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Purification of Human Intestinal Goblet Cell Antigen (GOA[1]), Its Immunohistological Demonstration in the Intestine and in Mucus Producing Gastrointestinal Adenocarcinomas

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Summary. Goblet cell antigen (GOA[1]) was purified from gastric signet ring cell carcinoma. It was immunogenic and was used to produce antisera which stained goblet cells of the small and large intestine and of intestinalized gastric mucosa by indirect immunological methods. Various types of gastric and colonic cancer contained GOA[1]. These findings demonstrate a histiogenic relationship between intestinal goblet cells, various gastrointestinal cancers and associated premalignant conditions.

Key words: Goblet cell antigen – Immunoenzymehistology – Intestinal metaplasia – Gastrointestinal cancer – Common stem cell.

Introduction

Intestinal goblet cells are normal constituents of the small and large intestine. Goblet cells secrete gel-like mucins which consist of complex glycoproteins (for ref. see Forster, 1978). By histochemical staining procedures two main types of intestinal mucins can be distinguished: the neutral mucins and the acid mucins. Neutral mucins stain with *p*eriodic *a*cid *S*chiff reagent (PAS), whereas the acid mucins, composed of acid sialo- and sulfomucins stain with cationic dyes, the most common of which is Alcian blue (for ref. see Filipe and Branfoot, 1976). Goblet cells are not found in normal gastric mucosa, but in intestinal metaplasia gastric mucosal cells are replaced by goblet cells, striated border and Paneth cells. Intestinal metaplasia of the stomach seems to be associated with gastric cancer (for ref. see Johansen, 1976) on both histological (Jarvi and Laurén, 1951; Morson, 1955; Elster, 1960; Nakamura et al., 1971; Morson and Dawson, 1972) and epidemiological grounds (Correa et al., 1970).

* Dedicated to Prof. Dr. W. Doerr on the occasion of his 65th anniversary

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Mucus producing tumors of the stomach and large intestine also stain with Alcian blue (Lev, 1965; Stemmerman, 1967; Goldman and Ming, 1968). However, no direct immunochemical relationship has yet been established between goblet cells and these mucus producing gastrointestinal tumors. For this reason we untertook a study to identify and purify immunogenic substances of goblet cells and mucus producing tumors of the intestine (Rapp and Wurster, 1978a).

In this paper we describe the purification procedure of an immunogenic *A*lcian *b*lue staining *s*ubstance (ABS) obtained from mucus producing gastric signet ring cell carcinomas. By immunoenzyme histology and application of specific antisera identical ABS could also be demonstrated in normal goblet cells of the small and large intestine and in gastric goblet cells of intestinalized mucosa. Hence we have called this antigenic substance *go*blet cell *a*ntigen (GOA^1) until definite chemical characterization permits precise definition. Preliminary studies have shown that GOA^1 is also present in mucin producing gastrointestinal tumors (Rapp and Wurster, 1978b).

Material and Methods

Five surgically resected stomachs from individuals with signet ring cell carcinoma were used for extraction. The tumors were localized in the antrum. The tumor and goblet cells of adjacent tissue stained with Alcian blue (AB) at pH 2.5.

Extraction of Tissue. Tumors and adjacent mucosa were dissected free from serosa and muscularis. One vol of minced tumor tissue was suspended in three vols of physiological buffered saline (PBS) and in one vol of crushed ice made from PBS and homogenized at 4° C for 5 min with an Ultraturrax at maximum speed. The homogenate was centrifuged at 10,000 g for 20 min and the supernatant filtered through teflon wool. The precipitate was homogenized and centrifuged twice as described above and finally discarded. The pooled supernatants were extracted with 0.2 M perchloric acid (PCA). For this purpose one vol of 1.8 M PCA was slowly added, dropwise, to 9 vol of the supernatant at 1° C. After stirring for 20 min at 1° C the precipitate was removed by centrifugation at 10,000 g for 20 min. The precipitate was discarded and the supernatant was neutralized by 1 M NaOH and dialyzed against PBS. Neutralized PCA extract was concentrated 20 to 30 fold by Visking dialysis bags in a negative pressure concentration unit (DESAGA, Heidelberg) to approx. 5 mg/ml protein and finally dialyzed against the starting buffer of the DEAE-cellulose column (see below).

DEAE-Ion Exchange Chromatography. This was performed in a 2.5×11 cm column of DEAE-cellulose (10 g Servacel, Type 32, Serva, Heidelberg) at room temperature, previously equilibrated with the 0.05 M phosphate buffer, pH 7.0. 29 ml of concentrated, neutralized PCA extract containing a total of 130 mg protein was applied for downward elution. A hydrostatic pressure of 40 cm H_2O and a flow rate of 50 ml/h was maintained. Discontinuous elution was performed with the phosphate buffer initially and then, with rising NaCl concentration in the original buffer at 0.1, 0.2, 0.3 M and with saturated NaCl-buffer solution. Elution was monitored at 280 mμ. Fractions of 15 ml were collected and pooled according to the elution patterns, concentrated 20 to 30 fold to approx. 1 to 4 mg protein and finally dialyzed against PBS.

Molecular Exclusion Chromatography. Gel filtration was performed in a Sephadex G-200 column of 5×80 cm equilibrated with PBS. The samples obtained from the DEAE-chromatography at 0.3 M and saturated buffer solution (1 ml; 2.0 mg protein) were applied for downward elution. A hydrostatic pressure of 18 cm H_2O was applied and a flow rate of 50 ml/h was maintained.

PBS was used as eluant. Elution was monitored at 280 mμ and fractions of 10 ml were collected. The column was characterized with Dextran blue. Eluted fractions were pooled according to the elution pattern and concentrated.

Preparative Polyacrylamide Gel Electrophoresis. Preparative polyacrylamide gel electrophoresis (PAGE) was performed as horizontal slab system using a hard glass cell with an interior dimension of $0.6 \times 9.0 \times 17.5$ cm and discontinuous voltage gradients as described by us in detail elsewhere (Rapp and Lehmann, 1976). The gel contained Tris-citric acid buffer of 0.15 M, pH 9.0. A 7.5% separation gel and a 3.5% spacer gel (1.5 cm length) were used. The electrode buffer solution was Tris-boric acid 0.75 M, pH 9.0. Samples of 1.5 ml containing 10 mg protein were mixed with sucrose and applied under the liquid spacer gel. Bromphenol-blue was added to the sample as a marker of the migrating boundary during electrophoresis. A 7.5% gel connected the cell with the electrophoretic tanks. Electrophoresis was performed at 4° C for 5 h with 350 volt and 55 mAmp. At the end of the separation the bromphenol-blue tracking had traversed 16.5 cm towards the anode. Gel strips of 3 mm were cut with a nylon thread on a standardized gel cutting device, shock frozen, thawed, homogenized manually with a glass rod, eluted with 100 ml PBS, dialyzed and concentrated to 2 to 4 ml (0.025 to 0.120 mg/ml) protein. Eluted fractions were pooled according to the staining patterns with AB after analytical PAGE and cleared by centrifugation at 10000 g for 20 min.

Analytical Techniques. Analytical PAGE was performed as vertical slab gel electrophoresis with the Ortec Model 4,200 and the 4,100 pulsed constant power supply (Ortec, 1970). For buffer and gel composition the ALLEN-System (Allen et al., 1969) and gel system no 2 (Maurer, 1971) were used. Electrophoresis was performed for 45 min applying rising pulses from 75 to 300. Proteins were stained with Coomassie Brilliant Blue R250, glycoproteins were stained with the periodic acid Schiff (PAS) reagent. Acid mucins were stained with AB at pH 1.0 and pH 2.5 (Wardi-Michas, 1972).

Immunochemical analysis, immunoelectrophoresis, radial immunodiffusion and counter immunoelectrophoresis was performed in 0.05 M Na-Veronal buffer, pH 8.2 in 1% Agarose (Serva, Heidelberg). Staining of precipitates were performed with Amido black. Protein was determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as standard.

Immunohistology. The modified ethanol-acetic acid (EA) fixation procedure was used. Tissue blocks were embedded in paraplast (Wurster et al., 1978). Gastric ($n=200$) and colonic ($n=40$) specimens of various diseases and of carcinoma were obtained by surgery. Serial paraplast sections of 4 to 6 μ were made, deparaffinized and stained routinely with hematoxylin eosin, PAS and AB at pH 2.5 and 1.0. The indirect immunoenzyme technique using peroxidase labelled anti-rabbit IgG (Miles) at 1/20 working dilution was applied. IgG fractions of the antiserum were used at 0.5 mg/ml protein concentration as first layer, washed in PBS and followed by the peroxidase labelled anti-rabbit IgG. Technical procedures and the criteria for specificity of immunoenzyme staining have been described by us in detail (Wurster et al., 1978).

For classification of gastric cancer the nomenclature of Laurén (1965) was used.

Antisera. Immunsera were produced in 4 rabbits using purified samples of ABS. Rabbits were immunized s.c. with 100 μg protein in complete Freund's adjuvant and boostered with the same amount i.m. 3 times at intervals of three weeks. Rabbits were bled and antisera were stored at −20° C. Contaminating anbibodies were absorbed by Sepharose bound plasma and by 0.2 M PCA extracts of normal fundal mucosa of the stomach (1 mg protein/ml antiserum). IgG fractions of the antisera were obtained by discontinuous DEAE-cellulose chromatography at 0.01 M and 0.04 M, pH 7.0. The protein concentrations of these fractions were 0.5 mg/ml.

Monospecificity of immunoglobulin fractions of antisera were tested by the analytical gel diffusion techniques already mentioned above, using various PBS and PCA soluble organ extracts of the stomach, small and large intestine, bone marrow, lung, spleen, kidney, liver at 10–20 mg/ml (PBS-extracts) and 5 mg/ml (PCA-extracts) protein concentration. Plasma, saliva and bile were also used for comparison. Further, antisera were tested by immunoenzyme histology using tissue sections of the organs mentioned.

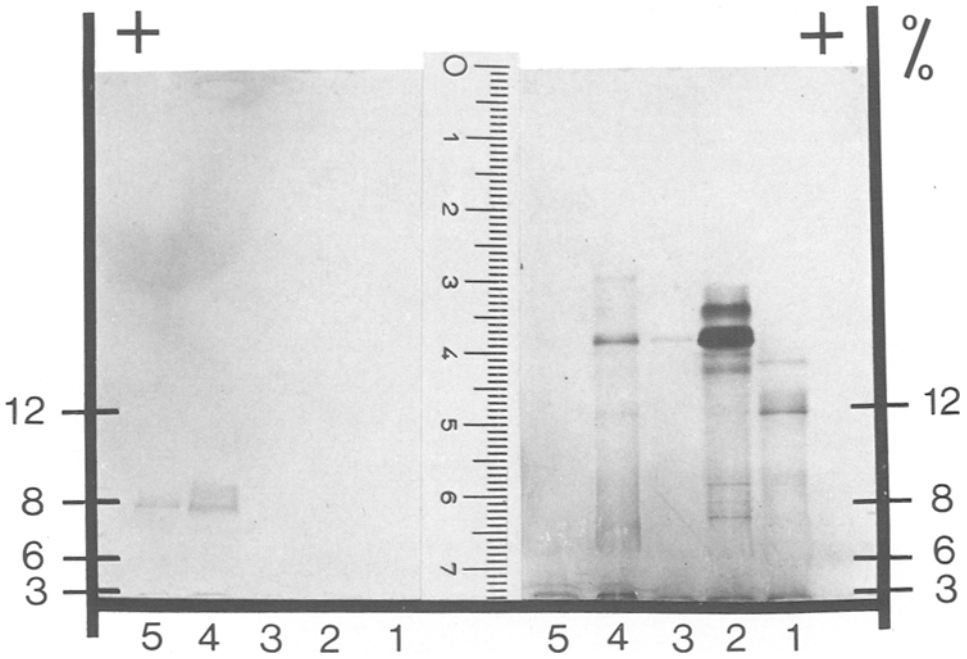

Fig. 1. Analytical Polyacrylamid Gel Electrophoresis of DEAE fractions. Perchloric acid extract of gastric signet ring cell carcinomas was separated on DEAE-cellulose chromatography at constant pH 7.0 and rising molarity of NaCl in a 0.05 M phosphate buffer (1 = 0.04, 2 = 0.1, 3 = 0.2, 4 = 0.3 M and 5 = saturated NaCl buffer solution). Alcian blue staining substances were eluted at high molarity. Left, Alcian blue staining, pH 2.5, right, protein staining with Coomassie R250

Results

Ion Exchange and Molecular Exclusion Chromatography

Soluble AB staining substances (ABS) could be extracted by PBS from gastric signet ring cell carcinomas. ABS were soluble in 0.2 to 2.0 M PCA. They migrated in the cathodic region in the 6% acrylamide gel when tested by PAGE at pH 9.0 (Fig. 1). When 0.2 M PCA gastric cancer extracts were applied to DEAE chromatography the bulk of ABS was eluted mainly at high molarity with the 0.3 M and saturated NaCl-buffer solution (Fig. 1). In some gastric specimens trace amounts of ABS were also observed at 0.05 M starting conditions and at 0.2 M. Together with ABS in the 0.3 M DEAE-fraction other substances staining for protein and PAS were observed in the more anodic gel zones of the 8 and 12% acrylamide gel concentration (Fig. 1). The ABS fractions of DEAE contained 3.6% applied protein.

When ABS containing DEAE-fractions were further separated by Sephadex G-200 molecular exclusion chromatography, ABS were eluted in the first peak with the void volume (Fig. 2). Two other peaks were observed which did not contain ABS but other substances staining for protein and PAS. In the ABS

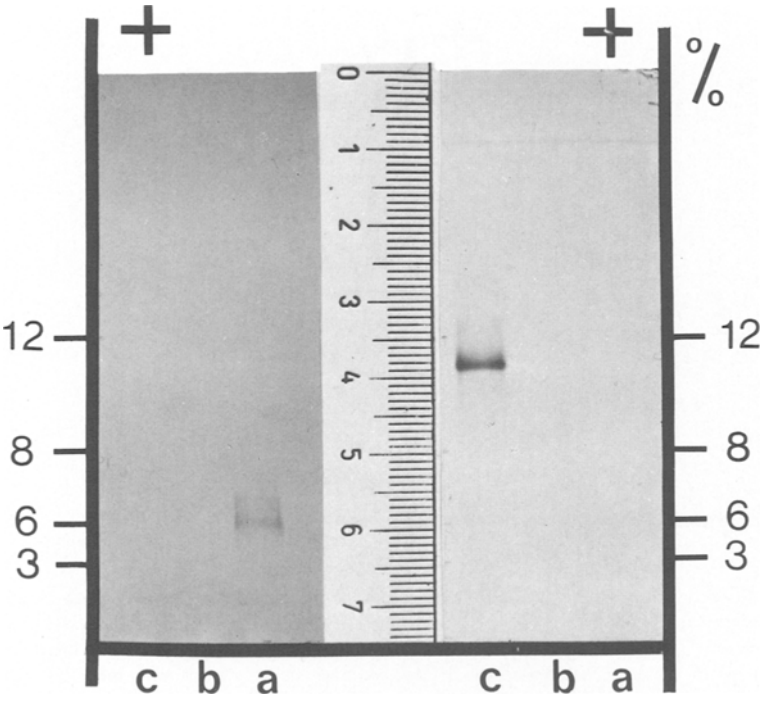

Fig. 2. Analytical Polyacrylamid Gel Electrophoresis after Sephadex G200 gel filtration of DEAE fractions eluted at 0.3 M, pH 7.0. Three protein peaks (**a, b, c**) were eluted. Alcian blue staining substances were eluted in the first peak (**a**) with the void volume. No other proteins were observed in this peak. Left, Alcian blue staining; right, protein staining

containing G-200 fractions no protein or PAS staining material was observed by analytical PAGE. ABS fractions contained 3.5% of the total protein applied to Sephadex G-200 column.

Preparative Polyacrylamide Gel Electrophoresis

When ABS obtained in the 0.3 M DEAE-fraction were submitted to preparative PAGE a more effective and economic separation was observed when compared with the G-200 chromatography. ABS were eluted from the cathodic gel strips number 3 to 10 in the 7.5% gel zones and resulted in a total of at least 4 distinct ABS bands when tested in analytical PAGE (Fig. 3). The recovery was 23% of protein applied and a total of 2.3 mg protein of ABS material was obtained from each electrophoretic run. Purified ABS showed in PAGE marked AB staining at pH 2.5 and 1.0, a very low protein and no PAS staining.

Immunochemical Characterization

Four rabbits immunized with a total of 400 μg protein of purified ABS gave rise to antisera which contained precipitating antibodies against the original

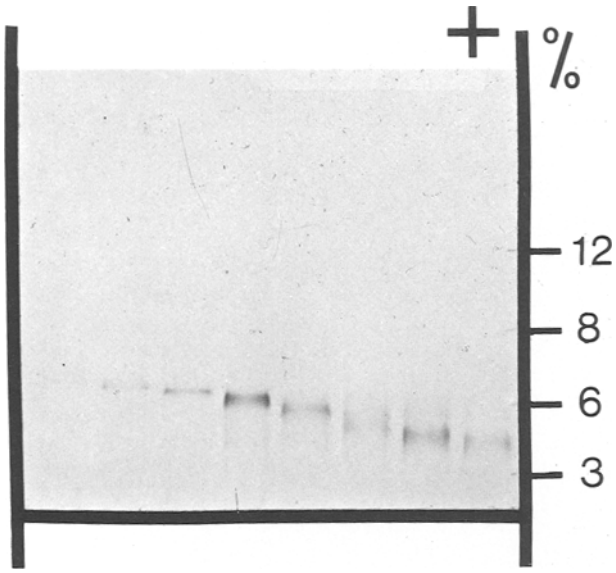

Fig. 3. Analytical Polyacrylamid Gel Electrophoresis of Alcian blue staining substances obtained by preparative polyacrylamid gel electrophoresis. The starting material were Alcian blue staining substances obtained by DEAE-chromatography at 0.3 M salt concentration. ABS were eluted in the cathodic gel strips in 7.5% gel. Various Alcian blue staining bands of different mobility can be seen. Alcian blue staining at pH 2.5

PBS and PCA extracts and against the purified material. In immunoelectrophoresis a very faint line was observed in the alpha-2 region. A second precipitating antibody system was directed against an alpha-2 serum protein, which was also present in saliva. This protein was not further characterized. Antibodies directed against this protein could be absorbed by solid plasma immunoadsorbents. Specific antiserum reacted also with extracts of the colon, the small intestine and of intestinalized gastric musoca containing goblet cells.

Immunohistological Demonstration of Goblet Cell Antigen

Rabbit immunoglobulins of the IgG type and directed against the purified ABS, stained goblet cells of the normal small and large intestine by indirect immunoenzyme techniques (Fig. 4). Weak staining of normal gastric surface epithelium could be suppressed by absorption of the antiserum with fundal extracts of normal gastric mucosa. After absorption with gastric mucosal extracts only the intestinal goblet cells reacted with the antibodies. The immunological contrast of this staining was excellent.

Staining was observed in the cytoplasma of the goblet cells, in mucinous substances being expelled through the cell apex, in mucinous layers within the lumen of the glands and also on the surface of the epithelium (Fig. 4). The immunoenzyme staining proved to be specific according to the various test systems for nonspecific staining (Wurster et al., 1978a).

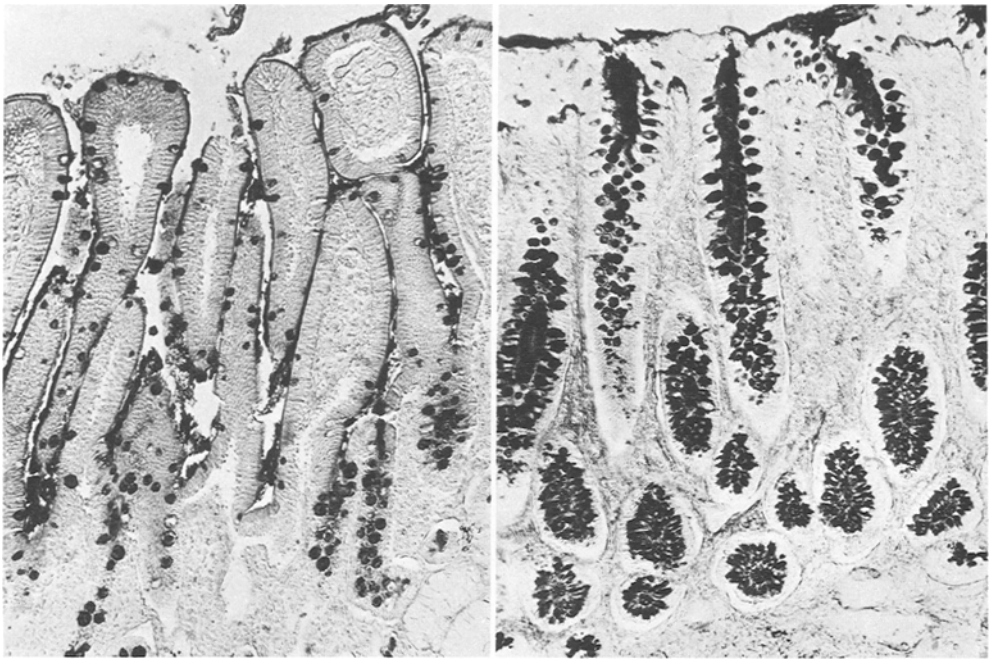

Fig. 4. GOA^1-positive goblet cells of the duodenum (left) and the colon (right). $\times 100$

In the diseased intestine we observed the following patterns and types of GOA^1-positive cells and mucins.

Stomach. Normal gastric epithelium of the corpus and the antrum did not contain GOA^1. In specimens with intestinal metaplasia the intracellular and the extracellular mucins of the majority of goblet cells stained for GOA^1, but the columnar absorptive cells surrounding the goblet cells did not (Fig. 5).

The following distribution patterns of GOA^1-positive goblet cells could be distinguished in gastric metaplasia: 1) single cells, 2) minor focal collections 3) focal collections and 4) diffuse patterns. The sell size, according to the immunological staining, ranged from small round goblet cells in the basal part of the glands to medium or large round goblet cells at the luminal aspect of the mucosa. In some specimens of tissue adjacent to the tumor we observed atypical columnar cells of the surface epithelium which contained small droplets of GOA^1 in their apical parts.

In gastric carcinoma various patterns and types of GOA^1-positive cells and mucins were observed: 1) no GOA^1 was present in gastric carcinoma of the pure intestinal type. 2) In carcinoma of the intestinal type small round or rod like GOA^1-positive immature goblet cells were interspersed between the GOA^1-negative intestinal tumor cells and further small lakes of GOA^1-positive mucins were observed (Fig. 6). 3) Gastric cancer cells of the signet ring cell type contained GOA^1 within their cytoplasm and also in the surrounding masses of

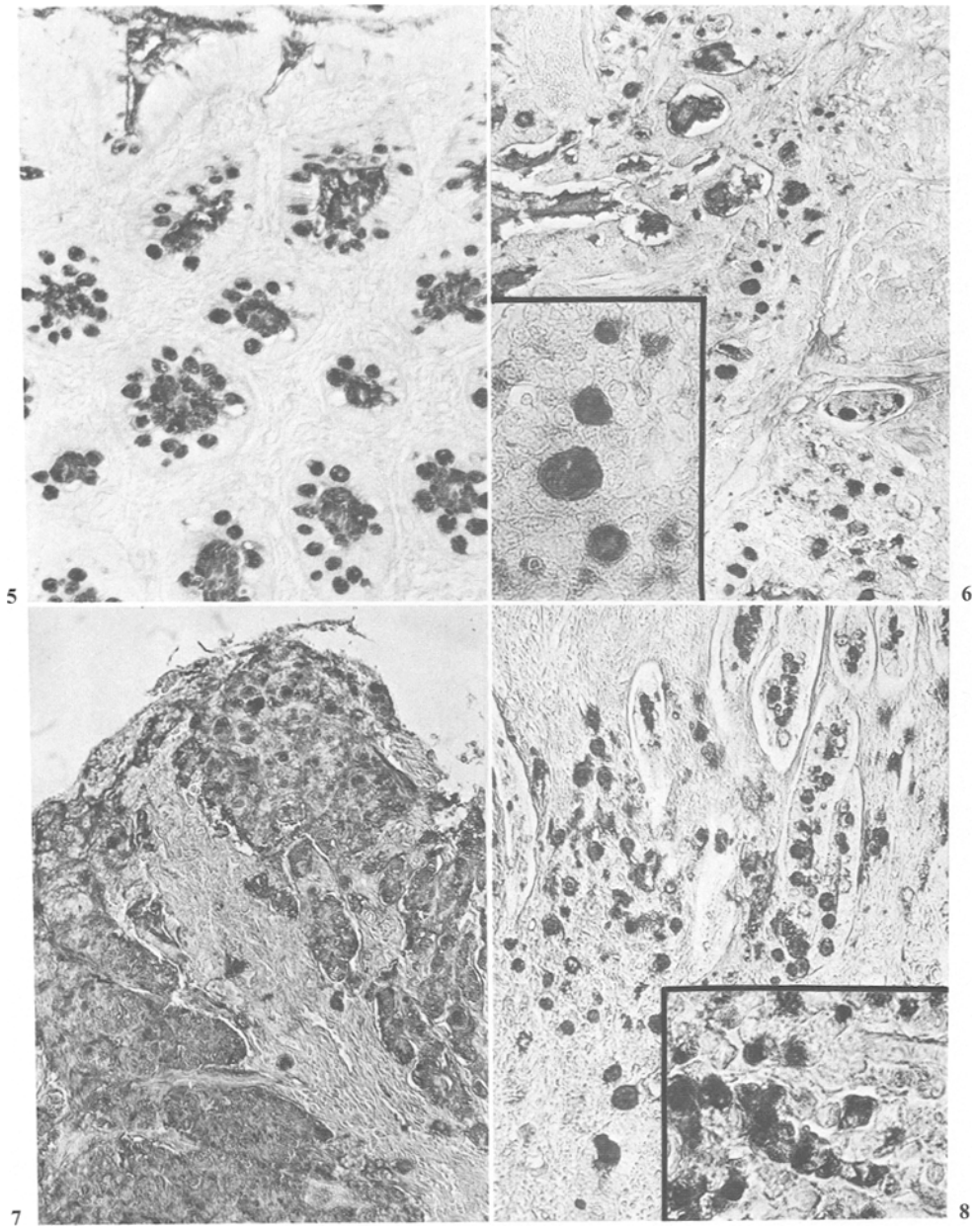

Fig. 5. Gastric intestinal metaplasia of the corpus. Diffuse distribution pattern. Goblet cells stain for GOA[1]. ×100

Fig. 6. Gastric carcinoma. Intestinal mixed type, low mucin production. Composed of GOA[1]-negative columnar cancer cells with interspersed GOA[1]-positive atypical cells. Small pools of mucus which contain GOA[1]. ×100 (×540)

Fig. 7. Gastric carcinoma. Signet ring cell type. Tumor cells are GOA[1]-positive. ×100

Fig. 8. Gastric carcinoma of diffuse type. Left, diffuse carcinoma cells (not all) are GOA[1]-positive. ×100 (×540). At the upper right, adjacent area with intestinal metaplasia

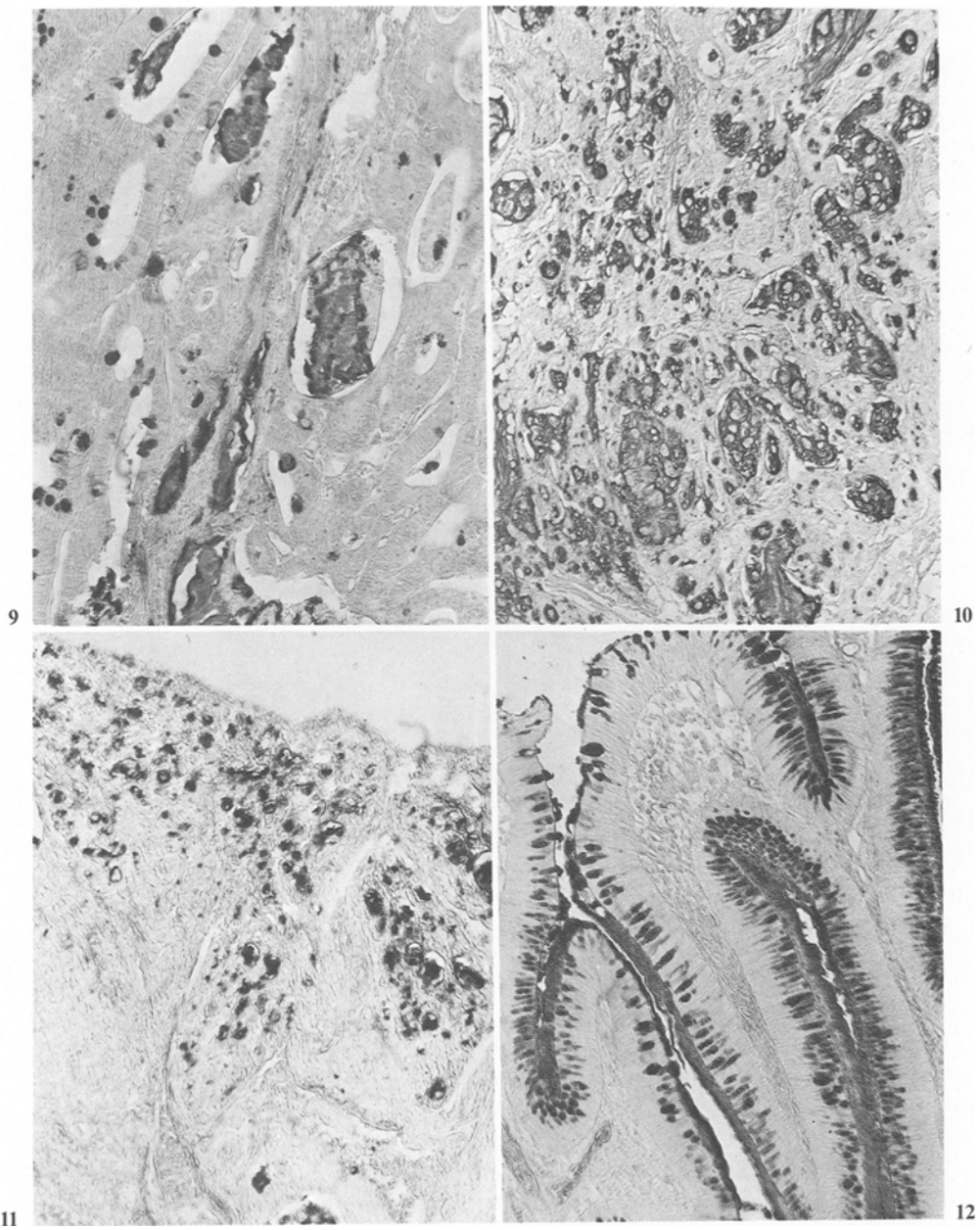

Fig. 9. Colonic adenocarcinoma. Intestinal mixed type of low mucus production. Composed mainly of GOA[1]-negative tumor cells with interspersed irregular GOA[1]-positive cells of various sizes. Large pools of mucus. × 100

Fig. 10. Colonic adenocarcinoma. Intestinal mixed type of high mucus production. GOA[1]-positive cancer cells of irregular form and GOA[1]-negative cancer cells. Larce pools of mucus, containing tumor cells. × 100

Fig. 11. Colonic adenocarcinoma of diffuse type. Diffuse carcinoma cells (not all) contain GOA[1]. × 100

Fig. 12. Tumor adjacent colonic tubular adenoma with GOA[1]-positive atypical cells. × 100

mucin (Fig. 7). In gastric cancer of the diffuse type GOA^1 was observed in the cytoplasm of single tumor cells (Fig. 8).

Colon and Rectum. In normal mucosa of the colon and rectum all goblet cells contained GOA^1. In some instances exhausted goblet cells with no, or only trace amounts of GOA^1, were observed. Immature goblet cells of the middle and basal part of the crypts also stained for GOA^1. The mucin within the lumen of the crypts and on the surface contained GOA^1 (Fig. 4).

In colorectal adenocarcinomas different distribution patterns of GOA^1-positive cells and mucins were observed: 1) No GOA^1 was seen in adenocarcinoma of differentiated intestinal type. 2) Adenocarcinoma of mixed type contained interspersed GOA^1-positive cells, which resembled more or less immature round goblet cells (Fig. 9). 3) Mucus producing adenocarcinomas contained large GOA^1-positive cells and mucin lakes (Fig. 10). 4) Adenocarcinoma of the colon of diffuse type contained GOA^1-positive cells (Fig. 11).

Tumor adjacent adenomas of the tubular, villous or mixed type showed GOA^1-positive immature goblet cells of different size and form (Fig. 12).

Discussion

The original aim of this study was the identification of immunogenic marker substances specific for gastric goblet cells, which are found in gastric intestinal metaplasia. In pursuing this aim we used a methodological approach which already proved to be useful in previous studies on marker substances of normal human gastric mucosa (Rapp et al., 1964): a combination of electrophoresis, immunoelectrophoresis and histochemical staining procedures.

We obtained a highly purified substance from gastric signet ring cell carcinomas which was soluble in perchloric acid and which stained with Alcian blue at pH 2.5 and 1.0. This substance proved to be immunogenic by immunization and antigenic by gel diffusion experiments and by immunohistology.This substance was localized in the cytoplasma of the goblet cells of the whole intestine, hence its designation as goblet cell antigen^1 (GOA^1).

Since Alcian blue staining acid mucosubstances are present in signet ring and goblet shaped tumor cells (Lev, 1965; Stemmerman, 1967; Goldman and Ming, 1968) we used these tumors as source for extraction. As extracted GOA^1 proved to be soluble in PBS and PCA, PCA extraction, – denaturating many other proteins, – was used as first step of purification, despite the fact that we observed a considerable loss of GOA^1 by this procedure. The second purification step was DEAE-cellulose chromatography by which the bulk of GOA^1 was eluted at high salt concentration quite in contrast to its apparent slow electrophoretic mobility in PAGE. This elution pattern, can be explained by the sulfo- and sialomucin type of GOA^1 with many negatively charged ionized groups, whereas the slow electrophoretic migration in PAGE was produced by the gel sieving capacity of this method and by the high molecular weight of GOA^1. One reason for the use of DEAE chromatography for purification was the clear cut separation of GOA^1 from carcinoembryonic antigen (CEA)

(Gold and Freedmann, 1965) and from CEA-crossreacting substances (Primus et al., 1977) which, in previous studies, were eluted at much lower salt concentrations (Rapp et al., 1976). By Sephadex G-200 gel filtration, GOA^1 was eluted with the void volume, a further indication of its high molecular weight. The recovery after gel filtration was extremely low, so that we assume a major loss of GOA^1 by nonspecific binding during this procedure. Therefore we used preparative PAGE as third step of purification. Purified GOA^1 could be resolved by analytical PAGE in at least 4 electrophoretic components. This electrophoretic diversity may reflect a charge heterogeneity of GOA^1 depending on the amount of sialic acid.

Purified GOA^1 showed predominant staining with AB at pH 2.5 and 2.0 in analytical PAGE. According to histochemical criteria (Filipe and Branfoot, 1976) GOA^1 of this type can be considered to be an acid sulfomucin. In the purified material we observed no PAS staining, which means that GOA^1 is devoid of neutral mucins and vic-glycol groups. The low, often non-existant protein staining can be explained by the predominance of carbohydrates in the molecule. For this reason protein staining as a criterion of purity, can no longer be accepted when intestinal components such as CEA and others are studied in polyacrylamide gel electrophoresis. Definitive quantitative measurements during purification of GOA^1 will also only be possible after complete chemical analysis of this substance. For this reason quantitative data obtained by the Folin reaction and mentioned here do not reflect the real amount of GOA^1 obtained. In analytical PAGE GOA^1 proved to be pure according to 3 different staining techniques. However, upon immunization we found indirect evidence for trace contaminants from plasma and from normal gastric epithelial components.

No relationship with CEA or its crossreactive substances was observed either in gel diffusion or by immunoenzyme histology (Rapp et al., unpublished). This comparison was made because staining of goblet cells of the small intestine with CEA has been reported recently (Isaacson and Judd, 1977). Because of similar physicochemical characteristics of GOA^1 and CEA (soluble in PCA, eluted with the void volume of Sephadex G-200, presence of GOA^1 at starting conditions in DEAE chromatography, same electrophoretic mobility), trace amounts of GOA^1 in CEA preparations might give rise to contaminating antibodies. Nevertheless, we have recently shown (Rapp and Wurster, 1978a) that some goblet cells of gastric tissue adjacent to carcinomas did stain with antibodies directed against CEA, whereas the same antibodies did not stain goblet cells of the large intestine.

GOA^1 was extracted from gastric signet ring cell carcinoma, a tumor which produces acid mucins. The antisera obtained reacted with goblet cells of the normal intestine, with goblet cells of intestinalized gastric mucosa and with mucin producing gastrointestinal tumors. These findings can be interpreted as immunochemical proof for a relationship between normal and pathological intestinal goblet cells and mucus producing gastrointestinal cancers.

A variety of more or less well defined "intestinal antigens" have been described (for ref. see Goldenberg, 1976). The first authors who reported the existence of immunogenic intestinal antigens by immunofluorescence techniques

were Nairn et al. (1962, 1966) and DeBoer et al. (1969), but no special mention was made as to their association with goblet cells. A substance, *i*ntestinal *m*ucosa specific *g*lycoprotein (IMG), comparable with our GOA[1] was identified, purified and studied in immunofluorescence by Kawasaki et al. (1971a, 1971b, 1972, 1974). Another substance from the colon, the *c*olonic *m*ucoprotein *a*ntigen (CMA) described by Gold and Miller (1974) proved to be PCA soluble and of high molecular weight. GOA might also be related to the *c*olon *s*pecific *a*ntigen (CSA) of Goldenberg et al. (1975) described by these authors as the high or very high molecular weight variant. According to the immunofluorescent studies of Häkkinen (1968) intestinal *f*eto*s*ulfoglycoprotein *a*ntigen (FSA, i-antigen) reacting with goblet cells and with striated absorptive cells of the duodenum, might also be associated with GOA. Other immunogenic and antigenic substances of human colon have been mentioned by various authors (Henry, 1968; von Kleist und Burtin, 1969; Norland, 1969; Burtin, 1971; Tappeiner et al., 1973). Whether GOA[1] is related to or even identical with the major glycoprotein described by Schrager and Oates (1973) cannot be determined at present.

*I*ntestinal *m*ucus *a*ntigen (IMA) as demonstrated recently by Mori et al. (1979) showed a histological distribution pattern which closely resembles the patterns of GOA[1] described in this paper.

Goblet mucins, the secretory product of goblet cells are of a complex chemical nature (Filipe and Branfoot, 1974, 1976) and may be composed of different antigenic entities, each entity with different varying immunogenic determinants depending on topographical distribution, state of metabolic maturation and ontogenetic differentiation. For this reason, direct immunchemical comparison and further chemical studies should be performed with the "intestinal antigens", hitherto described.

The immunohistochemical findings of this study led us to the following working hypotheses: 1) All intestinal goblet cells produce and secrete acid mucins which are immunochemically identical, or which share at least one common antigenic determinant. Until definitive immunochemical analysis is complete we call these immunogenic and related mucins of the intestinal goblet cells "goblet cell antigen[1]" (GOA[1]). We have, so far, no evidence that antibodies directed against GOA[1] distinguish between sialo- and sulfomucins. From current investigations we assume that GOA[1] related substances might be also present in other mucin producing organs and tumors. 2) Metaplastic goblet and tumor cells belonging to different types of adenocarcinoma contain GOA[1]. This may be further evidence for the existence of a metabolic association between intestinal metaplasia and gastric cancer (Wattenberg, 1959; Planteydt and Willinghagen, 1965). 3) GOA[1] is present in the colonic goblet cells and in tumor cells of different types of colonic cancers and in adenomas. This can be considered as evidence for a histogenetic relationship between goblet cells and these malignant and premalignant conditions. 4) Following the use of GOA[1] as a marker system we have observed a striking morphological and immunohistochemical resemblance between gastric- and colonic cancers. On the basis of current investigations on CEA and GOA[1] distribution patterns (Rapp and Wurster 1979; Wurster and Rapp, 1979) we anticipate a new system of immunochemical classification of gastrointestinal tumors complementary to that described by

Laurén (1975) and Ming (1977). Further, we speculate that certain CEA and GOA[1]-positive premalignant (transitional) and malignant gastrointestinal cell types originate from a common intestinal pluripotent stem cell.

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