Biological modeling of mucus to modulate mucus barriers

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Desseyn JL, Gouyer V, Gottrand F. Biological modeling of mucus to modulate mucus barriers. Am J Physiol Gastrointest Liver Physiol 310: G225–G227, 2016. First published December 10, 2015; doi:10.1152/ajpgi.00274.2015.—A recent study using a transgenic mouse, whose intestinal mucus contains a molecule made of 12 copies of a domain found in many gelling mucins, demonstrates that it is possible to strengthen mucus properties in situ, leading to promising new treatment strategies in diseases in which the mucosal barrier is impaired.

Mucus is a thick, slimy secretion that is essential for many biological functions including lubrication, hydration, and protection of the underlying epithelia (3, 22). Mucus is composed of water (~95%), salts, lipids, and proteins, but its viscous and gel-like properties are largely governed by O-glycoproteins named gel-forming/gelling mucins. The intestinal mucosa contains billions of commensal bacteria, which represent a permanent challenge to the integrity of the epithelial surface (36). However, commensal bacteria compete for nutrients and sites of epithelial adherence with unwanted bacteria, protecting the underlying epithelium from penetration by pathogenic bacteria (17). Modifications to the mucus properties can greatly affect mucous layer functioning. For example, an intestinal mucus layer that is too thin, as found in inflammatory bowel diseases (IBDs), will facilitate bacteria reaching the epithelial cells, which may trigger inflammation because of the dysregulated immune response to host intestinal microbiota. This has been demonstrated in several mouse models with a defective mucus layer, which leads to direct contact between bacteria and the epithelium associated with a severe intestinal inflammation (15, 19). Conversely, thick mucus in the lungs makes it difficult to expel, leading to lung obstruction as found in cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). Thicker secretions are also features in the gastrointestinal system in CF [for review, see Kelly and Buxbaum (18)]. Meconium ileus and distal intestinal obstruction syndrome develop when thick mucous secretions occlude the hollow gastrointestinal lumen. In the pancreas and bile ducts of CF patients, thickened secretions may cause obstruction and acute and chronic inflammation. Strategies aimed at modulating mucus properties in vivo are limited, partly owing to the complexity of mucin macromolecules.

The main characteristics of gelling mucins result from their large, heavily O-glycosylated region, where carbohydrates retain water, and from disulfide bonds between mucin monomers. Gel-forming mucins cross-link via their amino- and carboxy-terminal regions, which are enriched in cysteine residues to form either long polymers (28) or more complex structures responsible for netlike structures (16). Non-cross-linking interactions between mucin macromolecules seem also fundamental to mucus gelling (2, 3). Among the reversible cross-links within mucin polymers, intermolecular hydrophobic interactions are today the best characterized (7, 8, 10). However, few studies have investigated the mucus barrier or have attempted to modify mucin gelling in vivo. Several reports have suggested that a non-O-glycosylated domain interspersed within the O-glycosylated region participates in the mucin network (3, 14). This domain, named the CYS domain because it is enriched in cysteine residues, is ~110 amino acids long and is highly hydrophobic. The CYS domain has been found in two copies in human and mouse mucin MUC2, the major gelling mucin of the intestine, and in seven and nine copies, respectively, in the two respiratory mucins, MUC5B and MUC5AC (13). The CYS domain is not present in the two other gelling mucins, MUC6, which is expressed mainly in the stomach but also in deep glands of duodenum and ileum (34), and MUC19, which is expressed in submandibular glands and in trachea (11). Compared with other domain sequences of gelling mucins, the CYS domain is highly conserved, with a strong selective pressure on many amino acid residues, supporting a key role of the domain (13). The CYS domain is highly hydrophobic and ex vivo experiments suggested that CYS domains are able to interact with each other in a reversible manner (3, 8). Consequences of an increased number of reversible interactions between mucins on mucus properties in vivo have never been evaluated.

A transgenic mouse that secretes a recombinant molecule consisting of 12 consecutive identical copies of one CYS domain borrowed from human mucin MUC5B has been created (14). As expected, the gut mucus blanket is modified by the recombinant molecule. The mucus appears more robust and less permeable to inert particles (Fig. 1). In challenged mice, the transgene is associated with reduced susceptibility to chemically induced colitis, faster clearance of the pathogen Citrobacter rodentium administered by gavage, and better protection against bacterial translocation (14).

A compromised gut barrier function may facilitate the onset of many diseases in which increased bacterial translocation and/or microbial products are a key feature such as cachexia, chronic liver diseases (31), gut infection, IBD (4), intestinal obstruction (30), chemotherapy-induced mucositis (32), and acute pancreatitis (12). Bacterial translocation may also occur after epithelial cell hypoxic injury due to trauma or as a result of bacterial overgrowth after surgery, injury (30), or antibiotic use (21). Detergent action of bile acids throughout the gastro-
The intestinal tract has been suggested as a natural luminal aggressor. Many experimental data support that bile acids, and more especially deoxycholic acid, are cytotoxic for epithelial cells (9, 32). However, bile acids induce also mucus secretion and expression of MUC2, two mechanisms by which the intestinal epithelium protects itself (20, 23). To date, no disease has been described in which bile acids are the primary initiators of the epithelial damage (26), but we cannot rule out the exacerbation of epithelial damages by bile acids in several disease states where the mucus layer is less protective. The transgenic model shows that it is possible to reinforce the intestinal barrier by delivering a molecule made up of domains belonging to gelling mucins. This opens up new strategies to treat, limit, or prevent unwanted bacterial translocation, especially from the gut.

The recent report showed that delivery of poly-CYS molecules affects mucin O-glycosylation (14). This modification has been suggested to be linked to the higher load of beneficial *Lactobacillus* spp. found in the gut of transgenic mice (2.5 log/g of tissue). It is known that bacteria in the gut drive mucin maturation and use sugars from mucins as their energy source. Lactobacilli act as a primordial barrier to infection by competing for adhesion sites with pathogens, by producing lactic acid, bacteriocins, nonbacteriocin compounds, and nonproteinaceous molecules that exercise a direct bactericidal effect (24) and stimulating the production of antimicrobial molecules by the host. Consequently, the unexpected increase in *Lactobacillus* spp. in transgenic mice may strengthen the intestinal barrier. Similarly, delivering poly-CYS molecules into the vagina could increase the abundance of lactobacilli, thereby preventing or limiting infections such as bacterial vaginosis, yeast vaginitis, urinary tract infection, and sexually transmitted diseases (6). Delivering poly-CYS molecules into the cervical mucus may also represent a new method of contraception. In the cervical mucus, the poly-CYS molecule should favor reversible cross-links between mucin macromolecules that would change the mesh size of the mucin network and decrease its permeability to sperm (8).

At least two strategies can be envisaged to enrich a mucus gel with molecules made of CYS domains. The first one consists in delivering the recombinant molecule using food-grade living lactic acid bacteria (5) or nonpathogenic yeast strains, like *Yarrowia lipolytica* (25). Delivery would be more efficient in mucus gels housing the greatest abundance of microorganisms, i.e., colon, distal ileum, and vagina. The second strategy uses the CRISPR/Cas9 system, a genomic technology that is in its infancy (29). CISPR/Cas9 gene editing tool should enable to transactivate in the gut the expression of the respiratory genes *MUC5B* and *MUC5AC*, which have the particularity to encode gelling mucins with seven and nine copies of the CYS domain, respectively.

There are many potential applications of mucus enrichment with poly-CYS molecules. However, a high concentration of the domain in the mucus may be deleterious. For example, mucus makes it difficult for some compounds to reach the underlying epithelial cells, limiting the efficacy of drugs administered orally or as aerosols. In CF, abnormal dehydrated mucus tempered hopes for correcting the mutated CFTR gene, responsible for the disease. Perez-Villar and Boucher (27) hypothesized that the increased mucus concentration in CF may result from the formation of abnormal irreversible interchain bonds in airway mucins. Here, the increased production of respiratory mucins with seven or nine copies of the CYS domain may greatly favor interactions between CYS domains of gelling mucins, dangerously increasing mucus viscosity and stasis leading to obstruction of the airways. A better understanding of the interactions between CYS domains and amino acids engaged in these interactions would help identify new strategies to fluidify abnormal mucus in CF and COPD. Further studies of CYS domain properties will depend on the availability of recombinant molecules made of one or several copies of the domain, which seem particularly difficult to produce (1).

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