Microbial modulation of innate defense: goblet cells and the intestinal mucus layer

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ABSTRACT The gastrointestinal epithelium is covered by a protective mucus gel composed predominantly of mucin glycoproteins that are synthesized and secreted by goblet cells. Changes in goblet cell functions and in the chemical composition of intestinal mucus are detected in response to a broad range of luminal insults, including alterations of the normal microbiota. However, the regulatory networks that mediate goblet cell responses to intestinal insults are poorly defined. The present review summarizes the results of developmental, gnotobiotic, and in vitro studies that showed alterations in mucin gene expression, mucus composition, or mucus secretion in response to intestinal microbes or host-derived inflammatory mediators. The dynamic nature of the mucus layer is shown. Available data indicate that intestinal microbes may affect goblet cell dynamics and the mucus layer directly via the local release of bioactive factors or indirectly via activation of host immune cells. A precise definition of the regulatory networks that interface with goblet cells may have broad biomedical applications because mucus alterations appear to characterize most diseases of mucosal tissues. Am J Clin Nutr 2001;73(suppl):1131S–41S.

KEY WORDS Host-microbe interactions, intestinal innate defense, intestinal microbiota, goblet cells, mucins, mucus

INTRODUCTION

The intestinal microbiota serves as a primary stimulus for the development of both innate and acquired components of the mucosal immune system (1). Indeed, much of the structure and many of the functions of the mammalian intestine seem to have evolved to enable the host to tolerate the antigenic and chemical challenges associated with the permanent carriage of a complex microbiota. Innate and acquired components of the intestinal immune system interact to mediate homeostasis between the microbiota and the host under normal conditions. However, the antigenic nature of indigenous bacteria and the sensitive nature of host cells contribute to a precarious relation. A state of détente is evident.

The intestinal epithelium, or, more precisely, the overlying mucus gel layer, is the anatomical site at which the host first encounters gut bacteria. The protective functions of mucus are most often considered in the context of its chemical and physical properties and are viewed as static and constitutive. The concept of the mucus layer functioning as a dynamic defensive barrier is suggested by studies showing altered mucus-related indexes in germfree animals (1–6) and from consistent evidence of enhanced mucus secretion in response to intestinal microbes (7–9). Ontogenic changes in the composition of intestinal mucus that correlate with successional changes in the indigenous microbiota and with regional maturation of acquired immune functions are also consistent with the mucus layer playing a crucial role in intestinal homeostasis (10–12). The degree to which alterations in the mucus layer reflect direct effects of intestinal microbes compared with indirect signaling from activated epithelial or underlying lamina propria cells is not understood. Consistent changes in mucus-related indexes in a variety of intestinal and nutritional disorders, including enteric infections (13), inflammatory bowel disease (14–16), colon cancer (14, 16), and conditions requiring total parenteral nutrition (TPN) (17, 18) justify attempts to define how intestinal microbes, particularly mucore-sident commensal bacteria, regulate the development and maintenance of the mucus gel layer.

Here, we review the results of developmental and gnotobiotic animal studies and of in vitro studies with mucus-producing epithelial cell lines showing alterations in mucin gene expression, mucus composition, or mucus secretion in response to intestinal microbes or host-derived inflammatory mediators. Fundamental characteristics of the mucus gel layer and goblet cell biology are first reviewed briefly to provide background information. The dynamic nature of the mucus layer is shown, as is the deficiency in our knowledge of both bacterial cues and host-response pathways that mediate mucosal homeostasis in the antigen-dense environment of the intestine.

THE MUCUS LAYER AND GOBLET CELL BIOLOGY

The mucus gel layer is an integral structural component of the intestine, acting as a medium for protection, lubrication, and transport between the luminal contents and the epithelial lining (19). The viscoelastic, polymer-like properties of mucus are derived
from the major gel-forming glycoprotein components called mucins (19). Mucins consist of a peptide backbone containing alternating glycosylated and nonglycosylated domains, with O-linked glycosylated regions comprising 70–80% of the polymer. N-Acetylgalactosamine, N-acetylgalactosamine, fucose, and galactose are the 4 primary mucin oligosaccharides (19). Mucin oligosaccharide chains are usually terminated with sialic acid or sulfate groups, which account for the polyanionic nature of mucins at a neutral pH (19). Oligosaccharide chains are added to mucins individually by specific, membrane-bound glycosyltransferases that transfer monosaccharides from nucleotide sugar donors in the Golgi apparatus, whereas sulfate is transferred to peripheral or backbone oligosaccharide chains from 3′-phosphoadenosine-5′-phosphate (PAPS) by Golgi sulfotransferases (16). For a review of current knowledge of glycosyltransferases and subcellular mechanisms of glycoprotein synthesis, the reader is referred to Paulson and Colley (20) and Brockhausen et al (16), respectively.

Secretory mucins are secreted from the apical surface of specialized columnar epithelial cells (goblet cells) by 2 distinct processes, baseline secretion and compound exocytosis. Baseline secretion (or simple exocytosis) involves the constitutive release of newly synthesized mucin granules that preferentially move along the periphery of the apical granule mass (19). On exposure to a mucin secretagogue, goblet cells undergo compound exocytosis, an accelerated secretory event resulting in the acute release of centrally stored mucin granules (19). A wide array of bioactive factors, including hormones, neuropeptides, and inflammatory mediators (cytokines and lipids), can induce compound exocytosis. Detailed summaries of the molecular and subcellular basis of mucin secretion can be found in articles by Forstner et al (19), Laboisse et al (21), and Villalobo and Gabius (22).

The regulated expression of mucin (MUC) genes also contributes to mucin heterogeneity and the dynamic nature of the mucus layer. Nine epithelial MUC genes have been identified in humans (23). Gendler and Spicer (24), van Klinken et al (25), Gum (26), and Perez-Villar and Hill (27) provide detailed reviews of mucin gene structure and developmental patterns of expression.

GOBLET CELL ONTOGENY AND DEVELOPMENT OF THE MUCUS LAYER

Although the cytoarchitectural organization of goblet cells and their modes of secretion are relatively well described, less is known about factors contributing to glycoprotein heterogeneity in intestinal mucus or factors influencing the differentiation of stem cells toward the goblet cell lineage. A continuous mucus gel that varies in thickness covers the epithelial lining of the stomach and large intestine (19). The mucus layer can reach up to 450 μm in the stomach (19). In the colon, mucus thickness increases gradually from the ascending colon, reaching up to 285 μm in the rectum (28). The small intestine is covered with a thinner or discontinuous mucus layer; for example, Peyer’s patches are discontinuous mucus. Colonic mucins did not mix homogeneously but rather formed a stacked or laminated structure of alternating sialo- and sulphomucins. Bacteria were consistently observed within the laminated arrays of the outer layer, indicating the importance of the mucus gel in preventing direct adherence of even commensal gut bacteria to colonic epithelial cells.

The physiologic relevance of distinct mucin subtypes is not well understood. It is suggested that acidic mucins protect against bacterial translocation because particularly sulfated mucins appear less degradable by bacterial glycosidases and host proteases (34, 35). This idea is also consistent with the observation that goblet cells in intestinal regions densely populated by microbes express acidic mucins predominantly (12, 34, 36). However, acidic mucins appear to predominate throughout fetal life. Mucin sulfation starts as early as 14 wk of gestation in the human fetal colon (30), immediately after the first appearance of goblet cells (37), whereas O-acetylated sialomucins appear after 23 wk (30). The apparent programmed appearance of acidic mucins in the fetal, and hence sterile, colon is intriguing considering the suggested association between bacterial density and the presence of acidic mucins in postnatal life.

The mucin profile of the human colon at birth resembles that of the adult colon in that acidic mucins predominate (30). All goblet cells in the mouse colon appear to produce sulfated mucins at birth (31), and colonic mucins of newborn pigs are highly sulfated and sialylated (11). The presence of acidic mucins in early life stages may be of particular importance as an innate defense barrier because the acquired immune system is not fully functional in the neonatal intestine (38). Similarly, ontogenic changes in mucin subtypes may influence age-dependent patterns in the incidence and severity of intestinal infections (39–42).

Studies addressing mucin production during the birth-to-weaning transition are somewhat limited (11, 12, 31–33). However, the available data show clear developmental patterns during this stage of growth. As summarized in Table 1, the ratio of neutral to acidic mucins generally increases between birth and the weaning period and decreases after weaning.

We recently completed a mouse ontogeny study in which temporal and spatial mucin composition patterns were monitored from birth to maturity throughout the gastrointestinal tract (12; Figure 1). A developmental pattern of mucin distribution was obvious in the proximal and distal colon by 14 d after birth. Sialomucin-containing goblet cells were detected only in colonic crypts, whereas sulfomucin-containing goblet cells were observed on colonic cuffs but not in crypts (12). Hill et al (31) reported similar findings in mice. The separation of sialomucin-containing (crypts) and sulfomucin-containing (cuffs) goblet cells became more distinct in the
Human alters mucin composition comes from histochemical studies in ANIMALS MUCUS AND GOBLET CELL INDEXES IN GERMFREE birth. Much additional research is required to define the relation let cell mucins are predominantly sulfated in the fetus and at immune system, consistent with the observation that colonic gob- inantly sulfated (31). Mucin sulfation may constitute a default pathway that is operative in the absence of a well-developed immune system by colonizing bacteria. That hypothesis is sup-ported by the finding that the temporal development of sialo-
Figure 1) may therefore be linked to the activation of the immune system in the intes-
tine (1, 38). The appearance of sialomucin-containing goblet cells in lower crypts and the migration of sulfomucin-containing gob-
ertaining capacity of mucins (44). This morphologic difference, however, is thought to reflect the absence of mucus-degrading intestinal bacteria (45) rather than an increase in mucus production. Indeed, cecal mucins are rapidly degraded and cecum morphology normalizes with the introduction of commensal gut bacteria (46).

Mucin composition also differs significantly between germ-
free and conventionally raised animals, as summarized in Table 1. Under germfree conditions, the ratio of neutral to acidic mucins in the colon is higher, and sulfomucins appear to increase at the expense of sialylated mucins (2–4, 6; Table 1). Sharma and Schumacher (4) also reported fewer sialylated mucins in the small intestine of germfree rats than in conventionally raised rats, indicating greater mucus production (2, 3, 5, 6). A striking feature of germfree rodents is the proximal and distal colon could not be distinguished on the basis of the available data.

Indicates conflicting data between Turck et al (11) and Dunsford et al (32) compared with Brunsgaard (33). The proximal and distal colon could not be distinguished on the basis of the available data.

Includes the cuff surface.

1 The proximal and distal colon could not be distinguished on the basis of the available data.

2 Indicates conflicting data between Turck et al (11) and Dunsford et al (32) compared with Brunsgaard (33).

distal colon after weaning and remained constant at later ages (Figure 1). Independent of age, neutral mucins were usually associated with sulfomucins in goblet cells residing on colonic cuffs, whereas crypt goblet cells primarily harbored sialomucins.

The temporal sequence of mucin subtype development appears to parallel the establishment of climax microbial communities, with more dramatic differentiation events appearing during times of bacterial flux. Similarly, bacterial succession has a major effect on the development of the acquired immune system in the intestine (1, 38). The appearance of sialomucin-containing goblet cells in lower crypts and the migration of sulfomucin-containing goblet cells to upper crypts from 14 d after birth in the mouse colon (Figure 1) may therefore be linked to the activation of the immune system by colonizing bacteria. That hypothesis is supported by the finding that the temporal development of sialomucin and sulfomucin distribution observed in normal mice does not occur in germfree mice, in that crypt mucins remain predominantly sulfated (31). Mucin sulfation may constitute a default pathway that is operative in the absence of a well-developed immune system, consistent with the observation that colonic goblet cell mucins are predominantly sulfated in the fetus and at birth. Much additional research is required to define the relation between mucin development and bacterial succession.

MUCUS AND GOBLET CELL INDEXES IN GERMFREE ANIMALS

The most convincing evidence that the intestinal microbiota alters mucin composition comes from histochemical studies in which germfree animals were compared with conventionally raised controls or were inoculated with mixed microbial populations. Goblet cells of germfree rodents are fewer in number and smaller in size than those of conventionally raised mice (5, 43). As a result, the mucus layer may be up to twice as thick in conventionally raised than in germfree rodents, indicating greater mucus production (2, 3, 5, 6). A striking feature of germfree rodents is pronounced cecal enlargement due to the water-retaining capacity of mucins (44). This morphologic difference, however, is thought to reflect the absence of mucus-degrading intestinal bacteria (45) rather than an increase in mucus production. Indeed, cecal mucins are rapidly degraded and cecum morphology normalizes with the introduction of commensal gut bacteria (46).

Mucin composition also differs significantly between germ-

<table>
<thead>
<tr>
<th>Species and time</th>
<th>Ratio of neutral to acidic mucin</th>
<th>Ratio of sulfomucin to sialomucin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper crypt</td>
<td>Lower crypt</td>
</tr>
<tr>
<td></td>
<td>CV GF</td>
<td>CV GF</td>
</tr>
<tr>
<td>Mouse Fetus</td>
<td>&lt;&lt; 1</td>
<td>&lt;&lt; 1</td>
</tr>
<tr>
<td>Birth</td>
<td>&lt;&lt; 1</td>
<td>&lt;&lt; 1</td>
</tr>
<tr>
<td>Rat Preweaning</td>
<td>1 ND</td>
<td>1 ND</td>
</tr>
<tr>
<td>Postweaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal colon</td>
<td>&lt; 1 ↑</td>
<td>&lt; 1 ↑</td>
</tr>
<tr>
<td>Distal colon</td>
<td>&lt; 1 ↑</td>
<td>&lt; 1 ↑</td>
</tr>
<tr>
<td>Pig</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Birth</td>
<td>&lt; 1 ND</td>
<td>&lt; 1 ND</td>
</tr>
<tr>
<td>Preweaning</td>
<td>1 ND</td>
<td>&lt; 1 ND</td>
</tr>
<tr>
<td>Postweaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>&lt; 1/1</td>
<td>ND</td>
</tr>
<tr>
<td>Human Fetus</td>
<td>&lt;&lt; 1</td>
<td>&lt;&lt; 1</td>
</tr>
<tr>
<td>Birth</td>
<td>&lt;&lt; 1</td>
<td>&lt;&lt; 1</td>
</tr>
<tr>
<td>Preweaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postweaning</td>
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<td></td>
</tr>
<tr>
<td>Proximal colon</td>
<td>&lt; 1 ND</td>
<td>&lt; 1 ND</td>
</tr>
<tr>
<td>Distal colon</td>
<td>&lt; 1 ND</td>
<td>&lt; 1 ND</td>
</tr>
</tbody>
</table>

1 Data are summarized from the studies by Enss et al (3), Kendori et al (5), Turck et al (11), Deplancke et al (12), Sheahan and Jervis (29), Filipe et al (30), Hill et al (31), Dunsford et al (32), and Brunsgaard (33). We interpret the data as follows: << 1 or >> 1 indicates almost exclusive presence of one mucin subtype, 1 indicates equal density, and < 1 or > 1 indicates that one mucin subtype predominates. Arrows indicate changes (increase: ↑; decrease: ↓) in mucin subtype ratios in germfree (GF) compared with conventionally raised (CV) animals. ND, not determined.

2 Includes the cuff surface.

3 Indicates conflicting data between Turck et al (11) and Dunsford et al (32) compared with Brunsgaard (33).
FIGURE 1. Histologic analysis of mucin ontogeny in the distal colon of C57BL/6J mice. A through D: Periodic acid Schiff’s reagent–alcian blue was used to distinguish neutral (red) and acidic (dark blue arrow) mucins (magnification: 200×). A purple color indicates the presence of both neutral and acidic mucins (red arrow). E through H: High iron diamine–alcian blue was used to distinguish sialylated (light blue arrow) and sulfated (brown arrow) mucins (magnification: 200×). Mucins are brown when the sulfate concentration exceeds that of sialic acid. The area inside the box in H depicts a laminated structure of alternating sialo- and sulfomucins. The succession of mucin types in the distal colon is as follows: A and E = 14-d-old; B and F = 21-d-old; C and G = 60-d-old; D and H = 90-d-old mouse.
GOBLET CELL RESPONSES TO INTESTINAL MICROBES
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number in the large intestine (4). The authors suggested that the distinct mucin patterns observed in conventionally raised and human-microbiota-associated rats might reflect differential host responsiveness to specific bacterial communities or metabolites (4). Note that the germfree state affects numerous indexes that might indirectly influence goblet cell functions or mucin subtype patterns. Of particular significance is the underdeveloped nature of the intestinal immune system in germfree animals (47, 48) and the fact that germfree animals are routinely fed chemically defined, water-soluble diets (49).

Multiple studies have shown that dietary factors may affect goblet cell numbers and mucin heterogeneity (4, 18, 50–52) and modulate the secretory activity of goblet cells (53–55). However, the degree to which dietary effects are mediated by indigenous bacteria is difficult to determine, although comparisons of germfree and conventionally raised animals fed identical diets offer some insight. We compared goblet cell and immune-related indexes in neonatal piglets fed intravenously (by TPN) with those fed a milk replacer enterally (18). Total goblet cell numbers in the jejunum and ileum and sulfomucin-positive goblet cells within ileal villi were higher in the TPN-fed animals than in those fed enterally. Furthermore, goblet cell and mucin subtype alterations were significantly correlated with local expansion of T lymphocyte populations and observed only in inflamed intestinal segments. Preliminary evidence from related microbiological studies indicates that the growth of resident bacteria in TPN-fed animals is due in part to active mucolysis. Mucus degradation would compromise epithelial barrier function, leading to bacterial translocation into the lamina propria and subsequent recruitment of inflammatory cells, a working model consistent with the TPN data.

MICROBIAL OR HOST MODULATION OF GOBLET CELL FUNCTIONS

The regulatory networks that mediate goblet cell responses to intestinal signals are poorly defined. At least 2 scenarios may be envisioned. Intestinal microbes may directly affect goblet cell functions through the local release of bioactive factors. Alternatively, goblet cell functions may be altered in response to host-derived bioactive factors generated by activated epithelial or underlying lamina propria cells after their contact with intestinal bacteria. Evidence exists in support of both possibilities.

MICROBIAL-DERIVED FACTORS ALTERING MUCUS SYNTHESIS AND SECRETION

Mucus offers numerous ecologic advantages to intestinal bacteria. For example, mucin oligosaccharides represent a direct source of carbohydrates and peptides, and exogenous nutrients, including vitamins and minerals, are likely concentrated within the mucus matrix. Bacteria capable of colonizing mucus can avoid rapid expulsion via the hydrokinetic properties of the intestine. Bacterial colonization of mucus would thus impart a growth advantage. In fact, it is difficult to envision a more suitable bacterial niche than host mucus. In that regard, it is not surprising that mucus secretion is typically enhanced in response to intestinal microbes (7–9). Both commensal and pathogenic bacteria would derive significant benefit from an ability to chemically regulate mucous synthesis or secretion from host goblet cells. Our current knowledge of the biochemical basis of goblet cell sensitivity to microbial products is limited and is largely restricted to pathogens and their toxins.

A well-characterized mucin secretagogue is the cholera toxin of Vibrio cholerae, which triggers massive mucin release via a cAMP-dependent mechanism (56). Although it had been suggested that cholera toxin accelerates mucin secretion in intact mucosa by an indirect mechanism, perhaps mediated by mucosal nerves or other cell types (56), more recent work with HT29/B6 cells showed that cholera toxin can increase mucin secretion directly (57). The toxin listeriolysin O of Listeria monocytogenes also induces mucus exocytosis from HT29-MTX cells by binding to a brush border–associated receptor, after which membrane oligomerization of the toxin occurs (58). Listeriolysin O does not, however, induce an intracellular transducing signal known to regulate mucin exocytosis, and might interact directly with membrane components involved in the intracellular vesicular transport of mucins (58).

Another well-described example of direct microbial effects on mucus secretion is the enteric protozoan parasite Entamoeba histolytica. Examination of the progressive pathologic events in a gerbil model of amebic colitis showed that goblet cell mucin stores are depleted before amebic invasion of the epithelium (8). The cause of the mucin depletion is unknown, but it is speculated that parasite-derived secretagogues might eventually deplete mature mucin stores as a means of evading epithelial barrier functions (59). E. histolytica was shown to directly trigger mucin release from LS174T cells via a pathway dependent on protein kinase C (60). The entamoeba secretagogue, Chlostridium difficile toxin A exerts a rapid and dose-dependent inhibition of compound mucin exocytosis without altering baseline (constitutive) mucin secretion when added to HT29-Cl.16E cells (62).

Slomiany et al (63) examined the effects of Helicobacter pylori lipopolysaccharide on mucin synthesis and secretion from a human gastric mucosa biopsy segment. Although initial exposure to H. pylori lipopolysaccharide resulted in rapid mucin discharge, prolonged incubation led to a concentration-dependent decrease in mucin synthesis and secretion. Similar results were observed when H. pylori was exposed to viable H. pylori (64). Prolonged presence of viable H. pylori inhibited baseline mucin secretion from HT29-Cl.16E cells. Moreover, the addition of H. pylori not only inhibited total mucin synthesis but also suppressed MUC1 and MUC5AC gene expression in a human gastric cell line, which demonstrates the ability of microbes to directly alter MUC gene expression (65).

Exposure to both gram-positive (Staphylococcus aureus, Staphylococcus epidermis, and Streptococcus pyogenes) and gram-negative (Pseudomonas aeruginosa and Escherichia coli) bacteria increases MUC2 and MUC5A gene expression in mucin-producing NCIH292 epithelial cells (66). In a study by Li et al (67), P. aeruginosa, an opportunistic lung pathogen often associated with cystic fibrosis, activated MUC2 gene transcription in both airway and colonic goblet cell cultures through a nuclear factor κB signaling pathway. Data presented in that study indicated a major if not exclusive effect of P. aeruginosa lipopolysaccharide. Bordetella pertussis, the causative agent of whooping cough in humans, also induced MUC2 gene transcription when incubated with the human bronchial epithelial cell line BEAS-2B (68). Further investigation with the human colon
carcinoma cell line HM3 showed that *B. pertussis* could also induce *MUC5AC* gene transcription.

Exposure of HT29 cells to an enteropathogenic *E. coli* strain did not alter *MUC* gene expression (9). On the other hand, the probiotic strains *Lactobacillus plantarum* 299v and *Lactobacillus rhamnosus* GG increased expression of both *MUC2* and *MUC3* genes in HT29 colon cell cultures (9). The ability of these *Lactobacillus* strains to prevent enteropathogenic *E. coli* attachment to HT29 cells may then relate to the increased mucin expression induced by the *Lactobacillus* strains (9).

The list of bioactive components of bacterial cell walls continues to expand for both gram-positive and gram-negative organisms. Systematic research will be required to identify precisely those bacterial cell wall products that modulate goblet cell functions. The utility of the various mucus-producing epithelial cell lines to conduct such studies is clear given the difficulties associated with ex vivo research with intestinal tissues. However, as shown in Table 2, available epithelial cell lines vary considerably in their state of differentiation and few are well characterized. Goblet cell culture studies are, of course, also subject to the well-known limitations of transformed cell lines and do not reproduce the complex cellular environment of the intestinal epithelium. Nonetheless, given the current experimental inaccessibility of intestinal goblet cells, the epithelial cell lines represent a unique alternative to systematically investigate the molecular mechanisms regulating mucin synthesis and secretion.

**Table 2**

Characteristics of commonly used mucus-producing human colonic cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MUC genes expressed</th>
<th>Mucin composition</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT29-SB</td>
<td><em>MUC1</em></td>
<td><em>N</em>-Acetylgalactosamine, galactose, and sialic acid</td>
<td>Devine et al, 1991 (72), Devine et al, 1992 (73)</td>
</tr>
<tr>
<td>HT29-MTX</td>
<td><em>MUC1</em>, <em>MUC3</em>, <em>MUC5C</em></td>
<td>“Gastric-type mucins,” <em>N</em>-acetylgalactosamine, <em>N</em>-acetylglucosamine, galactose, mannose, sialic acid, and sulfate</td>
<td>Lesuffleur et al, 1990 (74), Lesuffleur et al, 1993 (75), Huet et al, 1995 (76)</td>
</tr>
<tr>
<td>HT29-FU</td>
<td><em>MUC1</em>, <em>MUC2</em>, <em>MUC4</em></td>
<td>“Colonic-type mucins”</td>
<td>Lesuffleur et al, 1991 (77), Lesuffleur et al, 1993 (75)</td>
</tr>
<tr>
<td>LS180</td>
<td><em>MUC2</em>, <em>MUC5A/C</em>, <em>MUC5B</em>, <em>MUC6</em></td>
<td><em>N</em>-Acetylgalactosaminol and sialic acid</td>
<td>Tom et al, 1976 (81), McCool et al, 1994 (82), Enns et al, 2000 (83)</td>
</tr>
<tr>
<td>LS174T⁴</td>
<td><em>MUC1</em>, <em>MUC2</em>, <em>MUC3</em>, <em>MUC5A/C</em>, <em>MUC6</em></td>
<td><em>N</em>-Acetylgalactosaminol, <em>N</em>-acetylgalactosamine, galactose, fucose, sialic acid, and sulfate</td>
<td>Tom et al, 1976 (81), van Klinken et al, 1996 (84), Göttke et al, 1998 (85)</td>
</tr>
<tr>
<td>HM3⁴</td>
<td><em>MUC2</em>, <em>MUC3</em></td>
<td><em>N</em>-Acetylgalactosaminol, <em>N</em>-acetylgalactosaminol, galactose, fucose, sialic acid, and sulfate</td>
<td>Kuan et al, 1987 (86), Ohara et al, 1994 (87), Cho et al, 1997 (88)</td>
</tr>
<tr>
<td>LIM 2463</td>
<td><em>MUC1</em>, <em>MUC2</em></td>
<td><em>N</em>-Acetylgalactosaminol, <em>N</em>-acetylgalactosamine, galactose, and sialic acid</td>
<td>Whitehead et al, 1991 (89), Devine et al, 1992 (73)</td>
</tr>
<tr>
<td>COLO 205</td>
<td><em>MUC1</em></td>
<td><em>N</em>-Acetylgalactosaminol, <em>N</em>-acetylgalactosaminol, galactose, fucose, and sialic acid</td>
<td>Semple et al, 1978 (90), Baeckström et al, 1991 (91), Devine et al, 1992 (73), Hanski et al, 1997 (92)</td>
</tr>
<tr>
<td>T84</td>
<td><em>MUC1</em>, <em>MUC2</em></td>
<td><em>N</em>-Acetylgalactosaminol, <em>N</em>-acetylgalactosaminol, galactose, fucose, and sialic acid</td>
<td>Reid et al, 1978 (93), Hanski et al, 1997 (92), Göttke et al, 1998 (85), Hong et al, 1999 (94)</td>
</tr>
</tbody>
</table>

¹Predominant *MUC* expression and mucin composition as described in the literature and according to analyses performed.

²The first reference describes the first isolation of the cell line and subsequent references collectively indicate the characteristics described.

³HT29 subclones were derived from the parental HT-29 colon cell line (95).

⁴Derived from the parental LS180 cell line.

Microbial-derived factors altering the chemical composition of mucus

The defensive nature of mucins lies in their capacity to entrap microbes (59). On the other hand, adhesion to specific mucin epitopes presumably facilitates mucus colonization by commensal bacteria, thereby providing the growth advantages discussed above (2, 59, 96–98). Accordingly, intestinal mucins likely dictate the composition of the bacterial community within the mucus layer, although the mechanistic details of this selection process are poorly characterized. Most efforts have focused on the identification of epithelial cell surface glycoprotein and glycolipid receptors for bacterial adhesins or toxins of enteric pathogens (42, 99). The mucus coat physically covers cell surface glycoconjugates and is thought to thereby prevent adhesion of enteric pathogens. Working models propose that soluble mucin epitopes bind specific bacterial adhesins and prevent their attachment to similar epitopes on host cell surface receptors (59, 100). Because enteric pathogens are typically transient residents of the gut, such a defensive strategy would require constitutive production by the host of carbohydrate epitopes capable of binding to pathogen adhesins.

The ecologic issues associated with mucus involvement in pathogen resistance compared with its mediation of colonization by commensal bacteria present an intriguing paradox. Who is most likely to control mucin composition: the host, resident commensals, transient pathogens, or some combination thereof? Goblet cell sensitivity to microbial products should not be surprising given that mucus is widely used as a medium for host-microbe interactions by many animals, including invertebrate species that do not possess an acquired immune system (101). As summarized below, the limited data available indicate that direct crosstalk between intestinal microbes and goblet cells is indeed likely.

When the lipopolysaccharide of an indigenous *E. coli* strain was administered to germfree rats, an increase of colonic neutral mucin was paralleled by enhanced attachment of the indigenous *E. coli* strain to secreted mucin, perhaps reflecting an ability of *E. coli* to alter mucin chemistry for their own benefit (102). This concept is consistent with remarkable data from Bry et al (103) showing that monoassociation (exposure to one defined bacterial
strain) of germfree mice with wild-type *Bacteroides thetaiotaomicron* (a gut commensal) induced host expression of α,1,2-fucosyltransferase messenger RNA and subsequent production of fucosylated glycoconjugates on small-intestinal epithelial cells. An isogenic mutant *B. thetaiotaomicron* strain that lacks the ability to utilize L-fucose did not restore the fucosylation program in monoassociated mice. The factors enabling *B. thetaiotaomicron* to regulate host cell fucosylation remain unidentified, but appear to be soluble and their effect is density dependent (103). It was proposed that low fucose concentrations induce bacterial genes whose products activate the host fucosylation program (104). Consequently, *B. thetaiotaomicron* would be able to trigger the host to produce a readily digestible substrate, fucose, for its own metabolism when fucose availability decreases. Bacterial substrates and metabolites produced from mucin digestion should also be investigated for their ability to exert feedforward control on goblet cell functions.

**Microbial mucolysis**

An ability to enzymatically degrade mucus was documented in pathogens and commensals alike and appears to be a common trait among bacteria (105). Enzymatic digestion of the mucus coat provides access to readily available sources of carbon and energy and enables bacteria to reach the epithelial surface. Mucin degradation is a multistep process that begins with proteolysis of the nonglycosylated “naked” regions of the mucin glycoproteins by host and microbial proteases (106). This initial step markedly reduces mucin gelation and viscosity and results in the accumulation of highly glycosylated subunits (>500 kDa) that are resistant to further proteolytic attack. Mucin glycopeptides are then degraded by various bacterial enzymes corresponding to the complexity of the oligosaccharide chains, which again differ in size, degree of branching, type of linkage, and the presence of terminal sialic acid or sulfate groups. For example, terminal sulfate and sialic acid residues are cleaved by bacterial sialidases and glycosulfatases, and oligosaccharide side chains are degraded by linkage-specific glycosidases (105).

There appears to be a correlation between the type of glycoconjugate to which microbes preferentially bind and the glycoconjugate-cleavage machinery of the respective organisms. For example, *E. histolytica* binds preferentially to N-acetylglucosamine and possesses the corresponding glycosidase (59). *Salmonella typhimurium* binds preferentially to a glycoprotein containing sialic acid (107) and possesses a sialidase (108). *H. pylori* binds to sulfated glycoconjugates (109) and harbors a glycosulfatase (110). *H. pylori* also possesses the enzymatic ability to disrupt the oligomeric structure of mucin, enabling the pathogen to move freely in the mucus layer, assisted by its highly active flagellum and its ability to down-regulate mucin synthesis (65, 111). Attachment of a mammalian reovirus to mucins is followed by expression of a mucolytic protein that facilitates penetration of the virus through the protective mucus barrier (112). Mucin adhesion may, in these examples, trigger the production of various mucolytic strategies to overcome mucus entrapment.

de Repentigny et al (113) showed that the ability of *Candida* species to bind to purified small-intestinal mucin correlates closely with their hierarchy of virulence. The production of a secretory aspartyl proteinase after adhesion enables *Candida albicans* to degrade and thus detach from the mucin, allowing the organism to move deeper into the mucus layer. A comparison of *S. typhimurium* strains also showed that mucus adherence was greater in more virulent strains (107). Expression of the *S. typhimurium* sialidase on binding to sialomucins may similarly enable this organism to penetrate mucus.

Virulence mechanisms may derive, in part, from the evolution of bacterial strategies to maximally exploit the mucus niche. Several pathogens, such as *V. cholerae*, possess virulence determinants that facilitate their penetration through the mucus coat (114, 115). Flagellated vibrios swim through the mucus coat, whereas nonmotile vibrios are avirulent (114–116). Flagellar movement through the mucus layer to the surface of underlying epithelial cells would increase encounters with novel substrates such as cell surface glycoconjugates and glycolipids. Motile organisms capable of using multiple nutritional substrates would be given further advantage over nonmotile organisms that rely on single or fewer substrates. The relation between nutritional strategies of mucus-resident bacteria and the evolution of virulence warrants closer inspection.

Also of interest is evidence from humans that resident mucolytic bacteria may differ among individuals according to the specific carbohydrate composition of intestinal mucins (eg, terminal sugars or branching patterns), which appears to vary by genetic background (117, 118). Evidence of host genetic background influencing bacterial community profiles has not been reported for other mammalian species. Such a finding would be consistent with increasing evidence of stable and host-specific microbial community profiles (119–122) and with evidence that endogenous substrates may have a significant influence on the spatial pattern of bacterial population profiles along the gastrointestinal tract (12).

Although it is not known whether goblet cell numbers or mucus secretion is increased directly in response to bacterial mucolysis, the sensitive nature of goblet cells to local perturbations such as inflammation is becoming increasingly clear. Certainly, host efforts to reconstitute or reinforce the mucus barrier in response to bacterial translocation into the lamina propria and subsequent subepithelial inflammation are easily perceived as being logical defensive responses.

**Host-derived factors altering mucus synthesis and secretion**

Most of our knowledge of the immunologic regulation of goblet cell differentiation and mucin secretion was gained through the use of goblet cell lines (Table 2). Jarry et al (123) showed that the proinflammatory cytokine interleukin 1 (IL-1) stimulates rapid mucin release from HT29-CL16E cells in a dose-dependent manner. The ability of IL-1 to trigger mucin release and to up-regulate MUC gene expression was later confirmed in studies of perfused rat colons (124) and the colonic LS180 cell line (83). Other proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and IL-6, also stimulate mucin secretion by LS180 cells and increase the expression of MUC2, MUC5AC, MUC5B, and MUC6 (83). The T cell cytokine interferon γ does not affect baseline mucin secretion or transcription of MUC2 in HT29-CL16E cells (125), but does inhibit cholera-toxin-induced mucin secretion and thus could potentially down-regulate the mucin secretion pathway.

Belley and Chadee (126) showed with both LS174T cells and a rat colon loop that the bioactive lipid prostaglandin E2, which is prominent during intestinal inflammation, enhances mucin release via a cAMP-dependent mechanism. IL-4 induces MUC2 expression and mucin production in NCI-H292 airway epithelial cells, indicating that CD4+ Tp1/2 lymphocytes are likely capable of stimulating goblet cell functions (127, 128). IL-9, another Tp1/2 cytokine, also stimulates mucin gene (*MUC5AC*) expression, and
may account for as much as 50–60% of the mucin-stimulating activity of lung fluids in allergic airway disease (129).

A role for goblet cells and enhanced mucus secretion in the elimination of the parasitic nematode 

\textit{Nippostrongylus brasiliensis} has long been documented (130). Data indicate that CD4+ T lymphocytes appear to orchestrate the host response to \textit{N. brasiliensis} because anti-CD4+ antibodies inhibit mucin secretory responses and goblet cell hyperplasia in mice infected with the nematode (131). Expression of the CD4+ T cell–derived cytokines IL-4 and IL-5 is also significantly up-regulated in mice during spontaneous recovery (ie, without medical treatment) from \textit{N. brasiliensis} infection (132, 133). Enhanced mucus release appears to be a common mechanism for the intestinal clearance of gut parasites (130, 134, 135) and is routinely mediated by cytokines produced by the Th2 subset of CD4+ T cells, which subsequently stimulate immunoglobulin E (IgE) production (136, 137). Lake et al (138) showed that IgE-mediated mast cell discharge of histamine enhanced the release of goblet cell mucus into the rat duodenum.

The ability of bioactive factors released by mucosal immune cells to regulate the composition of mucin subtypes has received more attention. Infection of rats with \textit{N. brasiliensis} increases the expression of the terminal N-acetyl-d-galactosamine epitope (139). However, enhanced N-acetyl-d-galactosamine expression is not observed in hyperthymic rats infected with this nematode, implicating the host immune system in the regulation of mucin composition (139). TNF-\alpha was shown to decrease mucin glycosylation by LS180 cells (83). On the other hand, TNF-\alpha specifically increases \alpha-2,3-sialyltransferase expression in HT29 cells (140), indicating increased synthesis of sialomucins. This finding is consistent with the hypothesis that the developmental increase in sialomucins in mouse colon crypts may be triggered by activated immune cells (Figure 1). In this regard, the presence of distinct subsets of immune cells in different intestinal regions may influence the mucin composition in the respective regions of the gastrointestinal tract. The various cytokine and T cell receptor gene knockout mouse strains represent a valuable resource for determining how specific cytokines or T cell subsets may influence the composition of intestinal mucins. Much could be learned with conventional mucin cytochemistry.

CONCLUSION

Collectively, the observations discussed throughout this review support the postulate that intestinal bacteria or host immune cells may each contribute bioactive factors affecting goblet cell dynamics and the mucus layer. The taxonomy as well as the temporal and spatial distributions of bacterial groups that preferentially reside within intestinal mucus must be defined to determine the role of normal gut bacteria in mucogenesis and mucolysis. At present, mucus-resident microbial populations are poorly characterized for all animal species. This limitation in our knowledge derives from the inherent biases of cultivation-dependent microbiological techniques (12, 141), as well as from the difficulties associated with preserving the mucus layer during tissue fixation (28). A precise definition of the regulatory networks that interface with goblet cells may have broad biomedical applications because mucus alterations appear to characterize most diseases of mucosal tissues.

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REFERENCES


GOBLET CELL RESPONSES TO INTESTINAL MICROBES


