Development of a monoclonal antibody specifically reactive to gastrointestinal goblet cells
(colon/mucus/M, 40,000 protein/ulcerative colitis)

M. Vecchi*, S. Sakamaki*, B. Diamond†, A. B. Novikoff‡, P. M. Novikoff‡, and K. M. Das*

Departments of *Medicine, †Pathology, ‡Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

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ABSTRACT A mouse monoclonal antibody (7E₆A₅) of IgG isotype, reacting specifically with mucus-producing goblet cells of the human gastrointestinal tract, has been developed. 7E₆A₅ reacts by an ELISA with colonic protein eluted from a DEAE column. A screening by immunoperoxidase assay of 76 specimens from 19 different human tissues showed that the immunoreactivity of 7E₆A₅ was confined exclusively to the gobules of goblet cells in the colon, the appendix, and the small intestine. Nonoblet small and large intestinal epithelial cells did not react. Immunoelectron microscopy demonstrated the reactivity with mucus droplets in a homogeneous granular pattern inside the gobles of goblet cells. Mucus-secreting cells from remaining parts of the gastrointestinal tract and other mucus-secreting organs such as respiratory, genitourinary tracts, salivary and mammary glands did not show any reactivity to 7E₆A₅. These findings indicate that the antigen recognized by 7E₆A₅ is shared by the goblet cells of both the small and large intestines and is unique to them. The monoclonal antibody may be useful in the study of function of mucus-secreting goblet cells and may represent an important tool in the evaluation of diseases such as ulcerative colitis, colon cancer, and intestinal metaplasia in gastric mucosa that are associated with qualitative changes in goblet cell numbers or with qualitative differences in mucin secretion.

Mucus-secreting cells can be observed in different human tissues, such as in the genitourinary, bronchial, and gastrointestinal tracts. However, goblet cells are mucus-secreting cells uniquely distributed along human small and large bowel. In the small intestine, they are interspersed among the more numerous columnar absorptive cells, but in the colon they represent <90% of crypt epithelial cells (1). Their most important known function is secretion of mucin, which provides a lubricating action for the passage of luminal content and a barrier to physical, bacterial, or viral aggression (2−4). The importance of normal mucin secretion seems to be indicated by the association between qualitative and quantitative changes in the goblet cell population and mucin production, and some colonic diseases such as ulcerative colitis (5−6) and colon cancer (7).

In an earlier paper, we reported the presence of a human colon-specific Mr 40,000 protein, which is recognized by an IgG antibody present only in patients with ulcerative colitis (8). We recently developed monoclonal antibodies that enabled us to localize the Mr 40,000 protein to the plasma membrane of colonic epithelial cells (9). Here we describe the development of another monoclonal antibody that reacts with the semi-purified Mr 40,000 protein and cross-reacts with the mucin contained in globules of goblet cells from both human small and large intestine, but not with other mucus-secreting cells in the remaining parts of the gastrointestinal tract and other nongastrointestinal organs.

MATERIALS AND METHODS

Preparation of Colon-Extracted Proteins. Normal segments of colon specimens from patients who underwent colectomy for colon cancer were freed of serosal fat, minced, and homogenized in phosphate-buffered saline (PBS) (10 mM sodium phosphate/0.15 M sodium chloride, pH 7.5) containing 2 mM EDTA, 0.025 M NaNO₃, and 2 mM phenylmethylsulfonyl fluoride, centrifuged sequentially at 4000 × g for 30 min, and at 10,000 × g for 60 min at 4°C. The supernatant was then subjected to DEAE ion-exchange chromatography. The proteins eluted with 0.35 M KCl contained an enriched amount of the Mr 40,000 protein(s) along with other proteins (8). This extract was used to inject the mice for the development of hybridomas.

Monoclonal Antibody Production. Six-week-old BALB/c mice were immunized with an emulsion of 100 µg of highly enriched Mr 40,000 protein (1 mg/ml) in an equal volume of complete Freund’s adjuvant given subcutaneously over the neck, in the footpad, and intraperitoneally. The mice were given 40 µg of highly purified Mr 40,000 protein intravenously 1 day prior to fusion. The splenic lymphocytes were mixed with cells of the nonspecific BALB/c-derived myeloma line (NSO) in the mid-logarithmic phase of growth in a spleen to myeloma cell ratio of 8:1. Fusion was performed with 50% polyethylene glycol (Mr 4000; Merck, Darmstadt, Federal Republic of Germany) using the standard technique (10). Screening for antibodies was performed in an ELISA and positive hybrids were cloned in soft agar. Cells were injected intraperitoneally into 2,6,10,14-tetramethylpentadecane (Pristane; Aldrich)-primed BALB/c mice for the production of ascitic fluid.

Isotype Analysis. For determination of the immunoglobulin class, the plates were coated with rabbit anti-mouse isotype-specific immunoglobulin (Zymed Laboratories, Burlingame, CA) using a 1:1000 dilution. After overnight incubation, the culture supernatant was added to the wells and the ELISA was developed with rabbit anti-mouse immunoglobulin alkaline phosphatase conjugate (Zymed Laboratories) p-nitrophenyl phosphate (Sigma) as substrate.

ELISA. ELISA plates were coated with 50 µl of the colon extract enriched with Mr 40,000 protein at different concentrations (1.5−50 µg/ml) in carbonate buffer (pH 9.3) overnight at 37°C. Nonspecific binding was blocked with 100 µl of 0.15 M PBS (pH 7.4) per well with 2% bovine serum albumin for 3 hr at 37°C. Culture supernatant, and a control unrelated monoclonal supernatant were incubated with different antigen concentrations for 3 hr at room temperature. After washings with PBS/0.05% Tween 20, a 1:500 dilution of alkaline phosphatase-conjugated rabbit anti-mouse IgG (Zymed Laboratories) was added (50 µl per well) for 1 hr at 37°C. After extensive washings with PBS/0.05% Tween 20, the substrate (disodium p-nitrophenyl phosphate in diethanolamine buffer, pH 9.8) was added. The plates were read at 405 nm.

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immunoglobulin dilution. An unrelated monoclonal antibody (\(\text{mAb}\)) of the same immunoglobulin isotype (4T001) did not show any reactivity. (B) 7E6A5 at 1:20 dilution was tested against different concentrations of the coated proteins (1.5–50 \(\mu\)g/ml). The decrease in OD readings paralleled the decrease of coated antigen concentration.

Immunocytochemical Experiments. Preliminary experiments were performed to evaluate the effect of several fixation procedures on antigen preservation. Since formalin fixation did not affect the antigen and gave the best structural preservation, formalin-fixed paraffin-embedded tissues from surgical or endoscopy samples were used. Sections (5 \(\mu\)m) were deparafinized, rehydrated, and incubated for 30 min in 0.1 M PBS (pH 7.4) containing 0.3% \(\text{H}_2\text{O}_2\) to block endogenous peroxidase activity. Sections were washed, incubated for 2 hr with diluted (1:50) normal horse serum to block nonspecific binding, incubated overnight with the monoclonal antibody and then with biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) for 90 min, washed in PBS, and incubated in avidin biotin peroxidase complex (Vector Laboratories) for 90 min. The reaction was revealed by 3,3'-diaminobenzidine hydrochloride (1 mg/ml) in 0.1 M Tris-HCl, pH 7.6/0.02% \(\text{H}_2\text{O}_2\) for 10 min in the dark. Slides were washed, dehydrated, and mounted. All incubations were performed at room temperature except for the overnight incubation that was done at 4°C. Other slides from the same tissue block were deparafinized, rehydrated, and stained with hematoxylin and eosin for routine histological analysis.

Immunoelectron Microscopy. Endoscopic colon biopsy specimens were fixed for 3 hr in 4% paraformaldehyde and washed in 0.1 M phosphate buffer, pH 7.4/7.5% sucrose. Sections (20–30 \(\mu\)m) were cut in a freezing microtome, washed in PBS with 7.5% sucrose, and successively incubated according to the floating sections technique with PBS and 0.3% \(\text{H}_2\text{O}_2\) for 30 min, normal horse serum (1:50) for 2 hr, 7E6A5 and control monoclonal antibody overnight, biotinylated horse anti-mouse IgG for 2 hr, avidin biotin peroxidase complex for 2 hr and 3,3'-diaminobenzidine hydrochloride for 15 min. Between each incubation, extensive washings in cold PBS with 7.5% sucrose were done. Some of the sections were examined by light microscopy after mounting in glycerol gelatin (Sigma). Other sections were processed for electron microscopy as described (11). Epon sections (500–800 \(\AA\) thick) were cut and examined with and without lead staining using an electron microscope.

RESULTS

A monoclonal antibody (7E6A5) was developed reactive with human colonic protein(s) eluted from a DEAE column.

![Figure 2](image-url)
Isotype analysis using double sandwich ELISA showed that 7E<sub>6</sub>A<sub>3</sub> belonged to the IgG<sub>1</sub> class. Fig. 1A shows the ELISA readings at different dilutions of the 7E<sub>6</sub>A<sub>3</sub> ascites compared to an unrelated ascitic fluid against the colonic protein(s). The titer of the 7E<sub>6</sub>A<sub>3</sub> ascitic fluid is at ≈1:50 dilution. Fig. 1B shows a 1:20 dilution of the monoclonal antibody when titrated against different concentrations of the coated antigen. The decrease in the OD reading paralleled the decrease of coated antigen concentrations.

To localize the immunoreactive protein in tissue by 7E<sub>6</sub>A<sub>3</sub>, a total of 76 biopsy specimens from different organs were examined by the immunoperoxidase method as shown in Table 1. A positive reactivity was detected only in mucosal biopsy specimens from the colon, the appendix, and the small bowel. All other tissues were uniformly negative. Figs. 2–5 show the results of immunoperoxidase studies in different biopsy specimens from large and small intestine. The monoclonal antibody reacted exclusively with the goblet cells of the colon (Fig. 2), the appendix (Fig. 3), and the small intestine (Fig. 4). The reactivity was localized in the globules of goblet cells in the colonic mucosal glands (Figs. 2, 3, and 5) and in the goblet cells lining the colonic lumen, which were interspersed among the absorptive epithelial cells (Fig. 5). Nongoblet colonic epithelial cells lining the lumen did not stain (Fig. 5). Small intestinal epithelial cells (enterocytes) did not show any immunoreactivity (Fig. 4). The reaction with mucin droplets by 7E<sub>6</sub>A<sub>3</sub> showed a homogeneous pattern, as was further confirmed by immunoelectron microscopy (Fig. 6).

A duodenal polyp showed the same reactivity as normal duodenal mucosa—that is, the staining was localized to

**Fig. 3.** Human appendix incubated with 7E<sub>6</sub>A<sub>3</sub>; homogeneous immunoreactivity of globules of goblet cells is seen. (a) Longitudinal section (×35); (b) cross section (×25). The remaining parts of epithelial cells and lamina propria cells did not react.
goblet cells only, while enterocytes were not stained (Fig. 7). More than 95% of goblet cell types showed reactivity, with no apparent differences between cells of the upper and lower portion of the crypts and cells from different parts of the small and large bowel. Nongoblet cells and lamina propria cells did not show any reactivity. Other mucous-secreting cells from remaining parts of gastrointestinal tract, hepatobiliary, pancreatic, respiratory, and genitourinary epithelium did not react with 7E6A5 (Table 1). Salivary and mammary glands also did not react.

**DISCUSSION**

Mucin, a high molecular weight glycoprotein, is the main component of mucus, a complex secretion of epithelial cells from the gastrointestinal, respiratory, and genitourinary tracts. It is not known yet whether the differences observed in the composition of mucin from different organs are due to the existence of distinct mucus protein cores identifying unique mucin molecules or to changes in the carbohydrate moieties caused by different forms and degrees of activity of glycosylating enzymes present in different cells. The functional significance of these structural differences remains to be clarified. While the stomach mostly contains neutral mucin, the small intestine contains sialic acid-rich mucin and the colon contains both sialomucins and neutral mucins together with sulfomucins (8). Furthermore, a fair amount of cytochemical, biochemical, and immunological data seem to indicate that mucin composition differs between goblet cells from the right and the left colon and between goblet cells from the upper and the lower portion of the same crypt (11–16). From the study of the three major components of colonic mucins, 21 different oligosaccharide chains have been identified, some of them being shared by the three major colonic mucins, others being specific for a given mucin species (17). Immunofluorescence experiments with mouse monoclonal antibodies reacting with different mucin specificities have shown that many heterogeneous goblet cell populations can be recognized on the basis of their pattern of reactivity with the monoclonal antibodies (18). These findings indicate a broad heterogeneity of colonic mucins.

The monoclonal antibody 7E6A5 reported here, however, reacts with all populations of goblet cells present in both small and large intestine. No differences have been observed in the staining pattern or in the cellular distribution of the specific immunoreactivity throughout the intestine. More than 95% of goblet cells in the duodenum, jejunum, and ileum, as well as in the right and left colon, are recognized by the monoclonal antibody with a diffuse and uniform staining of the globules. It was interesting that the goblet cells in the duodenal polyp were also stained with 7E6A5. The reactivity of 7E6A5 differs, on the basis of the staining pattern and/or the cellular and tissue distribution, from what has been observed by Podolsky et al. in the study of their anti-mucin monoclonal antibody library (18, 19). Our findings indicate that the antigenic determinant(s) recognized by the monoclonal antibody 7E6A5 is shared by all goblet cells in the small and large intestine irrespective of their location in the crypts and luminal surface. However, no reactivity was observed when other mucous-secreting tissues (e.g., nasal mucosa, respiratory, and genitourinary tracts) were studied by immunoperoxidase techniques with this monoclonal antibody. The staining was also absent in the remaining parts of gastrointestinal tract, salivary and mammary glands, and in squamous epithelium such as esophagus and skin. This finding, together with the presence of the specific reactivity in all goblet cells of the gastrointestinal tract indicate that this monoclonal antibody recognizes an antigen that is common to all gastrointestinal goblet cells and unique to them.
7E6A5 has been developed using an antigen of Mr 40,000 enriched preparation of colon extract. However, it is unknown whether 7E6A5 reacts with the Mr 40,000 protein. In ELISA, 7E6A5 reacts with the colon extract enriched with Mr 40,000 protein, but in immunotransblot experiments, the monoclonal antibody neither reacts with the Mr 40,000 protein nor with any of the other proteins present in the extract. This suggests that the immunoreactive protein in the colon extract recognized by 7E6A5 is denatured by NaDodSO4 treatment. The monoclonal antibody against Mr 40,000, 7E12H12, however, reacts with the Mr 40,000 protein in immunotransblot (9). There is a clear difference in cellular reactivity of 7E12H12 and 7E6A5, as demonstrated by immunoperoxidase assay. 7E12H12 specifically reacts with colonic epithelial cells but not small intestine, and the staining is localized along basolateral domains of the plasma membrane (9). In contrast, 7E6A5 stains only the globules of the goblet cells present in both large and small intestine. Indeed, in the colonic mucosa, 7E6A5 does not stain the absorptive epithelial cells lining the lumen (Fig. 5), whereas these cells are clearly stained by the anti-Mr 40,000 monoclonal antibody (9). Because of the specific staining of the mucus globules, it appears that 7E6A5 may be directed against a glycopeptide or sugar residue specific to intestinal goblet cells. The enriched preparation of Mr 40,000 protein may contain this component as a contaminant or 7E6A5 may be cross-reactive to the Mr 40,000 protein. The explanation for staining of the small intestinal goblet cells by 7E6A5 may be due to the common glycopeptide or sugar residue shared by the goblet cells to which 7E6A5 reacts but 7E12H12 does not. The absence of Mr 40,000 protein from the small intestine is shown by immunotransblot using the natural antibody against Mr 40,000 protein eluted from ulcerative colitis colon mucosa (8) and by immunoperoxidase assay with 7E12H12.

Further studies are needed to determine whether the observed reactivity of 7E6A5 is due to an epitope present in the protein core or in the carbohydrate side chains of goblet cell mucins and if there is a relation to the Mr 40,000 protein associated with ulcerative colitis (8). The characterization of the reactive antigen(s) may clarify some of the structural and functional differences between goblet and absorptive cells in the colon and between mucin from different organs. In addition, clinically the monoclonal antibody can be applied in studies of colonic diseases associated with either quantitative changes in goblet cell numbers or with qualitative differences in mucin secretion, such as ulcerative colitis and colonic neoplasia. It may also be useful in the study of extracolonic conditions in which the appearance of metaplastic goblet cells may help identify a possible precancerous lesion, such as intestinal metaplasia in gastric mucosa, or demonstrate colonic metastases, particularly in the liver.

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