Advances in Research on Intestinal Mucus Barrier Function: Postprint

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Abstract

The mammalian intestine harbors multiple defense mechanisms that protect the host from pathogenic insults while simultaneously preventing aberrant responses to the endogenous gut microbiota. Intestine-secreted mucus constitutes the first line of defense among these protective mechanisms. Through its continuous secretion, the mucus layer separates bacteria from epithelial cells and facilitates bacterial clearance, thereby mitigating intestinal inflammation and enteric infections. Recent studies have revealed that pathogenic microorganisms have evolved sophisticated mechanisms to circumvent this mucus-based protective system. This review synthesizes recent research advances on the fundamental properties of the intestinal mucus barrier and its multifaceted roles in establishing commensal microbiota, defending against pathogenic colonization and invasion, thereby providing insights for a deeper understanding of the mucus barrier' s contribution to intestinal homeostasis.

Full Text

Research Progress on Intestinal Mucus Barrier Function

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Abstract: Mammalian intestines possess multiple defense mechanisms to protect the organism from pathogenic invasion while preventing aberrant responses to the commensal microbiota. Intestinal mucus serves as the first line of defense among these mechanisms. Through continuous secretion, the mucus layer separates bacteria from epithelial cells and facilitates bacterial clearance, thereby reducing intestinal inflammation and enterogenic infections. Recent studies

have revealed that pathogenic microorganisms have evolved sophisticated mechanisms to bypass this mucus protective system. This review summarizes research progress on the fundamental characteristics of the intestinal mucus barrier and its roles in establishing commensal microbiota and defending against pathogenic colonization and invasion, providing insights for better understanding the mucus barrier' s function in maintaining intestinal health.

Keywords: intestinal mucus; barrier function; intestinal microbiota; inflammation

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The intestine represents one of the largest and most complex organs in mammals, serving not only digestive and absorptive functions but also providing a mucosal barrier that prevents bacterial translocation and endotoxin invasion, thereby maintaining internal homeostasis and normal physiological activities. The intestinal mucosa forms a sophisticated structure separating the internal environment from the intestinal lumen, comprising mechanical, chemical, biological, and immunological barriers. Once this barrier' s integrity is compromised, exogenous harmful substances (bacteria, toxins, etc.) can invade host intestinal tissues, causing inflammation and tissue damage. The chemical barrier primarily consists of the mucus layer covering intestinal epithelial cells, digestive fluids, and antimicrobial peptides—collectively termed the mucus barrier. This barrier separates bacteria from the intestinal mucosa, preventing direct bacterial contact with epithelial cell surfaces and forming the first line of innate defense. It functions to block pathogenic microbial colonization and invasion, establish commensal microbiota, prevent damage, and provide immunological defense, effectively maintaining intestinal homeostasis and health. Disruption of intestinal homeostasis can impair the mucus barrier, and increased permeability may ultimately lead to intestinal mucosal inflammation and injury. The dynamic equilibrium among the mucus layer, intestinal epithelial cells, microorganisms, and host immune defense represents the key to maintaining intestinal homeostasis.

1.1 Composition of Intestinal Mucus

Intestinal epithelial cells, goblet cells, Paneth cells, and enteroendocrine cells constitute the main cell types of the intestinal epithelium. Intestinal mucus primarily comprises mucins secreted by goblet cells, along with approximately 2% lipids and 90-95% salts, cells, electrolytes, other cellular debris, and water. In addition to mucins, goblet cells secrete bioactive factors including trefoil factors, resistance molecules, -defensins, and Fc- binding proteins. These molecules are secreted through two main pathways: constitutive and regulated secretion. Constitutive secretion depends on cytoskeletal movement of secretory granules and exhibits low-level continuous secretion, whereas regulated secretion involves exocytosis triggered by external granule stimulation. The structural and functional integrity of intestinal mucosal epithelial cells depends on coordinated regula-

tion of the mucus layer, intercellular tight junctions, and host adaptive immune responses.

After secretion from goblet cells, mucus rapidly expands to form a dense layer attached to the epithelium, isolating luminal parasites from epithelial cells and preventing bacteria from reaching the epithelial surface. The mucus layer gradually thickens from the small intestine to the colon. Since the small intestine' s primary function is nutrient absorption, a thick mucus layer would hinder nutrient uptake, resulting in a relatively thin mucus layer in this region. Small intestinal mucus contains antimicrobial substances such as defensins and secretory immunoglobulin A (sIgA) that inhibit bacterial contact with epithelial cells. Studies using mechanical spectroscopy on porcine small intestinal and colonic mucus gels have revealed that both secretions exhibit viscoelastic gel properties, though small intestinal mucus is less robust. Small intestinal mucus gels are readily disrupted by acids, detergents, and protein denaturants, whereas colonic mucus remains relatively stable.

1.2 Structure of Intestinal Mucus

Intestinal mucus secreted by goblet cells, together with water, inorganic salts, and antimicrobial peptides, forms the intestinal mucus barrier, which spatially presents as a viscous gel-like network structure. The intestinal mucus can be broadly divided into inner and outer layers. The outer layer, facing the intestinal lumen, serves as the colonization site for commensal bacteria and is termed the loose mucus layer. The inner layer is the firmly attached mucus layer that acts as a "filter" to prevent microbial penetration toward intestinal epithelial cells. Both layers share similar components, but the inner layer is more dense. Through proteolytic action, the inner mucin network structure becomes diluted and transitions into the outer loose mucus layer.

Mucins represent the main component of intestinal mucus. Based on structure and localization, mucins can be classified as secreted or membrane-bound. The secreted mucins forming the intestinal mucus gel consist of five oligomeric mucins (Muc2, Muc5AC, Muc5B, Muc6, and Muc19) and one non-oligomeric mucin, Muc7. Except for Muc19, the other four are clustered on chromosome 11p15.5. In both the small intestine and colon, Muc2 is the predominant secreted protein and was the first secreted mucin identified. The major intestinal mucin Muc2 is a massive O-glycosylated gel-forming protein containing a highly glycosylated central region of variable length. Through C-terminal dimerization and N-terminal trimerization, it forms an enormous dense two-dimensional network structure, with each mucin monomer linked by disulfide bonds. All mucins possess long, elastic "PTS" domains (comprising proline, threonine, and serine). Threonine and serine O-linked glycans are heavily glycosylated, accounting for 40-80% of the mucin weight. Most glycans terminate with negatively charged carboxyl groups (sialic acid), and each mucin monomer is attached to negatively charged sulfate groups, rendering the PTS domain highly negatively charged.

1.3 Barrier Properties of Intestinal Mucus

Goblet cells continuously secrete intestinal mucus daily, with nearly 10 liters of mucus secreted into the human gastrointestinal tract each day. The mucus layers are thickest in the stomach (approximately 180 m) and colon (110-160 m). The mucus layer functions as a dynamic semipermeable barrier that enables exchange of nutrients, water, gases, hormones, and other substances while obstructing most bacteria and various pathogens. Understanding this dynamic barrier function is crucial for establishing pathways for drug transport across intestinal mucosa and nutrient translocation. Florey et al. reported that when India ink particles were injected into cat stomachs, the particles gradually coalesced into small clumps due to mucus entrapment and were ultimately firmly fixed in feces. Gruber et al. observed various microparticles in the gastrointestinal tract and concluded that regardless of size, density, or composition, all become "clumps" covered by mucus. Intestinal mucus can encapsulate particulate matter, preventing direct contact with epithelial cells. The intestinal mucus layer isolates the organism from the external environment, forming a barrier that resists bacterial, antigenic, and toxin invasion while assisting in water, ion, and nutrient uptake. Early studies indicated that small molecules diffuse more easily through mucus, whereas larger molecules such as globular proteins cannot reach epithelial cells through intestinal mucus. Recent work has demonstrated that particles much larger than digestive enzymes, even nanoparticles exceeding 500 nm, can diffuse through the mucus gel to epithelial cells, proving that particle penetration through the mucus layer depends on both size and charge.

Intestinal barrier function regulation primarily depends on the paracellular pathway. The spaces connecting adjacent epithelial cells selectively control passive diffusion of ions and other small solutes through paracellular routes, making intercellular tight junctions crucial for maintaining intestinal barrier integrity. Tight junctions are multi-protein complexes composed of transmembrane proteins and regulatory molecules (including kinases) that are selective regarding the size and charge of diffusing substances. Generally, diffusion rates decrease with increasing particle size. Studies have shown that capsid viruses can readily pass through the mucus layer, whereas polystyrene particles of the same size cannot. However, when polystyrene particles are densely coated with polyethylene glycol, they can diffuse through mucus to epithelial cells. Capsid viruses possess a net neutral surface that neither repels nor attracts the negatively charged glycan domains of mucins and lack exposed hydrophobic surface groups, enabling smooth passage through the mucus layer. In contrast, polystyrene particles with hydrophobic surfaces are blocked. Mucus viscoelasticity is key to mucociliary transport and is regulated by ionic environment changes that modulate mucus hydration. Moderate increases in viscoelasticity can significantly impair ciliary clearance of respiratory mucus and hinder bacterial motility, while excessive viscoelasticity impedes nutrient delivery. Conversely, moderate reductions in viscoelasticity promote bacterial movement, but excessively low viscoelasticity weakens the barrier against pathogens. Therefore, the selective properties of

mucus relate to particle charge polarity, particle size, and mucus viscoelasticity.

2.1 Bacteria in Intestinal Mucus

The mammalian intestine provides an excellent environment for various facultative and strict anaerobes to survive, metabolize, and reproduce, with composition influenced by intrinsic factors (host genetics) and extrinsic factors (diet, environment, stress). These resident or transient bacteria metabolize nutrients within the intestine, simultaneously influencing metabolism of luminal components, intestinal tissue cell renewal, and immune responses. The intestinal mucosa represents the body' s largest immune system, where anaerobes and facultative anaerobes can induce mucosal immune responses upon pathogen invasion, enhancing host resistance and preventing intestinal damage. Nutrient supply, energy metabolism, immune function, and inflammatory development are all linked to metabolites or signaling molecules produced through interactions between bacteria and the intestine or among bacteria themselves.

The human gut harbors enormous bacterial populations, reaching ten times the number of host cells, though small intestinal microbiota exhibit lower diversity and stability. Under normal physiological conditions, these bacteria reside in the intestine in a symbiotic relationship with humans. In the colon, bacteria colonize the outer mucus layer, using mucin glycans as attachment sites and nutrient sources. Bacteria can utilize various glycans in the intestine, providing microbes with a stable energy source; indeed, mucin glycans serve as the sole energy source for certain bacteria. Most bacteria preferentially utilize various undigested dietary polysaccharides, and changes in food composition promote expansion of bacteria capable of using mucin glycans, thereby influencing intestinal bacterial composition. In populations consuming fiber-rich diets, intestinal bacteria must rely more heavily on mucin glycans as an energy source, impacting mucus homeostasis.

Mucus also influences bacterial function in the intestine. Motile bacteria can maintain planktonic and mobile states within mucus, preventing biofilm formation and adhesion to underlying surfaces. Maintaining intestinal mucosal homeostasis represents an adaptive strategy of the gut microbiota. The stable intestinal mucosa provides a convenient long-term habitat for these microorganisms, while microbes reciprocally maintain intercellular connections and promote intestinal epithelial repair to preserve barrier integrity. Studies on porcine intestinal mucus layers have found that mucus thickness significantly affects mucosal adhesion function; thicker mucus increases diffusion of exogenous polymer chains and entanglement with various mucus molecules, though many other factors also influence this process.

2.2 Bacterial Influence on Small Intestinal Mucus Development

Under normal physiological conditions, small intestinal mucus does not completely adhere to the epithelium, whereas in germ-free conditions, it cannot be

completely removed from the epithelium. The normal intestinal lumen harbors abundant bacteria that serve as the primary stimulus for sIgA production by intestinal mucosa. Research has shown that both commensal and exogenous pathogenic bacteria stimulate intestinal mucosal sIgA secretion. Following bacterial colonization in germ-free animals, defensins and sIgA are secreted within the first few weeks. After four weeks of colonization, small intestinal bacterial composition changes significantly, with decreased relative abundance of Clostridium, increased Bacteroides and Bacillus, and undetectable segmented filamentous bacteria. After four weeks, mucus structure transitions to the normal two-layer configuration, and microbial composition resembles that of wild-type animals, indicating that dynamic mucus secretion maintains small intestinal microenvironment homeostasis. Studies in Muc2-deficient mice have also demonstrated mucus importance in the small intestine; these mice exhibit increased intestinal bacteria, causing dynamic changes in gut microbiota that lead to tumorigenesis and inflammation.

2.3 Bacterial Influence on Colonic Mucus Development

For bacteria or similarly sized particles, colonic mucus is generally impenetrable. However, under germ-free conditions, such particles can penetrate the mucus to reach epithelial cell surfaces. In germ-free mice, colonic mucus only becomes impenetrable after five weeks of bacterial colonization. While gut microbiota in germ-free animals can normalize approximately two weeks after bacterial colonization, normal mucus systems and stable colonic microbial communities require approximately eight weeks or longer to establish. Changes in intestinal bacterial composition and mucus first appear in the small intestine, followed by the colon, indicating that the small intestine plays an important role in bacterial selection. Recent research suggests that only certain bacterial types possess mucus-stimulating properties, and bacterial effects on host mucus properties may be mediated by small compounds diffusing across the inner mucus layer.

2.4 Bacterial Products Affecting Mucus Production

Toll-like receptors (TLRs) are key regulators between microorganisms and hosts, playing important roles in promoting homeostasis and mucus formation. In hosts, bacterial cell wall component peptidoglycan can maintain tight junctions and reduce apoptosis through TLR2 signaling pathways, thereby promoting intestinal epithelial integrity. Other microbial components have also been shown to maintain intestinal epithelial homeostasis and repair mucosal damage through TLR pathway signaling. Bacterial metabolites such as short-chain fatty acids (SCFAs) also influence intestinal epithelial barrier protection. In the distal colon, butyrate provides an energy source for goblet cells, maintaining Muc2 secretion. As a major component of intestinal SCFAs and the preferred energy source for colonic epithelial cells, reduced butyrate absorption correlates with intestinal inflammation and carcinogenesis.

2.5 Intestinal Pathogens and Mucus

Gastrointestinal mucus is continuously secreted, particularly evident in the small intestine. As the body' s absorptive organ, the small intestine requires a loose and permeable mucus layer to facilitate nutrient absorption. Bacteria can interact with mucus through several mechanisms to prevent epithelial contact. First, despite larger mesh pores in small intestinal mucus compared to colonic mucus, bacteria can still become trapped. Second, although mucins are largely hydrophilic due to glycan modifications, certain structures confer hydrophobic properties that primarily account for mucus viscosity. Third, polymorphic and variable mucin glycans bind bacteria bearing specific adhesins for these glycan structures. However, these mechanisms are imperfect, as pathogens have evolved evasion strategies. For example, Salmonella enterica serovar Typhimurium, *Shigella*, and *Vibrio cholerae* penetrate the mucus layer through flagellar motility and proteolytic degradation to reach the epithelium, while *Listeria* exploits rapidly secreting goblet cells for transcytosis to penetrate the mucus layer. The colonic mucus layer serves as an effective physical barrier against both commensal bacteria and pathogens. Th2 cell-mediated goblet cell proliferation demonstrates mucus importance in pathogen expulsion during helminth co-infection. For colonic pathogens, the inner mucus layer presents challenges to maintaining intestinal barrier stability. Current research has limited understanding of this process, but in murine models of *Citrobacter rodentium* infection, bacteria persist in the colonic inner mucus layer through mechanisms similar to those described for *Helicobacter pylori*. Additionally, larger parasites such as *Entamoeba histolytica* can degrade Muc2 proteins through hydrolysis, disrupting colonic mucus and enabling parasite penetration of the inner mucus layer and invasion of intestinal epithelium.

3.1 Role of Bacteria in Inflammation

Both inner and outer layers of gastrointestinal mucus contain abundant Muc2 mucins. The dense inner mucus layer adheres to epithelial cell surfaces, while the outer mucus layer represents a soluble, less organized structure derived from the inner layer through proteolytic action that provides a habitat for commensal bacteria. Intestinal microorganisms first contact the outer mucus layer but are blocked by the inner mucus layer, demonstrating that the dense inner mucus layer protects epithelial cells from damage by preventing bacterial adhesion. Oligosaccharide Muc2 mucins in the outer mucus layer provide an energy source for microbial colonization. Muc2-deficient mice exhibit thinner, more permeable intestinal mucus layers with increased bacterial migration to epithelial cells, predisposing to intestinal inflammation. Goblet cells, the primary secretory cells of intestinal mucus, have functions influenced by multiple factors that can alter mucus barrier integrity. Intestinal microorganisms, microbial toxins, and certain cytokines can stimulate or inhibit goblet cell mucus synthesis and secretion, altering mucus chemical composition or causing mucus layer degradation that impairs barrier function. Once damaged, the mucus barrier can

lead to various chronic inflammatory diseases. During pathogenic infection or ischemia, intestinal bacteria penetrate the inner mucus layer to contact epithelial cells, activating host immune responses and inducing inflammation. Studies in spontaneous colitis animal models with Muc2 expression defects show that lack of mucus layer protection allows frequent bacterial penetration into intestinal epithelium or crypt structures, triggering intestinal inflammation and even tumorigenesis. Research indicates that germ-free mice have relatively thinner inner mucus layers compared to conventionally raised mice, but exposure of germ-free mouse intestinal mucosa to bacterial products (peptidoglycan or lipopolysaccharide) rapidly restores inner mucus layer thickness to levels comparable to conventionally raised mice, while these bacterial products have no effect on mucus layer thickness in conventionally raised mice. The dextran sulfate sodium (DSS)-induced murine colitis model, commonly used to study colitis pathogenesis, operates on the principle that bacterial penetration of the inner mucus layer and contact with intestinal epithelium induces colitis within 3-5 days, demonstrating the protective importance of the inner mucus layer. Muc2-deficient mice lacking mucus secretion develop diarrhea, colonic bleeding, rectal prolapse, and epithelial hyperproliferation. Furthermore, direct bacterial contact with intestinal epithelium readily triggers intestinal mucosal immune responses, causing pronounced immune reactions.

3.2 Intestinal Mucus and Inflammatory Bowel Disease

Inflammatory bowel disease primarily includes ulcerative colitis (UC) and Crohn' s disease (CD). Classical Crohn' s disease is associated with nucleotide-binding oligomerization domain protein 2 (NOD2) and ATG16L1 protein function, whereas ulcerative colitis pathogenesis remains less clearly understood. Current evidence suggests UC has individual predisposition and develops through combined environmental factors and microbial exposure. Studies indicate that UC patients have reduced rectal adherent mucus layers compared to normal controls, while CD patients exhibit thicker mucus layers with abundant bacterial colonization in the distal colon. Normally, these bacteria do not cause inflammatory responses, but under certain circumstances, they can trigger excessive immune activation leading to severe inflammation.

In murine models with deficiencies in Muc2, core 1 O-glycans, TLR5, interleukin-10 (IL-10), solute carrier family 9 member A3 (SLC9A3), and DSS-induced ulcerative colitis models, bacterial invasion of intestinal epithelial cells is observed. In IL-10 knockout mice (with blocked inflammatory processes), intestinal mucus layer thickness is significantly higher than in wild-type mice, but mucus quality is altered, demonstrating that both Muc2 quantity and quality are crucial for mucus layer protective properties. In vivo experiments using these colitis models reveal bacterial mucus penetration, while in vitro studies using fluorescent microspheres show they can penetrate inflamed intestinal mucus layers. In normal human colonic biopsy specimens, mucus layers clearly separate bacteria or fluorescent microspheres from epithelial cells, whereas in ulcerative colitis or acute

enteritis patients, bacterial mucus penetration is observed. In healthy individuals, a non-penetrable mucus layer exists on the sigmoid colon mucosal surface, while in spontaneous colitis mouse models and ulcerative colitis patients, colonic mucus layers become thin or disappear, allowing bacterial epithelial invasion and damage. Therefore, Muc2 alterations contribute to inflammatory bowel disease, while inflammatory bowel disease also affects mucin expression and goblet cell differentiation, with their reciprocal interactions influencing disease pathogenesis. Phospholipids constitute a small portion of mucus lipids, with phosphatidylcholine accounting for 90% of phospholipid components. Phosphatidylcholine forms a hydrophobic lamellar structure on hydrated mucus surfaces that prevents bacterial invasion. In ulcerative colitis patients, phosphatidylcholine is significantly reduced in mucus layers regardless of inflammation status, and oral administration of delayed-release phosphatidylcholine improves intestinal inflammation and induces remission. Supplementing sIgA, an important antibody component of the intestinal mucus layer, can also enhance mucus layer protection.

4 Conclusion

The intestine harbors the largest microecological environment in mammals, making normal or dysbiotic intestinal ecology critically important for organismal health and longevity. Mucins secreted by intestinal goblet cells constitute the main component of the intestinal mucus barrier, providing intestinal protection. The outer mucus layer provides a suitable symbiotic environment for intestinal microorganisms, while the inner mucus layer prevents microbial penetration to intestinal epithelium and crypt regions through its dense network structure, effectively maintaining dynamic equilibrium between intestinal microorganisms and the host. Mucus barrier defects can increase intestinal permeability, causing inflammatory damage to intestinal epithelial cells. A deeper understanding of intestinal mucus characteristics and its relationship with intestinal inflammation, further investigation of mucus barrier damage mechanisms, and enhanced mucus barrier protection may provide new therapeutic strategies for intestinal inflammatory diseases. Additionally, the physiological and biochemical properties of mucus can be utilized to promote nutrient absorption and prevent pathogenic damage, or to modify nutrient absorption states in the intestine by adjusting food texture and composition.

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