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Mini Review

Mucosal barriology: The molecular machinery and physiological significance of multiple epithelial barriers

Yuuki Obata[1,2] and Koji Hase[1,3,*]

[1)]Division of Mucosal Barriology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

[2)]Department of Immune Regulation, Graduate School of Medical and Pharmaceutical Sciences, Chiba University, Chiba, Japan

[3)]Department of Biochemistry, Faculty of Pharmacy, Keio University, Tokyo, Japan

"Barriology" is newly emerging research field initially defined by The Late Prof. Shoichiro Tsukita as the science of barriers in multicellular organisms. The mucosal surface of the gastrointestinal tract is continuously exposed to a wide variety of foreign antigens. The intestinal mucosa is covered by a single layer of intestinal epithelial cells (IECs), which establish the first line of defense against these diverse microorganisms on the luminal surface. Compromised integrity of the IEC layer leads to the development of intestinal inflammation and systemic diseases. Recent studies have demonstrated the active contribution of commensal bacteria and their metabolites in the enhancement of epithelial barrier functions. In this review, we discuss the mechanisms by which the function of the barrier is being regulated and physiological significance of epithelial barriers in the gastrointestinal tract.

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*Correspondence should be addressed to:
Koji Hase, Ph. D., Department of Biochemistry, Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan. Phone: +81-3-5400-2484, Fax: +81-3-5400-2484, E-mail: hase-kj@pha.keio.ac.jp

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Introduction

In multicellular organisms, epithelial sheets cover the body surface to separate the body from the external milieu. The tight adhesion of the epithelial cells not only prevents leakage of internal components but also protect the body against environmental stress. Accumulating evidence has shown that epithelial cells perform multiple barrier functions and play critical roles in the maintenance of immunological homeostasis in the mucosal tissues (Fig. 1). It has been particularly well documented that a monolayer of intestinal

epithelial cells (IECs) serves as a sentinel for detecting potentially hostile antigens present in the gastrointestinal lumen. IECs provide a tightly sealed physical barrier by forming tight junctions (TJs) between adjacent IECs and regulate epithelial permeability. The luminal secretion of mucins and antimicrobial proteins (AMPs) by secretory epithelial cells, such as goblet and Paneth cells, contributes to the establishment of a biochemical barrier that prevents microbial attachment to IECs. In addition, IECs actively transport secretory immunoglobulin A (S-IgA) from the lamina propria to the intestinal lumen by expressing polymeric immunoglobulin (poly-Ig) receptor on the basolateral membrane. IEC progenitors vigorously proliferate to maintain a rapid turnover of the epithelium, which is important for the establishment of a mucosal barrier and for epithelial restitution. Furthermore, IECs possess an immunoregulatory function by controlling the development and activity of intestinal immune cells. Impaired integrity of the IEC layer has been shown to increase the risk of inflammatory disorders, such as inflammatory bowel disease (IBD). In this review, we discuss key components of epithelial barrier functions and the molecular machineries that regulate these barrier functions. We also describe some links between epithelial barrier dysfunction and disease development.

Component of epithelial barrier function

Epithelial stem cells located at the base of the crypts of the small intestine continuously produce transit-amplifying (TA) cells, which divide several times before giving rise to terminally differentiated epithelial cell lineages: absorptive enterocytes and three types of secretory epithelial cells, namely goblet cells[1], enteroendocrine cells[2] and Paneth cells[3]. Intestinal goblet cells form the first line of defense by producing mucins, mainly the heavily glycosylated MUC2 protein. There are two mucus layers in the colon: an outer layer where intestinal microbiota infiltrate and an stratified inner layer that is devoid of the microbiota[4, 5] (Fig. 1). In mice, the thickness of the inner and outer mucus layers is estimated to be about 50 and 100 μm, respectively[4, 6]. The important role of the mucus layer in intestinal homeostasis has been demonstrated by studies of *Muc2*-deficient mice, which lack a normal intestinal mucus layer. In wild-type mice, commensal bacteria are confined to the outer mucous layer. However, bacteria are abundant in contact with the epithelial surface of the lumen as well as in the crypt region of *Muc2*-deficient mice[4]. Eventually, these mice spontaneously develop severe colitis due to aberrant

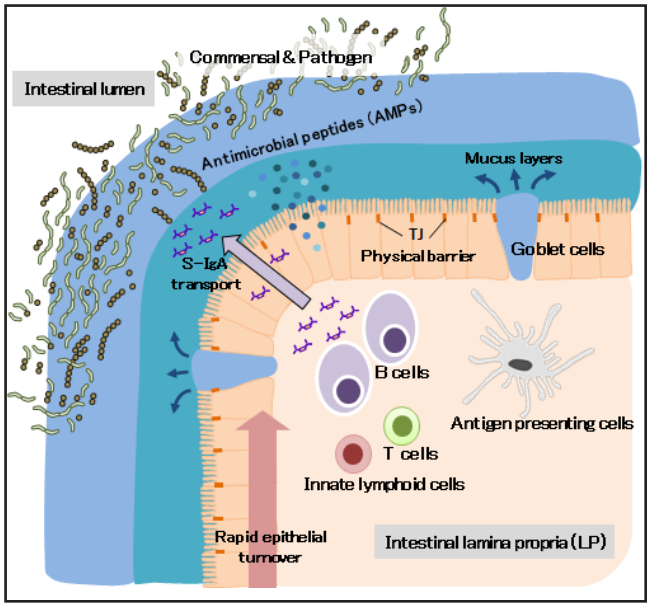

Fig. 1 Component of intestinal epithelial barriers

A single layer of intestinal epithelial cells (IECs) covering the intestinal mucosa serves as a barrier to separate environmental antigens from the underlying intestinal lamina propria (LP). IECs provide a tightly sealed physical barrier by forming tight junctions (TJs), which connect adjacent IECs and regulate epithelial permeability. The luminal secretion of mucins and antimicrobial proteins (AMPs) contribute to the establishment of a biochemical barrier, which prevents the attachment of microbes to the IECs. There are two mucus layers in the colon: an outer layer that contain intestinal microbiota and an inner layer that is devoid of microbiota. IECs directly transport secretory immunoglobulin A (S-IgA) across the epithelial barrier. IEC progenitors rapidly proliferate to maintain rapid turnover of the epithelium. IECs also regulate the mucosal immune response by producing various cytokines and chemokines in the lamina propria.

immune responses to the commensal microbiota[7]. Similarly, *Atoh1*$^{IEC\text{-}KO}$ mice, which have an IEC-specific deletion of *Atoh1*, a gene that encodes a master regulator for secretory cell differentiation, also spontaneously developed severe colitis due to a lack of mucus (Obata et al, unpublished observation). These observations demonstrate the critical role of the mucus layer in the avoidance of intestinal inflammation. Furthermore, *Muc2*-deficient mice are extremely susceptible to *Citrobacter rodentium* infection[8]. Importantly, the non-pathogenic microorganisms also abundantly penetrate into the colonic mucosa during *C. rodentium* infection[8]. This indicates that MUC-dependent mucus formation is essential for the containment of intestinal bacteria. Intestinal goblet cells also produce intestinal trefoil factor 3 (TFF3)[9] and resistin-like molecule β (RELMβ)[10], which contributes to epithelial repair and barrier functions[9]. The importance

of TFF3 is emphasized by the increased susceptibility of *Tff3*-deficient mice to colitis induced by dextran sodium sulfate (DSS)[11]. Conversely, colonization of the mucosa by commensal bacteria[10] and infection with intestinal nematodes[12] induce RELMβ to promote the release of MUC2 from goblet cells[13]. Intrarectal administration of RELMβ has been shown to significantly ameliorate inflammatory symptoms in TNBS-induced colitis[13]. Notably, goblet cells may also contribute to immunosurveillance on the mucosal surface by transferring soluble luminal antigens to $CD103^+$ dendritic cells (DCs) in the intestinal lamina propria[14]. This mode of antigen transfer is called a goblet-cell-associated antigen passage (GAP), and it is thought to mediate the acquirement of innocuous luminal antigens by intestinal tolerogenic $CD103^+$ DCs. Furthermore, goblet cell-derived MUC2 imprints $CD103^+$ DCs with immunoregulatory features that are characterized by the increased expression of IL-10, TGFβ and RALDH; these molecules are important for the induction of $Foxp3^+$ regulatory T (T_{reg}) cells[15]. These studies imply that goblet cells actively contribute to the induction of oral tolerance. Taken together, intestinal goblet cells play a critical role in not only the mucosal defense mechanism but also in the maintenance of gut immune homeostasis.

Paneth cells located primarily in the crypts of the small intestine but not of the colon are another critical component of the epithelial barrier. Paneth cells shape the composition of the microbiota by secreting antimicrobial peptides (AMPs) into the intestinal lumen[16]. Defensins are a major family of AMPs and are classified into two subfamilies: α-defensins and β-defensins. Both types of defensins exhibit antibacterial activity against gram-negative and gram-positive bacteria[16]. Paneth cells store α-defensins (also termed cryptdins) in intracellular granules, and undergo degranulation in response to cholinergic stimulation or exposure to microbial components[17]. The local concentration of cryptdins in the crypt microenvironment is estimated to be as high as 15-100 mg/ml[17]. Paneth cells also express matrix metalloproteinase 7 (MMP7), which cleaves the precursors of cryptdins to generate functionally mature antimicrobial peptides[18]. *Mmp7*-deficient mice are more susceptible to *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) compared with wild type mice[18], and have an altered intestinal microbiota[19]. In contrast, transgenic mice that express a human α-defensin gene (*DEFA5*) in their Paneth cells exhibit much greater resistance to *S*. Typhimurium infection than wild-type mice[20], and show an altered commensal microbiota characterized by a decreased abundance of segmented filamentous bacteria (SFB)[19]. SFB are strong inducers of intestinal T_H17 responses, and so IECs may indirectly regulate intestinal T helper cell development by controlling microbial composition. In addition to Paneth cells, absorptive enterocytes are also capable of producing AMPs such as RegIIIγ, a secreted C-type lectin. Microbial colonization induces the expression of epithelial RegIIIγ through epithelial cell-autonomous MyD88 activation[21-23]. The antibacterial activity of RegIIIγ is restricted to gram-positive bacteria[21]. RegIIIγ-deficient mice exhibited a marked increase in the number of gram-positive bacteria attached to the epithelial surface[23]. Importantly, aberrant expansion of IFNγ-expressing T_H1 cells was observed in the intestinal lamina propria of RegIIIγ-deficient mice[23], suggesting that RegIIIγ-dependent exclusion of the gut microbiota is critical for the avoidance of excessive immune responses in the gut.

Enteroendocrine cells sense intraluminal toxins (e.g. cholera toxin) during acute infections and secrete serotonin and motilin. These paracrine mediators induce diarrhea to expel the toxic agents[24]. Enteroendocrine cells also contribute to the maintenance of epithelial barrier function[2, 25]. Glucagon-like peptide 2 (GLP-2) produced by L cells, which are one of the major populations of enteroendocrine cells, plays a significant role in the promotion of epithelial proliferation[26, 27]. GLP-2 activates subepithelial myofibroblasts via GLP-2 receptor (GLP2R), leading to the release of epithelial repair factors, such as insulin-like growth factor 1 (IGF1) and ErbB ligands. These factors bind to their receptors that are expressed on the surface of crypt epithelial cells and promote cell proliferation[28]. Ablation of GLP-2 receptor increases the severity of intestinal inflammation induced by indomethacin, and the frequency of bacterial translocation into the small intestinal mucosa[29].

Diverse environmental factors including physical stress, digestive enzymes, drugs, and infectious agents cause epithelial cell damage in the gut. To deal with this damage, epithelial progenitors vigorously proliferate to totally renew IECs within 3-4 days. This rapid turnover of IECs enables prompt wound healing after the removal of damaged cells. Mice with a heterozygous deletion of *Klf5*, a transcription factor responsible for proliferation of crypt epithelial cells, have been shown to exhibit increased sensitivity to DSS due to a slower rate of proliferation and migration of epithelial cells in the injured region of the mucosa[30]. The rapid epithelial turnover is also essential to expel invasive intestinal

pathogens such as *Trichuris trichuria*[31, 32] and *Shigella flexneri*[33]. Therefore, if these mechanisms are impaired during the epithelial renewal, there is an increased risk of enteric infection and inflammatory disorders[34]. In support of this notion, ablation of Notch signaling, which is important for the proliferation of epithelial progenitors, leads to spontaneous colitis due to the translocation of bacteria into the colonic mucosa[35]. The role of epithelial Notch signaling in the maintenance of gut immune homeostasis is described in a detail in section 3.

Epithelial cells at barrier sites constitutively express alarmin, including IL-1 family member IL-33[36]. During tissue damage, IL-33 is released extracellularly and alerts immune cells to promote cytokine responses[37]. IL-33 activates group 2 innate lymphoid cells (ILC2s) which express a receptor for IL-33 (ST2)[38] and facilitates the production of type2 cytokines, leading to the enhancement of T_H2 responses as well as the activation of eosinophils and mast cells[39]. Recently, Schiering et al has reported that ST2 is also expressed on colonic T_{reg} cells, and contributes to maintain the stability and function of T_{reg} cells[40]. ST2-deficient T_{reg} cells showed attenuated function to suppress colitis induced by adoptive transfer of naïve T cells into $Rag^{-/-}$ mice[40]. The expression level of IL-33 is upregulated in the intestinal mucosa of patients with ulcerative colitis (UC)[41]. These observations indicate that the IL-33-ST2 signaling is important for the maintenance of colonic T_{reg} homeostasis under inflammatory conditions. Thus, epithelial alarmin contributes to both the onset and regulation of immune responses at barrier sites.

Commensal-bacteria-dependent regulation of intestinal barrier function

Compelling evidence has demonstrated that commensal bacteria play a critical role in the establishment of epithelial barrier function. Early studies showed that germ-free mice exhibit lower intestinal epithelial proliferation than conventional mice[42], and are more susceptible to DSS-induced colitis[43]. These observations suggest a link between commensal bacteria and the intestinal epithelial barrier. Commensal-bacteria-derived signals are mainly recognized by pattern recognition receptors (PRRs) on IECs, such as Toll-like receptors (TLR) and NOD receptors[44]. Evidence of the role of commensal bacteria in the regulation of epithelial homeostasis emerged from studies of mice deficient in TLR2, TLR4 and MyD88, an adaptor molecule essential for TLR-mediated signal transduction. $TLR2^{-/-}$, $TLR4^{-/-}$ and $MyD88^{-/-}$ mice showed severe levels of mortality, morbidity, and epithelial injury after administration of DSS[45]. The same effects were also observed in wild-type mice that were devoid of commensal bacteria after treatment with antibiotics. These results suggest that commensal bacterial products confer protection against DSS-induced epithelial destruction in a TLR-dependent manner. In support of this notion, oral administration of ligands for TLR2 and TLR4 was found to completely protect mice from DSS-induced colonic injury in the antibiotic-treated mice[45]. What is the mechanism of the TLR-dependent maintenance of epithelial integrity? TLR2 signaling ensures epithelial integrity by regulating PKCα/β-mediated apical tightening and sealing of zonula occludens 1 (ZO-1), which serves as a major TJ protein[46]. Therefore, TLR2-deficient mice showed higher susceptibility to TJ destruction after the administration of DSS[47]. Conversely, treatment with the TLR2 agonist PCSK in drinking water significantly ameliorated inflammatory symptoms in a DSS-induced colitis model by restoring TJ integrity[47]. Epithelial TLR4 signaling induces the release of EGFR ligands, such as amphiregulin (AR), which can activate EGFR signaling on IECs, leading to the proliferation of epithelial cells[48, 49]. TLR4 signaling also induces the expression of cyclooxygenase 2 (COX2) and the secretion of prostaglandin E_2 (PGE_2), which can further stimulate the expression of AR[44, 48]. This positive feedback loop is thought to be important for the maintenance of rapid epithelial turnover. Furthermore, intestinal epithelial TLR4 signaling facilitates the production of secretory IgA (S-IgA). Transgenic mice expressing an active form of TLR4 in their IECs displayed an increased expression of APRIL (a proliferation-inducing ligand) and chemokines CCL28 by their epithelial cells. This lead to the promotion of immunoglobulin (Ig) class switching to IgA and the recruitment of IgA^+ cells in the gut[50]. These observations demonstrated the contribution of the gut microbiota to the enhancement of the mucosal barrier function.

Commensal bacteria can also secrete AMPs called bacteriocins, which are known to play an important role in the protection of an established microbial community from invasion by other strains or species of bacteria[51]. For example, *Lactobacillus salivarius* strain UCC118 provided protection against *Listeria monocytogenes* infection by producing a bacteriocin Abp118[52], which is active against *L. monocytogenes*[53]. In addition to bacterially derived AMPs, various metabolites have been linked to the epithelial barrier, to immune regulation, and to inflammation. These

metabolites include bile acids, vitamins, and short chain fatty acids (SCFAs), namely acetate, propionate, and butyrate. Certain *Bifidobacterium spp*. actively produce folate (vitamin B9)[54], and its derivative 6-formyl pterin (6-FP), which is one of ligands of the MHC class I-like molecule MR1 that presents an antigen to mucosal-associated invariant T (MAIT) cells[55]. Interestingly, several riboflavin (vitamin B2)-based metabolites, which have structures that are closely related to 6-FP with an extra ribityl moiety, activate MAIT cells to produce TNF and IFN-γ in an MR1-restricted manner. Given that B-group vitamins, including riboflavin, are synthesized *de novo* by certain commensal bacteria, it is possible that MAIT cells will detect the growth of riboflavin-producing bacteria on the mucosa.

Commensal microbe-derived metabolites regulate mucosal barrier functions. For instance, indole, which is a quorum-sensing molecule derived from tryptophan by tryptophanase from the gut microbiota, enhances epithelial barrier function *in vitro* and *in vivo* through upregulation of components of TJ complexes[56, 57]. Treatment of germ-free mice with indole significantly ameliorated DSS-induced colitis[57]. A recent study has shown that indole 3-propionic acid (IPA) is produced by commensal bacteria and regulates intestinal barrier function through pregnane X receptor (PXR). PXR-deficient (Nr1i2$^{-/-}$) mice showed an increased intestinal permeability and were susceptible to intestinal injuries induced by indomethacin, anti-CD3 antibody or LPS[58]. Another study showed that lactobacilli and the metabolite lactic acid trigger epithelial turnover in a starvation-refeeding model[59].

Acetate is another metabolite that enhances gut epithelial barrier function. Germ-free mice succumb to a lethal infection of *Escherichia coli* O157:H7. However, inoculation of these mice with certain bifidobacterial strains prevents *E. coil* O157-induced death[60]. These protective strains efficiently import carbohydrates such as fructose via an ATP-binding-cassette (ABC)-type carbohydrate transporter and actively produce acetate. Bifidobacteria-derived acetate inhibits translocation of luminal Shiga toxin from the gut lumen to the blood by improving epithelial defense function and suppressing colonic inflammation. In addition to barrier enhancement, acetate seems to mediate anti-inflammatory responses in the gut and the other peripheral tissues. Treatment of the drinking water of germ-free mice with acetate significantly improved the inflammatory symptoms induced by DSS treatment[61]. This therapeutic effect of acetate is canceled by the deletion of Gpr43, which serves as a receptor for SCFAs. Furthermore, deficiency of Gpr43 exacerbates the severity of K/BxN-serum-induced arthritis and ovalbumin (OVA)-induced allergic airway inflammation[61]. These observations reflect the biological significance of the acetate-Gpr43 axis in the avoidance of inflammation[62]. Another SCFA, butyrate, also plays a pivotal role in the establishment of mucosal barrier function. An *in vitro* study demonstrated that butyrate enhanced the transepithelial electrical resistance (TER) by facilitating the TJ protein assembly in an AMPK-dependent manner[63]. Butyrate also upregulates the expression of cathelicidin antimicrobial peptides[64] and MUC2 in the epithelial cells of the proximal colon[65]. Recently, Singh et al have shown that activation of Gpr109a, a receptor for butyrate and niacin (Vitamin B3), induces the expression of epithelial IL-18[66], which contribute to inhibit the intestinal inflammation[67]. Gpr109a-deficient mice (Niacr1$^{-/-}$) showed severe inflammation, extensive mucosal damages, decreased expression of TJ protein claudin-3 and increased translocation of bacteria into systemic organs after the treatment with DSS and azoxymethane (AOM)[66]. These observations indicate that the butyrate-Gpr109a axis plays an essential role in the maintenance of epithelial barrier function. Remarkably, SPF mice fed with a diet containing butyrylated resistant starch[68] had elevated expression levels of Ang4, which is a host-defense-related RNase that has antibacterial and antiviral activities (Obata et al, unpublished observation). These findings provide insight into the mode of action through which probiotic bacteria exert a protective effect against pathological infections, and they open up a new question of how bacterial metabolites regulate epithelial barrier function.

Epithelial barrier dysfunction and disease development

Under physiological conditions, the cellular composition of the intestinal epithelium is kept in balance. However, disruption of this homeostatic state has been associated with various gastrointestinal diseases, including ulcerative colitis (UC)[69], experimental colitis induced by DSS[70], and an infection model with *C. rodentium*[71, 72]. These findings raise the possibility that a balanced composition of the four types of IECs is essential for the maintenance of intestinal homeostasis and host defense function. The balance of epithelial cell composition is regulated by the Notch signaling pathway[73]. Notch proteins function as receptors for transmembrane ligands, Jagged and Delta-

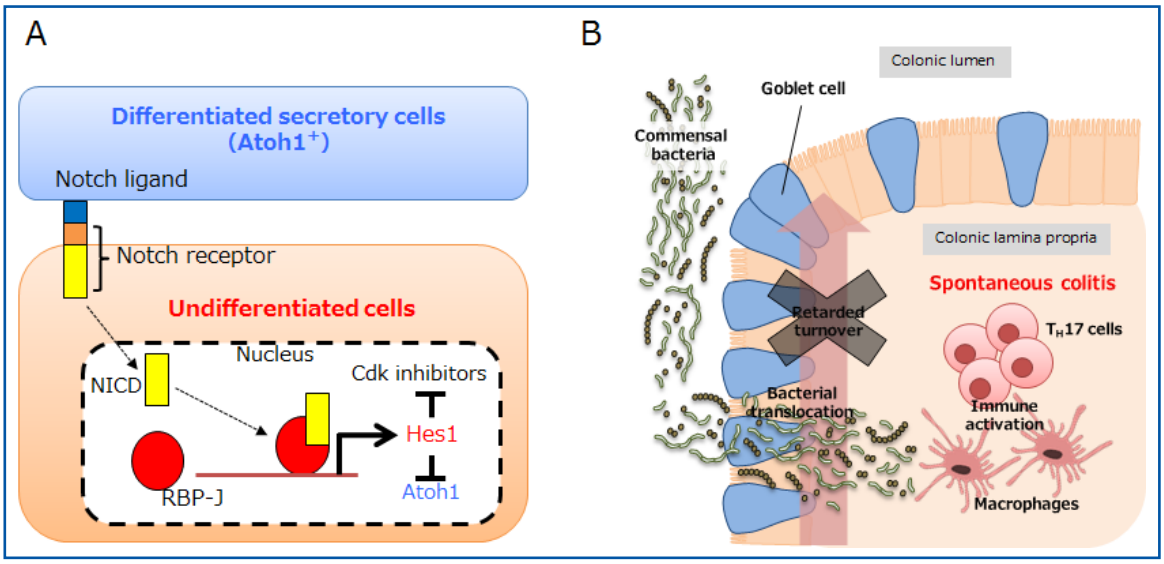

Fig. 2 An essential role of Notch signaling in intestinal immune homeostasis

(A) Schematic model for Notch signaling pathway in IECs. Differentiated secretory cells express Notch ligand, which bind to Notch of adjacent epithelial cells. After the binding, Notch intracellular domain (NICD) translocates into the nucleus and forms a complex with transcription factor RBP-J, which activates the expression of Hes1. Hes1 represses the transcription of Atoh1 and cyclin-dependent kinase (Cdk) inhibitors. Notch activation promotes cell proliferation and inhibits the differentiation of secretory cells. Atoh1: A responsible transcription factor for the differentiation of secretory cells.

(B) Schematic model for spontaneous colitis developed in RBP-JIEC-KO. Commensal bacteria translocate into the colonic lamina propria (LP) due to the retarded epithelial turnover. This leads to the activation of immune cells in the LP, resulting in the chronic inflammation.

like (Dll) proteins, expressed on the surface of differentiated secretory cells and intestinal stromal cells. Upon ligand activation, Notch receptors undergo γ-secretase-mediated proteolytic cleavage to release the Notch intracellular domain (NICD). The NICD is translocated into the nucleus, where it forms a transcriptional activator complex with RBP-J (recombination signal binding protein for immunoglobulin κ J region)[35, 73, 74]. This complex activates the Notch target gene *Hes1*, which in turn represses the expression of *Atoh1*, a master regulator of the differentiation of secretory cell lineages[75, 76]. Hes1 also represses the expression of *Atoh1* as well as the cyclin-dependent kinase (CDK) inhibitors $p27^{Kip1}$ and $p57^{Kip277)}$ (Fig. 2A). Consequently, Notch signaling secures vigorous epithelial proliferation. Therefore, mice with an IEC-specific deletion of *Rbpj* (RBP-J^{IEC-KO}) were found to harbor a greater number of secretory epithelial cells due to the upregulation of *Atoh1*, whilst their epithelial proliferation was defective[35]. Strikingly, RBP-J^{IEC-KO} mice spontaneously developed chronic colitis. Similar symptoms were observed in mice with an IEC-specific deletion of protein *O*-fucosyltranferase 1 (Pofut1^{IEC-KO})[78], which is required for the binding of Notch ligands to Notch receptors[79]. The development of chronic colitis in the RBP-J^{IEC-KO} and Pofut1^{IEC-KO} mice are attributed to an enhanced rate of bacterial translocation into the colonic mucosa due to the impairment of the epithelial integrity and cell proliferation (Fig. 2B).

Mice deficient in an epithelium-specific protein sorting adaptor AP-1B complex were also found to spontaneously develop chronic colitis soon after weaning[80]. AP-1B is responsible for basolateral sorting of certain cytokine receptors such as the interleukin-6 signal transducer (IL-6st) as well as poly-Ig receptor. These receptors are mislocalized in the colonic epithelial cells of AP-1B-deficient mice. These changes probably cause immune dysfunction, namely the reduction of antimicrobial peptide expression due to compromised cytokine responses, and the impairment of S-IgA transcytosis. As a result, the barrier function of the colon is severely affected, leading to the enhanced translocation of bacteria into the mucosa, which causes chronic inflammation in the AP-1B-defiient colon.

These studies demonstrate the physiological significance of epithelial barriers in the maintenance of immunological homeostasis in the gut. The phenotypes of IEC-specific gene-deficient or transgenic mice, as well as the susceptibility of the experimental colitis model, are summarized in the Table 1[7, 70, 78, 80-98].

A recent study indicated that intestinal epithelial barrier

Table 1 The phenotypes of IEC-specific gene-deficient or transgenic mice, as well as the susceptibility of the experimental colitis model

Model	Genotype	Affect of epithelial function	Phenotype	References
Physical barrier				
Dextran sodium sulfate (DSS)-induced colitis	Wild-type	Destruction of colonic epithelial cells	Colitis Loss of goblet cells	(81) (70)
2,4,6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis	Wild-type	Destruction of colonic epithelial cells	Colitis	(81)
JAM-A-deficient mice	*F11r* deficiency	Impaired TJ barrier	High susceptibility to DSS induced colitis Increased IgA level	(82) (83)
FoxO4-deficient mice	*Foxo4* deficiency	Impaired TJ barrier due to reduced expression of ZO-1 and occluding	High susceptibility to TNBS-induced colitis	(84)
Constitutive active MLCK transgenic (Tg) mice	*IECs-specific* Truncated *Mylk transgenic*	Increased intestinal permeability	Increase in the production of inflammatory cytokine by colonic $CD4^+$ T cells; Exacerbation of $CD45RB^{hi}$ transfer colitis	(85)
Chemical barrier				
Muc2-deficient mice	*Muc2* deficiency	Impaired mucin production	Spontaneous colitis	(7)
Winnie mice	*Muc2* mutation	Impaired mucin production ER stress	Spontaneous colitis	(86) (87)
$Dicer^{IEC\text{-}KO}$ mice	IECs-specific *Dicer* deficiency	Loss of goblet cells	Spontaneous colitis	(88)
$XBP1^{IEC\text{-}KO}$ mice	IECs-specific *XBP1* deficiency	ER stress Apoptosis of Paneth cells Loss of mature goblet cells	Spontaneous enteritis High susceptibility to DSS induced colitis	(89)
$C1galt1^{IEC\text{-}KO}$ mice	IECs-specific *C1galt1* deficiency	Impaired of mucus layer	Spontaneous colitis	(90)
$STAT3^{IEC\text{-}KO}$ mice	IECs-specific STAT3 deficiency	Reduction in the IL-22-dependent production of AMPs Impaired epithelial repair	High susceptibility to DSS induced colitis	(91)
AP-1B deficient mice	*Ap1m2* deficiency	Reduction in the production of AMPs; Defect in IgA transcytosis	Th17-dominant spontaneous colitis	(80)
$HDAC3^{IEC\text{-}KO}$	IECs-specific *HDAC3* deficiency	Loss of Paneth cells Increased intestinal permeabilitiy	Spontaneous inflammation Increased susceptibility to DSS induced colitis and *Listeria monocytogenes* infection	(98)
Cell proliferation · Apoptosis · Autophagy				
Tff3-deficient mice	*Tff3* deficiency	Impaired epithelial turnover	High susceptibility to DSS induced colitis	(11)
$Pofut1^{IEC\text{-}KO}$ mice	IECs-specific *Pofut1* deficiency	Defect in the epithelial proliferation Goblet cell hyperplasia Increased intestinal permeability	Spontaneous colitis Alteration of composition of gut microbiota	(78)
$NEMO^{IEC\text{-}KO}$ mice	IECs-specific *kbkg* deficiency	Increase in TNFα-dependent apoptosis Reduction in the production of AMPs	Spontaneous colitis	(92)
$TAK1^{IEC\text{-}KO}$ mice	IECs-specific *Map3k7* deficiency	Increase in TNFα-dependent apoptosis	Spontaneous ileitis and colitis	(93)
$A20^{IEC\text{-}KO}$ mice	IECs-specific *Tnfaip3* deficiency	Increase in TNFα-dependent apoptosis	High susceptibility to DSS induced colitis	(94)
$FADD^{IEC\text{-}KO}$ mice	IECs-specific *Fadd* deficiency	Increase in RIP3-dependent necrosis Loss of Paneth cells	Spontaneous ileitis and colitis	(95) (96)
$Caspase\text{-}8^{IEC\text{-}KO}$ mice	IECs-specific *Casp8* deficiency	Increase in RIP3-dependent necrosis in Paneth cells; Reduction in goblet cells	Spontaneous ileitis	(97)
$RBP\text{-}J^{IEC\text{-}KO}$ mice	IECs-specific *Rbpj* deficiency	Defect in the epithelial proliferation Goblet cell hyperplasia Increased intestinal permeability	Th17-dominant spontaneous colitis	(35)

dysfunction and alteration of the gut microbiota are associated with the pathophysiology of experimental autism spectrum disorder (ASD) model, which is induced by the administration of the immunostimulant polyinosinic-polycytidylic acid (poly I:C) during pregnancy. Abnormal gut microbiota produce autism-associated metabolites, such as 4-ethylphenylsulfate (4EPS). Increased intestinal permeability leads to systemic leakage of these metabolites from intestinal lumen[99]. Oral treatment with *Bacteroides fragilis* corrected the intestinal permeability and microbial composition, and ameliorated the symptoms of ASD[99]. Based on these observations, IECs can be considered as a potential therapeutic target for ASD.

Concluding remarks

The studies highlighted in this review demonstrate the contribution of IECs and the gut microbiota in the maintenance of mucosal barrier function. According to these reports, IECs serve as not only a physical barrier but also a frontline sensor to convert luminal environmental signals into antimicrobial and immunoregulatory responses. Furthermore, commensal-dependent signals including TLR ligands and metabolites also contribute to the enhancement of epithelial barrier function. The importance of epithelial barriers is emphasized by the development of multiple types of disease as a result of the impairment of the integrity of IECs. Hence, IECs can be considered as a novel therapeutic target for these diseases. At this time, the current knowledge about “mucosal barriology” has been mainly derived from studies using experimental animal models. Therefore, in the future we must translate this basic understanding into human systems in order to develop novel IEC-based therapeutics against human diseases.

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Conflict of interests

None

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