

REVIEW

Roles of intestinal epithelial cells in the maintenance of gut homeostasis

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The intestine is a unique organ inhabited by a tremendous number of microorganisms. Intestinal epithelial cells greatly contribute to the maintenance of the symbiotic relationship between gut microbiota and the host by constructing mucosal barriers, secreting various immunological mediators and delivering bacterial antigens. Mucosal barriers, including physical barriers and chemical barriers, spatially segregate gut microbiota and the host immune system to avoid unnecessary immune responses to gut microbes, leading to the intestinal inflammation. In addition, various immunological mediators, including cytokines and chemokines, secreted from intestinal epithelial cells stimulated by gut microbiota modulate host immune responses, maintaining a well-balanced relationship between gut microbes and the host immune system. Therefore, impairment of the innate immune functions of intestinal epithelial cells is associated with intestinal inflammation.

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INTRODUCTION

The gastrointestinal tract is an organ that takes in food, digests it and absorbs food-derived nutrients. Therefore, exogenous microorganisms, such as bacteria, fungi and viruses, can also enter the gut, accompanying food intake. Some of the microorganisms inhabit the intestine symbiotically and form an ecological community called the gut microbiota. However, intestinal microbiota does not just reside inertly in the gut; rather, it confers vital benefits to the host by digesting dietary fibers to short-chain fatty acids (SCFAs) that can be used as an energy source by the host, synthesizing vitamin B and vitamin K, and metabolizing bile acids. Recent evidence has indicated that intestinal microbiota also influences host immunity by directly interacting with host cells or producing several metabolites, including SCFAs and adenosine triphosphate (ATP).^{1,2}

Between intestinal environmental factors including gut microbes and host immunity, there exist intestinal epithelial cells and several mucosal barriers covering epithelial cells, such as the mucus layer containing antimicrobial molecules. Intestinal epithelial cells, which include absorptive epithelial cells, goblet cells and Paneth cells, have two major roles, 'segregation' and 'mediation,' to maintain a healthy relationship between gut microbiota and host immunity.

'Segregation' is defined as the separation of the gut microbiota and host immune cells. Intestinal epithelial cells construct

two types of mucosal barriers, physical barriers and chemical barriers, to spatially segregate gut microbiota in the intestinal lumen and immune cells in the lamina propria. These barriers prevent conflict between gut microbiota and host immune cells that would result in intestinal inflammation.

'Mediation' is defined as the delivery of signals between gut microbes and host immune cells. Intestinal epithelial cells react to gut microbes or their metabolites and produce mediators, including cytokines and chemokines, to induce T-cell immune responses or deliver antigens to antigen-presenting cells (APCs) in lymphoid tissues, contributing to antigen-specific IgA responses and the oral tolerance to food antigens. Activated T cells produce several cytokines, including interleukin (IL)-17 and IL-22, which promote the production of antimicrobial molecules by intestinal epithelial cells to regulate the overgrowth of pathogenic opportunistic microbes.

Inflammatory bowel diseases (IBD) include ulcerative colitis (UC) and Crohn's disease (CD). IBD involves the chronic inflammation of all or part of the digestive tract. Recent evidence has revealed that the dysfunction of intestinal barriers is one of the causes for IBD development. Indeed, the reduced production of mucus or antimicrobial peptides is observed in some IBD patients, and mice genetically defective in mucosal barrier components show a high sensitivity to intestinal inflammation.

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In this review, we focus on the two roles of intestinal epithelial cells, 'segregation' and 'mediation,' in the maintenance of gut homeostasis and the prevention of intestinal inflammation.

MUCOSAL BARRIERS CONSTRUCTED BY INTESTINAL EPITHELIAL CELLS SEGREGATE GUT MICROBES AND THE HOST IMMUNE SYSTEM

Intestinal epithelial cells generate various types of barriers to protect the intestinal mucosa from commensal microbes or invading pathogenic microorganisms. These barriers are divided into two subtypes, physical barriers and chemical barriers.

Physical barriers include the mucus layer covering the intestinal mucosa, the glycocalyx on the microvilli of absorptive intestinal epithelial cells, and the cell junctions firmly linking intestinal epithelial cells. These barriers physically inhibit the invasion of the mucosa by intestinal microorganisms.

Mucus is a viscous fluid secreted by goblet cells that is enriched in mucin glycoproteins that form large net-like polymers. Mucus secretion from goblet cells is regulated by the host sensing gut microbes or their metabolites, such as SCFAs or Th2 cytokines, including IL-5 and IL-13.^{3–6} In addition, recent studies revealed that the activation of the inflammasome mediated by NOD-like receptor family pyrin domain-containing 6 (NLRP6), a member of the NOD-like receptor family, drives mucus granule exocytosis from goblet cells by promoting autophagy.⁷

In the large intestine, where there are tremendous numbers of intestinal bacteria, the number of goblet cells is much higher than in the small intestine. Therefore, the mucus layer in the large intestine is very thick. It is composed of two layers, the inner, firm mucus layer and the outer, loose mucus layer.⁸ These mucus layers are organized by gel-forming MUC2, which is a highly O-glycosylated protein produced by goblet cells. The inner mucus layer is a stratified mucus layer anchored to the intestinal epithelia, which contains polymerized MUC2, and it does not allow microorganisms to easily invade the intestinal epithelia.⁸ Thus, the inner mucus layer is free from intestinal bacteria. Notably, many bacteria are able to invade the colonic epithelia in Muc2-deficient mice lacking the inner mucus layer.⁸ The inner mucus layer is converted into the outer mucus layer by the proteolytic processing of polymerized MUC2 by the host or bacteria. The outer mucus layer is inhabited by a large number of intestinal microbes. These microbes utilize polysaccharides of MUC2 as an energy source; therefore, the absence of dietary fiber, which is the main energy source of intestinal bacteria, leads to the expansion of mucin-degrading species, resulting in the increase of inner mucus degradation.⁹

Regarding the mechanism by which the inner mucus layer segregates intestinal bacteria and epithelial cells in the large intestine, various antimicrobial molecules, including immunoglobulin A (IgA) and the defensin family of proteins, that are transported or produced by intestinal epithelial cells are thought to be involved in the protection against bacterial invasion of the inner mucus layer. However, in the large

intestine, there are no Paneth cells, which is a specialized intestinal epithelial cell type that produces antimicrobial molecules; thus, the expression level of antimicrobial molecules is not high compared with that in the small intestine.¹⁰ It is unclear what molecules are critical in the segregation between intestinal microbes and intestinal epithelia in the large intestine.

In this context, a recent study identified a highly glycosylated Glycosylphosphatidylinositol (GPI)-anchored protein called Ly6/Plaur domain-containing 8 (Lypd8) as a novel molecule contributing to the segregation of intestinal bacteria and intestinal epithelia in the large intestine.¹¹ Lypd8, which is anchored to the intestinal epithelial cells in the uppermost epithelial layer, is constitutively shed into the intestinal lumen and preferentially binds to flagellated bacteria from genera such as *Escherichia*, *Proteus* and *Helicobacter*, thereby inhibiting the bacterial invasion of the colonic epithelia. Mice lacking Lypd8 demonstrate the disappearance of the bacteria-free space just above the epithelial layer of the colon, indicating that Lypd8 is critical for the segregation of intestinal bacteria and colonic epithelia.¹¹

Even if bacteria can penetrate the inner mucus layer, the glycocalyx, a meshwork of carbohydrate moieties of glycolipids or glycoproteins, including transmembrane mucins, faces invading bacteria as a barrier on the epithelial cell surface. In addition, cell junctions, such as the tight and adhesion junctions linking epithelial cells, physically hamper microbial invasion through the paracellular pathway. Recent reports revealed that the expression of cell junction-associated molecules, including occludins and claudins, is upregulated by indole, a metabolite of dietary tryptophan from commensal bacteria possessing tryptophanase after Pregnane X receptor stimulation.^{12,13}

In the small intestine, where there are fewer goblet cells than in the large intestine, chemical barriers, including antimicrobial peptides (AMPs) and the regenerating islet-derived 3 (Reg3) family of proteins produced by intestinal epithelial cells, particularly Paneth cells, have critical roles in the segregation of intestinal bacteria and epithelial cells.^{14,15} AMPs are small, basic amino acid-rich cationic proteins that are evolutionally conserved in a wide range of organisms. They include the defensin family of proteins and cathelicidins, both of which interact with the negatively charged microbial cell membrane and cause membrane disruption by forming pore-like structures.¹⁶ Defensins are classified into α -, β - and θ -defensins, among which α -defensin (cryptdin) mainly protects against pathogenic bacterial infection. Pro-cryptdin is converted into mature-cryptdin by matrix metalloproteinase-7 (MMP-7); therefore, MMP-7-deficient mice lacking mature-cryptdin are highly susceptible to *Salmonella typhimurium* infection.¹⁷

The Reg3 family of proteins was recently identified as novel antimicrobial molecules mainly produced by Paneth cells. In particular, Reg3 γ is active against Gram-positive bacteria and has a critical role in the spatial separation of the intestinal bacteria and intestinal epithelia in the small intestine.^{15,18,19}

Intestinal epithelial cells express pattern recognition receptors, including Toll-like receptors (TLRs) and nucleotide-

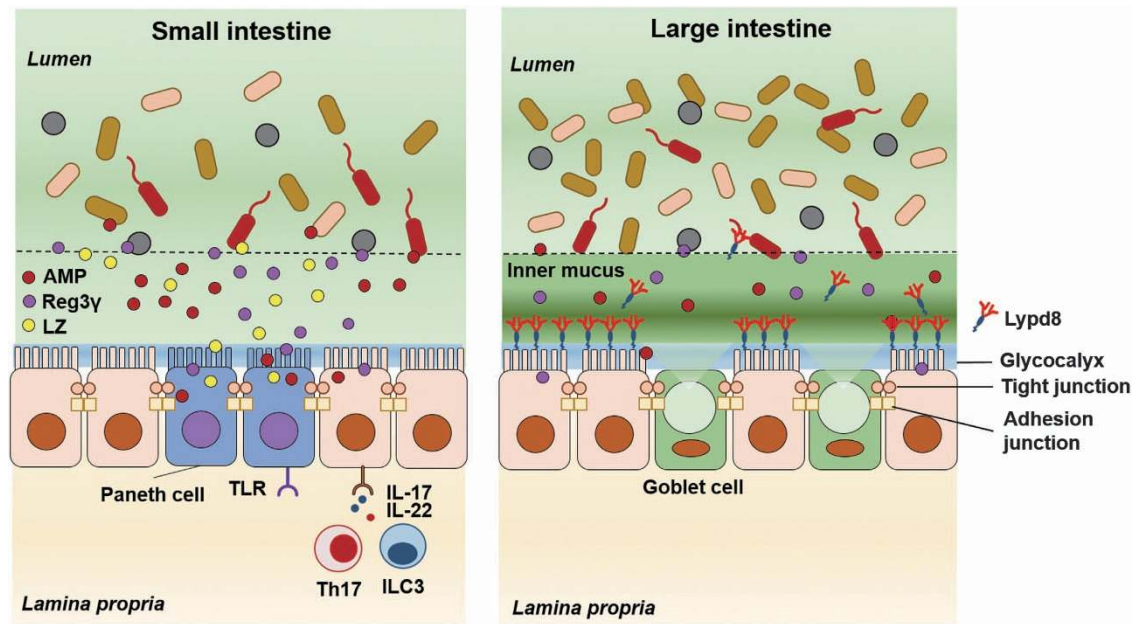


Figure 1 Mucosal barriers in the gut. In the small intestine, chemical barriers, including AMPs produced by Paneth cells, have major roles in the segregation of intestinal bacteria and intestinal epithelial cells. In contrast, in the large intestine, which is inhabited by a tremendous number of bacteria, intestinal bacteria and intestinal epithelial cells are separated by the inner mucus layer containing polymerized MUC2. Lypd8, a GPI-anchored protein expressed in the intestinal bacteria, especially flagellated bacteria. AMP, antimicrobial peptides; LZ, lysozyme; TLR, toll-like receptor; ILC3, type 3 innate lymphoid cell.

binding oligomerization domain-containing proteins (NODs). The production of antimicrobial molecules by Paneth cells is regulated by TLR4/MyD88 signaling and NOD2 signaling driven by gut microorganisms.^{14,15,20} In mice lacking NOD2, which recognizes muramyl dipeptides, which are conserved structures in bacterial peptidoglycans, the expression of defensins is substantially reduced, resulting in high susceptibility to *Listeria monocytogenes* infection.²⁰

Gut immune cells also influence mucosal barriers through the production of cytokines or direct cell–cell contact. IL-17 and IL-22 produced by Th17 cells or type3 innate lymphoid cells (ILC3) upregulate the secretion of AMPs and Reg3 family proteins by intestinal epithelial cells.²¹ In addition, IL-6 derived from intraepithelial lymphocytes enhances intestinal epithelial cell proliferation and contributes to healing from mucosal injury.²² Conversely, other pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α and interferon (IFN)- γ , inhibit epithelial cell proliferation through the suppression of β -catenin/T cell factor (TCF) signaling.²³ In mucosal injury, activated macrophages differentiated from monocytes recruited to the mucosal wound site trigger the colonic epithelial progenitor niche with direct cell–cell contact to promote epithelial regeneration, which helps in the recovery of the mucosal barrier.²⁴ Th2 cytokines, such as IL-5 and IL-13, promote colonic wound healing by inducing the alternative activation of macrophages, which contributes to epithelial cell proliferation.²⁵ In contrast, IL-13, which is upregulated in the colon of patients with UC, has been shown to promote the

apoptosis of intestinal epithelial cells, leading to mucosal barrier disturbance.^{26,27}

As described above, intestinal epithelial cells produce both physical barriers, such as the mucus layer, glycocalyx and cell junctions and chemical barriers, including AMPs and the Reg3 family of proteins, which are regulated by intestinal environmental factors and immune cell-derived cytokines. These barriers ingeniously segregate commensal microbes and host immune cells to prevent unnecessary conflict between symbiotic microbes and host immune cells, thereby maintaining their symbiotic relationship in the intestine (Figure 1).

INTESTINAL EPITHELIAL CELLS MEDIATE THE CROSSTALK BETWEEN GUT MICROBES AND THE HOST IMMUNITY

The intestinal microenvironment, including gut microbiota and their metabolites, is easily and rapidly altered by diet, drugs, stress, infection with bacterial or viral pathogens and even jet lag.^{28–33} Thus, host immunity must adapt to alterations in the gut environment, including dysbiosis and pathogenic bacterial infections. Therefore, intestinal epithelial cells stimulated by gut environmental factors interact with host immune cells and modulate gut immune cell responses.

Segmented filamentous bacteria (SFB) are commensal bacteria found in the mouse or rat intestine, most of which attach to intestinal epithelial cells in the ileum. The attachment of SFB strongly facilitates Th17 cell differentiation by inducing the production of serum amyloid A (SAA) by intestinal

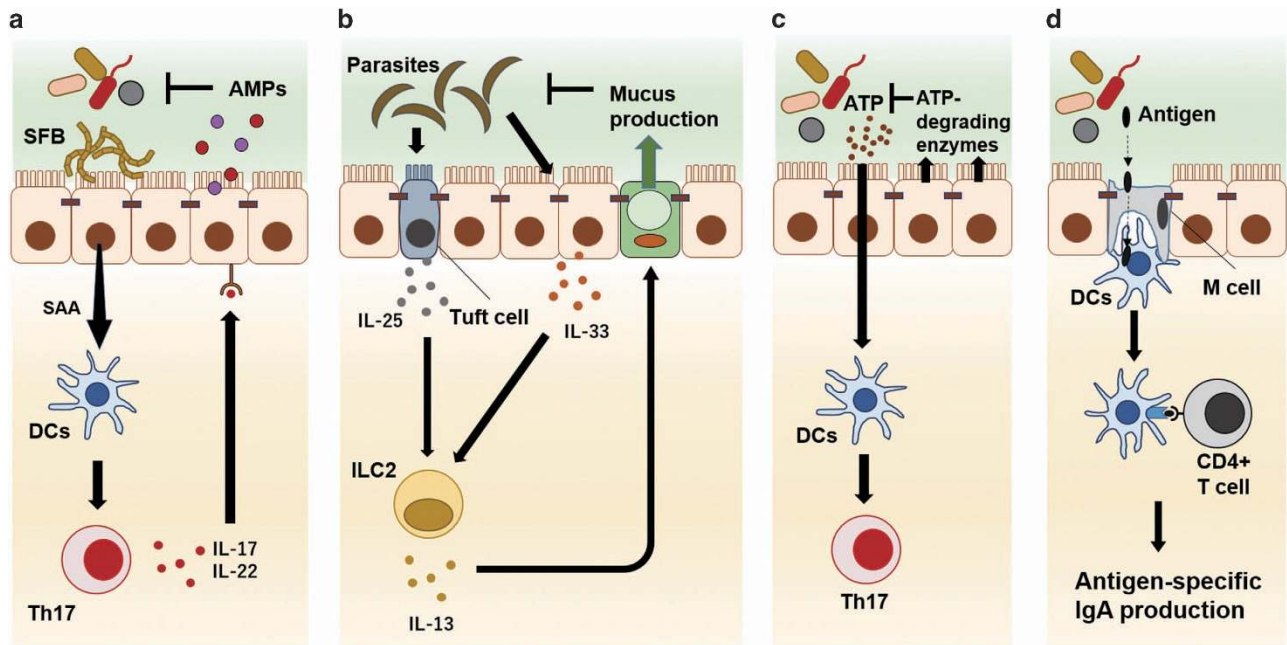


Figure 2 Intestinal epithelial cells mediate the crosstalk between gut microbes and the host immunity. Intestinal epithelial cells stimulated by gut environmental factors modulate gut immune cell responses by secreting humoral factors, such as serum amyloid A (SAA) and several kinds of cytokines (**a**, **b**). Intestinal epithelial cells also inactivate bacteria-derived stimulators to host immune cells, such as adenosine triphosphate (ATP), to regulate excessive host immune responses (**c**). In addition, M cells, which specialize in the uptake and delivery of antigens in the intestinal lumen, contribute to the induction of antigen-specific IgA responses by delivering bacterial or dietary antigens to dendritic cells (DCs) (**d**).

epithelial cells (Figure 2a).^{34,35} In addition, SFB stimulate IL-23 receptor-dependent IL-22 production by activated ILC3.³⁶ Furthermore, the attachment to intestinal epithelial cells of *Citrobacter rodentium*, an enteric pathogen, upregulates the expression of *Nos2*, *Duoxa2* and *Duox2*, which are involved in the production of reactive oxygen species, facilitating Th17 cell differentiation in the colonic lamina propria.³⁵ IL-17 and IL-22 from Th17 cells or ILC3 promote the production of antimicrobial molecules, including AMPs and Reg3 family proteins, to control gut microbiota. IL-22 also affects the glycosylation of intestinal epithelial cell-surface proteins by inducing the expression of fucosyltransferase 2 (Fut2).³⁷ The fucosylation of membrane proteins by Fut2 in intestinal epithelial cells is critical for protection against infection by enteric pathogens, such as *S. typhimurium*.^{37–39}

Intestinal epithelial cells also modulate host immune responses by secreting cytokines and chemokines. The TLR5/MyD88 signaling stimulated by the flagellin proteins of Gram-negative rod bacteria, such as *Escherichia* and *Proteus*, promotes the production of IL-8 by epithelial cells, which recruits neutrophils to the lamina propria.^{40,41} Recent studies demonstrated that tuft cells, taste-chemosensory epithelial cells, contribute to the elimination of parasites by producing IL-25, which activates ILC2 to secrete IL-13 inducing Th2 responses, resulting in an enhancement of tuft cell and goblet cell differentiation (Figure 2b).^{42–44} IL-33, a member of the IL-1 family, which is released from damaged intestinal epithelial

cells, promotes Th2 responses by activating ILC2 to produce IL-5 and IL-13 during parasitic infections (Figure 2b).^{4,45} In addition, recent studies found that IL-33 from intestinal epithelial cells also promotes the suppressive function of colonic regulatory T (T_{reg}) cells expressing ST2, an IL-33 receptor.^{46,47} Thymic stromal lymphopoietin (TSLP), a four-helix bundle cytokine that is produced by intestinal epithelial cells, contributes to protection against helminth infection by inducing Th2 immune responses.⁴⁸ In addition, intestinal epithelial cells drive IgA class switching in B cells present in the intestinal lamina propria by releasing a proliferation-inducing ligand (APRIL) through TLR-inducible signaling.⁴⁹

Intestinal epithelial cells also produce mediators other than cytokines and chemokines that modulate gut immune responses. MUC2, which is produced by goblet cells, not only organizes the mucus layer but also constrains the immunogenicity of gut antigens by delivering tolerogenic signals to dendritic cells (DCs).⁵⁰ Some peptide hormones, including cholecystokinin, glucagon-like peptide (GLP) 1 and 2, and serotonin, are secreted by intestinal endocrine cells in response to luminal nutrients and gut microbiota; they both directly or indirectly affect gut immunity.⁵¹ Cholecystokinin regulates the differentiation and cytokine production of $CD4^{+}$ T cells and B cells, and it also controls macrophage activation by inhibiting inducible nitric oxide synthase (iNOS) production.^{52–55} In addition, cholecystokinin indirectly inhibits the release of

inflammatory cytokines from macrophages by triggering the secretion of acetylcholine (ACh) from vagal afferents.⁵⁶

Recent studies revealed that some metabolites produced by commensal microbes directly influence T cell immune responses. ATP derived from commensal bacteria drives Th17 differentiation through the activation of CD70^{high} CD11b⁺ DCs in the intestine.² In terms of ATP-dependent Th17 cell differentiation, intestinal epithelial cells regulate excessive Th17 cell activation by controlling the ATP concentration in the intestinal lumen through the expression of ATP-degrading enzymes, such as ecto-nucleoside triphosphate diophosphohydrolase (E-NTPD) 7 (Figure 2c).⁵⁷

In addition to the production of humoral factors that regulate gut immunity, intestinal epithelial cells can contribute to gut adaptive immune responses by delivering antigens to gut immune cells. M cells, which are found in follicle-associated epithelia, specialize in the uptake and delivery of antigens from the lumen to APCs, including DCs, and have a critical role in the induction of antigen-specific IgA (Figure 2d).⁵⁸ Glycoprotein 2 (GP2) is a transcytotic receptor on M cells that participates in the uptake of bacterial antigens; therefore, antigen-specific IgA responses are attenuated in mice lacking GP2.^{59,60} Recent reports indicate that Spi-B, an Ets transcription factor, is a master regulator for M cell functional and structural maturation, and M cell-dependent T cell activation in Spi-B-deficient mice is impaired.^{61,62}

Thus, between intestinal environmental factors and gut immunity, intestinal epithelial cells operate as sensors to signals from gut microbes and host immune cells and mediate the balance between both players by secreting cytokines, chemokines and hormones.

INTESTINAL INFLAMMATION CAUSED BY THE DYSFUNCTION OF MUCOSAL BARRIERS

IBD is a group of intractable diseases characterized by chronic inflammation of the digestive tract and includes UC and CD. The incidence and prevalence of IBD are increasing with time and in different regions around the world, indicating that the elucidation of the pathogenesis of IBD is a matter of great urgency.⁶³ Recent evidence has revealed that both gut environmental factors and host immune dysfunction based on genetic predisposition contribute to the development of IBD.⁶⁴ With regard to genetic predisposition underlying the pathogenesis of IBD, recent genome-wide association studies using next-generation sequencing technology identified various IBD susceptibility genes, which include the mucosal barrier-related genes *FUT2*, *MUC19* and *NOD2*.^{65–68} Indeed, the reduced production of mucosal barrier-related molecules, such as AMPs and mucins, is observed in the intestines of IBD patients.⁶⁹ In addition, the expression of LYPD8, which inhibits bacterial invasion in the colon, was found to be severely decreased in the colonic mucosa of some UC patients.¹¹

Many studies using genetically modified mice with mucosal barrier dysfunction have demonstrated the relationship between mucosal barrier dysfunction and intestinal

inflammation. Mice lacking MUC2 are missing the inner mucus layer physically segregating gut microbes and colonic epithelia and develop spontaneous colitis because of the bacterial invasion of the colonic mucosa.^{8,70} In accordance with the deficiency of MUC2, the lack of cooperation of core 1 synthase (C1galt), which synthesizes the major constituent of the O-glycan core structure, allows bacteria to penetrate the inner mucus layer, resulting in spontaneous colitis. Mice devoid of Fut2, which mediates the transfer of fucoses to the terminal galactose on glycans in cell-surface glycoproteins, are highly susceptible to *S. typhimurium* infection.³⁷ In mice deficient in Lypd8, a larger number of flagellated bacteria, such as *Proteus* and *Escherichia*, can invade the inner mucus layer, which causes high susceptibility to dextran sulfate sodium (DSS)-induced intestinal inflammation.¹¹ In mice lacking NLRP6, an inflammasome-associated molecule, mucus secretion from goblet cells is impaired, and these mice show the disappearance of the bacteria-free zone in the inner mucus layer and thus are highly sensitive to DSS-induced intestinal inflammation and infection with bacterial pathogens such as *Citrobacter rodentium*.^{7,71} Interestingly, wild-type mice cohoused with NLRP6-deficient mice, which have dysbiosis of the intestine, also show high susceptibility to DSS-induced intestinal inflammation, indicating colitogenic dysbiosis is transmissible between individuals.⁷¹ The dysfunction of cell junctions also causes intestinal inflammation. The intestinal deletion of Claudin-7, which is an important component of tight junctions, enhances paracellular organic solute flux and causes colitis in mice.⁷²

The impairment of chemical barriers also causes high susceptibility to intestinal inflammation. Mice lacking MyD88 in intestinal epithelial cells show decreased production of AMPs and mucus by intestinal epithelial cells, and consequently, they are highly susceptible to experimental colitis and enteric bacterial infection.^{73,74} Mice deficient in IL-22 that promotes the production of AMPs by intestinal epithelial cells, also show a high sensitivity for DSS colitis.⁷⁵ In addition, intestinal epithelial cell-specific inhibition of nuclear factor (NF)- κ B through the conditional ablation of NEMO, an I κ B kinase subunit essential for NF- κ B activation, causes the spontaneous development of intestinal inflammation in mice because of the impaired expression of antimicrobial peptides and bacterial translocation into the colonic mucosa.⁷⁶ Mice deficient in NOD2, which is a susceptibility gene for CD, do not show spontaneous intestinal inflammation but show severe Th1-driven granulomatous inflammation of the ileum induced by *Helicobacter hepaticus* because of the reduced production of AMPs by Paneth cells.^{77–79} Deficiency in adaptor protein (AP)-1B, which mediates the sorting of membrane proteins, causes epithelial immune dysfunction, such as the reduced expression of antimicrobial proteins and the impaired secretion of IgA, leading to chronic colitis with an enhanced Th17 response.⁸⁰

It has been recently indicated that mucosal barrier dysfunction based on genetic predisposition leads to the alteration of gut microbiota composition, dysbiosis, and enhanced

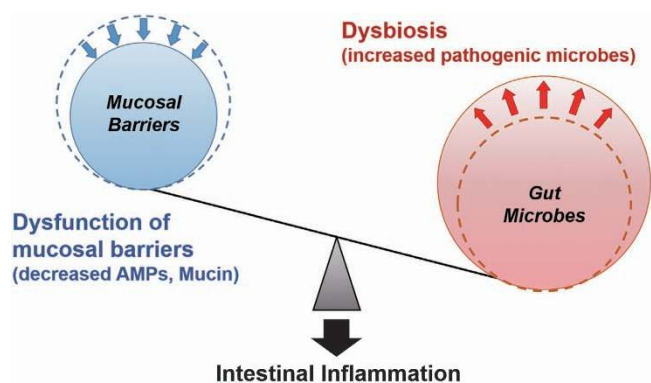


Figure 3 The imbalance between mucosal barriers and gut microbes promotes susceptibility to intestinal inflammation. Dysfunction of mucosal barriers because of genetic predisposition, such as the decreased production of AMPs and mucin, allows intestinal bacteria to gain access to gut immune cells, thereby contributing to the development of intestinal inflammation. Dysbiosis induced by environmental factors, such as a high-fat diet and various medicines, accelerates intestinal inflammation in situations in which the mucosal barrier is disrupted. AMPs, antimicrobial peptides.

susceptibility to intestinal inflammation and the infection of pathogens. Furthermore, dysbiosis induced by environmental factors, such as a high-fat diet and some medicines, accelerates intestinal inflammation in genetically predisposed individuals (Figure 3).

CONCLUSIONS

Intestinal epithelial cells create various kinds of mucosal barriers to 'segregate' gut microbes and gut immune cells, and sense signals from both populations and secrete humoral factors to 'mediate' the balance between both populations, both of which contribute to the maintenance of gut homeostasis. Accordingly, defects in these functions of intestinal epithelial cells in mice promote the development of disorders demonstrating intestinal inflammation, similar to human IBD. Thus, the dysfunction of the intestinal mucosal barrier system is thought to cause IBD. At present, therapies for intractable IBD are limited, and several different therapies, including immunosuppressive treatment, are required. A more detailed knowledge of the mechanisms regulating gut mucosal barrier system will certainly lead to novel therapeutic approaches for IBD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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