Biochimica et Biophysica Acta 1788 (2009) 864–871

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Cytokine regulation of tight junctions

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ARTICLE INFO

Article history:
Received 12 March 2008
Received in revised form 26 August 2008
Accepted 27 August 2008
Available online 8 October 2008

Keywords:
Tight junction
Cytokine
IFN-gamma
TNF-alpha
Claudin
Interleukin
ZO-1
Paracellular permiability

ABSTRACT

Epithelial and endothelial tight junctions act as a rate-limiting barrier between an organism and its environment. Continuing studies have highlighted the regulation of the tight junction barrier by cytokines. Elucidation of this interplay is vital for both the understanding of physiological tight junction regulation and the etiology of pathological conditions. This review will focus on recent advances in our understanding of the molecular mechanisms of tight junctions modulation by cytokines.

Contents

1. Introduction

Cytokine mediated changes in paracellular permeability contribute to a multitude of pathologic conditions including inflammatory bowel disease (IBD), airway inflammation in asthma [1] and cystic fibrosis [2], and diseases that perturb the blood-brain barrier (BBB) [3]. Cytokines also regulate diverse physiological, developmental, and non-inflammatory processes such as spermatocyte transmigration across the blood testis barrier [4] and mammary epithelial cell differentiation [5]. Epithelial and endothelial barrier function is maintained by intercellular Tight Junctions (TJs), multi-protein complexes that seal the space between adjacent cells. It is therefore easy to envision that cytokine mediated perturbation of TJ function results in enhanced paracellular permeability and increased exposure of tissues to luminal antigens in organ systems such as the gastrointestinal and respiratory tracts. While the molecular mechanisms that regulate these processes are incompletely understood, our knowledge is rapidly expanding through the use of reductionistic model cell culture systems of inflammation and epithelial/endothelial barrier function. This review will focus on recent findings that clarify the signaling processes underlying cytokine modulation of epithelial and endothelial barrier function.

As a case in point, chronic recurring inflammation of the intestinal mucosa and loss of the epithelial barrier is observed in IBD. One of the major clinical manifestations of IBD, which encompasses both Crohn's

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doi:10.1016/j.bbamem.2008.08.027

disease (CD) and ulcerative colitis (UC), is chronic relapsing diarrhea. While the pathophysiology of these disorders is complex, an important underlying basis of these diseases is the existence of an abnormal "leaky" epithelial barrier that results in aberrant tissue exposure to luminal antigens and pathogens. Increased epithelial paracellular permeability has been documented in epithelium from acutely inflamed and chronically damaged intestinal mucosa. Furthermore, enhanced epithelial barrier dysfunction has been observed in first-degree relatives of patients with Crohn's disease, which suggests that a genetic component contributes to loss of barrier function and the pathophysiology of this disorder [6,7]. In animal models of IBD such as the SAMP/Yit model, increased epithelial paracellular permeability precedes chronic intestinal mucosal inflammation [8]. Additionally, in animal models such as the mdr1a−/−mouse, altered epithelial barrier function has been associated with the subsequent development of colitis [9]. These observations further support the critical role of epithelial TJ protein complexes in maintaining mucosal tissue homeostasis. A broad array of cytokines perturb epithelial and endothelial barrier function by influencing the structure and function of the TJ. Table 1 contains a list of cytokines that influence epithelial/endothelial permeability to ions (*), and/or small molecules (#), and highlights postulated cytokine mechanisms of action. Experimentally, TJ barrier function is assessed by measurement of transepithelial (or endothelial) electrical resistance (TER), and the ability of TJs to restrict the passage of small molecules such as inulin, mannitol, or dextran through the paracellular space. Elucidating the molecular mechanisms behind the interplay between cytokines and epithelial permeability is vital for understanding the causes and complications of inflammatory disorders such as IBD.

Table 1
Paracellular permeability changes due to cytokine treatment

Cytokine	Permeability	Cells	Mechanism
IFN-γ	Increased* #	T84	Actin reorganization, ↓ ZO-1 [21]
	Decrease* #	Calu-3	Unkn [14]
	–	T84	Myosin II-dependent vacuolarization, MLC/Rho/ROCK [25]
	Increase* #	T84	↔ JAM-A, occludin, claudins1/4 [22]
	Increase* #	T84	Unkn [17,86]
	Increase*	MVECs	Actin structure [20]
	Increase* #	Cholangiocytes	Unkn [60]
	Increase*	HUVECs	↓ Occludin, ↓ E-Cadherin [27]
TNFα	Increase#	HUVECs	↓ Occludin, ↔ claudin 5 and JAM-A [40]
	Increase#	BPAEC	Actin restructuring [107]
	Increase*	Caco-2	Unkn [108]
	Increase*	Caco-2	NF-κB, MLCK [38]
	Increase* #	Caco-2	NF-κB, ↓ ZO-1 [36]
	Decrease*	UEC	Unkn [32]
	Decrease*	LLC-PK1	Unkn [31]
	Increase* #	HT29/B6	Lowered TJ complexity [99]
	Increase* #	MVEC	Actin restructuring [20]
	Increase* #	LLC-PK1	Apoptosis [34]
	Increase* #	LLC-PK1	Unkn [33]
	Increase* #	cholangiocytes	Unkn [60]
IFN-γ + TNF-α	–	HEC	Unkn [6], ↔ JAM-A [40]
	Increase*	PAC	Unkn [2]
	Increase* #	T84	Unkn [43],↓ Claudin 2,3 ↔ Claudin 4 [41]
	Increase*	T84	Altered lipid composition [46]
	Increase* #	T84/Caco-2	MLC/MLCK [42]
	Increase* #	Caco-2	MLCK [44]
	Increased#	MVECs	↔ Claudin 5 [45]
IFN-γ + LIGHT	Increase*	Caco-2	MLCK, caveolar endocytosis [47]

IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells; *, transepithelial resistance; #, small molecule flux; HUVECs, human umbilical vascular endothelial cells; BPAEC, bovine pulmonary artery endothelial cell; Caco-2, human colonic adenocarcinoma; UEC, uterine epithelial cells; T84, human colonic epithelial cells; Calu-3, human lung epithelial cells; MVEC, microvascular endothelial cells; PAC, primary airway cells; LLC-PK1, porcine renal epithelial cells; ↔, change in localization; ↓, decrease protein or mRNA levels; ↑, increased protein or mRNA levels; Unkn, unknown; JAM-A, junctional adhesion molecule A; NF-κB, nuclear factor-kappa B; MLCK, myosin light chain kinase; ZO-1, zonula occludins 1; ROCK, Rho associated kinase.

2. IFN-γ

Interferon gamma (IFN-γ) is a Th1 pro-inflammatory cytokine found at elevated levels in the intestinal mucosa of IBD patients [10–12]. In addition to its immunomodulatory role during inflammation, IFN-γ acts to modify epithelial and endothelial barrier function. In model cell culture systems of inflammation, direct treatment with IFN-γ increases the paracellular permeability of endothelial and epithelial monolayers (see Table 1). However, in airway epithelial cells, IFN-γ exposure has anti-inflammatory properties and promotes epithelial barrier function; indicative of pleiotropic effects [13,14]. The reason for this discrepancy is unclear, although it should be noted that airway inflammatory episodes are considered primarily a Th2 mediated response [15]. The mechanisms through which IFN-γ influences epithelial/endothelial permeability are beginning to be understood at the molecular level. First observed in endothelial cell cultures by Stolpen et al., treatment with recombinant IFN-γ causes actin rearrangement into stress fibers [16]. More subtle changes in actin structure were observed in T84 epithelial cells which, with IFN-γ treatment, show contraction of cortical actin co-incident with epithelial barrier dysfunction [17]. TJ transmembrane proteins are linked by scaffolding proteins to the actin–myosin cytoskeleton, and disruption of acto-myosin structures has long been understood to modulate paracellular permeability [18,19]. These observations are consistent with the hypothesis that actin–myosin restructuring plays a central role in cytokine mediated permeability changes.

Endothelial and epithelial cells respond to IFN-γ by restructuring actin and by decreasing protein levels or subcellular localization of the scaffolding protein ZO-1 [20,21]. In T84 epithelial cells, the TJ transmembrane proteins, claudin, occludin, and junction adhesion molecule A (JAM-A), are internalized away from cell–cell contact regions [22,23]. When visualized by immunofluorescence microscopy, these TJ components become disorganized and discontinuous at the lateral membrane after IFN-γ treatment [21,22]. This may indicate an enhancement of constitutive TJ remodeling as opposed to gross TJ dissolution. Indeed, internalization of TJ proteins in response to IFN-γ proceeds by macropinocytosis into early recycling endosomes and requires acto-myosin based contraction [24,25]. It is therefore feasible that IFN-γ increases acto-myosin contractility, promoting endocytosis of TJ structures. Endothelial cells treated with IFN-beta 1a and 1b blocked IFN-γ induced endocytosis of cadherin based junctions and maintained barrier integrity, suggesting a conserved mechanism between endothelial and epithelial responses [26]. Interestingly, mucosal biopsies from patients with actively inflamed UC show internalized sub-apical vesicles, similar to those found in T84 cells treated with IFN-γ, that contain TJ transmembrane proteins. This suggests that vesicle mediated internalization of TJ proteins is an in-vivo mechanism involved in permeability changes [24].

Recent studies have explored in greater depth the molecular mechanisms behind actin contractility, TJ protein endocytosis, and barrier function. IFN-γ exposure was found to activate the small GTPase RhoA and increase the expression of Rho associated kinase (ROCK), which in turn phosphorylates and activates myosin light chain (MLC) [25]. ROCK can also regulate MLC through inactivation of MLC phosphatase [27]. RhoA is a powerful regulator of actin remodeling associated with the formation of stress fibers (reviewed in [28]). Importantly, these data help to explain two consistently observed cellular responses to IFN-γ treatment: actin restructuring and acto-myosin contractility. However, the signaling pathways by which IFN-γ activates Rho/ROCK and the mechanisms of acto-myosin induced macropinocytosis remain unclear.

3. TNF-α

The pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α), like IFN-γ, is implicated in IBD pathogenesis and is found in increased levels in the pulmonary sputum of cystic fibrosis patients [29,30]. Using in-vitro model systems, TNF-α has been shown to directly impair TJ function in a number of epithelial and endothelial cell lines (see Table 1). Yet conflicting reports exist in several systems that complicate mechanistic interpretations of TNF-α responses. These may reflect cell type specific variation, as well as differences in the length and dose of cytokine treatment [31,32]. TNF-α mediated increases in permeability were first described using the porcine renal cell line LLC-PK1, which shows an early (<3 h) transient increase in permeability that quickly returns to normal levels (~5 h) [33]. A more recent study by this group implicated cell apoptosis, although the authors speculated that barrier dysfunction was due to actin and TJ rearrangement as apoptotic cells were extruded from the monolayer [34]. Through pharmacological inhibition and the over-expression of dominant negative I kappa B alpha (IκBα) mutants, the subsequent barrier recovery was shown to be mediated by nuclear factor-kappa B(NF-κB) [35]. TNF-α-stimulated NF-κB signal transduction has garnered much attention in recent studies. In the intestinal epithelial cell line Caco-2, TNF-α exerts a delayed effect on cell permeability. This results in an increase in small molecule flux within 24 h of treatment, and alters TER by 48 h post-treatment [36]. The authors point to a decrease in ZO-1 protein levels at both these time points, indicating an additional mechanism may be involved. The role of NF-κB was investigated by pharmacological inhibition, which ameliorated barrier defects, and stabilized ZO-1 subcellular localization and protein levels. A thorough investigation over several studies revealed that NF-κB acts to increase myosin light chain kinase (MLCK) transcription during TNF-α treatment [36–38]. Moreover, this correlated with increases in MLCK protein levels, MLC hyperphosphorylation, and increased paracellular permeability. In endothelial cells, MLC phosphorylation and RhoA activation are early events after TNF-α treatment and correlate with increased permeability [39]. Together, these findings are suggestive of a mechanism involving enhanced actin contractility similar to IFN-γ. However, in endothelial cells, pharmacological inhibition of ROCK or MLCK altered the early morphological changes observed but failed to improve barrier function [39]. Permeability changes in endothelial cells, which occur after several days of treatment, do not correlate with RhoA or MLC activation, but rather with decreased occludin levels and mislocalization of TJ transmembrane components claudin 5 and JAM-A. The authors conclude that long-term barrier dysfunction caused by TNF-α treatment is therefore due to TJ remodeling rather than acto-myosin contractility. The reason for the discrepancy between epithelial and endothelial systems is unclear, but may be indicative of cell-specific mechanisms for TJ remodeling during acute or chronic exposure.

4. Combined effects of TNF-α and IFN-γ

Under inflammatory conditions, target cells are exposed to a variety of cytokines. While model cell culture systems exposed to multiple cytokines may complicate mechanistic interpretation, it likely reflects many endogenous cellular environments. Both epithelial and endothelial cell culture systems exposed to TNF-α and IFN-γ simultaneously show increased paracellular permeability (see Table 1). Investigations into the molecular pathways involved report findings that are largely consistent with studies using either cytokine alone. These include altered actin structure, displacement or down regulation of TJ proteins, and activation of acto-myosin contractility pathways [16,40–42]. Interestingly, cotreatment reveals a synergy between TNF-α and IFN-γ, with relative increases in barrier dysfunction and sensitization of airway epithelial cells to IFN-γ [2,43,44]. Combined treatment with TNF-α and IFN-γ results in mislocalization into the cytoplasm of tight junction proteins such as JAM-A , claudin 4 and claudin 5 [40,41,45]. Indeed, TJ proteins such as occludin and JAM have decreased membrane raft association in model intestinal epithelial cells exposed to a combination of TNF-α and IFN-γ [46]. The lymphotoxin-like inducible protein LIGHT, a TNF family member, also synergizes with IFN-γ to increase paracellular permeability [47]. MLC phosphorylation increases with combined TNF-α and IFN-γ treatment in Caco-2 epithelial cells [42]. The engagement of the myosin motor is essential for IFN-γ/TNF-α induced permeability changes in T84 cells, as myosin inhibition with pharmacological inhibitors reduces endocytosis in cytokine treated cells [42]. Although not required for protein internalization in T84 cells, MLCK is upregulated in Caco-2 cells treated with IFN-γ and TNF-α [25,42]. Consistent with these observations, MLCK transcript was found to be upregulated by priming cultured cells with IFN-γ prior to TNF-α exposure [48]. This response, as with TNF-α alone, proceeds in a NF-κB dependent manner [44]. These data suggest that IFN-γ and TNF-α signal through independent pathways that converge at MLC phosphorylation (see Fig. 1.2). The mechanism of IFN-γ and TNF-α synergy

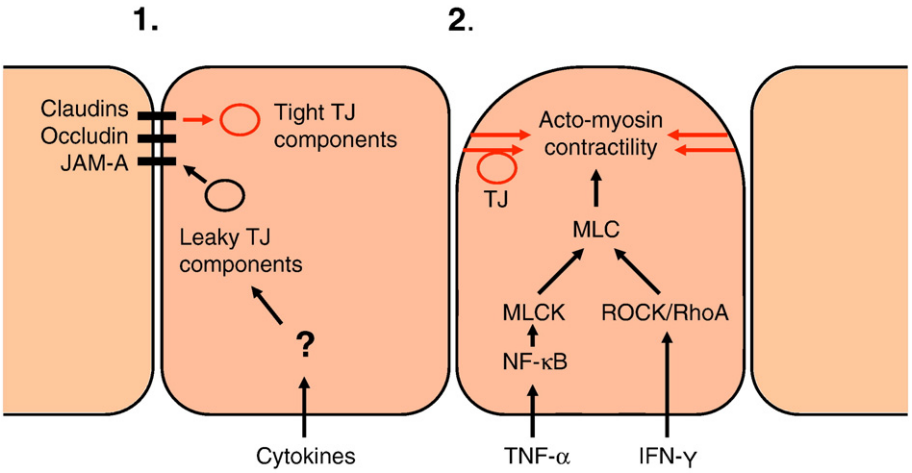

Fig. 1. Mechanisms of paracellular permeability modulation by cytokines. The figure depicts cells in a monolayer undergoing stimulation and tight junction remodeling that results in barrier dysfunction. (1) In response to cytokine activity, tight junction structure is maintained but tight junction protein composition is altered. Tight junction proteins that confer "tight" barrier properties are replaced with those with "leaky" properties. (2) Internalization of TJ structures is due to acto-myosin contractility. The pathways are cytokine specific yet converge on MLC.

is unclear, yet it is interesting to note that IFN-γ is believed to prime cultures for TNF-α treatment by upregulating the TNF-α cell surface receptor [49].

5. Interleukins

Interleukins (IL) are a large family of cytokines, and several have been studied for effects on paracellular permeability in-vitro. These include IL-1, 2, 4, 6, 8, 10, and 13, all of which have been found to have a variety of effects on epithelial and endothelial paracellular permeability (Table 2). Interleukin-1 (IL-1) is type 1 pro-inflammatory cytokine that is elevated in the intestinal mucosa of patients with IBD and in the bronchoalveolar lavage fluid from asthma patients [50,51]. In both epithelial and endothelial in-vitro cell culture systems, IL-1 addition to growth media directly increases paracellular permeability to ions and small molecules [52,53]. In the model intestinal epithelial cell line Caco-2, IL-1 treatment decreases occludin protein levels due, at least in part, to the reduction of occludin mRNA levels [53]. This is consistent with previous findings in astrocytes, where IL-1β treatment suppressed occludin protein levels [54]. While occludin knockout mice have normal intestinal permeability (as measured by both transepithelial resistance (TER) and mannitol flux) they develop chronic inflammation, gastritis, and bone defects [55–57]. Indeed, exogenous occludin expression in MDCK cells increased TJ strand complexity and number, as well as decreasing cell permeability [58]. While the role of occludin in regulation of TJs is still incompletely understood, these studies clearly highlight a relationship between aberrant occludin expression and TJ permeability. IL-2 knockout mice exhibit spontaneous UC-like symptoms, yet specific effects on TJ protein composition and morphology have not shown direct effects by IL-2 [59,60]. IL-4 treatment increases permeability in model intestinal epithelial T84 cells as well as in Calu-3 airway epithelial cells, which after 24 h of treatment demonstrate decreased TER [14,61]. Small molecule flux across the epithelium increases with extended IL-4 treatment (48 h), which corresponds with a decrease in the protein levels of ZO-1 and occludin [14]. Interestingly, an IL-4-induced increase in intestinal epithelial permeability has also been associated with increased protein expression of claudin 2 [41,62]. Increases in claudin 2 alter cell permeability, as exemplified by claudin 2 overexpression in epithelial cells, which lowers transepithelial resistance (TER) and confers increased Na^+ conductance [63–65]. In IL-6 knockout mice, increased intestinal permeability to small molecules has been linked to stability of ZO-1 in TJs [66]. Consistent with these findings, IL-6 treatment in-vitro also increases permeability across endothelial cells and produces ZO-1 mislocalization, actin structure remodeling, and increased actin contractility [67]. In many circumstances IL-10 opposes the influence of pro-inflammatory cytokines such as IFN-γ on the barrier properties of the epithelium or endothelium [68,69]. Mazzon et al. have observed that IL-10 knockout mice, a model of spontaneous colitis, have increased levels of pro-inflammatory cytokines TNF-α, IL-1, and IL-6 [70]. IL-10 ablation also correlates with mislocalization of ZO-1 and claudin 1 away from TJs and may reflect the action of increased pro-inflammatory cytokines [70]. Direct treatment of airway epithelial cells with IL-13 causes decreased TER and enhanced manatol flux and lower ZO-1 protein levels [14]. IL-13 mediated barrier dysfunction in T84 cells also correlates with increased claudin 2 protein levels [41]. IL-13 and IL-4 act synergistically to stimulate the classical STAT6 pathway, although the involvement of this pathway in TJ structure and function has not been directly evaluated [71].

Many interleukins act as modulators of TJ components, controlling occludin, claudin, and ZO-1 protein levels. These in turn form TJs with altered permeability characteristics. Alternatively, interleukins 2 and 10 act to antagonize the action of pro-inflammatory cytokines on TJ permeability, although the mechanisms involved are poorly understood.

Table 2
Permeability modification by interleukins and growth factors

Cytokine	Permeability	Cells	Mechanism
IL-1	Increase* #	HUVECs	Unkn [52]
	Increase* #	Caco-2	NF-κB, ↓ occludin [53]
	–	Astrocytes	↑ Claudin 1, ↓ occludin [54]
	Increase*	PAE	Unkn [2]
IL-4	Increase* #	Calu-3	↓ ZO-1, ↔ occludin [14]
	Increase* #	T84	Unknown [61] ↑claudin 2 [62]
IL-6	Increase#	Intestine	↓ ZO-1 [66]
	Increase*	HUVECs	Actin restructuring, ↔ ZO-1, PKC (α or β) [67]
IL-10	Decrease#	Liver	↑ ZO-1, ↔ claudin 1 [70]
	Decrease* #	T84	Antagonizes IFN-γ [68]
	Decrease* #	HUVECs	Antagonizes IFN-γ, ↑ occludin [69]
IL-13	Increase* #	T84	↑ Claudin 2 [41]
	Increase* #	Calu-3	↓ ZO-1, ↔ occludin [14]
TGFβ	Increase*	UEC	Unkn [32]
	–	hepatocytes	↓ Claudin 1, ↑ claudin 2, SMAD [79]
	Decrease*	T84	↑ Claudin 2, ERK, MAPK, SMAD [72,73]
HGF/SF	Increase#	Cerebrovascula	↓ Occludin, ↓ ZO-1 [81]
	Decrease*	UEC	Unkn [32]
HB-EGF	Decrease*	MDCK	↓ Claudin 2 [83]
PDGF	Increased#	MDCK	TJ structure, ↔ occludin [84]

IL-1, interleukin 1; TGF-β, transforming growth factor beta; HGF/SF, hepatocyte growth factor/scatter factor; HB-EGF, heparin-binding-epidermal growth factor; PDGF, platelet derived growth factor; *, transepithelial resistance; #, small molecule flux; HUVECs, human umbilical vascular endothelial cells; Caco-2, human colonic adenocarcinoma; UEC, uterine epithelial cells; T84, human colonic epithelial cells; Calu-3, human lung epithelial cells; PAC, primary airway cells; MDCK, Madin–Darby canine kidney cells; ERK, extracellular signal-regulated kinases; MAPK, mitogen-activated protein kinase; SMAD, small mothers against decapentaplegic; PKC, protein kinase C; TJ, tight junction; ZO-1, zonula occludins 1; NF-κB, nuclear factor-kappa B.

6. Growth factors

Growth factors have a variety of effects on paracellular permeability, either increasing or decreasing permeability, depending on the cell environment (Table 2). Transforming growth factor beta (TGF-β) is a multifunctional cytokine that has been shown to enhance epithelial barrier properties in-vitro [72,73]. TGF-β binding to its cell surface receptor TGFRβI/II promotes SMAD mediated signaling to the nucleus. Recently, a SMAD independent pathway for TGF-β function was linked to partitioning defective protein 6 (Par6) through SMURF1 ubiquitination and degradation of RhoA [74]. Par6, an evolutionarily conserved regulator of cell polarity, is believed to act as a negative regulator of TJ establishment, yet may support TJ integrity through RhoA degradation [74,75]. Par6 is located at TJs and is in a constitutive complex with atypical protein kinase C (aPKC), which together forms a complex with Par3 and Cdc42 [76]. Cdc42, a small Rho family GTPase, is thought to act together with Par6 as a GTP dependent molecular switch for the activation of aPKC [77]. Interestingly, aPKC activity is required for SMURF1 degradation of RhoA [78], presenting the possibility of a counter-acting mechanism to proinflammatory cytokines. Like many cytokines, TGF-β exhibits pleiotropic effects, as it increases permeability in uterine epithelial cells [32]. This is consistent with studies in rat hepatocytes, which show decreases in claudin 1 and increases in claudin 2 protein expression following exposure of cells to TGF-β [79]. TGF-β regulation of claudin 1 was found to proceed by a SMAD-dependent mechanism [73]. This may reflect multiple functions for TGF-β that are dependent on the biological setting. Indeed, TGF-β treatment is a model for the study of cell–cell contact disruption during epithelial-mesenchymal transition (EMT). The factors that dictate which of these pathways predominate after TGF treatment are unknown. Variable effects in cell barrier function are also seen with Hepatocyte Growth Factor/Scatter Factor (HGF/SF) treatment. An HGF-induced increased permeability is observed in epithelial and endothelial cells, and correlates with a

decrease in occludin and ZO-1 protein levels [80,81]. In contrast, HGF/SF exposure decreases uterine epithelial cell monolayer permeability [32]. HGF/SF is also used to study EMT and acts to internalize junction proteins in a clathrin dependent mechanism in Madin–Darby canine kidney cells (MDCK) [82]. Heparin binding epidermal growth factor (HB-EGF) decreases permeability in MDCK cells, which is correlated with suppression of claudin 2 protein levels [83]. Platelet-derived growth factor (PDGF) increases small molecule flux in MDCK cells through modulation of TJ structure and displacement of occludin [84]. Although varied in their effects on barrier properties, growth factors and cytokines act by similar mechanisms, including displacement or down regulation of TJ protein components and regulation of RhoA. Further studies will be needed to determine the signaling pathways that mediate these effects.

7. Mechanisms of tight junction regulation by cytokines

Although cellular responses to cytokines show cell type specific, pleiotropic, and time and dose-dependent effects, common mechanisms for TJ modulation emerge. These include cytokine induced actin remodeling and changes in TJ structure. By and large, in-vitro cell culture systems have shown that cytokines alter TJs independent of apoptosis. Epithelial and endothelial barrier continuity, when disrupted by cell apoptosis, leads to increases in tissue permeability. Cytotoxic effects due to cytokines have been described in a variety of cell lines and are dependent on the dose and duration of TNF-α and IFN-γ exposure [85]. Yet under experimental conditions that increase cell permeability, apoptosis was excluded as a mechanism in T84 cells treated with TNF-α and IFN-γ [17,22,36,86]. For example, pharmacological inhibition of apoptosis fails to attenuate increases in monolayer permeability [46]. Therefore, cytokines are capable of directly modifying TJ composition and structure through signaling pathways independent of cell death.

7.1. Claudin/occludin turnover at the tight junction

A recent study by Zeissig et al. found aberrant expression of claudin 2, 5 and 8 in Crohn's disease patients. Surprisingly, these claudins were found to be increased in non-inflamed segments of the intestine [87]. Similar changes in claudin expression were not found in the same patients during periods of remission, suggesting that cytokines induce TJ remodeling and changes in claudin expression [88]. Indeed, recent studies in our laboratory demonstrated that increased susceptibility to experimentally induced colitis in JAM-A knockout animals correlated with increased expression of pore forming claudins (10 and 15) prior to experimental injury [89]. Changes in the claudin compliment within TJs affects the size and charge selectivity of the paracellular pathway as well as the structure of the TJ stands. A review of recent data groups claudins into two types, pore-forming claudins (2, 7, 10, 15, and 16) and sealing claudins (1, 4, 5, 8, 11, 14 and 19) [90]. Most of these claudins remain to be investigated and it is unclear by what mechanism claudin exchange takes place after cytokine exposure. It is believed that claudins are constitutively internalized and recycled in epithelial cells during normal junction maintenance and homeostasis [91]. Changes in TJ components could be due to increases in turnover rate at the expense of integration. Paracellular pore characteristic may therefore be altered through the exchange of transmembrane components, or by increasing/decreasing the number of existing pores (Fig. 1.1). Indeed, cell line specific paracellular permeability has recently been attributed to changes in the density and number of pores between cells [92]. Therefore, both the number and the character of claudins expressed in a cell after cytokine treatment have the potential to alter paracellular barrier function.

Occludin and claudin protein function and stability can be regulated by post-transcriptional modification of C-terminal residues [93–95]. Phosphorylation in these domains has been shown to increase protein internalization. For example, EphA2 phosphorylation of the claudin 4 C-terminus disrupts the interaction of ZO-1 and claudin 4 localization in the membrane [96]. Claudin C-terminal cytoplasmic domains have PKC and Protein kinase A (PKA) phosphorylation sites within the C-terminal cytoplasmic domain [90,97]. PKA-dependent phosphorylation of the serine residue in position-2 of the K^+ channel Kir 2.3 disrupts interaction with PSD-95 [98]. These modifications may therefore increase protein turnover or disrupt interactions with scaffolding proteins. Both are thought to disrupt TJ structure and function. Interestingly, TNF-α induced permeability changes can be ameliorated by the PKA inhibitor H-8, and cytokine effects can be attenuated by PKC inhibitors [2, 99]. Cytokine-induced changes in TJ permeability have previously been linked to alterations in tyrosine kinases and PKC [67,99]. Indeed, IL-6 induced permeability increases can be attenuated by pharmacological inhibition of PKC and with a PKC pseudosubstrate [67].

Cytokine regulation of transmembrane protein transcription would be a powerful mechanism for TJ remodeling. Recent studies indicate that occludin downregulation by IL-1 is dependent on NF-κB signaling [53]. NF-κB is a co-factor for transcription and a classic mediator of inflammatory signals to the nucleus. NF-κB activation within the first hour of IL-1β treatment is vital for TER disruption [53]. Therefore, NF-κB activation occurs prior to permeability increases and mediates the observed changes in occludin and claudin protein levels. SMAD4, a mediator of TGF-β signaling, suppresses claudin 1 levels in SW480 cells, although the link to cytokine signaling and claudin 1 expression is unclear [100].

7.2. Actin cytoskeletal contraction and endocytosis of tight junction proteins

Wholesale disassembly of TJs is studied experimentally by incubation of cells in low or calcium-free media (calcium switch), which internalizes both adherens junctions and TJs. In low calcium conditions, endothelial cell monolayers lose barrier function through actin mediated internalization of E-cadherin [101]. Epithelial cultures lose barrier properties after calcium reduction and this has been shown to proceed via clathrin mediated membrane vesicle endocytosis [102]. Interestingly, endocytosis in both calcium depletion experiments and cytokine treatments lead to actin reorganization and activation of myosin dependent contractility [103]. In this model, peripheral actin ring structures contract in a non-muscle myosin dependent manner, which coincides with internalization of TJ structural proteins [103]. Cytochalasin D treatment increases permeability in MDCK cells and inhibits TJ restructuring after calcium depletion [104]. The same is true for Cytochalasin B, indicating actin related processes are required for endocytosis as well as junction reassembly [105]. Blebbistatin treatment, which inhibits the myosin II motor, also attenuates loss of junction complexes in the calcium switch model of junction disassembly [103]. Enteropathogenic bacteria induce increases in epithelial permeability in a manner similar to cytokine exposure, and requires MLC phosphorylation [42,106]. These experimental systems are models of junction disassembly and barrier dysfunction, and share several features seen in cytokine treated cells. These include vesicle endocytosis and contraction of acto-myosin based cytoskeletal networks.

Cytokine mediated restructuring of TJs proceeds through a variety of different signaling pathways to affect paracellular permeability. A synthesis of current data with the aim of distilling common mechanisms, is complicated by pleiotropic cytokine actions as well as temporal and dose dependent variation in cell culture systems. For example, endothelial cells in-vivo may be required to maintain barrier function during exposure to chronic low-levels of systemic cytokines during an inflammatory response, and therefore respond

differentially to low cytokine levels in model cell culture systems. Yet modulation of TJ properties by cytokines appears to proceed through two distinct processes, the remodeling of TJs by selectively removing or introducing TJ components (Fig. 1.1) or the wholesale restructuring of TJ and actin networks (Fig. 1.1). Several lines of evidence indicate that these processes may function simultaneously or sequentially. Although it should be recognized that during in-vivo inflammatory events the cytokine environment experienced by the cell is quite complex and the processes of cytokine modulation of TJs are unclear, our understanding of the molecular mechanisms involved is expanding rapidly through the use of reductionistic cell culture systems.

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