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Research Article

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Human Colonic Goblet Cells

Demonstration of Distinct Subpopulations Defined by Mucin-specific Monoclonal Antibodies

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Abstract

We studied glycoprotein content of human colonic goblet cells, using a library of monoclonal antibodies (MAbs) directed against purified human colonic mucin (HCM). Using indirect immunofluorescence (IIF), we found that 17 of 23 anti-HCM MAbs stained some or all goblet cells of normal human colonic mucosa. We observed a variety of cellular staining patterns, including (*a*) diffuse (homogeneous) staining of intracellular mucin, (*b*) speckled (inhomogeneous) staining of mucin droplets, (*c*) peripheral staining of intracellular droplets, (*d*) cytoplasmic staining of goblet cells, and (*e*) apical (luminal) surface staining. Staining patterns were not associated with particular HCM species. In addition to variable patterns of IIF within individual cells, anti-HCM MAbs varied in the proportion of goblet cells stained. Some MAbs stained all goblet cells, while others stained a limited number of goblet cells. Although each goblet cell contained more than one type mucin, HCM species III, and IV and V appeared to exist in mutually exclusive goblet cell populations and it was possible to define at least seven subpopulations of goblet cells in colonic mucosa by their content of various combinations of HCM species.

Anti-HCM MAbs stained goblet cells from other sites within the gastrointestinal tract to a varying extent. Anti-HCM MAbs also showed extensive cross-reactivity with rodent, rabbit, and monkey colonic mucosa. However, several anti-HCM MAbs stained only human colonic mucosa. These data show that human colonic mucosa contains discrete subpopulations of goblet cells that produce distinctive combinations of specific mucin glycoprotein species.

Introduction

The colonic epithelium is an important site of interaction between man and his environment. In addition to containing smaller populations of endocrine cells, the colonic mucosa appears to be composed of seemingly homogeneous populations of epithelial colonocytes and goblet cells (1–4). Despite the recognition that alterations may exist in goblet cell glycoproteins in association with a variety of disease processes (5–11), there is little basic understanding of normal goblet cell function (12–14). It is unclear whether the enormous population of goblet cells found throughout the colon is functionally homogeneous or instead contains distinct subpopulations. Recent morphologic studies suggest that epithelial cells themselves may represent a subpopulation of colonic goblet cells (12). Regional differences between goblet cells of the ascending and descending colon have been suggested on the basis of histochemical staining patterns (2, 10, 12, 15) and within individual colonic crypts, goblet cells from the upper portion of the gland may be distinguished from those of the lower crypt using fluoresceinated lectin probes (16, 17). Nonetheless, it remains unclear whether goblet cells are functionally homogeneous or heterogeneous.

While it has long been recognized that mucin glycoprotein is the most abundant goblet cell product, there has been little detailed information on its composition and structure. Recent work in this laboratory has shown that the colonic mucosa collectively produces a complex mixture of at least six compositionally and structurally distinct mucin glycoproteins (mucin species I–VI) (9, 18, 19). However, the cellular basis of mucin glycoprotein heterogeneity remains unknown. It is unclear whether the spectrum of mucin species I–VI are produced coordinately by all goblet cells or whether alternatively they represent the products of discrete subpopulations of colonic crypts or individual goblet cells. In this report we describe the results of studies in which the heterogeneity of colonic goblet cells and their content of mucin glycoprotein has been examined using a library of anti-human colonic mucin (HCM)[1] monoclonal antibodies (MAbs) (20). These studies suggest that there are functionally distinct subclasses within otherwise morphologically homogeneous populations of human colonic goblet cells.

Methods

Preparation and characterization of anti-HCM MAbs. A library of 23 anti-HCM MAbs (7 IgM, 7 IgG_1, and 9 IgG_2) was isolated by double cloning at limiting dilutions of hybridomas produced from splenocytes of Balb/c mice immunized with pure whole human colonic mucin (20). The characteristics of this library may be briefly summarized: all MAbs bound pure HCM in a solid-phase radioimmunoassay (RIA). Recognition of separated HCM species I–VI by individual MAbs was assessed by solid-phase sandwich RIA: four bound single HCM species, five did not appear to bind any separated HCM species, and the remainder recognized various combinations of two to six of the individual HCM species. Structural determinants specified by some MAbs were identified by competitive RIA using oligosaccharides isolated from HCM species. Among the 18 MAbs that bound one or more isolated HCM species, it was possible to define the structural determinants recognized by 12 MAbs. These encompassed a range of intact oligosaccharides or components of oligosaccharides of defined structure isolated from the predominant HCM species III, IV, and V. In addition to the 12 MAbs specifying defined oligosaccharide determinants, four anti-HCM MAbs appeared to be directed to peptide cores or determinants requiring an intact peptide configuration. The structural determinants specified by the remainder of the

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1. *Abbreviations used in this paper:* FITC, fluorescein isothiocyanate; HCM, human colonic mucin; IIF, indirect immunofluorescence; MAbs, monoclonal antibodies.

anti-HCM MAbs could not be defined. Spent MAb containing media or MAbs purified from ascites were prepared as described (20).

Tissue samples. Pinch mucosal biopsy specimens were obtained from patients undergoing diagnostic flexible sigmoidoscopy, colonoscopy, or esophagogastroduodenoscopy at Massachusetts General Hospital, Boston, MA. Biopsy specimens were obtained at the same time that sampling was performed for routine histologic examination. Diagnostic classification of samples included in these studies as normal was made on the basis of the examining physicians' reports and the official interpretation of diagnostic biopsy specimens by members of the Pathology Department at Massachusetts General Hospital. Tissue samples from other sites (e.g., small intestine, gallbladder) were obtained from fresh surgical specimens. These studies were approved by the Human Studies Committee of Massachusetts General Hospital.

Samples of colonic and small intestinal mucosa were obtained from outbred rats (Charles River Breeding Laboratories, Wilmington, MA) and New Zealand white rabbits, after sacrifice by intraperitoneal injection of sodium pentobarbital; samples from cotton-top tamarins (*S. oedipus*) were obtained after anesthetization as previously described (19). All samples were promptly processed for immunofluorescent studies as described below.

Indirect immunofluorescence. Multiple frozen sections (2 μm) were prepared from tissue specimens embedded in OCT compound (Miles Laboratories, Naperville, IL) for indirect immunofluorescent (IIF) staining. Initial pilot studies showed no improvement in IIF staining characteristics in sections fixed in 1% formaldehyde before staining when compared with unfixed sections, and therefore prestaining fixation was not routinely employed. After equilibration at room temperature, one drop of anti-HCM MAb containing ascites, diluted 1:100 with phosphate-buffered saline (PBS), or spent anti-HCM MAb culture medium supernatant was added to cover individual tissue sections, and slides were placed in a moist chamber at room temperature for 30 min. Control sections were incubated with ascites or media derived from the parent NS1 myeloma line. Subsequently, ascites or media were aspirated and sections were washed three times by immersion in excess PBS. After air drying, sections were routinely stained by addition of fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse Ig (Cappel Laboratories, Cochranville, PA) diluted 1:25 with PBS. In double-labeling experiments, staining was accomplished using FITC and rhodamine red-conjugated goat anti-mouse $F(ab')_2$ specific for mouse immunoglobulin heavy chains (gamma or mu chain). Pilot studies demonstrated equivalent IIF staining in double-labeling experiments if conjugated antisera were added sequentially or simultaneously, and the latter was therefore used routinely. After incubation at room temperature for 30 min in a moist chamber, excess reagent was aspirated and sections were washed again as before. Tissue was counterstained with Evans Blue and fluorescent staining evaluated using a fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY). Quantitative estimates of goblet cell and crypt staining were determined as the percentage of cells stained in 10 high power fields (40 × objective) or lower power fields (10 × objective), respectively. The coefficient of variation between fields was found to be ±12%.

Results

In these studies, anti-HCM MAbs were used to examine tissue localization of HCM species by means of indirect immunofluorescent techniques. Among 23 anti-HCM MAbs, 17 demonstrated appreciable staining of the normal human colonic sigmoid mucosa by IIF. The properties of anti-HCM MAbs, including their ability to effect immunofluorescent staining of colonic mucosa, are summarized in Table I. Of note, several anti-HCM MAbs (2, 10, 8, 12, 15) demonstrated significant IIF staining although they failed to recognize individual mucin species. Conversely, staining could not be demonstrated using these IIF techniques by six anti-HCM MAbs that bound specific combinations of mucin species in solid-phase RIA, including two MAbs that bound single mucin species (MAbs 19 and 21). The failure to demonstrate staining by IIF did not seem to be associated with any particular HCM species.

Several distinct patterns of staining of individual cells in the normal human sigmoid colon were observed when the various anti-HCM MAbs were studied. Many anti-HCM MAbs stained mucin droplets within the goblet cells in a dense homogeneous manner designated "diffuse" (Fig. 1 *A*). These MAbs usually also stained some of the extracellular materials overlying the mucosa, which presumably represented secreted mucin glycoproteins. A number of other MAbs stained intracellular mucin droplets, but in an inhomogeneous pattern, designated "speckled" (e.g., MAb 4, Fig. 1 *B*). Other MAbs stained the "peripheral" margin of intracellular mucin droplets (e.g., MAbs 7 and 10) as depicted in Fig. 1 *C*; while the remainder stained the goblet cell "cytoplasm" (MAb 15) or "apical" membrane (MAb 8) as illustrated in Fig. 1 *D* and 1 *E*, respectively. Staining patterns of

Table I. Properties of Anti-HCM Monoclonal Antibodies

MAb*	Mucin species specificity‡	Isotypes§	Antigenic determinant‖	Immuno-fluorescence¶
1	IV, V	IgG_1	O	+
2	0	IgM	P	+
3	I, II, III, IV, V	IgG_{2B}	O	+
4	III, IV, V	IgG_{2B}	O	+
5	IV, V, VI	IgM	O	+
6	V	IgG_{2B}	O	+
7	III	IgM	UNK	+
8	IV, VI	IgG_1	P	+
9	0	IgG_{2A}	UNK	+
10	0	IgG_1	P	+
11	III, IV, V, VI	IgM	O	+
12	0	IgG_{2A}	UNK	+
13	III, IV	IgG_{2B}	O	+
14	IV	IgM	UNK	+
15	0	IgG_1	UNK	+
16	V, VI	IgM	O	+
17	IV, V	IgG_1	P	+
18	IV, V	IgG_{2B}	O	−
19	VI	IgG_1	UNK	−
20	I, II, III, IV, V, VI	IgM	O	−
21	III	IgG_{2B}	UNK	−
22	III, IV, V	IgG_1	O	−
23	II, VI	IgG_{2B}	UNK	−

* Monoclonal antibodies demonstrating binding to unfractionated pure human colonic mucin in solid-phase sandwich RIA (20).
‡ HCM species specificity determined by solid-phase sandwich assay using panel of polystyrene beads coated with separated mucin species as detailed in text (20). All MAbs bound unfractionated mucin.
§ Isotypes of anti-HCM MAbs determined by sandwich RIA using heavy chain specific ^{125}I-labeled goat anti-mouse Ig or peroxidase immunoassay.
‖ Antigenic determinant recognized by MAb: O, oligosaccharide (see 20 for specific studies); P, protein (protease sensitive); UNK, unknown.
¶ Staining of goblet cells in normal human sigoid colonic mucosa assessed by indirect immunofluorescent techniques using FITC-conjugated rabbit anti-mouse Ig as described in Methods. Characteristics of staining depicted in Figs. 1 and 2 and Table II.

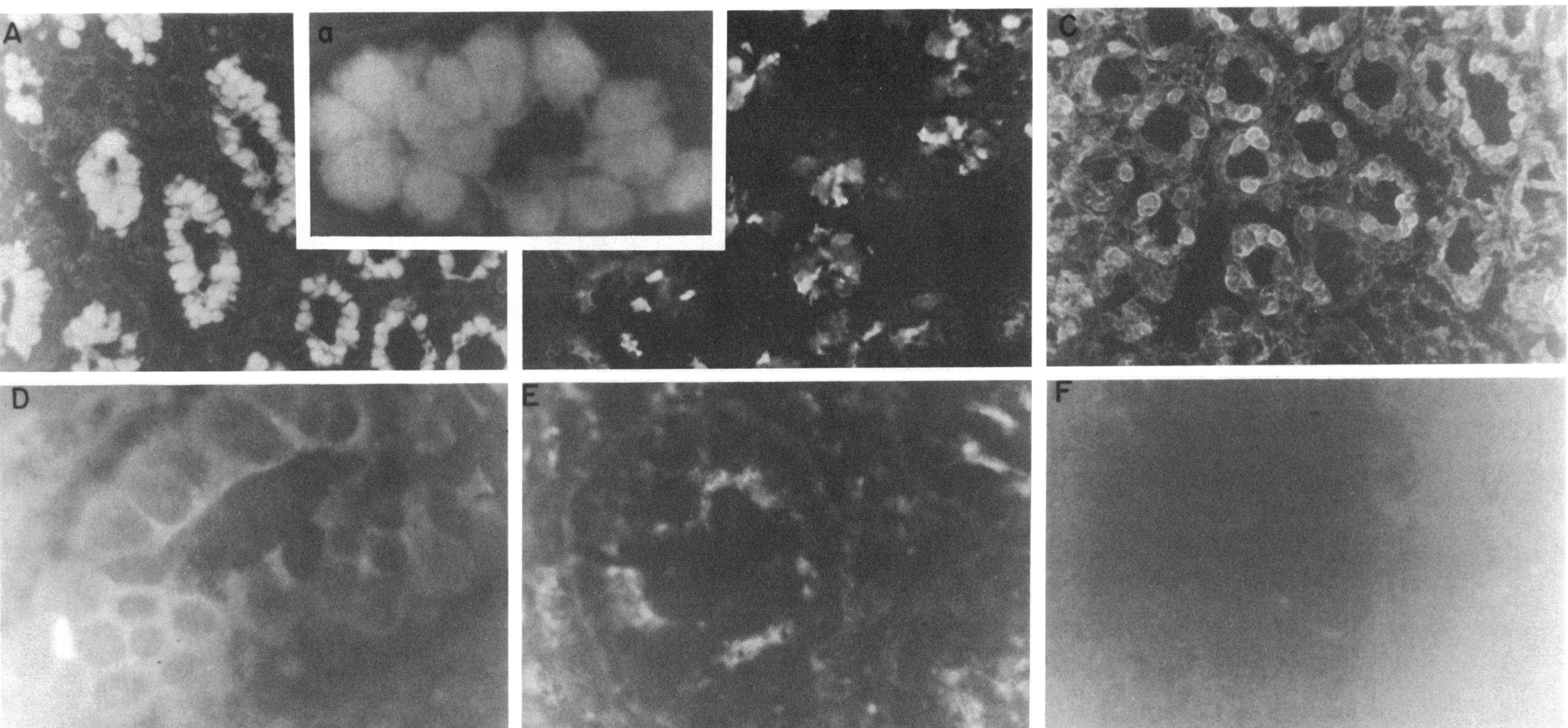

Figure 1. Representative patterns of indirect immunofluorescent staining of normal colonic goblet cells by anti-HCM MAbs. Frozen sections (2 μM) were prepared from mucosal biopsies of normal human sigmoid colon and incubated with anti-HCM MAb ascites (1:100 dilution) at room temperature for 30 min. After washing, the immunofluorescent probe (FITC-conjugated rabbit anti-mouse Ig) was added (30 min) and fluorescent staining was assessed. Note most glands seen in transverse cross section. (*A*) *Diffuse* goblet mucin staining (MAb 2); × 450 (insert *a*, × 1,150). (*B*) *Speckled* goblet mucin staining (MAb 4); × 450. (*C*) *Peripheral* goblet mucin staining (MAb 7); × 450. (*D*) *Cytoplasmic* goblet cell staining (MAb 15); × 450. (*E*) *Apical* goblet cell staining (MAb 8); × 1,150. (*F*) Control (NS1 myeloma); × 450.

individual cells were consistent within specimens; e.g., all cells stained by MAb 2 showed diffuse staining of the intracellular mucin droplet and not other staining patterns. Specific staining characteristics of each anti-HCM MAb were also consistent among mucosal samples of normal sigmoid colon from different patients ($n = 9$). The staining patterns of individual anti-HCM MAbs in normal human sigmoid colon are summarized in Table II. There were no consistent associations between species specificities of MAbs as determined by solid-phase RIA and particular patterns of cellular IIF staining (cf. Tables I and II). The latter point is illustrated by MAbs 4 and 5, which both bind species IV yet showed different cellular staining patterns. In this context, it should be noted that these MAbs recognize different and distinct epitopes as indicated by the different patterns of binding to other HCM species in solid-phase assays. Some anti-HCM MAbs that were specific for single HCM species failed to demonstrate any appreciable IIF staining; and MAbs that were positive for IIF staining but failed to bind separated mucin fractions in solid-phase RIA, demonstrated a range of staining patterns.

In addition to the differences in the manner of staining of individual cells, anti-HCM MAbs varied in the proportion of goblet cells stained within and between individual colonic crypts as shown in the representative photomicrographs of Fig. 2. 10 MAbs were found to effect "uniform" goblet cell staining defined as >95% of cells stained in >95% of crypts of normal sigmoid mucosa (e.g., MAb 3; Fig. 2 *A*). Three anti-HCM MAbs stained goblet cells in every crypt (>95%) but within individual crypts not all goblet cells were stained (<70%) (e.g., MAb 7; Fig. 2 *B*), a crypt staining pattern designated "dispersed." Finally, four anti-HCM MAbs stained mucosa in a "limited" pattern defined as staining <70% of individual cells, with fewer than 70% of crypts containing stained goblet cells. Thus, there was complete

Table II. Pattern of Cellular Staining of Normal Colonic Mucosa by Anti-HCM MAbs

MAb*	HCM species	Staining pattern
1	IV, V	Cytoplasmic
2	0	Diffuse
3	I, II, III, IV, V	Diffuse
4	III, IV, V	Speckled
5	IV, V, VI	Diffuse
6	V	Diffuse
7	III	Peripheral
8	IV, VI	Apical
9	0	Speckled
10	0	Peripheral
11	III, IV, V, VI	Diffuse
12	0	Diffuse
13	III, IV	Peripheral
14	IV	Speckled
15	0	Cytoplasmic
16	V, VI	Peripheral
17	IV, V	Peripheral

* Anti-HCM MAbs with varying HCM species binding specificities. Staining pattern of normal sigmoid mucosa by indirect immunofluorescence using FITC-conjugated rabbit anti-mouse Ig as detailed in Methods. ($n = 9$); cellular pattern classification as depicted in Fig. 1 reflecting manner of staining of individual cells within crypts.

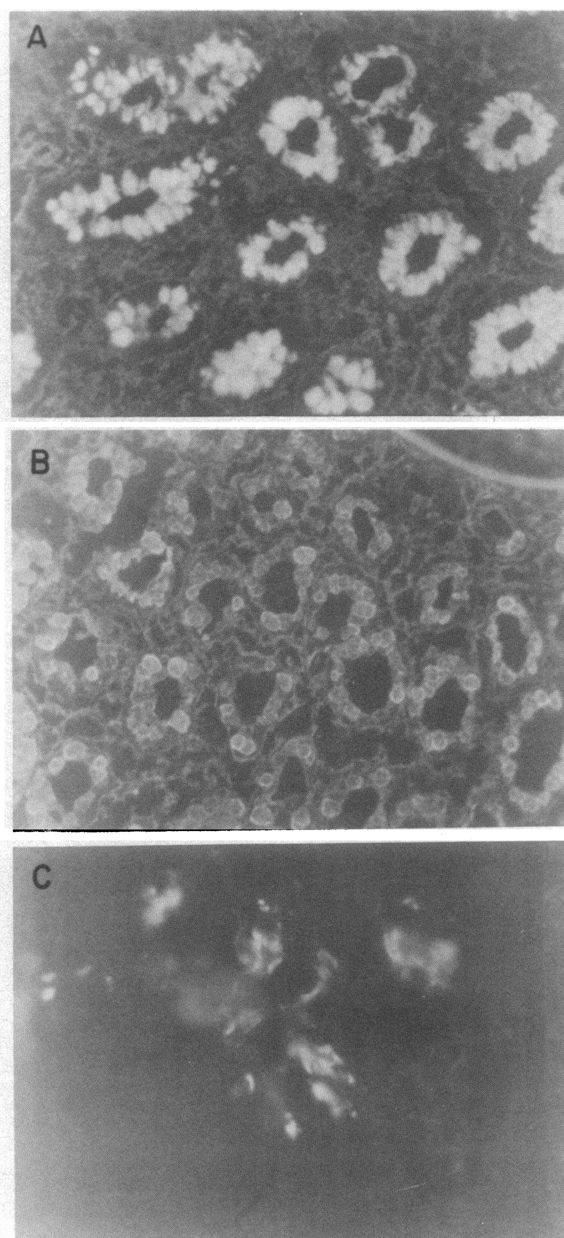

Figure 2. Heterogeneity of IIF staining of colonic mucosa by anti-HCM MAbs. IIF staining was performed as detailed in Methods and legend to Fig. 1 using normal sigmoid mucosa. (*A*) *Uniform* staining of crypts (MAb 3); × 450 (>95% cells stained in >95% crypts). (*B*) Staining of goblet cells *dispersed* in all crypts in a peripheral pattern (staining in >95% crypts, in each crypt <70% cells stained) (MAb 7, see Fig. 1 *C*); × 450. (*C*) Staining of scattered goblet cells in *limited* number of crypts (staining in <70% crypts: in each crypt <70% cells stained: MAb 6). For control see Fig. 1 *F*; × 450.

absence of staining in some crypts by MAbs exhibiting the "limited" staining pattern.

Patterns of crypt staining observed for the library of anti-HCM MAbs, referred to as "uniform," "dispersed," and "limited," and the data from which these designations were derived, are detailed in Table III. Variations in the pattern of staining appeared unrelated to the manner of staining of individual cells (i.e., diffuse, speckled, peripheral, etc.) (Tables II and III). Furthermore, heterogeneity of goblet cell staining by those MAbs

Table III. Pattern of Crypt Staining in Normal Colonic Mucosa by anti-HCM MAbs

MAbs*	Staining‡ Within crypts (% staining)	Whole crypts (% staining)	Designation
	n = 9	*n* = 9	
1	29±18	42±11	Limited
2	95±3	>99	Uniform
3	100	100	Uniform
4	97±3	95±5	Uniform
5	96±4	>99	Uniform
6	41±9	94±2	Dispersed
7	24±12	>99	Dispersed
8	32±7	31±15	Limited
9	98±3	96±4	Uniform
10	51±14	22±8	Limited
11	>99	100	Uniform
12	>99	100	Uniform
13	97±2	93±4	Uniform
14	46±10	100	Dispersed
15	>99	100	Uniform
16	21±6	27±14	Limited
17	98±2	>99	Uniform

* Anti-HCM MAbs with varying species binding specificities detailed in Table I and (23).
‡ Indirect immunofluorescent staining of normal sigmoid mucosa using FITC-conjugated rabbit anti-mouse Ig as detailed in Methods. Values reflect mean±SD of percentage of cells stained in 10 high power fields (× 40 objective) within individual crypts ("within crypts") or of crypts containing stained cells within 10 low power fields (× 10 objective) "whole crypts". Staining characteristics of individual cells are summarized in Table II.

yielding dispersed or limited patterns could not be associated with specific regions within colonic glands of the normal sigmoid colon, as indicated in Table IV. Thus, the percentage of cells stained in the upper half of crypts was equivalent to the extent of cellular staining within the lower crypts. Anti-HCM MAbs that were both mucin species-specific and exhibited IIF staining demonstrated either dispersed or limited staining patterns, with intense staining of some goblet cells and lack of staining of others (see Table I and III). This finding is consistent with the presence of compositionally and/or functionally discrete goblet cell subpopulations (see below). In contrast, less mucin species-specific anti-HCM MAbs, recognizing relatively small oligosaccharide structures, more commonly stained the mucosa in a diffuse and uniform pattern.

To further assess the relationship of staining heterogeneity to mucin species content, double-labeling IIF studies were carried out using anti-mucin MAbs with mutually exclusive species specificities as determined by solid-phase assay. These studies depended upon the use of heavy chain-specific fluorescent probes to permit simultaneous assessment of IIF staining, and were therefore limited to comparison of MAbs of different Ig classes. Multispecies-specific anti-HCM MAbs could not be uniformly used to effectively assess the relative distribution of the different species recognized by that MAb. These latter MAbs could only be used in conjunction with other MAbs directed to nonoverlapping subsets of mucin species.

As demonstrated in a representative photomicrograph (Fig. 3 *A*), goblet cells exhibited simultaneous staining for two anti-HCM MAbs of differing species specificity, which suggests that these cells contain more than one mucin. Therefore, individual goblet cells seemed to be able to produce more than one mucin species. Note that anti-HCM MAbs used in double staining studies included those with demonstrated specificity for more extended intrinsic structural components of HCM (i.e., whole oligosaccharide side chain), and these served as reliable probes for the presence of specific HCM species. Nonetheless, pairs of mucin species were not invariably associated in every goblet cell; some goblet cells in the same tissue section were stained by only one of the pair of anti-HCM MAbs. Additional double-labeling experiments suggested that some mucin species were found in mutually exclusive subpopulations of goblet cells; e.g., simultaneous incubation with MAbs selective for HCM species III (MAb 7) and V (MAb 6) showed staining of different goblet cells by the two probes (Fig. 3 *B*).

In summary, it appeared that species III did not coexist with IV or V within single goblet cells, but seemed to be restricted to nonoverlapping goblet cell populations. However, goblet cells containing each of these species did consistently contain other remaining HCM species (I, II, and VI). Collectively, the double IIF staining studies using pairs of anti-HCM MAb allowed delineation of a number of subsets of goblet cells distinguished by their content of different combinations of HCM species (see Table V). None of these subpopulations were localized to specific areas of the mucosal surface or crypts, but rather, they appeared dispersed throughout the colonic surface. In view of the limitations of the IIF technique described above, the variety of sub-

Table IV. Anti-HCM MAb Goblet Cell Staining within Crypts

Region	MAb* goblet cell staining (%) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Upper crypt	27±15	98±2	>99	98±2	>99	44±6	25±0	36±7	97	53±9	>99	100	96±2	49±7	>99	15±8	97±2
Lower crypt	33±9	94±4	>99	96±2	97±3	40±5	21±11	30±4	>99	45±8	>99	>99	98±2	38±9	>99	23±7	100

* Anti-HCM MAbs with varying HCM species binding specificites summarized in Table I. Indirect immunofluorescent staining assessed as described in Methods and legend to Table III. Values represent mean±SD of percentage of goblet cells stained derived from >10 crypts from each of nine specimens of normal sigmoid colonic mucosa. Upper crypt arbitrarily defined as upper 50% of gland and lower crypt as base and lower 50% estimated from overall crypt length.

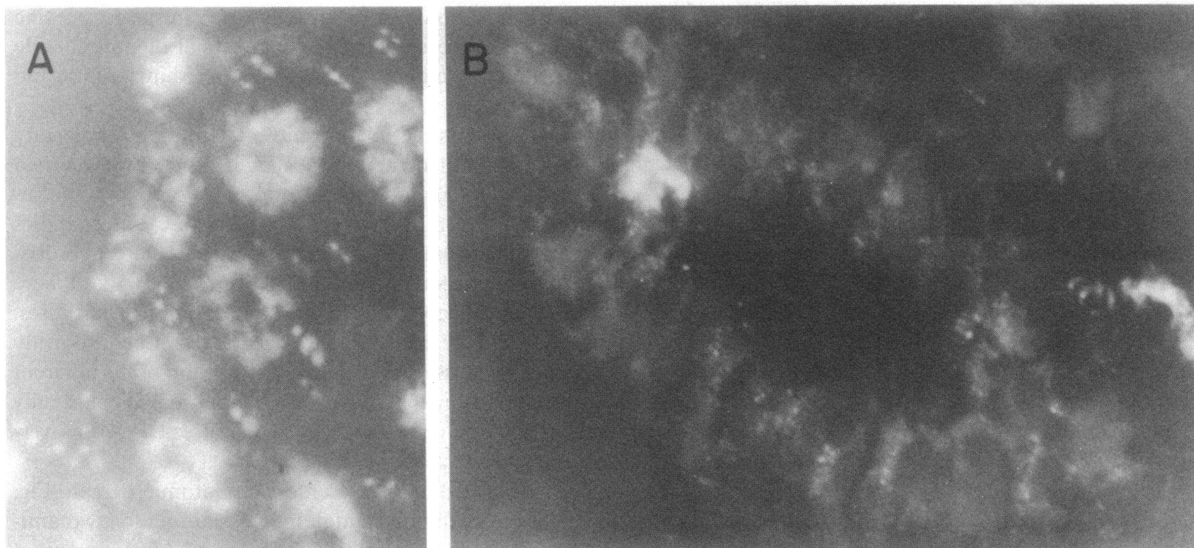

Figure 3. Double IIF staining of normal colonic mucosa using two anti-HCM MAbs. Normal human mucosa was incubated simultaneously with two MAbs, one of IgG class and the other of IgM class, followed by sequential incubation with FITC conjugated goat anti-mouse IgG and rhodamine red conjugated $F(ab')_2$ fragment goat anti-mouse IgM as detailed in Methods. (*A*) Double labeling with MAb 6 (IgG recognizing HCM species V) and MAb 14 (IgM recognizing HCM species IV) × 1,150. Note that goblet cells are stained by both MAbs. (*B*) Double labeling with MAb 6 (IgG specific for HCM species V) and MAb 7 (IgM specific for HCM species III) × 450. Note goblet cells showing mutually exclusive staining.

classes of goblet cells summarized in Table V must represent a minimum estimate of the extent of goblet cell heterogeneity, although collectively they accounted for 72±11% of goblet cells in normal colonic mucosa.

Subsequently, IIF staining by anti-HCM MAbs was assessed in tissue samples from different regions of the colon. As indicated in Table VI, staining patterns were nearly consistent throughout the colon. However, a few instances of statistically significant reductions were encountered, e.g., staining by MAbs 1 and 12 was diminished in the transverse colon and staining by MAbs 1, 7, and 12 was reduced in the cecum. Staining by 14 of the anti-HCM MAbs was comparable in tissue samples from all areas of the large bowel. Cellular and crypt staining characteristics of each anti-HCM MAb were found to be the same in mucosal samples from the other areas of the colon as those observed in the sigmoid colon (summarized in Tables II and III). Therefore, despite complex heterogeneity of colonic goblet cells at the microscopic level, the mixture of subpopulations appeared to be generally consistent throughout the large intestine. However, there was a gradient in representation of goblet cells staining by MAbs directed against HCM species IV (i.e., MAbs 1, 3, 4, 5, 8, 11, 13, 14, and 17). The proportion of cells containing HCM species IV appeared to increase progressively in more distal sites, with greatest numbers of positive cells in the rectum and sigmoid colon. However, the difference between proximal and distal colonic mucosa in the aggregate extent of staining by these MAbs did not achieve statistical significance ($P > 0.05$ and <0.10) and despite this possible gradient, there were still a large number of goblet cells that contained species IV in more proximal regions of the colon. Because these techniques permit only a semiquantitative assessment of mucin species, it is possible that there may be subtle regional variations in the number of cells stained by each MAb and/or the quantity of mucin glycoprotein antigen present within individual goblet cells.

Table V. Colonic Goblet Cell Subpopulations Defined by HCM Species

Goblet cell type*	HCM species‡					
	(I)‡	(II)‡	III	IV	V	VI
1	(+)	(+)	+			
2	(+)	(+)		+		
3	(+)	(+)			+	
4	(+)	(+)				+
5	(+)	(+)				+
6	(+)	(+)		+		+
7	(+)	(+)			+	+

* Goblet cell types defined by presence of the indicated HCM species in double IIF staining experiments as described in text using MAbs summarized in Table I.
‡ The presence of HCM species I and II could not be determined independently of other species with available MAbs.

Finally, the similarity of human colonic mucin glycoprotein to that in goblet cells in other sites of the human gastrointestinal tract as well as other animal species was examined using the library of anti-HCM MAbs and IIF techniques. As indicated in Table VII, a continuum of cross-reactivity between human colonic mucin and goblet cells in other areas of the gastrointestinal tract was observed. Overall, there was a progressive rise in the number of anti-HCM MAbs staining goblet cells in more distal areas of the digestive tract. While only one anti-HCM MAb stained gastric mucin, eight MAbs were found to stain goblet cells in the human ileum. Although there was some similarity between distal small intestinal goblet cells and colonic mucin as assessed by IIF, a number of the anti-HCM MAbs stained only the large intestinal cells. In this respect it is noteworthy that there appeared to be closer antigenic similarity between human

Table VI. Regional Variations in Immunofluorescent Staining of Colonic Mucin by Anti-HCM MAbs

Region	Anti-HCM MAb* goblet cell staining																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Rectum (*n* = 9)	32±12	92±5	100	98±2	93±5	38±12	21±7	43±8	95±4	46±9	>99	>99	98±2	50±11	>99	18±7	95±3
Sigmoid (*n* = 9)	29±18	95±3	100	97±3	96±4	42±9	24±12	32±7	98±3	51±14	>99	>99	97±2	46±10	>99	21±6	98±2
Transverse (*n* = 6)	<5‡	>99	>99	95±3	92±6	33±6	19±6	37±9	92±9	44±10	92±10	85±9§	89±8	39±7	>99	27±29	87±9
Cecum (*n* = 6)	<5‡	97±3	>99	96±2	94±7	80±8	<5	25±10	96±10	57±12	87±12	6±5†	93±7	33±8	>99	25±10	92±5

* Indirect immunofluorescent staining of goblet cells as discussed in Methods, expressed as percent±SD cells stained per 10 high power field/specimen. Pattern of staining for individual MAbs as detailed in Tables II and III. ‡ $P < 0.005$. § $P < 0.05$.

colonic goblet cells and those in the colon of a non–human primate (*S. oedipeus*, cotton top tamarin) and rodents than more proximal sites of the human gastrointestinal tract as judged by the staining of some anti-HCM MAbs (Table VII). These data suggest that there are structural features of mucin glycoproteins that may be organ-specific and presumably related to colonic function. However, as shown in Table VII, despite the similarities of staining by a number of anti-HCM MAbs in colonic mucosa from humans and a variety of animal species, concordance was not uniform and several MAbs appeared to recognize determinants expressed only in the human large intestine.

Discussion

It has long been recognized on the basis of histochemical studies that mucin glycoproteins are the most abundant products of colonic goblet cells (21–24). Early studies suggested that these substances were heterogeneous but it was unclear whether polydispersity detected by conventional analytic tools (e.g., sedimentation centrifugation) reflected important biological diversity (25–29). More recent studies from this laboratory showed the presence of at least six chromatographically distinct HCM subclasses (9, 18). Partial structural analysis of the oligosaccharides of the most abundant HCM species has confirmed the presence of some distinctive structural components on each of these HCM species (30). The recent development of a library of anti-HCM MAbs with defined HCM species specificities and defined antigenic determinants has further substantiated the presence of discrete HCM species (20).

Our library of anti-HCM MAbs, with a range of defined species binding patterns, has permitted evaluation of the cellular basis of HCM species distribution in colonic mucosa using IIF.

Table VII. Tissue Immunofluorescence using Anti-HCM MAbs

Species/Tissue	Anti-HCM MAbs*																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Human																	
Colon																	
Rectum (*n* = 9)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Sigmoid (*n* = 9)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Transverse (*n* = 6)		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Cecum (*n* = 6)		100	100	100	100	100		100	100	100	100		100	100	100	100	100
Ileum (*n* = 7)		100	100	100		100		100			86		100			72	
Duodenum/Jejunum (*n* = 4)		100	100	75							75		100				
Stomach (*n* = 4)		100	75														
Gallbladder (*n* = 3)		100															
Rat																	
Colon (*n* = 3)		100	100	100				100	67		100		100	67		67	
Small intestine (*n* = 3)		100	100	67				33			67						
Rabbit																	
Colon (*n* = 3)		100		100				100			100						
Small intestine (*n* = 3)		100		67													
Monkey																	
Colon (*n* = 2)		100		100	100	100	100	100			100		100	50		100	

* Properties of individual MAbs described in Table I. Pattern of staining by individual MAbs described in Table II. Data expressed as percentage of tissue specimens exhibiting cellular staining. Blank indicates no detectable specific immunofluorescence.

Anti-HCM MAbs with limited or unique species specificity in solid-phase binding assays stained goblet cells selectively within the mucosa, indicating that each individual HCM species is not produced by all goblet cells. Conversely, goblet cells did show simultaneous staining by MAbs directed against distinct species, which suggests that individual goblet cells produce more than a single mucin specie. However, some combinations of HCM species were never observed within the same goblet cells, indicating their production by mutually exclusive subpopulations. On the basis of these observations, it appears that colonic mucosa contains subpopulations of morphologically similar goblet cells, which produce distinctive combinations of mucin species.

Goblet cell subpopulations defined by anti-HCM MAbs were distributed throughout the colonic crypts. Each crypt appeared to contain all types of goblet cells, and similar patterns of goblet cell heterogeneity were found in mucosa from all regions of the large bowel. Thus, the organization of goblet cell heterogeneity does not appear to involve regional or glandular concentration of particular cell types. These observations suggest that all HCM species or the coordinate pairs of HCM species produced by different goblet cell populations are important for normal colonic function.

A number of MAbs that bound HCM in solid-phase assays failed to exhibit immunofluorescence in tissue studies, while a number of anti-HCM MAbs stained goblet cells specifically, yet did not recognize separated HCM species. These disparities likely reflect limitations of the methods employed to detect antigenic structures in either solid-phase binding or IIF assays. Failure to observe staining by IIF could reflect lack of accessibility of the antigenic structures or unfavorable configurations related to spatial relationships, suboptimal fixation, or intrinsic properties of the MAbs. Therefore, failure of staining or alterations in intensity of fluorescence cannot be construed as unequivocal proof of the absence of the relevant structures.

Findings obtained with anti-HCM MAbs differ from those of earlier studies using lectins, histochemical stains, or conventional antisera, which suggested the presence of gradations of mucin glycoproteins both along the length of the colon (e.g., increasing acidic mucosubstances in the left colon and neutral in the right) or within individual colonic crypts (2, 6, 15, 16, 31–33). However, these earlier approaches used reagents directed against limited peripheral structural components, such as sulfate or terminal carbohydrate residues. Solid-phase RIA binding studies with anti-HCM MAbs suggest that probes which recognize only limited peripheral structural determinants may lead to distinctions unrelated to significant structural differences. Interestingly, anti-HCM MAbs directed to nonspecific peripheral oligosaccharide determinants showed some regional variations in staining patterns comparable to those observed with these earlier methods. These findings emphasize the importance of defining the structural antigenic determinants specified by probes before the biological significance of staining or binding phenomena can be meaningfully assessed.

Despite the differences between patterns of mucin heterogeneity observed with anti-HCM MAbs and other methods within the colonic mucosa, the increasing cross-reactivity of anti-HCM MAbs at progressively more distal sites of the gastrointestinal tract is comparable to the results of earlier studies (29, 34). The present studies confirm the impression that some structural features are common to all gastrointestinal tract mucin, while other components of mucin may represent organ specific determinants. Note that the similarity between animal and human colonic mucin was greater than that observed between human colon and more proximal areas of the human digestive tract, which supports the concept that colonic mucin structure is related to specific colonic functions.

Although no HCM species was found to be localized to a specific region of the colon, HCM species IV containing goblet cells appeared to be present in greater numbers at more distal sites although the difference did not achieve the level of statistical significance. The distribution of mucin species IV containing goblet cells is interesting in view of the demonstrated association between reductions in this component and ulcerative colitis, a disorder in which there is preferential involvement of the distal colon. This observation raises the intriguing possibility that there may be a reduction or functional alteration of particular goblet cell subpopulations in association with specific disease processes; and studies are currently underway using these techniques to examine goblet cell heterogeneity in patients with ulcerative colitis as well as in other disorders. However, the limitations of the essentially qualitative methods used in these studies should be emphasized. The present methods do not precisely quantify all anti-HCM MAb defined goblet subtypes. They also do not quantify the amount of each substance within individual cells.

Despite the demonstrated association of a particular disease process with selective reduction in one mucin species, the functional roles of discrete mucin species remain unknown. Neutra and co-workers (35–38) have demonstrated that mucin production and secretion may be subject to a variety of neural and hormonal control mechanisms. They have suggested that patterns of responsiveness to various mediators may define functional subclasses of colonic goblet cells. It will be interesting to determine whether the goblet cells producing different HCM species are subject to different control mechanisms. Insight into the functional significance of these distinctions will also depend on more detailed understanding of the peptide and oligosaccharide structures of HCM species. Anti-mucin MAbs may facilitate attempts to prepare enriched subpopulations of colonic goblet cells. Collectively, the present studies indicate the presence of previously unappreciated heterogeneity among colonic goblet cells.

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