Influences of the colonic microbiome on the mucus gel layer in ulcerative colitis

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Abstract

The colonic mucus gel layer (MGL) is a critical component of the innate immune system acting as a physical barrier to microbes, luminal insults, and toxins. Mucins are the major component of the MGL. Selected microbes have the potential to interact with, bind to, and metabolize mucins. The tolerance of the host to the presence of these microbes is critical to maintaining MGL homeostasis. In disease states such as ulcerative colitis (UC), both the mucosa associated microbes and the constituent MGL mucins have been shown to be altered. Evidence is accumulating that implicates the potential for mucin degrading bacteria to negatively impact the MGL and its stasis. These effects appear more pronounced in UC. This review is focused on the host-microbiome interactions within the setting of the MGL. Special focus is given to the mucolytic potential of microbes and their interactions in the setting of the colitic colon.

Keywords: colon, mucus gel layer, bacteria, mucins, ulcerative colitis

Introduction

The gastrointestinal tract is a natural reservoir for bacteria as it is a rich source of nutrients that can be used for bacterial metabolism in a temperature environment ideal for bacterial growth. An estimated 1013–1014 bacterial cells reside within the adult intestine, a number that exceeds the total number of cells in the human body by a factor of 10. With such favorable conditions for bacterial growth, the human host has had to evolve mechanisms that control bacterial growth, while maintaining the presence of symbiotic bacteria. These symbions perform vital roles in energy extraction from foods otherwise indigestible by the host while exerting trophic effects on the host epithelium and immune structure and function. Although commensal bacterial species exist in symbiosis with their host and are typically well tolerated, the potential for deleterious effects on the host still remains, owing to their sheer numbers and large surface area of the intestinal epithelium. The mechanisms by which the bacteria are maintained in homeostasis with the MGL are not fully understood; however, two principles are involved: physical separation of the bacteria from the host epithelium by the MGL and tolerance of the host immune system for the presence of bacteria.

The physical separation of the bacteria from their host depends on a continuous MGL secreted by host goblet cells, which serves as a barrier between the outer luminal contents and the host epithelium. The MGL also contains anti-microbial peptides and IgA, both of which serve to limit the number of bacteria that reach the host epithelium. In cases where bacteria breach the MGL, host adaptive immune responses mediated by dendritic cells are employed to eliminate the bacteria. In health, this process occurs without an overt immunological reaction whereas this response is amplified in UC and other forms of colitis. The importance of the adaptive immune system within the colon have been discussed in a number of recent review articles, and therefore will not be discussed here. The focus of this discussion is on the physical separation of the host epithelium and bacterial cells, and the potential for the bacteria to modulate this physical barrier leading to inflammatory consequences. Particular emphasis is placed on the discussion of these properties within the setting of UC.

Properties and Function of the Colonic Mucous Gel Layer

The MGL serves to protect the colonic epithelium from physical, chemical, and biological damage. Its functionality and efficacy are dependent not only on the thickness of the MGL but also on its chemical composition. The MGL is produced and secreted by goblet cells resident in intestinal crypts. The number of goblet cells per crypt is greater in the colon than the small bowel reflecting the greater concentration of bacteria within the colonic microbiome. The viscoelastic properties of the MGL derive from its glycoprotein constituents (mucins). These mucins consist of a peptide backbone containing serine or threonine residues upon which N-acetylgalactosamine residue can be added. This structure is then modified by core transferases, which serve to elongate
the oligosaccharide side chains. These side chains are often terminated with sialic acid, which can be post translationally modified to also contain a sulfate group. The core structures are formed by the core 1 enzyme β1,3-galactosyltransferase to form the core 1 glycan Galβ1–3GalNAcα-Ser/Thr, the core 3 β1,3 N-acetylgalactosaminyltransferase to form a GlcNAcβ1–3GalNAcα-Ser/Thr core, and additional core 2 and core 4 transferases, which serve to act on these precursors with the addition of N-acetylglucosamine. (Fig. 1A).

Alterations to the structure or composition of MGL mucins have the potential to affect the protective barrier and play a role in the pathogenesis of inflammation of the underlying epithelium. In UC a number of specific changes have been reported. During active inflammation the goblet cell population is depleted, and individual goblet cells contain less mucin than those of healthy controls. Additionally the MGL is reduced in thickness. In UC, a number of specific changes have been reported. During periods of disease remission both the number and appearance of goblet cells return to normal. In UC changes in O-glycosylation and shortening of the oligosaccharide side chains of MGL mucins have been reported, along with alterations in glycosylation and sulfation. In vivo mouse models lacking functional core 2 and 3 enzymes displayed increased susceptibility to colitis in the dextran sulfate sodium colitis model, while mice lacking core 1 enzymes developed spontaneous colitis. These alterations are thought likely to lead to a diminished protective capacity of the MGL.

The presence of sulfate groups is thought to confer a resistance to enzymatic degradation of the mucin, thus the reduced content observed in the colitic colon is likely to be disadvantageous. Interestingly bacteria which are capable of cleaving sulfate and utilizing it as a metabolite have been found to be overrepresented in the colitic colon. Their presence may offer an explanation for the reduced sulfated content of mucin in the colitic colon.

**Mucous Gel Layer Organization**

Development of methods to measure and characterize the MGL in vivo in animal studies has allowed better understanding of the MGL architecture. The MGL is now known to be organized into a two layer structure; a loosely adherent outer layer and an inner layer which is firmly attached to epithelial cells. In mouse models, the thickness of this two layered structure is estimated at 150 µm, with the inner layer measuring 50 µm and the outer layer 100 µm. The thickness of the MGL in humans remains to be clarified however it is thought to measure between 107 and 155 µm. Both layers are composed of MUC2 type mucin, with a well-organized stratified inner layer and a loosely adherent, non-stratified outer layer. A clear border exists between the inner and outer layers suggesting a controlled transition of mucus between the layers. Analysis of the mucosa associated microbiome within the two layers of the MGL has clearly demonstrated that in health bacteria reside in the outer mucus layer only, leaving the inner layer sterile and devoid of bacteria. In a dextran sulfate sodium mouse model of colitis this mucosal homeostasis was absent. Bacteria were found to be resident within crypt structures, and bacteria (mainly *Bacteroides*) were both in contact with and invading host mucosa. This direct contact between bacteria and their host is now known to negatively impact the host cells. Animal models of MUC2 deficient mice, which render the MGL absent and bacteria in direct contact with the colonic epithelium, have resulted in abnormal

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**Figure 1.** Schematic representation of a mucin structure and organization of the MGL. The four major core types are synthesized by specific glycotransferase enzymes following transfer of N-acetylgalactosamine to serine or threonine. The mucin molecule is then elongated by the addition of galactose or N-acetylgalactosamine and terminated with sialic acid and a sulfate group (A). Schematic representation of the two layered organization of the MGL (B).
mucosal morphology, increased cellular proliferation of epithelial cells, decreased apoptosis, development of spontaneous colitis, and increased migration of epithelial cells leading to intestinal tumor formation and subsequent spontaneous progression to invasive carcinoma. Given that it is known that the normal colonic microbiome stimulates cell proliferation in colon, it has been hypothesized that the increased cell proliferation observed in MUC2 deficient animals could be explained by overt bacterial stimulation due to the diminished MGL. In addition to the presence of a MGL, the integrity of its mucin components and its stratified organization is also vital to maintenance of mucosal homeostasis. Misfolding of the MUC2 mucin has been shown to lead to endoplasmic reticulum (ER) stress which in the microbial milieu associated with the colon leads to epithelial cell dysfunction and intestinal inflammation. Nhe-3 (a sodium hydrogen transporter necessary for normal mucus expansion and organization) deficient mice have shown the MGL to be intact with a two layer organization similar to that of wild type mice. However bacteria were found to be capable of penetrating both the outer and inner MGL resulting in an inflammatory response, suggesting that defects in the MGL have the potential to trigger inflammatory responses. Likewise IL-10 deficient mice were found to have an intact but penetrable MGL which yielded a phagocyte mediated innate inflammatory response, suggesting a link between the MGL integrity, cytokines produced and the immune response.

Mucosa Associated Microbiome of the Colon

An accurate description of the community composition and structure of mucosa associated bacteria has until recently been difficult to generate. However recent advances in electron microscopy technologies, molecular techniques such as fluorescent in situ hybridization (FISH) and metagenomic sequencing methods have now enabled the mucosal communities to be identified and their distribution mapped without disrupting MGL architecture. The colonic microbiota is unevenly distributed along the longitudinal axis of the colon, with spatial segregation occurring between the luminal and mucosa adjacent regions. In health this spatial segregation can be further characterized by the existence of bacterial colonization in the lower outer regions of the MGL and a sterile inner region of densely packed mucins which are adjacent to the mucosa.

The recent advances in molecular techniques and metagenomic studies based on 16S rRNA gene sequences have now made it possible to fully interrogate the resident mucosa associated bacterial communities. A clear differentiation between luminal and mucosa associated communities has been widely reported both in health and diseased states, with Firmicutes and Bacteroidetes reported as the dominant phyla in both locations. In humans the precise spatial differences in bacterial communities remains to be clearly defined. Initial studies suggest a variety of bacteria show a predisposition for colonization of the human mucosa (Table 1). Included among these is Akkermansia muciniphila, which is known to express mucin degrading enzymes. Their presence as part of the healthy mucosa microbiome likely reflects the dualisms that exist between host and microbes, and may have protective or anti-inflammatory roles in health. Murine and rodent models have indicated that the mucosa harbors increased abundances of Lachnospiraceae genera, along with Blautia/Butyrivibrio longum and Ruminococcus torques, both of which possess mucolytic properties, have been shown to be increased in the mucosa of patients with UC. A. muciniphila was found to be decreased in the same cohort of patients, suggesting that A. muciniphila was a constituent of the homeostatic MGL environment. The increased presence of butyrate producing bacteria, along with decreased levels of degrading bacteria (MDB), along with decreased levels of butyrate producing bacteria (Table 1). Ruminococcus gnavus and torques, both of which possess mucolytic properties, may offer an explanation for the reported increase total mucosa-associated bacteria in UC. Additionally the same study showed that these MDB are capable of providing substrates for other non-MDB through degradation of MUC2, providing evidence of the possible syntrophic relationships occurring between colonic bacteria within the MGL.

Alterations in Ulcerative Colitis

The MGL of patients with UC is thinner than in health, with alterations to intracellular mucin content, mucin glycosylation, and a reduction in sulfation coincident with an increase in mucosa associated bacteria and a dysbiosis of the constituent microbes.

Studies have revealed an increase in some but not all mucus degrading bacteria (MDB), along with decreased levels of butyrate producing bacteria (Table 1). Ruminococcus gnavus and torques, both of which possess mucolytic properties, have been shown to be increased in the mucosa of patients with UC. A. muciniphila was found to be decreased in the same cohort of patients, suggesting that A. muciniphila was a constituent of the homeostatic MGL environment. The increased presence of butyrate producing bacteria (MDB), along with decreased levels of degrading bacteria (MDB), along with decreased levels of butyrate producing bacteria (Table 1). Ruminococcus gnavus and torques, both of which possess mucolytic properties, may offer an explanation for the reported increase total mucosa-associated bacteria in UC. Additionally the same study showed that these MDB are capable of providing substrates for other non-MDB through degradation of MUC2, providing evidence of the possible syntrophic relationships occurring between colonic bacteria within the MGL.

MGL-Microbe Interactions

The MGL provides the first line of defense between bacteria in the lumen and the host cells, and, as such, it also acts as the primary site of host-microbe interactions through mucin binding. The diverse range of carbohydrate groups that form the peripheral structure of mucins offers a multitude of binding sites for bacteria (both commensal and pathogenic). Bacteria, which are capable of such binding, are likely to gain an advantage over the luminal bacteria through access to additional nutrient sources provided by the MGL itself. Indeed in health the MGL provides a dual role, serving to protect the mucosa from potential pathogens while providing a state of mutualism between the host cells and the MGL residing bacteria. In addition to a nutrient source, the MGL provides an initial binding site for selected bacteria upon which they can colonize. Through the provision of such binding sites for commensal bacteria, the MGL can circumvent the interaction of pathogenic bacteria with the host cells and prevent further translocation of pathogens into the mucosa. Additionally the short chain fatty acids produced by
fermentation of these commensal bacteria can be utilized as an energy source by the host cells (Fig. 2).

The mechanisms by which commensal bacteria bind to the MGL have been studied in members of the *Lactobacillus* and *Bifidobacterium* genera, both of which are common constituents of probiotic formulas. Within the *Lactobacillus* genera several binding factors have been identified including the mucus-binding protein (MUP) and its homolog MucBP,59,60 the mannose-binding protein Msa,61 a cell wall bound pilli protein SpaC,62 and the cell surface protein GroEL.63 Within the *Bifidobacterium* genera type IV pilus-type proteins, 64 glycoprotein-binding fimbriae protein 65 have been identified as potential mucus binding proteins. It should be noted that these proteins have been identified through sequence predictions, and these attributes remain to be validated. An example of the beneficial effects offered by binding of these bacteria is displayed by *Lactobacillus johnsonni* and its GroEL surface protein. The binding of *L. johnsonni* to mucins results in aggregation of *Helicobacter pylori*, decreased *H. pylori* load, and facilitation of the clearance of the pathogen, thereby suggesting that the binding of such bacteria to mucins may play a role in gastrointestinal homeostasis.63

It is likely that other gut bacteria will present with additional mucus binding proteins, as suggested by Huang et al. in the case of *Bacteroides fragilis*.66 However, information pertaining to the nature of these binding proteins is absent. Identification of these biologically important motifs will allow recognition bacterial genera and species of biological importance for both health and diseased states. Mucin Degradation Certain microbes are known to have the potential to degrade the colonic MGL.67-69 The oligosaccharide side chains of mucins account for 70–80% of the mucin structure and have the potential to act as a significant nutrient source for bacteria that are capable of cleaving these linkages.13 In order to utilize these oligosaccharides as nutrient sources, the bacteria are required to perform extensive degradation of the oligosaccharides to their constituent monosaccharides. This occurs through enzymatic cleavage with linkage-specific glycosidases.68,70,71 The composition of the mucin sugars in particular glycosylation and terminal linkages are highly variable.15 Mucin degradation involves proteolytic cleavage of the non-glycosylated regions from the peptide backbone, degradation of the sugar side chains through a range of glycosidase enzymes tailored to the oligosaccharide constituents, and sialidase and sulfatase enzymes that are capable of degrading the acidic groups on the oligosaccharide chains. Approximately 1% of enteric bacterial species possess the ability to produce the requisite extracellular enzymes,73 and specific

| Bacteria                       | Mucin binding protein | Mucolytic enzyme | Health/Disease association | Reference |
|-------------------------------|----------------------|------------------|---------------------------|-----------|
| Akkermansia muciniphila       | Unknown              | Glycosidase      | Decreased in UC           | 54, 93    |
| Ruminococcus gnavus           | Unknown              | α-galactosidases | Increase in active UC     | 54, 94    |
| Ruminococcus torques          | Unknown              | α-N-acetylglactosaminidase | Increase in active UC | 54, 95    |
| Desulfovibrio desulfuricans   | Unknown              | Sulfatase        | Increase in active UC     | 31, 32    |
| Bacteroides thetaiotaomicron  | Unknown              | Sulfitase, neuraminidase, α-fucosidase, β-galactosidase, α-N-acetylglactosaminidase, β-N-acetylglucosaminidase | Decreased mucin sulfation in animal models | 86, 96    |
| Bacteroides fragilis           | Unknown              | Neuraminidase, sulfatase, proteases, α-fucosidase, β-galactosidase, β-N-acetylglucosaminidase, α-fucosidases | Increase in active UC | 97–99    |
| Bacteroides vulgatus           | Unknown              | Neuraminidase, α and β-galactosidases, α-fucosidase, β-N-acetylglucosaminidase, α and β-N-acetylglactosaminidase | Increased in UC | 100      |
| Lactobacillus reuteri          | NUC/MucBP            | Unknown          | Probiotic                 | 59, 60    |
| Lactobacillus plantarum        | Mua                  | Unknown          | Probiotic                 | 61        |
| Lactobacillus rhamnosus        | SpaC                 | Unknown          | Probiotic                 | 62        |
| Lactobacillus johnsoni         | GroEL                | Unknown          | Probiotic                 | 63        |
| Bifidobacterium breve          | Type IV pilus        | Unknown          | Probiotic                 | 64        |
| Bifidobacterium longum         | Glycoprotein-binding fimbriae protein | Unknown          | Probiotic                 | 65        |
enzymes are found in bacteria spanning several genera (Table 1). However, complete degradation of mucin requires the action of a consortium of bacteria as few intestinal bacteria produce all of the requisite glycosidase, sulfatase, and sialidase enzymes.74 Mucin degradation is often thought of as the primary step in bacterial pathogenesis as it disturbs the physical barrier between the lumen and host mucosa. However, this may only apply to excessive degradation as mucin turnover is a normal part of the colonic ecosystem. MGL homeostasis, with mucin degradation through both bacterial enzymatic processes and peristalsis, occurs shortly after birth.55,75 However in chronic inflammation such as that associated with UC, there is an increased presence of the MDB *R. gnavus* and *torques*,26,54 offering a possible explanation for increased mucosa associated bacteria found in IBD.

Following binding to, colonization of, and degradation of the MGL, invading microbes have been shown experimentally to have the potential to continue to influence the MGL and exert an effect on the host cells. In a macaque model of the gastric mucosa, invading *H. pylori* have been shown to signal the host cells to both downregulate expression of mucin genes and to alter the glycosylation profile of the mucins which are expressed.75 While in a *Drosophila* model a transcriptomic analysis of the host intestinal genes suggested that the mucus barrier was remodeled following infection with *Erwinia carotovora*.77

**Commensal and Pathogen Utilization of Mucin**

The ability of enteric bacteria (both commensal and pathogenic) to utilize mucin oligosaccharides as energy sources is now beginning to be elucidated. While only a small proportion of the enteric commensals are predicted to poses the requisite glycosidase enzymes necessary for such processes,73 those which do, appear to be adept to this purpose. *Bacteroides thetaiotaomicron* and *Bifidobacterium bifidum* (both common gut commensals) have been extensively studied for their mucin degrading capacity. Transcriptomic analysis has shown both to poses an array of glycosidase genes as well as several carbohydrate transporters which may aid in the import of degraded mucin oligosaccharides.78,79 *B. thetaiotaomicron* has also been shown to adaptively direct its glycan foraging to mucin polysaccharides when polysaccharide availability from the diet is reduced thereby contributing to the homeostasis of the gut microbiome.79 In contrast to this it appears that enteric pathogens may be poorly adapted to utilization of mucin and rely on commensal mucin oligosaccharide liberation of metabolites. Both *Clostridium difficile* and *Salmonella typhimurium* possess the ability to catabolize mucin sialic acid despite the absence of sialidase enzymes in their genome, suggesting an ability to exploit the sialidase activity of other commensal bacteria.79 Similarly

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*Figure 2.* Schematic representation of the different association between the MGL and gut microbes. Mucins can act as a barrier to both pathogenic and commensal bacteria. Some commensal bacteria are capable of binding to the MGL and in do so act as antagonists to the binding of pathogen (1). The MGL can provide a source of nutrients for some commensals through mucin degradation; these MDB in turn generate SCFAs which serve as an energy source for the host epithelium (2). The MGL can provide a source of nutrients for MDB, which in turn generate nutrients for other commensal bacteria, thereby offering them an ecological advantage (3). Pathogenic bacteria bind to and degrade the MGL, thereby allowing the pathogens access to the host mucosa where they can exert a negative effect on the host cells.
The microbial diversity that exists within the colonic microbiome which functions as a “microbial organ,” contribute to a multitude of processes and functions. Its composition is a result of co-evolution between the microbial communities and the host. It is highly likely that a similar co-evolution has occurred between the mucosa associated bacteria and is key to maintaining mucosal homeostasis. Given the selective pressures under which these microbes are placed, development of syntrophic and antagonistic relationships is not surprising. One such syntrophic relationship exists between the B. thetaiotaomicron and Faecalibacterium prausnitzii. B. thetaiotaomicron possesses a repertoire of genes encoding mucin degrading proteins and its presence has been shown to result in a decrease in sulfated mucins in rodents. However, a co-culture model of both B. thetaiotaomicron and F. prausnitzii results in an increase in the presence of sulfated mucins and maintenance of mucosal homeostasis. These bacteria may be metabolically complementary, with F. prausnitzii metabolizing acetate produced by B. thetaiotaomicron that in turn produces butyrate. This butyrate is then utilized by host cells and stimulates synthesis of mucin serving to maintain mucosal homeostasis. F. prausnitzii counts are decreased in patients with UC, however no information is currently available regarding the co-colonization rates of these bacteria in a UC cohort.

A second aspect of syntrophy is that which exists between MDB and sulfate reducing bacteria (SRB). This relationship has been characterized in an in vitro model, in which a three-stage continuous culture model of the colon was infused with pig gastric mucin. The SRB alone were found to be incapable of directly metabolizing mucin, however when co-cultured with other faecal bacteria an increase in SRB numbers was observed. Similarly B. fragilis has been found to release sulfate groups from sulfated mucin in order to utilize the desulfated mucins as an energy source. The released sulfate may then be utilized by Desulfovibrio indicatring that SRB may be dependent on other intestinal bacteria to cleave and release sulfate, necessary as a metabolite. Within the setting of UC, an increased presence of SRB has been identified by several groups, with an association between D. desulfuricans and active inflammation being demonstrated by Rowen et al. SRB have also been found to be exclusive to ileal pouches fashioned for UC, with their presence corresponding to the expression of sulfated mucins. Further studies focusing on the co-colonization rates of MDB and SRB during both active and quiescent disease are necessary in order to fully elucidate the role SRB may play in the pathogenesis of UC.

**Future Directions of Investigation**

Much information relating to host microbe interaction within the MGL remains to be established. To date most studies have focused on single bacterial species within a colonic mucin model or establishing the presence of one or a limited number of mucosa associated bacteria in clinical samples. While helpful in establishing the ability of a given organism to metabolize mucin, such studies do not reflect the complex interactions needed for mucin degradation in the colon. Co-culture experiments with a consortium of bacteria with synergistic profiles will be necessary. Use of metagenomic sequencing to study the microbiome of specific niches will allow co-colonization patterns to be identified and provide the essential background data to allow clinically appropriate studies to be performed. Additionally, metatrascriptomic sequencing will provide information relating to the expression mucolytic enzymes within MDB.

**Summary**

The MGL is the first line of defense for the mucosa, acting as a physical barrier between microbes and the host epithelium. In health it is in a state of equilibrium allowing resident microbes to colonize and utilize metabolites, while in return obtaining nutrients that were otherwise inaccessible to the host. The diverse nature of the mucin composition has yielded a niche environment for microbes which have co-evolved with the host to survive. The presence of MDB are tolerated by the host, however in the colitic colon this symbiotic relationship is unbalanced. The increased presence of MDB and their symbiotes results in a degradation of the MGL and a reduction in its protective capacities. Future metagenomic sequencing studies will allow elucidation of the dysbiotic mucosa associated microbiome in UC, particularly when micro-dissection of the MGL is applied prior to sequencing. Moreover to fully address the relationship between host and microbes in health and disease their precise interactions need to be identified. Characterization of mucins and their glycan profiles is needed along with the mucin binding preferences of MDB during periods of both active and quiescent UC.

**Disclosure of Potential Conflicts of Interest**

No potential conflict of interest was disclosed.

**Acknowledgments**

This manuscript was prepared using funding from Science Foundation Ireland, Grant number 09/IN.1/B2606.
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