Radioimmunoassay of Human Intestinal Goblet Cell Mucin

INVESTIGATION OF MUCUS FROM DIFFERENT ORGANS AND SPECIES

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ABSTRACT We have developed a double-antibody radioimmunoassay for the quantitative measurement of human goblet cell mucin (GCM) in order to study intestinal mucus in human and other species. The assay used ^{3}H-labeled mucin as the antigen, rabbit antisera, and sheep anti-rabbit IgG antisera as the second antibody. A number of applications of the assay were investigated. A survey of human tissues revealed that mucins of the rectum, colon, and small intestine had identical affinity for the rabbit antibody, whereas lung, eyelid conjunctiva, esophagus, and stomach reacted less strongly. GCM concentration ranged from 1.9 to 14 μg mucin protein/mg tissue protein in the small and large intestine, respectively. The radioimmunoassay was also found to be useful as a marker during the isolation of GCM from human ileal extracts, where it indicated that a 10,000-fold purification had been achieved. Antigenic determinants of the mucin did not rely upon ABH blood group-specific terminal sugars in oligosaccharide chains. A comparison of mucins among various species revealed a partial species specificity of the GCM antibody. Human GCM cross-reacted with dog, monkey, and rabbit mucins, but not with mucins of rat, pig, toad, and oyster. Organ distribution of cross-reactive mucins in rabbit tissues indicated a pattern that was qualitatively similar to that seen in human tissues. Possible implications of these findings for autoimmune diseases are briefly discussed.

INTRODUCTION

High molecular weight mucus glycoproteins (mucins) are presumed to be responsible for lubricating and protecting epithelial surfaces (1). As a class they are distinguished by a high sugar content, the absence of mannose, alkali-labile *O*-glycosidic linkages between oligosaccharide side chains and the peptide core, and a high proportion of serine and threonine. In spite of these identifying characteristics, mucins are very difficult to study at the molecular level because of chemical heterogeneity within the oligosaccharide side chains and their physical polydispersity (2). Mucins also have no recognized enzymatic or easily measured biological activity to facilitate their recognition and isolation.

Identification and characterization have also been a source of difficulty in studying mucin synthesis and secretion in tissue slice (3), organ culture (4), and intestinal loop systems (5). A heavy reliance has been placed upon labeling with radioactive precursors (6–9), but these precursors are also incorporated into nonmucin-glycoproteins, often more actively than into mucins (10). As a result, one cannot be certain that contamination of mucin preparations by small amounts of nonmucin glycoproteins might not account for much of the radioactive incorporation data.

Comparison of mucins from different sources has also been difficult because of the variety of methods used in their isolation, and their uncertain purification. Thus, although mucins within a single organ, such as the gastrointestinal tract, may have a similar function and share antigenic characteristics (11), there is continuing controversy as to the similarity of their structures.

In this paper we describe a sensitive radioimmunoassay for human goblet cell mucin (GCM)[1] from the small intestine, and explore its usefulness in identifying, measuring, and comparing related mucin antigens in human and other animal tissues.

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[1] *Abbreviations used in this paper*: GCM, goblet cell mucin; RIA, radioimmunoassay.

METHODS

Preparation of the antigen. Goblet cell mucin (GCM) was purified from human small intestinal (ileal) scrapings of a single donor (blood type A) by methods described previously (12). The void volume peak obtained from chromatography on Sepharose 4B columns (Pharmacia Fine Chemicals, Piscataway, N. J.) was centrifuged at 30,000 *g* for 30 min, and the supernate, containing soluble GCM, was used throughout this study as the "standard purified GCM." It was used to immunize rabbits and as the stock for unlabeled standards in the radioimmunoassay (RIA).

The GCM molecule was a high molecular weight glycoprotein containing 13% protein (by weight) of which serine, threonine, and proline made up 51% of the total residues. The molar ratio of sugars, based on *N*-acetylgalactosamine as 1.0, was *N*-acetylneuraminic acid:fucose:galactose:*N*-acetylglucosamine:*N*-acetylgalactosamine, 0.23:0.94:1.23:0.95:1.0, respectively. Mannose and uronic acid were not detected. GCM contained blood group A (Le^{b+}) activity measured by hemagglutination inhibition studies. Except for blood group specificity and secretor status, these characteristics are almost identical to those published earlier for another human GCM preparation from a nonsecretor having Le^{a+} and no A, B, or H antigens in the mucin (12). The absence of mannose and uronic acid rules out significant contamination of the mucin by membrane or serum glycoproteins, or acid mucopolysaccharides. Details of methods for amino acids, hemagglutination inhibition, sugar composition, and immunodiffusion are given in references 12–14.

For some experiments the antigen was further fractionated by application of GCM to DEAE-Bio-Gel (A) columns (Gel Filtration Materials, Bio-Rad Laboratories, Richmond, Calif.) and elution with 0.1 M NaH_2PO_4-Na_2HPO_4 pH 6.0 containing 6 M urea. The first peak from this column contained no sialic acid, but retained 75% of the total hexoses (and thus the majority of the carbohydrate) of the applied GCM. The total protein (by weight) was 6%, and serine, threonine, and proline made up 53% of residues. The molar ratios of sugars were *N*-acetylneuraminic acid:fucose:galactose:*N*-acetylglucosamine:*N*-acetylgalactosamine, 0:1.25:1.68:1.26:1, respectively. This product represents the most highly glycosylated glycoprotein species of GCM, and is designated "peak I" in this paper.

Labeling of the antigen. GCM was labeled using the technique of mild periodate oxidation and reduction with sodium [^{3}H]boro hydride as described by van Lenten and Ashwell (15), and applied successfully to rat GCM in an earlier study (13). The specific activity of the final tritiated human GCM was 7×10^6 dpm/μg GCM protein. Labeled GCM had identical chromatographic (Sepharose 4B), immunodiffusion, and ultracentrifugal properties to those described earlier for unlabeled human GCM (12). Between 85 and 90% of the ^{3}H was removed from the antigen by digestion with *N*-acetylneuraminidase, type VI (Sigma Chemical Co., St. Louis, Mo.) at 37°C for 24 h at pH 5.0. No other sugar and no protein were lost, confirming that the location of the label was mainly on sialic acid in the mucin.

Antibody generation. Standard purified GCM was injected subcutaneously into three New Zealand rabbits (using 400 μg antigen/rabbit) together with Freund's complete adjuvant. Injections of antigen were repeated at 4 and 6 wk and thereafter every 3 mo. Antisera production was monitored by Ouchterlony double-immunodiffusion analysis. One rabbit died of an unrelated respiratory infection, but antisera from the other two were pooled, and immunoglobulin-rich fractions prepared by precipitation with 40% saturated ammonium sulfate. After dialysis against phosphate-buffered saline (0.05 M Na_2PO_4-NaH_2PO_4, 0.1 M NaCl) pH 7.0, the antibody fraction was adjusted to a final concentration of 5 mg protein/ml. Throughout this study the immunoglobulin-rich fraction was used in the RIA and is referred to as the "antibody" to GCM. On double immunodiffusion against the antibody, a single precipitin line was observed for standard purified GCM. No reaction was observed with normal human serum, immunoglobulin A, or intestinal brush border membranes.

Preparation of tissue samples for assay. Tissue samples (mucosal scrapings or whole wall thickness) were homogenized at 4°C in 5 mM EDTA-NaOH pH 7.0 (1:10 wt/vol), and heavy debris cleared by a brief centrifugation at 1,000 *g* for 10 min. Supernatant solutions were mixed with ice-cold trichloroacetic and phosphotungstic acids (final concentrations 10 and 1%, respectively), and after standing for 15 h at 4°C, the acid pellet was packed by centrifugation at 2,000 *g* for 20 min. The supernate was discarded, the acid pellet was solubilized by adding ≅5 vol of phosphate-buffered saline, and neutralized with 0.5–1.0 M NaOH. After appropriate dilution, these solubilized, neutralized acid-pellet solutions were tested in the RIA for human GCM. Serum samples were diluted and assayed directly without prior acid treatment. Sputum samples obtained from patients with cystic fibrosis were precipitated by acid in the same way as tissue homogenates, before use in the RIA.

Tissues and other samples used in the assay included human lower eyelid conjunctiva provided by Dr. Alistair Adams, Princess Alexandra Eye Pavilion, Edinburgh, Scotland; dog tracheal pouch mucus from Dr. Allen Baker, Smith Kline & French, Philadelphia; *Crassostrea virginica* oyster gill mucus from Dr. Barbara Bowman, University of Texas Medical Branch, Galveston, Tex.; highly purified pig gastric mucin from Dr. Adrian Allen, University of New Castle-upon-Tyne, England. All other samples were obtained locally and frozen at −20°C until use. Most human tissues were obtained within 5 h postmortem. In the case of intestinal tissue, however, some samples were also obtained from a kidney transplant donor at the time of surgery.

Antibody titer curve. ^{3}H-Labeled human GCM (27,000 dpm, 3.5 ng protein) was incubated with increasing volumes of diluted rabbit anti-GCM antibody (20 mg protein/ml) for 30 min at 37°C. A second antibody system was added, using normal rabbit serum (10 μl) and sheep anti-rabbit IgG antisera (100 μg) as described earlier (13). After 48 h at 4°C, the pellet and supernate were separated by centrifugation (1,000 *g*, 20 min, 4°C), the pellet washed twice, and the combined supernate counted in Aquasol-2 (New England Nuclear, Boston, Mass.) in a Beckman 8100 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Control incubations containing no antibody revealed nonspecific binding to range between 5 and 10%.

RIA procedure. Incubation and assay procedures were similar to those outlined for the RIA of rat GMC (13). Briefly, incubations at 37°C contained 27,000 dpm of [^{3}H]GCM (170 μl), unlabeled standard GCM (0–200 ng protein), and anti-GCM antibody (100 μl of 1:1,000 dilution). The ^{3}H-labeled GCM was added 30 min after unlabeled standard GCM, and 15 min later the second antibody system was added. All constituents were buffered to pH 7.0 with phosphate buffered saline, giving a final volume of 0.6 ml. The remaining 48-h incubation at 4°C and separation steps were identical to the procedure described above for the antibody titer curve. Whenever tissue samples were assayed, separate control incubations containing tissue without antibody were included to monitor nonspecific binding (which was found to be negligible in all cases). If the displacement of ^{3}H-labeled GCM by a tissue sample was parallel to that of unlabeled standard GCM, the reaction was considered one of identity. A non-

parallel curve was considered to be cross-reactive. A negative reaction was defined as one showing no displacement of labeled antigen from the antibody. Quantitation of the curves was achieved by calculating the "normalized" slope of test samples, which was the ratio of the slope of the test sample divided by the slope of the standard curve. Samples exhibiting displacement curves identical to the standard GCM therefore exhibit a normalized slope of 1.0. Concentrations of mucin are presented under Results in terms of protein concentration despite the much greater proportion of carbohydrate in the mucin. This was done instead of measuring "total hexoses" because the anthrone assay for the latter (11) showed interference by the presence of high concentrations of protein. Tissue extracts tended, therefore, to give variable degrees of inaccuracy in assays for hexose, whereas total protein was consistent, reliable, and conveniently simple.

RESULTS

Antibody titer and standard curves. Using tracer amounts of ^{3}H-labeled GCM, and increasing amounts of the rabbit antibody to GCM, an antibody titer curve was constructed (Fig. 1). Even in the presence of a second antibody system, a maximum of only 60% of the labeled mucin could be precipitated. As noted earlier for purified rat GCM (13), only 60% of the ^{3}H-labeled mucin could be precipitated by a rabbit antibody to the mucin. The same rat mucin, after in vivo precursor labeling of peptide and carbohydrate components by [^{3}H]threonine and [1-^{14}C]glucosamine, respectively, was also tested against the antibody in incubations containing a much higher concentration of both antigen (20 μg protein) and antibody (0.6 ml). In this case, 80% of the antigen (both isotopes) was precipitated. Therefore, either the borohydride labeling technique "destroyed" antigenic determinants in the mucin, or the dilutions involved in using tracer amounts of ^{3}H-labeled antigen resulted in less precipitation of antigen-antibody complex. As 60% precipitation of purified mucin antigen, labeled by the borohydride technique, was also observed in the present study, we assume that the same phenomenon occurred. Fortunately, this did not preclude the usefulness of human [^{3}H]GCM in the development of a specific RIA. The standard curve is shown in Fig. 2. Bo in most experiments ranged between 35 and 38%. With each doubling dose of unlabeled GCM antigen, there was a decrease in binding of ≅14%, indicating that both labeled and unlabeled GCM bound to the antibody with the same affinity (16). The intraassay variation of each point ranged between 1 and 3%, and the most reliable range of measurement of unlabeled antigen was between 4 and 100 ng protein. The slope of the standard curve exhibited minor variations from day to day (average slope 4.0 ±0.7, total range). Thus, a standard curve was always performed at the same time as tissue samples for purposes of comparison.

Recovery of pure GCM in tissue extracts. Measurements of the mucin content of intestinal tissue or scrapings were made with the RIA on solubilized and neutralized acid pellets prepared from human jejunal homogenates as outlined under Methods. It was first established that purified GCM standards (5–2,000 ng protein) were fully precipitated by acid and recovered by RIA of the solubilized pellet. The accuracy of the RIA was checked by adding increasing amounts of standard pure GCM preparations to fixed amounts (50 μl) of solubilized jejunal pellets. As shown in Table I, the recovery of pure GCM was 95% on average.

Purification of GCM. The RIA was used to monitor the purification of human intestinal mucin from crude

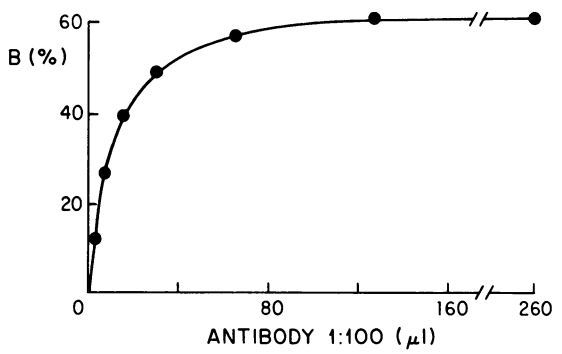

FIGURE 1 Antibody titer curve. [^{3}H]GCM was incubated with increasing volumes of rabbit anti-GCM antibody and a second antibody system of normal rabbit serum (10 μl) and sheep anti-rabbit IgG (100 μl) as described in Methods. %B refers to the antigen bound to antibody, calculated from the percentage of total radioactivity found in the pellet after centrifugation and washing. Half-maximal binding occurred with 10 μl of antibody (diluted 1:100).

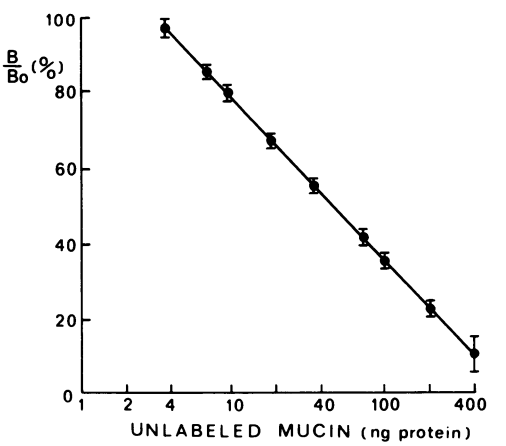

FIGURE 2 Standard curve of RIA of human GCM. Incubations contained [^{3}H]GCM antigen (27,000 dpm), unlabeled GCM (0–400 ng), and rabbit anti-GCM antibody (10 μl of 1:100 dilution) in phosphate-buffered saline, total volume 0.6 ml. Procedural details are given in Methods. B/Bo (%) is the percentage of radioactivity bound to the antibody at each concentration of unlabeled GCM, divided by the percentage bound in the absence of unlabeled GCM (Bo was 35%). Each value is the average ± SEM calculated from 20 standard curves.

TABLE I
Recovery of GCM Added to Incubations of Human Jejunal Tissue

Standard GCM added	Total GCM expected	GCM measured	Recovery
ng protein	*ng protein*	*ng protein*	*%*
0	—	4.0	—
0	—	4.0	—
4.0	8.0	8.2	102
4.0	8.0	8.2	102
7.2	11.2	10.5	94
40.0	44.0	44.09	102
72.0	76.0	73.5	97
72.0	76.0	63.0	83

Incubations contained 50 μl of jejunal acid pellets (prepared as described in Methods), plus 0–72 ng protein of unlabeled standard GCM, and the usual mixture of anti-GCM antibody and second antibody system. Recovery of added GCM was calculated from the concentration of GCM determined in each RIA.

tissue extracts through various chromatographic steps. Fig. 3 illustrates the displacement of labeled [^{3}H]GCM from its antibody by preparations representing three different stages of purification of ileal mucin. The crude sample of soluble neutralized acid pellet (Ileum) contains mucin plus all other acid-precipitable protein and glycoprotein in the mucosal scrapings. To achieve 50% displacement of antigen required 11 μg of sample protein. The purified, standard GCM required 0.016 μg of mucin protein for the same degree of displacement, whereas peak I required <0.001 μg of protein. Therefore the extent of total purification of antigen in peak I mucin was ≅10,000 times.

Comparison of mucins within the human intestinal tract. The RIA was applied to different concentrations of intestinal tissue samples (i.e., solubilized pellets) taken at autopsy (<5 h post-mortem) from a patient with no intestinal disease. Fig. 4 shows the slope of each sample compared with that of purified, standard GCM. Samples of the small and large intestine were identical in slope to the standard GCM, whereas those of the stomach and esophagus were significantly less steep. Thus, antigenic determinants of mucins in the small intestine, colon, and rectum appeared to be identical, although geographically more distant regions appeared to share some determinants but to have a weaker overall affinity for the antibody.

Cross-reactivity of human samples. Table II summarizes data obtained by multiple samples from different patients and tissues. The normalized slopes of specimens from the jejunum to the rectum were remarkably similar for most specimens, clustering close to 1.0, and therefore indicative of an extreme degree of antigenic similarity among mucins from these areas. Specimens from the stomach, eyelid, and lung (sputum) gave consistently lower slopes, indicating lesser affinity for the antibody than the intestinal mucins, and therefore a degree of structural dissimilarity. All specimens from these areas were cross-reactive however, implying that their mucin antigens shared important structural characteristics with those of the intestine. Interestingly, the variation in the normalized slopes of the four stomach specimens and the two eyelid specimens was small, suggesting that the antigen in these tissues varied little from person to person. In contrast, the sputum samples had markedly varying slopes. These specimens were all obtained from patients with a bronchopurulent disease (cystic fibrosis), and the

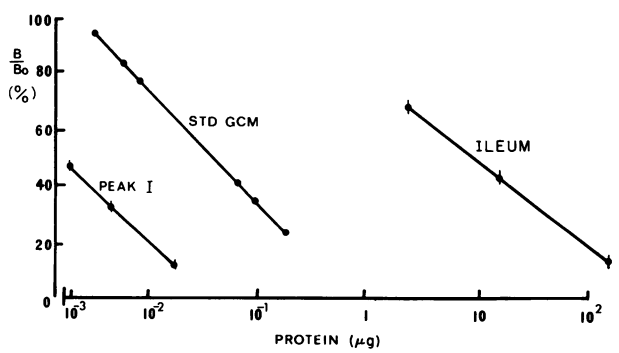

FIGURE 3 RIA of GCM fractions during purification. Varying concentrations of GCM-containing solutions were included in the RIA. Ileum, solubilized neutralized acid pellet of crude human ileal homogenate. STD GCM, standard purified GCM preparation used routinely for the RIA. Peak I is the major glycoprotein species of GCM isolated on DEAE-Bio-Gel (A) columns.

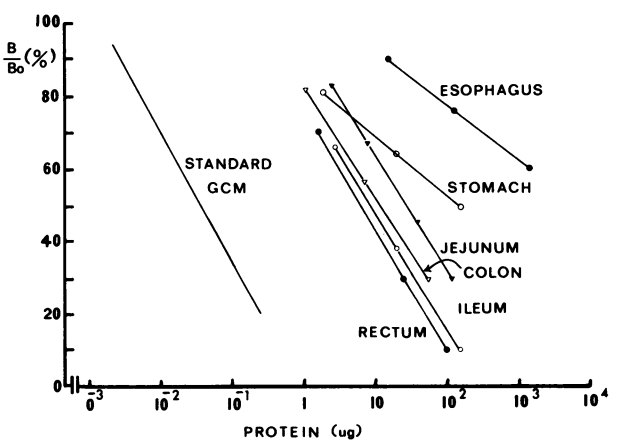

FIGURE 4 GCM RIA on human gastrointestinal tissues. The standard curve is the same as shown in Fig. 2 and is presented for comparison with values obtained using intestinal samples (neutralized acid pellets) taken from a single patient. The abscissa records the amount of sample protein used in the assay volume of 0.6 ml.

TABLE II
RIA of GCM in Human Tissues

Patient	Tissue sample	Normalized slope	Protein required for 50% displacement	Mucin concentration	Blood group activity in erythrocytes (and mucin extracts)
			μg	*μg mucin protein/ mg tissue protein*	
	Jejunum				
M.S.	Scrapings	1.04	20	2.1	A (A)
T.S.	Scrapings	1.00	45	0.93	B (−)
	Ileum				
M.S.	Scrapings	1.04	12.5	3.4	A (A)
T.S.	Scrapings	1.33	9.0	4.7	B (−)
H.H.	Scrapings	0.93	9.0	4.7	A (A)
D.B.	Scrapings	0.98	12.0	3.5	O (H)
U.K.	Section (surgical)	0.97	22.0	1.9	O (−)
	Colon				
M.S.	Section	1.04	11.0	3.8	A (A)
T.S.	Section	0.89	16.0	2.6	B (−)
D.	Section	1.10	20.0	2.1	O (−)
	Rectum				
M.S.	Section	0.92	9.0	4.7	A (A)
T.S.	Section	1.21	30.0	14.0	B (−)
	Stomach				
M.S.	Scrapings	0.67	85.0		A (A)
T.S.	Scrapings	0.76	250.0		B (−)
D.	Section	0.65	1,000.0		O (−)
P.	Section	0.65	1,100.0		O
	Sputum				
D.N.		0.72	10,500.0		A
J.K.		0.63	22,000.0		O
K.L.		0.39	20,000.0		O
D.T.		0.33	8,000.0		ND
B.W.		0.27	11,000.0		B
	Eyelid Conjunctiva				
A.		0.60	450.0		ND
B.		0.63	45.0		ND
B.R., C.	Sections of Pancreas, liver, spleen, brain, thymus, heart, kidney, skeletal muscle, serum (1–400 μg protein assayed)				No displacement

Solubilized neutralized acid pellets were made from homogenates of tissue mucosal scrapings or whole tissue sections. At least three dilutions of each sample were assayed in duplicate incubations. Normalized slopes, slope of sample/slope of standard curve. 50% displacement, the amount of sample required to achieve a B/Bo value of 50%. ND, not determined. Blood group activity was determined for patients' erythrocytes and tissue extracts (given in brackets) by hemagglutination inhibition assays (12). (−) means that the extract was negative for A, B, and H antigens. The Lewis specificity of the extract of patient U.K. was Le^{a+}; and that of H.H. was Le^{b+}.

variation quite likely represents postsecretion artifact as a result of degradative alteration of the antigen.

Tissue concentrations of mucin were surprisingly uniform within the intestine, ranging from 0.93 to 4.7 μg mucin protein/mg of tissue protein in scrapings from the small intestine. In whole thickness sections, concentrations varied between 1.9 μg in the ileum and 14.0 μg/mg tissue protein in the rectum. Because human intestinal mucin contains ≅10% protein by weight (12), the total concentrations of mucin in the

intestine are ≅10 times higher than those listed in Table II.

The affinity of tissue mucins for the GCM antibody was independent of the A or H bloodgroup status of the samples. (Unfortunately, B-reactive intestinal samples were not available.) These findings help to confirm our previous observations that the rabbit antibody does not form in response to blood group-specific sugar residues of intestinal mucins (12).

Multiple nonmucus-producing tissues and serum were tested, and despite the use of high concentrations, none exhibited any evidence of cross-reactivity (Table II). This was also true for seven other samples of serum (using as much as 1 mg protein/assay) obtained from normal volunteers (not shown).

Species cross-reactivity. A number of mucus-producing tissues or secretions of other species were surveyed. The results in Table III indicate that cross-reactivity with mucins of other species occurs, but is rather selective. Of a variety of samples tested, rabbit and monkey small intestine, and dog tracheal mucus contained cross-reactive antigenic material, although rat, toad, oyster gill, and pig mucus samples did not.

Canine tracheal mucus was a fairly pure preparation, derived from surgical tracheal pouches, which may help to explain the low concentration of protein required for 50% displacement.

The results of various (nonimmune) rabbit samples included in the RIA are presented in Table IV. Although much higher concentrations of rabbit ileum (compared with human ileum) were required to cause 50% displacement of labeled antigen, the amount of rabbit tissue required was roughly the same (350–450 μg protein) in all the intestinal samples tested. The slopes of the rabbit intestinal tissues were also quite uniform, indicating a similar degree of cross-reactivity and antibody affinity. Cross-reactivity was also seen in the case of rabbit respiratory and eyelid epithelial tissues, indicating that the organ distribution of immunoreactive mucins was qualitatively similar to that seen in human tissues.

TABLE III
Species Specificity of the RIA of Human GCM

	Normalized slope	Protein required for 50% displacement
		μg
Purified human GCM	1.00	0.042
Human ileum (mucosal scrapings)	0.98	11.0
Rabbit ileum (mucosal scrapings)	0.69	350.0
Monkey ileum (mucosal scrapings)	0.73	1,000.0
Canine tracheal mucus	0.80	11.0
Rat ileum (mucosal scrapings)	0	No displacement
Toad intestine (whole thickness)	0	No displacement
Pig gastric mucin (purified)	0	No displacement
Oyster gill mucus (secretions)	0	No displacement

See legend of Table II for method of assessment of test samples. Three concentrations or more of test samples were included in the RIA in duplicate incubations. Up to 500 μg of protein was used per assay in those samples showing no displacement of labeled antigen.

TABLE IV
Cross-reactions of Rabbit Tissues in RIA of Human GCM

Rabbit tissue	Normalized slope	Protein required for 50% displacement of labeled antigen
		μg
Lower eyelid conjunctiva	1.00	260.0
Duodenum (whole thickness)	0.55	450.0
Jejunum (whole thickness)	0.78	450.0
Ileum (whole thickness)	0.68	350.0
Rectum (whole thickness)	0.55	400.0
Trachea (whole thickness)	0.60	540.0
Bronchi (whole thickness)	0.63	1,250.0
Human ileum (whole thickness)	1.00	22.0

DISCUSSION

These experiments indicate that mucins from the human small intestine and colon are very similar immunologically, and also point to the rather widespread occurrence of cross-reacting antigens in mucus-secreting tissues in man and other animals. Very few previous studies have compared mucins from various tissues. On the basis of composition and size it has been suggested that gastric and colonic mucins are considerably different (17, 18). Our studies provide immunological support for such a conclusion. Interestingly, the normalized displacement slopes varied little from person to person for the stomach, small intestine, and colon samples, suggesting that the primary structure responsible for the antigenicity of a mucin from a particular organ displays little individual variation.

The cross-reactivity of epithelial tissues or secretions in the dog, monkey, and rabbit indicates that structural features characteristic of human mucins are not confined to human tissues. It is important to note that mucins from the rat, pig, toad, and oyster were unreactive. These differences have not been disclosed previously by biochemical or physical examination. They indicate that all mucins cannot be assumed to be structurally similar. Crude hog mucin has provided the basis for many studies of mucus rheology (19), and interaction with drugs (20), from which findings have been extrapolated to human clinical conditions. The fact that pig and human gastric mucins appear to be quite different immunologically suggests that such extrapolation may carry a risk.

Inasmuch as circulating antibodies to mucins are not found in either human or rabbit species under normal conditions, mucins are probably confined to their epithelial organs and do not gain access to the circulation. We did not detect evidence of immunoreactive mucins in the nine normal serums tested. However, autoantibody formation against colonic antigens can be easily triggered in rabbits if they are given rat (21) or human (22) colonic extracts, or even bacterial antigens which cross-react with rabbit colonic antigens (23). In certain human diseases characterized by intestinal epithelial damage, one might expect that mucins could gain access to the circulation and trigger autoantibody formation. This may explain the presence of autoantibodies to colonic tissue (24–27) and immune complexes (28, 29) observed by others in chronic ulcerative colitis. Recently, Gold and Miller (30) have shown that antisera from patients with this disease react with purified mucin antigens isolated from various segments of normal human intestine. The implication that circulating mucins and/or autoantibodies may exacerbate or perpetuate the pathophysiology of this disease, therefore, needs further investigation.

Although we did not have a tissue extract of a B-secretor individual, we found no evidence that the antigenic determinants of human mucin relied upon their content of A or H blood group-specific sugar residues because mucins from several donors with different blood groups were equally active in the RIA. This same lack of association was noted using hemagglutination inhibition methods in an earlier study (12). It has also been noted by others for salivary mucin antigens (31). L'hermitte et al. (32) showed that for a human neutral bronchial mucin both peptide (C-terminal amino acids) and carbohydrate (galactose) residues were important to its antigenicity. More recently, Roche et al. (33) have indicated that for ovine submaxillary mucin, antibodies form against mucin structures in which threonine(serine)-*N*-acetylgalactosamine linkages are preserved. In the present study, the patterns of organ and species specificity make it clear that the antigenicity of human GCM must involve more than just the *O*-glycosidic (glycopeptide) bond structure, which is common to all epithelial mucins. It would seem likely that specific sugar residues and/or amino acids close to, as well as directly involved in, the glycopeptide bond may be required.

The development of a RIA for intestinal GCM has provided for the first time a quantitative measurement of a human mucin in tissues or secretions. The sensitivity of the assay is high enough (nanogram range) to provide an accurate measurement in tissue samples containing as little as 1 mg (or less) of total protein. The RIA would therefore be applicable to intestinal biopsies or organ cultures. Because we have until this time had to rely mainly upon morphological examination of goblet cells to identify conditions of goblet cell hyperplasia or depletion (34, 35), the RIA may be useful in adding precision to studies of mucus in human disease.

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