Serum Protein Content of Rat Small-Intestinal Mucus

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This investigation was performed to determine whether serum proteins which might contribute to mucosal protection are present in rat small intestinal mucus. Rats were fed an elemental diet, their small intestines removed, perfused, everted, and the surface mucus gently collected. Mucus was centrifuged to express its aqueous component (sol), whose serum protein content was analyzed by immunodiffusion in agarose gel. Immunoglobulins G and A were present in intestinal mucus sol; however, M and E could not be demonstrated. Serum albumin was present in mucus sol, but could be detected only following luminal perfusion with protease inhibitors. Although the role of serum albumin in intestinal mucus function is not clear, the presence of immunoglobulins G and A may contribute to the protective role played by mucus in the small intestine.

Mucus helps to protect epithelial surfaces; how it performs this important task is incompletely understood. In the gastrointestinal tract, mucus occupies the critical interface that exists between the epithelial surface and a potentially harmful luminal mixture of microorganisms and digestive enzymes. Mucus forms an adhesive, viscoelastic gel as a result of noncovalent intermolecular attraction between mucin molecules in the presence of water (1). The existence of a highly hydrated gel at this important interface may be particularly appropriate. The gel matrix will present a physical barrier to luminal microorganisms, thereby helping to prevent infection. The gel must, however, remain sufficiently permeable to allow the products of digestion access to the underlying intestinal surface. The high hydration of mucus should allow soluble nutrients to pass through gel water in order to reach the epithelial surface. It will also permit the entry of biologically protective solutes into mucus, as has already been demonstrated in the respiratory tract (2).

Despite its high hydration, the gel structure of the mucus layer effectively impedes water movement through its substance (3). Restriction of free fluid movement through mucus reflects the presence of unstirred conditions within mucus water which may conserve useful solutes at the intestinal surface in the face of free luminal flow. Such an effect has been proposed to occur in the stomach and duodenum where the small amount of bicarbonate secreted into the mucus layer is conserved at the epithelial surface for acid neutralization (4, 5). Although the solute content of small intestinal mucus has not been characterized, luminal fluid contains immunoglobulins (6), which may also enter the mucus layer. Immunoglobulins protect the gastrointestinal epithelial surface by interfering with antigen absorption and inhibiting the attachment of microorganisms to the intestinal surface (6). Their presence within intestinal mucus would add these protective immune processes to those protective physical properties of the intestinal mucus layer referred to previously.

The purpose of this investigation was to characterize the serum proteins present in rat small intes-
tinal mucus. Albumin, IgA, and IgG proteins were detected. Their presence may augment the protective role of the mucus layer in the small intestine.

**MATERIALS AND METHODS**

Male Wistar rats weighing between 150 and 200 g were housed in wire-bottom cages and fed full-strength Vivonex (Norwich Eaton, Paris, Ontario) for 48–72 hr prior to sacrifice to eliminate small intestinal intraluminal residue. Animals were killed by stunning and cervical fracture; the small intestine was removed and rinsed in ice-cold 0.15 M NaCl. The intestinal lumen was then slowly perfused so as not to disrupt the mucus layer with 50 ml of either ice-cold 0.15 M NaCl or 0.15 M NaCl containing 0.94 mM α-toluenesulfonfyl fluoride (Eastman Kodak Co., Rochester, New York) and 46.5 μM soybean trypsin inhibitor (Worthington Biochemical Corp., Freehold, New Jersey). The perfusate was discarded and the intestine everted over a glass rod. Surface mucus was then removed by gently drawing the everted intestine between two gloved fingers, then transferred to a 1.5-ml polypropylene centrifuge tube and centrifuged at 30,000g at 4°C for 4 hr. The clear bile-stained mucus sol (7) was removed and either analyzed immediately or stored at −20°C. Each rat produced between 400 and 800 μl of mucus sol.

The protein content of the mucus sol was analyzed by double immunodiffusion in 1% (W/V) agarose gels containing 0.12 M NaCl, 0.03 M Tris HCl (pH 7.4), and 1.5 mM Na azide. Reactants were infused into 700-μm thick gels with the aid of Plexiglas templates (8). Nonimmunized sera were obtained locally from goats, sheep, and rabbits which had not been exposed to rat proteins. Antisera to rat plasma proteins were obtained from Miles Laboratories Inc. (Rexdale, Ontario), except rabbit antiserum to rat serum albumin which was obtained from Nordic Immunological Laboratories (El Toro, California) and goat antiserum to rat IgG (Fc fragment) from Jackson ImmunoResearch Laboratories (Avondale, Pennsylvania). All antisera to rat immunoglobulins were heavy-chain-specific except the anti-IgG from Miles Laboratories which exhibited activity against both heavy and light chains. Immunodiffusion was carried out overnight in a moist chamber at room temperature. Following incubation, points of antiserum and mucus sol infusion into the gel were marked by wells cut prior to template removal. Precipitin lines were stained with amido black 10B after washing.

Protease activity of the mucus sol was estimated by caseinate proteolysis in agarose gel. The technique of Bjerring et al (9) was used except for substitution of the milk powder with light soluble casein (BDH Chemicals Ltd., Poole, England).

**RESULTS**

When studied by double-immunodiffusion in agarose gel, precipitin lines developed in reaction between components of intestinal mucus sol and a number of antisera to rat serum proteins. Not all precipitin lines observed were the result of known antigen–antibody interaction, as mucus sol obtained following perfusion of the intestine with 0.15 M NaCl produced precipitin lines during immunodiffusion against goat and sheep serum devoid of antibody activity against rat serum proteins. Such nonimmunologic precipitin line formation (10) is illustrated in Figure 1. A heavy precipitin reaction was observed between mucus sol and goat serum. A faint precipitin reaction was also observed in reaction between intestinal mucus sol and sheep serum. No precipitin line formation was observed between intestinal mucus sol and either rabbit or rat serum (data not shown).

Addition of α-toluenesulfonfyl fluoride plus soybean trypsin inhibitor to the intestinal perfusate prevented the development of these nonimmunologic reactions and is also illustrated in Figure 1. These protease inhibitors were subsequently added to all intestinal perfusion fluids prior to the collection of intestinal mucus unless otherwise indicated. Their presence substantially reduced but failed to completely eliminate protease activity in mucus sol when measured by casein proteolysis and...