* afadin DIL domain-interacting protein, *LM07* LIM domain only

In addition, the cystic fibrosis transmembrane conductance regulator (CFTR) is localised at the apical membrane of epithelial cells and a link between CFTR and epithelial cell polarity has long been recognised. CFTR is also required for the organisation and integrity of TJs. CFTR was shown recently to interact with a novel intracellular junction protein AF-6/afadin, which interacts with the immunoglobulin-like nectin cell adhesion molecules at AJs (Sun et al. 2014). Nectins form AJs in cooperation with cadherins interacting in trans with each other via their extracellular regions and with afadin through their cytoplasmic tails. Afadin links nectins to the actin cytoskeleton (Takai and Nakanishi 2003; Harris and Tepass 2010; Kurita et al. 2011). As a consequence of the E-cadherin-based AJs and cell–cell adhesion organisation, the afadin/nectin interaction plays a role in the regulation of cellular activities, such as directional motility and proliferation (Takai and Nakanishi 2003).

The relationship between adherens junction and inflammatory bowel disease

Idiopathic IBD encompasses UC and CD, and is associated with recurrent episodes of chronic intestinal inflammation followed by resolution, mucosal healing and epithelial regeneration during periods of remission. Insights in understanding the pathophysiology of the disease are rapidly evolving, although the exact etiology remains poorly understood and a cure is elusive. IBD is a multifactorial disease with environmental and genetic contributions associated with dysbiosis of the intestinal microbiota and dysregulation of the mucosal immune system. Although the epithelial cells within the


Tight junction
Adherens junction
Desmosome
Epithelial cell
IFNgamma + TNFalpha
Cytoplasmic E-cadherin
Cytoplasmic B-catenin
B-cat
S1P
JAK3
AJ complex dissipation
Membranous E-cadherin
Actin filaments
a-cat
p120 Cat
p120 Cat
B-cat
a-cat
B-cat
a-cat
Myosin II ATPase function
a-actinin
Vinculin
Ponsin
ADIP
LMO7
CKM, CKB, CKMT1A
CD97
B-cat
Nectin
Nectin
Afadin
PDGF
HIF2
oxygen radicals
Ca2+ depletion
Hypoxia
Endosomes
Epithelial cell

mucosal layers of the gut represent a physical barrier between the gut lumen and the underlying tissues, epithelial cells are shed continuously from the intestinal surface. The epithelium maintains the integrity of the epithelial barrier, in part, by reorganisation of TJ and AJ proteins (Watson and Hughes 2012). This process fills any gaps left following cellular ejection from the epithelium, but inflammation can cause serious disturbance of this dynamic process by a marked increase in rates of cell shedding compromising the maintenance of barrier function. The importance of this is highlighted by the consequences of abnormal intestinal permeability seen in CD (Peeters et al. 1997; Breslin et al. 2001) and UC (Schmitz et al. 1999).

As discussed above, cadherin is the main transmembrane glycoprotein localised in AJs and is fundamental in mediating cell-to-cell adhesions through the extracellular domain. In a classic experiment, Hermiston and Gordon (1995) used chimeric mice that expressed the dominant-negative cadherin in some intestinal epithelial stem cells to demonstrate the disruption of AJs and numerous other epithelial cell defects: incomplete polarisation; disruption to both the brush border and actin cytoskeleton; enhanced crypt–villus migration and premature apoptosis. In addition, the mucus layer was disrupted where underlying epithelial cells expressed the dominant negative cadherin; numerous adherent bacteria were present at these sites (Hermiston and Gordon 1995). These features mirror closely characteristics of IBD pathology. Interestingly, genome-wide association studies have identified multiple susceptibility loci for both CD (Duerr et al. 2006; Hampe et al. 2007; Wellcome Trust Case Control Consortium 2007; Barrett et al. 2008) and UC (Dubois and van Heel 2008; UK IBD Genetics Consortium et al. 2009; McGovern et al. 2010), which include members of the cadherin families, *CDH1* and *CDH3* (van Heel et al. 2003), that encode E-cadherin and p-cadherin, respectively. More recently, Elding and colleagues have confirmed this causal variant link to *CDH1* and *CDH3* (Elding et al. 2011), indicating that alterations in these genes may be important for the development of IBD.

Early ex vivo experiments performed on isolated crypts and epithelial cells from mucosa of UC, CD and control patients showed a perturbation of the epithelial barrier in IBD (Gibson et al. 1988, 1995). Protein expression of AJ molecules was investigated in a number of studies using human mucosal samples from IBD patients and healthy controls. For example, Doğan and colleagues showed strong expression of E-cadherin in normal and inflamed tissues, but a significant reduction of staining was observed in ulcer-associated cell lineage (UACL) cells bordering ulcerated mucosa in CD patients (Doğan et al. 1995). This cell lineage arises from mucosal stem cells and is associated with chronic inflammation and ulceration stimulating cell proliferation, regeneration and ulcer healing (Wright et al. 1990). The phenomenon of E-cadherin loss around ulcerated mucosa in both UC and CD was confirmed by subsequent studies (Karayiannakis et al. 1998; Demetter et al. 2000; Kucharzik et al. 2001; Jankowski et al. 1998; Gassler et al. 2001). Along the gastrointestinal tract, mucosal ulceration is linked with the migration of epithelial cells as part of a well-recognised repair mechanism to reconstitute the damaged epithelial barrier. Down-regulation of the E-cadherin–catenin complex during this process allows regenerating cells to differentiate and migrate over the denuded mucosal areas (Hanby et al. 1996). However, this absence of E-cadherin–catenin expression becomes a double-edged sword in the context of IBD when this loss of cell–cell adhesion further impairs the integrity of the mucosal barrier allowing the exposure of the luminal content to the underlying mucosal immune system which can lead to disease relapse particularly in CD (Wyatt et al. 1993; Arnott et al. 2000).

Other catenin proteins associated with AJs such as α-catenin (Gassler et al. 2001), p120-catenin (Karayiannakis et al. 1998) and β-catenin (Kucharzik et al. 2001) were found to be decreased around regions of ulceration in IBD mucosa compared to control samples. β-catenin cytoplasm and nuclear expression can distinguish the two subtypes of IBD as this protein was significantly increased in UC compared to CD (Soletti et al. 2013). β-catenin is a key player of the Wnt signalling pathway and its dysregulation is highly associated with colorectal cancer (CRC) and likely to play a role in IBD-associated CRC in particular in UC-CRC (Aust et al. 2001; Kinugasa et al. 2010; Shenoy et al. 2012). Whether this is due to activation of the canonical Wnt signalling pathway or if membrane-bound β-catenin plays a role is under debate. However, a recent study indicated that Wnt signalling activation could destabilise AJs to increase cytoplasmic β-catenin and further activate Wnt signalling (Vlad-Fiegen et al. 2012).

Despite being linked with both CD and UC by GWAS, expression levels of p-cadherin (*CHD3*) has not widely been investigated in IBD. P-cadherin is usually expressed in normal mammary epithelial cells and breast cancer tissue, although reports have shown aberrant expression at the early stages of abnormal colon crypt formation (Hardy et al. 2002). In ex vivo IBD samples, p-cadherin was up-regulated in both UC and CD mucosa compared to control mucosa, in particular in dysplastic regions of UC (Jankowski et al. 1998). In addition, p-cadherin's promoter is activated by β-catenin in a LEF/TCF-independent manner (Faraldo et al. 2007). Therefore, it is tempting to speculate that p-cadherin is involved in UC-associated dysplasia, although more ex vivo and in vitro cell experiments are needed to elucidate the exact role for p-cadherin and how its dysregulation alters AJ function in early neoplastic changes in IBD.

Taken together, maintaining intestinal barrier integrity is crucial to preserve a selective barrier permitting nutrients and electrolytes yet protecting us against exposure to the external environment and avoiding infiltration of toxins and enteric flora. The next section will focus on the role of AJ proteins in intestinal inflammation in animal models and how inflammatory mediators can disrupt the homeostasis of these proteins.

Inflammatory mediators and adherens junctions

The AJ proteins play a vital role in the maintenance of epithelial cellular contact and adhesion. However, cellular adhesion in the gut is often compromised during episodes of inflammation, resulting in increased epithelial permeability. Acute inflammatory infiltrates of neutrophils, monocytes, macrophages, eosinophils and T cells within the gut wall characterise many intestinal disorders including those caused by infection and infarction. Non-resolution of intestinal permeability and non-removal of these infiltrates drives chronic inflammatory cascades. Therefore, it is necessary to understand not only the effects of inflammation on the AJ protein regulation that serves to maintain AJs and barrier function but also how non-resolution of intestinal permeability impacts on failure to achieve remission in IBD patients. An appreciation of the mechanism of endocytosis of these proteins during normal cellular homeostasis is fundamental. Endocytosis is the process used by cells to internalise macromolecules and particles into transport vesicles that are derived from the plasma membrane. This process is important for embryological development and tissue morphogenesis (Polak-Charcon et al. 1980; Miller and McClay 1997; Oda et al. 1998). Endocytosis also controls pathogen entry and the immune response, neurotransmission, intercellular communication, signal transduction, and cellular and tissue homeostasis. Defects in endocytosis have been linked to cancer and heart disease (Conner and Schmid 2003). In general, endocytosis mechanisms can be grouped into three classes: clathrin-mediated endocytosis, caveolar-mediated endocytosis and macropinocytosis. A comprehensive overview of these processes is beyond the scope of this article, but has been examined previously (Mukherjee et al. 1997; Utech et al. 2010). Physiological turnover involves constitutive internalisation of AJ proteins followed by either degradation by late endosomes or recycling to the membrane by recycling endosomes. Ongoing internalisation and processing does not compromise membrane integrity.

A number of studies have demonstrated that endocytosis of TJ proteins occurs under various physiological and pathological stimuli, both in vivo and in vitro, including inflammatory cytokines (Kevil et al. 1998; McNamara et al. 2001; Harhaj et al. 2002; Talavera et al. 2004; Wrobel et al. 2004). TJ protein loss increases membrane permeability and compromises membrane barrier function. Studies on AJ protein endocytosis are more limited, but increased AJ protein degradation does occur in intestinal epithelial cells exposed to calcium depletion (Ivanov et al. 2004a), treatment with platelet-derived growth factor (Harhaj et al. 2002) or oxygen radicals (Rao et al. 2002; Ivanov et al. 2004b).

Again, much is known about the effects of the inflammatory milieu on the distribution and function of TJ proteins, whilst there are far fewer studies examining the effects on AJs. One such study found no difference in E-cadherin or β-catenin expression in intestinal epithelial cells treated with IFN-γ. However, combined cytokine treatment of IFN-γ plus TNF-α resulted in reduced E-cadherin membrane localisation as assessed by immunofluorescence with unchanged total cellular E-cadherin levels (Table 1). This was accompanied by an increase in paracellular permeability in vitro, unrelated to apoptosis, as measured by trans-epithelial electrical resistance (TER) (Bruewer et al. 2003). Since IFN-γ and TNF-α are two of the dominant pro-inflammatory cytokines in IBD, and anti-TNF-α agents have clinical efficacy in the treatment of IBD, it is possible that this mechanism is not only relevant pathophysiologically but also therapeutically.

Another molecule implicated in the reduction of intestinal permeability associated with AJs and inflammation is CD97 (Table 1) (Becker et al. 2010), a member of the epidermal growth factor receptor transmembrane (EGF-TM7) family of adhesion G-protein coupled receptors (Yona et al. 2008). Transgenic mice overexpressing CD97 in intestinal epithelial cells are relatively protected from dextran sodium sulfate (DSS)-induced colitis, both in terms of clinical parameters and histological severity (Becker et al 2010). Mice exposed to azoxymethane/DSS-induced tumorigenesis also developed significantly fewer inflammation-associated CRCs. Interestingly, cytoplasmic segments of AJs were strengthened in CD97-overexpressing mice and characterised by dense arrangements into which thickened cytoskeletal microfilaments were anchored (Becker et al 2010). Conversely, CD97-deficient mice displayed much weaker AJs and showed dissipation of microfilaments, which appeared confined to AJs, with preservation of TJ function and TER. Moreover, CD97 was found to co-localise with both E-cadherin and β-catenin with little or no co-localisation with TJ proteins such as desmoplakin. CD97 appeared to be related to higher amounts of membranous β-catenin but not cytoplasmic or nuclear β-catenin, which were related to alterations in Akt/GSK-3β signalling. Interestingly, a genetic susceptibility locus for IBD has been identified close to the EGF-TM7 cluster on chromosome 19p13 (Rioux et al. 2000; Mathew and Lewis 2004). Therefore, CD97 appears to exert a protective effect on the conservation of AJ function, contrasting with the many other molecules studied which tend to act in the opposite manner. Further studies confirming this work and exploring the potentially therapeutic value of this pathway are merited.

Hypoxia is a key feature of inflamed tissue and hypoxic signalling mechanisms, including the hypoxia-induced factor (HIF) complex, have been implicated in intestinal epithelial barrier regulation during inflammation (Schofield and Ratcliffe 2004; Majmundar et al. 2010). A recent study examining epithelial junctional responses to hypoxia demonstrated creatine kinase (CK) metabolism as a distinctive downstream target of HIF-2 in Caco-2 intestinal epithelial cells, using ChIP analysis (Glover et al. 2013). CK isozymes were increased at

Table 1 The mediators of adherens junction protein localisation and function and downstream clinical effects

Mediator	Model	Effect on AJ proteins	Downstream effects	Reference
IFN-γ+TNF-α	T84 human CRC cell line	↓ Membranous e-cadherin	↓ TER ↑ Intestinal permeability	Bruewer et al. 2003
CD97	Murine intestinal CD97 overexpression	Co-localisation with AJ proteins ↑ Membranous β-catenin ↑ Strengthening of cytoplasmic AJs	Protection from DSS-colitis (clinically and histologically) ↓ Inflammation-associated CRC	Becker et al. 2010
HIF-2 HIF1α	Caco-2 human CRC cell line exposed to hypoxia	↑ CK isoenzymes co-localising with AJ proteins ↑ CK isoenzymes promoting myosin II ATPase function	–	Glover et al. 2013
	HIF-2 deficient Caco-2 human CRC cell line exposed to hypoxia	–	↓ TER ↑ Intestinal permeability	
	HIF-2 deficient Caco-2 human CRC cell line exposed to hypoxia, treated with CK inhibitor	↓ Recovery of AJ assembly during calcium switch		
	DSS-induced colitis in HIF1α KO mice	–	↑ Intestinal permeability ↑ Clinical severity of colitis	
JAK3	JAK3 KO mice	↓ AJ localisation of β-catenin	↑ Histological colonic inflammation ↑ Colonic MPO, IL-6 and IL-17a	Mishra et al. 2013
	DSS-induced colitis in JAK3 KO mice	–	↑ Clinical severity of colitis	
	Murine intestinal epithelial cell lysates	Co-localisation with β-catenin	–	
p120 catenin	p120 catenin KO mice	↓ Small and large intestinal cellular adhesion ↓ Membranous E-cadherin, β-catenin and α-catenin (with intact TJs)	↑ Colonic inflammatory exudates early death due to severe colitis	Smalley-Freed et al. 2010

AJs adherens junctions, *IFN-γ* interferon-gamma, *TNF-α* tumour necrosis factor α, *TER* trans-epithelial resistance, *DSS* dextran sodium sulphate, *CRC* colorectal cancer, *HIF* hypoxia inducible factor, *CK* creatine kinase, *ATPase* adenosine triphosphatase, *KO* knockout, *JAK3* Janus kinase 3, *MPO* myeloperoxidase, *IL-6* interleukin 6, *IL-17a* interleukin 17a, *TJs* tight junctions

both the mRNA and protein levels, and were inducible in a HIF-2-dependent fashion. These same CK isozymes (CKM, CKB and CKMT1A) co-localised with AJs and cadherin proteins, and were found to play a role in myosin II ATPase function, known co-ordinators of E-cadherin-dependent AJ utility (Table 1). HIF-2-deficient cells displayed increased intestinal permeability (with increased TER), and CK inhibition was found to impair epithelial barrier recovery during calcium switch. Finally, murine colitis induced by administration of DSS led to greater intestinal permeability in HIF1β knockout mice compared to WT mice, and was associated with a more severe clinical colitis picture. The hypoxic environment generated by inflammation, therefore, may independently be responsible for AJ dysfunction and increased epithelial permeability, which are both hallmarks of IBD.

JAK3 is a tyrosine kinase expressed in the gut among other tissues. JAK3 mediates cytokine signalling through activation of a number of cytokine receptors including IL-2, IL-9 and IL-15 (Safford et al. 1997). Recent work has suggested a role for JAK3 in intestinal barrier function in the context of inflammation (Table 1). Mishra and colleagues found that the colons of JAK3 knockout mice displayed higher levels of inflammation at baseline, characterised by increased levels of myeloperoxidase activity and IL-6 and IL-17a expression (Mishra et al. 2013). Upon treatment with DSS, JAK3 knockout mice suffered a more severe colitis phenotype than wild-type controls. Co-immunoprecipitation of JAK3 with β-catenin from intestinal epithelial cell lysates suggested co-localisation, and the association of β-catenin with AJs appeared to be impaired in JAK3 knockout mice colonic epithelia, suggesting a contributory role of JAK3 in β-catenin–AJ interactions (Mishra et al. 2013). Further studies to explore this mechanism in human ex vivo models are warranted.

The final pathway implicating a link between inflammation and AJs relates to p120-catenin (p120) (Table 1). P120 stabilises E-cadherin interactions in AJs and is an important regulator of cellular adhesion (Hu 2012). However, the literature also suggests a role of p120 as a functionally relevant anti-inflammatory mediator in different organ systems. For example, p120 deficiency in murine pulmonary vasculature has been shown to predispose to greater inflammatory responses to endotoxin exposure characterised by neutrophil homing and increased expression of TNF-α and Il-6 (Wang et al. 2011). Similarly, work with intestine-specific p120 knockout mice has demonstrated an association with increased colonic inflammatory exudates (Smalley-Freed et al. 2010). Indeed, the majority of the mice in this study died within 23 days from the sequelae of severe colitis. Defects in cellular adhesion were apparent in both the small and large intestines, with preserved TJ structure. Although the focus of the majority of studies examining AJ function during inflammation has been on the effects on E-cadherin and β-catenin, it would be worthwhile considering the function of the entire AJ complex, including p120; the biological relevance of p120 may be greater than has been previously realised.

Whether modulation of AJ protein levels at cellular junctions can be used as a therapeutic option remains to be seen. Greenspon and colleagues showed that increasing levels of E-cadherin at the cell–cell junctions led to improved barrier integrity in cultured intestinal epithelial cells (Greenspon et al. 2011). They showed that treatment with sphingosine-1-phosphate (S1P), a protein which is highly expressed in the intestine and has a pivotal role in enhancing barrier function in several non-intestinal tissues, can increase levels of E-cadherin mRNA and protein in intestinal epithelial cells leading to strong E-cadherin localisation to the cell–cell border. S1P treatment also improved the barrier function by decreasing permeability and increasing transepithelial electrical resistance (Greenspon et al. 2011). Once again, more focus is required to identify further modulators of AJ protein localisation and function, as well as examining effects on intestinal permeability and ultimately gut inflammation.

Therapeutic strategies to strengthen epithelial barrier function

Targeting AJ proteins and restoring epithelial barrier function are attractive treatment concepts in IBD, given that mucosal healing is associated with improved patient outcomes (Frøslie et al. 2007). Characteristics of any ideal AJ-modulating agent in IBD are shown in Table 2. Very few studies have been conducted in this area and none have identified a suitable therapeutic option, although the studies themselves have been heterogenous.

Vitamin D and the vitamin D receptor (VDR) have key roles in human disease pathogenesis. Significantly, vitamin D deficiency is a critical factor in IBD (Holick 2004; Narula and Marshall 2012), and other autoimmune diseases, as well as numerous additional pathologies including cancer (Holick 2004). Zhang and colleagues have recently drawn attention

Table 2 The characteristics necessary for adherens junction-modulating treatment for patients with inflammatory bowel disease

Characteristics of an ideal AJ-targeting treatment in IBD
Stabilises AJ proteins at lateral membranes
Provides complementary stabilising effects on TJ proteins
Increases trans-epithelial resistance
Modulates cytokine expression to favour resolution of inflammation
Reduces histological inflammation
Improves clinical parameters
Can be delivered topically as well as systemically
Carries no or minimal off-target effects

AJ adherens junction

to an interesting link between vitamin D and Vitamin D receptor (VDR) signalling and barrier function (Zhang et al. 2013). Vitamin D, taken in the diet, has potential in treating defective tissue barriers as observed in the IBD intestine (Zhang et al. 2013). Currently, there is only limited and indirect data from colon cancer cells that suggest a role for vitamin D and VDR in regulating the adhesion complexes and AJs. For example, β-catenin expression can be repressed by VDR activation (Shah et al. 2006), and both vitamin D and VDR affect the Wnt signalling in colon cancer cells (Pendás-Franco et al. 2008; Larriba et al. 2007). Furthermore, 1,25(OH)2D3, the active metabolite of vitamin D, can promote the differentiation of colon carcinoma cells via induction of E-cadherin and inhibition of β-catenin signalling in SW480 cells that express VDR, but not in a malignant or metastatic sublines which lack VDR (Pálmer et al. 2001). Kong and colleagues used the DSS-induced colitis model to investigate the role of the vitamin D receptor (VDR) in mucosal barrier homeostasis (Kong et al. 2008). VDR null mice exposed to 2.5 % DSS had a marked colitic response with severe ulceration and impaired wound healing and diarrhea, rectal bleeding, and body weight loss culminating in death within 2 weeks. In contrast, wild-type mice were significantly more resistant to the DSS dose. Greater loss of TER and severe disruption in epithelial junctions was observed in colons of VDR−/− animals compared to VDR+/+ mice (Kong et al. 2008). Furthermore, administration of 1,25(OH)2D3 enhances E-cadherin expression in CaCo-2 human epithelial colorectal adenocarcinoma cells (Kong et al. 2008). These observations suggest that VDR plays a critical role in mucosal barrier homeostasis by preserving the integrity of junction complexes and the healing capacity of the colonic epithelium.

Vitamin D deficiency may compromise the mucosal barrier, leading to increased susceptibility to mucosal damage and increased risk of IBD. Clearly, further studies are needed on the function of vitamin D and VDR in murine and cellular colitis models, which should also include culturing of ex vivo IBD biopsies, to assess the link with AJs and the associated barrier repair and stability. Such models can also be used to elucidate new therapeutic targets for IBD patients with defective barrier functions. Furthermore, if vitamin D is to be used in IBD chemoprevention then further study is necessary. For example, the optimal level of the active vitamin D in patients with IBD is currently unknown, as is the best therapeutic modality. Understanding the role of AJs and the mechanisms critical to barrier function will allow more effective and efficient use of this relatively inexpensive and easy to use supplement.

Another class of agent, similarly inexpensive, safe and subject to much recent research in the IBD field is probiotics. Khailova et al. (2009) examined the effects of *Bifidobacterium bifidum* on intestinal integrity in a neonatal rat model of necrotising enterocolitis (NEC). NEC is a significant cause of mortality in preterm infants with parallels to IBD in that altered intestinal permeability leading to bacterial invasion is a key feature. Compared to premature rats (NEC-model) given standard feed, those given feed supplemented with *B. bifidum* showed less histological mucosal damage and reduced mucosal IL-6 expression within the terminal ileum when exposed to asphyxia and cold stress to develop NEC. In addition, in the intestinal epithelium of NEC-rats on standard feed, both E-cadherin and α-catenin were predominantly cytoplasmic in location, whereas in the *B. bifidum* supplemented group, both AJ proteins were demonstrated to be mainly membranous, resembling the distribution observed in healthy animals. Overall, *B. bifidum* supplementation was associated with reduced ileal inflammation, maintenance of the mucosal layer, and improved intestinal integrity (Khailova et al. 2009). Although clinical trials have been undertaken to assess the efficacy of different probiotics in IBD, the majority were unsuccessful. Some probiotics have been found to reduce intestinal permeability in IBD models (Carlsson et al 2013; Hering et al 2014) by altering TJ function and expression, but alterations in AJ complexes have not been studied. Examining the effects of probiotics on AJ structure, distribution and function in models closer to IBD may provide insights into how some of the less efficacious therapies could be improved, for example in combination with other agents.

Other studies examining the effects of therapies on AJs have been performed in murine intestinal tumour models. Both carnosol (a constituent of the herb rosemary) (Moran et al. 2005) and post-ovariectomy estradiol (Javid et al. 2005) reduced tumour burden in an APC-mutation mouse model of intestinal polyposis, when delivered orally. Both treatments led to a reversal of intestinal epithelial AJ protein distribution associated with tumours: namely, increased membranous E-cadherin and an increased association of E-cadherin with β-catenin. Although the improved function of AJs in this setting was inferred, this was not demonstrated explicitly.

Finally, inhibition of Jak has been considered as a treatment modality in IBD patients. The Jak1/Jak3 inhibitor tofacitinib has been evaluated in a recent phase II clinical trial in UC patients. Targeting both Jak1 and Jak3 will block the action of numerous Jak1/Jak3-dependent cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) (Liao et al. 2011; Sandborn et al. 2012) and may influence the function of cytokines dependent on Jak1/Jak2 (IL-6, IL-11, IFN-g, and IFN-a/IFN-b). Although broad pleiotropic effects on multiple cytokines may raise drug safety concerns, response and remission rates were significantly higher in tofacitinib-treated UC patients compared with placebo-treated controls (Sandborn et al. 2012). Interestingly, endoscopic assessment of patients revealed that tofacitinib treatment induced mucosal healing in many patients. These observations appear to run counter to those of Mishra and colleagues who showed that JAK3 knockout mice suffered a severe colitis phenotype on exposure

to DSS (Mishra et al. 2013). The possibility that the association of β-catenin with AJs was impaired in JAK3 knockout mice colonic epithelia, suggesting a contributory role of JAK3 in β-catenin-AJ interactions (Mishra et al. 2013), also runs contrary to the observations in tofacitinib treated UC patients. Clearly, further studies are necessary to resolve this paradox.

Conclusion

The AJs form an integral and critical component of the selective epithelial barrier in the intestine. Much remains to be discovered about AJ structure and function in the developing and normal gut, and how homeostasis of the barrier is maintained throughout life. Significant disruption of AJs occurs during the inflammatory response. How early this happens in the disease process or whether an unknown underlying weakness of the AJ barrier contributes to disease pathogenesis requires investigation. Restoration of barrier integrity is a key goal for therapy in IBD patients. The identification and design of novel therapies will require detailed observational studies on IBD mucosa during the disease process to inform the design of better model systems. A much more comprehensive understanding of AJ barrier function and dysfunction has the potential to provide key inputs to improve the clinical management of IBD patients.

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