Human Pancreatic Cancer-associated Antigens Detected by Murine Monoclonal Antibodies

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ABSTRACT

Two monoclonal antibodies, YPan1 and YPan2, were produced from spleen cells of mice immunized against a human pancreatic cancer cell line, Capan-2, which also reacted with eight other human pancreatic carcinoma cell lines and with sections of cancerous human pancreas tissue. Reactivity was also found with colon and stomach carcinoma tissues. Whereas YPan1 reacted strongly with normal pancreatic tissue, it bound weakly, if at all, to a variety of other normal tissues. YPan1 reacted with both the membranes and cytoplasm of pancreatic adenocarcinoma cells and with constituents of normal pancreatic ductal cells and duct luminal contents. YPan2, on the other hand, reacts to a greater degree with intracytoplasmic constituents in pancreatic adenocarcinoma cells. Unlike YPan1, YPan2 reacted weakly with only one of 16 cases of normal pancreas. Pretreatment of the tissue with neuraminidase abolished YPan1’s activity, which indicated that sialic acid may be involved in the antigenic activity of the molecule(s) recognized by YPan1. Neuraminidase pretreatment had no effect on YPan2 reactivity. Neither YPan1 nor YPan2 competed with 19-9 monoclonal antibody in binding to soluble CA 19-9 antigen. These results suggest that these monoclonal antibodies are of potential use in the diagnosis of pancreatic carcinoma.

INTRODUCTION

The incidence of pancreatic cancer has increased steadily over the past 40 years so that pancreatic cancer now is the fourth most common cause of death from all cancers in the United States (4). Early detection of pancreatic cancer is difficult for several reasons. The pancreas is relatively inaccessible and easily traumatized so that it is frequently not advisable to examine this organ directly. Another hindrance to early detection has been the lack of a specific marker for this disease. Thus a blood test that is highly sensitive and specific for early pancreatic cancer would be a major advance in the diagnosis of this disease. The techniques which make it possible to produce monoclonal antibodies of predetermined specificity by means of hybridomas (5, 6) have greatly improved the possibility of mapping the surface antigenic determinants of tumor cells and of cataloguing cellular components that may be released into the bloodstream. Furthermore, an obstacle to an understanding of the histogenesis of pancreatic cancer has been that any pathological process, such as malignant transformation, may result in alterations in the production of cell markers such as zymogen granules, mucin, and enzymes in a given cell type. Thus the proper histotypical identification of pancreatic cancer cells is often difficult. The availability of monoclonal antibodies that are pancreatic cancer associated and/or are specific for a particular pancreatic cell product would facilitate the histological classification of pancreatic cancer tissues.

Several pancreatic cancer-associated antigens defined by monoclonal antibodies have been described recently. One of these, 19-9, was originally isolated for its reactivity with human colon cancer cells (7), but it has since been shown to also react with gastric and pancreatic cancer tissues (8, 9) and cell lines (10) and with sera from patients with gastric and pancreatic cancer (11). Reactivity with normal pancreas has also been reported (8, 9). Monoclonal antibodies DU-PAN-1, 2, 3, 4, and 5 were elicited against the human pancreatic cell line HPF and were found to have varying specificities toward the human pancreatic cancer cell lines examined (12). The tissue specificity of DU-PAN-2 has been reported recently (13), and DU-PAN-2 antigen has been shown to be present in serum and ascites of patients with adenocarcinoma (14). Two other pancreas-associated monoclonal antibodies have been reported recently. One appears to be specific for acinar cells and human acinar cell carcinoma (15), and the other appears to be specific for ductal cells and human duct cell carcinoma (16).

In the present study, we report the production of two monoclonal antibodies against human pancreatic cancer cell line Capan-2. One of these, YPan1, reacts with a mucin-like glycoprotein (1, 2) and the other, YPan2, appears to react preferentially with cancerous cells of the human pancreas.

MATERIALS AND METHODS

Cell Lines and Tissues. Human pancreatic carcinoma cell lines used were: Capan-1 (J. Fogh); Capan-2 (J. Fogh); PANC-1 (W. A. Nelson-Rees); RWP-1 (D. L. Dexter); RWP-2 (D. L. Dexter); Hs766T (W. A. Nelson-Rees); UCVA-1 (developed in the Gastrointestinal Research Laboratory, Veterans Administration Medical Center, San Francisco, CA); SW1990 (W. B. McCombs, III); and MIA PaCa-2 (A. A. Yunis). The human colon carcinoma cell lines used were: HRT-18 (R. M. Schultz); HT-29 (J. Fogh); CaCo-2 (J. Fogh), SKCO-1 (W. A. Nelson-Rees); HCT-28 (R. M. Schultz); HCT-48 (R. M. Schultz); LS174T (B. D. Kahan); and SW1116 (A. Leibovitz). Miscellaneous human carcinoma cell lines included: breast cell lines Hs905T (W. A. Nelson-Rees), MDA-436 (D. Oda), ZR-75-30, and ZR-75-31A (R. Stems; originated by L. Engel); urethra Hs769T (W. A. Nelson-Rees), stomach HuTu80 (W. A. Nelson-Rees), and lung cell lines A549 (American Type Culture Collection), Calu-3, SK-MES-1, and A427 (J. Shellito); and melanoma cell lines A375 and A101D (W. A. Nelson-Rees). Normal human bladder cell line Hs767BL and normal human skin fibroblast cell line Hs27F were obtained from W. A. Nelson-Rees. Mouse myeloma cell line Sp2/O-Ag14 (17) was obtained from the
American Type Culture Collection. All of these cell lines were maintained as monolayer cultures in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). Many of these cell lines have been described in previous publications from this laboratory (18–20).

Tissue specimens were obtained from the Pathology Department of the Veterans Administration Medical Center, San Francisco, CA.

Immunization of Mice. BALB/c mice were given intraperitoneal injections of 2 x 10^6 Capan-2 human pancreatic carcinoma cells (21) which were suspended in 0.25 ml PBS. The animals were given a booster injection 3 weeks later.

Production of Hybridomas. Cell fusion was performed 3 days after the booster injection according to the procedure described by Oi and Herzenberg (22) with minor modifications. Briefly, mouse immune spleen cells (1.7 x 10^7) were fused in 50% polyethylene glycol (M, 1500; BDH Chemical Co.) in complete medium without fetal bovine serum [Dulbecco’s modified Eagle’s medium supplemented with 1 mm sodium pyruvate, 2 mm glutamine, 5 x 10^-5 M 2-mercaptoethanol, penicillin (100 units/ml), and streptomycin (100 μg/ml)] with Sp2/0-Ag14 mouse myeloma cells (1.7 x 10^5). Fused cells were distributed in 24-well tissue culture plates (10^5 total cells/well) and cultured in complete medium containing hypoxanthine/aminopterin/thymidine (Ref. 6; Sigma Chemical Co., St. Louis, MO) at 37°C with 5% CO2 in a humid atmosphere. Two to 3 weeks later, hybridoma cultures were screened by the peroxidase ELISA. Colonies producing antibodies which bound to Capan-2 cells but not to normal skin fibroblasts were selected for limiting dilutions in the presence of BALB/c mouse spleen cells as a feeder layer. After two limiting dilutions, those colonies producing antibodies of the desired specificities were expanded in complete medium supplemented with 10% heat-inactivated fetal bovine serum.

Production of Ascitic Fluid. Ascitic fluid was produced by the injection of 10^7 hybridoma cells i.p. into BALB/c mice preconditioned 7 days earlier with an i.p. injection of 0.5 ml of 2,6,10,14-tetramethylpentadecane (Pristane; Aldrich Chemical Co., Milwaukee, WI).

Cell Coated 96-well Plates. Medium (100 μl) containing target cells were seeded into each well of a 96-well culture plate and incubated at 37°C overnight or until the cells were confluent. Cells were then routinely fixed in 10% formalin in PBS and incubated with bovine serum albumin according to the method of Huang et al. (23).

Enzyme-linked Immunosorbent Assay. Cells in the 96-well plates were washed three times with PBS containing 0.05% Nonidet P-40 and then incubated with 50 μl of supernatant fluid from hybridoma cultures or of different dilutions of ascitic fluid or of partially purified 19-9 monoclonal antibody (33.3 mg/ml; gift from V. R. Zurawski, Jr.) for 60 min at room temperature. After washing three times in PBS-Nonidet P-40, 100 μl of PBS or a 1:10 dilution (in PBS) of YPan1 or YPan2 ascitic fluid or unlabeled purified 19-9. After an incubation of 3 h at 37°C, each well/bead was washed twice with 5 ml H2O. The beads were then transferred to 12 x 75-mm polystyrene test tubes and counted for 10 min in a Beckman Gamma 7000 counter.

Immunofluorescent Staining of Tissues. Sections of surgical specimens obtained from formalin-fixed, paraffin-embedded tissues, were deparaffinized in xylene, rehydrated, washed three times with PBS, and incubated for 60 min at room temperature with undiluted supernatant fluids from hybridoma cultures. As controls, tissue sections were treated in the same way as described above, except that culture medium was replaced with PBS. The sections were then washed three times with PBS, incubated for an additional 60 min with fluorescein isothiocyanate-labeled rabbit anti-mouse IgG+IgA+IgM (Zymed Laboratories, Inc.) diluted 1:50 with PBS, again washed three times with PBS, mounted on slides, and then examined under a Zeiss Universal microscope fitted with an epi-fluorescence condenser III 72.

Isotyping of Monoclonal Antibodies. Supernatant fluids from hybridoma cultures were tested by ELISA (peroxidase) with rabbit class-specific antiserum to mouse IgA, IgG1, IgG2a, IgG2b, IgG3, and IgM obtained from Zymed Laboratories, Inc.

RESULTS

Cell Fusion. Hybridomas (195) were obtained from the fusion of SP2 mouse myeloma cells with spleen cells isolated from mice immunized with human pancreatic carcinoma cell lines. Thirty-three of the hybridomas which produced antibodies that bound to Capan-2 cells, but not to normal human skin fibroblasts, were selected for the first limiting dilution. More than 90% of the colonies derived from the last cloning were shown to have an identical reactivity with target cells. After this selection, three colonies, YPan1, YPan2, and YPan3, which had a restricted range of reactivities with various human cell lines, were cultured in complete medium, and the antibodies which they produced were further characterized. The reactivity characteristics of two of these, YPan1 and YPan2, are the topic of the current article.
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Immunoglobulin Class. The class of immunoglobulins to which YPan1 and YPan2 belonged was determined by reacting them with rabbit antisera to mouse immunoglobulins. The monoclonal antibodies reacted with rabbit anti-IgM but not with rabbit antisera to the other immunoglobulin groups. Thus the monoclonal antibodies produced by these hybridomas were of the IgM class.

Reactivity with Cell Lines. The specificity of the monoclonal antibodies was characterized by peroxidase ELISA using undiluted spent culture medium on a panel of human normal and cancer cell lines (Table 1). The monoclonal antibodies were found to bind to all nine human pancreatic carcinoma cell lines. YPan1 reacted with four, and YPan2 with three, of nine colon carcinoma cell lines.

Saturation Curves for YPan1, YPan2, and 19-9 Against Capan-2 and SW1116 Cells. Saturation curves for YPan1 and YPan2 ascitic fluids and of purified 19-9 monoclonal antibody were determined against both Capan-2 and SW1116 fixed cell lines by the β-galactosidase ELISA (Chart 1). YPan2 ascitic fluid reached maximal reactivity at a dilution of 1:6400 for both Capan-2 and SW1116 cells. In the case of Capan-2 cells there was a decrease in measurable reactivity with higher concentrations of YPan2. No such decrease in reactivity with increasing concentrations was observed with either YPan1 or 19-9 up to a dilution of 1:400 (Chart 1) or even up to a dilution of 1:100 (data not shown).

The titers of spent media of YPan1 and YPan2 hybridomas were compared to those of their respective ascitic fluids (Chart 2). With both types of medium, up to an 8-fold dilution resulted in almost the same reactivity as the maximal saturating reactivity of the respective ascitic fluids (Chart 1). Thus undiluted culture media...

Table 1

<table>
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<th>Monoclonal antibody</th>
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* Immunogen used to produce YPan1 and YPan2.

** Color intensity: +, weak; ++, intermediate; ++++, strong; and 0, no color.

*** Undiluted spent culture media were used in these studies.
MONOCLONAL ANTIBODIES TO Pancreatic Carcinoma

Reactivity with Cancerous Tissue Sections. To further characterize the specificity of the monoclonal antibodies, human tissue sections were screened using an indirect immunofluorescence staining technique (Table 2). In some cases in which no reactivity was detected using the immunofluorescence technique, the immunoperoxidase method was also used. In all cases in which both techniques were used, those tissues which were found to be negative by the immunofluorescence method were also found to be negative with the immunoperoxidase method.

YPan1 was expressed in well and moderately differentiated pancreatic carcinoma but not in the two cases of poorly differentiated carcinoma that were tested. If, as we have reported (1, 2), YPan1 reacts with a mucin-like glycoprotein, the lack of reactivity with poorly differentiated pancreatic carcinoma may be due to the absence of such secretory products in these carcinomas. As determined by the immunoperoxidase method, there was intense staining of the luminal border and contents of welldifferentiated carcinoma (Fig. 1). Staining of the region of the cell nearest the lumen, when it occurred, was weak, and only occasionally were the supranuclear regions stained. In moderately differentiated carcinoma (Fig. 2), reactivity was with the whole membrane, and staining was diffuse throughout the cytoplasm of the cells. YPan1 also reacted with colonic and gastric cancer sections, and in several cases staining was intense (Table 2).

Some weak reactivity was detected with cancerous bladder, prostate, kidney, and breast tissues. YPan1 was also expressed in the secretory part of signet ring cell carcinoma and intestinal metaplasia of gastric mucosa (3 of 4 cases). Subcellular distribution of YPan1 reactivity was the same in colonic and gastric carcinoma as in pancreatic carcinoma.

YPan2 was expressed in well-, moderately, and poorly differentiated pancreatic carcinoma. Usually it was present diffusely in the cytoplasm. Occasionally staining was present in the apical membrane of well-differentiated carcinoma. Staining of luminal contents was not often seen (Figs. 3 and 4). Cytoplasmic staining of moderately differentiated carcinoma appears to be more intense than that of the cytoplasm of well-differentiated carcinoma (compare Figs. 3 and 4). YPan2 did not have the same intense staining of colonic and gastric cancer tissues that YPan1 had, and it reacted to some degree with cancerous prostate and kidney but not with bladder and lung tissues (Table 2). YPan2 did not react with signet ring cell carcinoma or intestinal metaplasia of gastric carcinoma as did YPan1. Subcellular distribution of YPan2 reactivity in colonic and gastric carcinoma resembled that in pancreatic carcinoma.

Reactivity with Normal Tissue Sections. YPan1 reacted with normal pancreatic tissue sections (Fig. 5 and Table 3). Staining of the luminal borders of ducts and ductules was moderate, in contrast to the intense staining of the luminal borders in cancerous pancreatic tissues (Figs. 1 and 2). Staining of the upper part of epithelial cells was weak. The surface of the inner layer of cells of the acinus was focally and weakly stained. No staining of islet cells was observed.

YPan1 also reacted with duodenum, bile duct, and mucus glands of the trachea. Staining was of the luminal border of epithelial cells of the bile duct, as well as of the luminal contents. In the duodenum, apical membranes of villi and the apical portions of the epithelium were also stained. Some kidney tissue was also weakly stained, principally in the region of the proximal tubule. In kidney, luminal borders and occasionally tubule luminal contents were stained. Prostate tissue was also weakly stained. In the lung only bronchial glands and the surface of epithelial cells of the bronchi were stained. YPan1 did not react with any other normal tissue sections tested, including various gastrointestinal tissues such as colon, stomach, appendix, and liver (Table 3).

YPan2 was found to react with only one case of normal pancreas when either the immunofluorescence or immunoperoxidase method was used. Thus the lack of YPan2 reactivity was not a function of the staining technique. Staining was weak and reactivity was with the surface and upper part of epithelial cells of ducts and ductules and focally in acinar cells. YPan2 did not react with any of the other normal tissues tested.

Neither of the monoclonal antibodies reacted with connective tissue of any of the sections.

Effect of Neuraminidase on Cell and Tissue Reactivity. Table 1 shows that both YPan1 and YPan2 reacted with SW1116 cell lines, the immunogen used for the production of monoclonal antibody 19-9 (7). We have reported that YPan1 reacts with a mucin-like glycoprotein present in Capan-2 cells and medium (1), while Magnani et al. (11) have reported that 19-9 reacted with a mucin-like substance in patients' sera. Both YPan1 (Figs. 1 and 2) and 19-9 (8) react with the luminal border of ductal epithelium. These results would suggest that YPan1 and YPan2 may react with the same or similar antigens as 19-9. Therefore several
experiments were designed to compare the nature of the reactivities of the three monoclonal antibodies to characterize similarities and differences. The first of these was to determine the effect of neuraminidase digestion on their reactivities with fixed cells. Chart 3 shows that 19-9 and YPan1 reactivities with SW1116 cells were lost if the cells were pretreated with neuraminidase (0.05 units/ml). However, reactivity of YPan2 was not affected by treatment with neuraminidase, even when incubations were for as long as 90 min at 0.25 units/ml (results not shown). Thus YPan1 and 19-9 reactivities both are neuraminidase sensitive, but YPan2 reactivity is insensitive. If pancreatic cancer tissue was first incubated with neuraminidase prior to reaction with YPan1, no immunoperoxidase staining of tissue is observed (Fig. 6B). This is in contrast to a similar tissue section which had not been treated with neuraminidase (Fig. 6A), where staining is pronounced. Thus, YPan1 reactivity with pancreatic cancer tissue sections also probably depends on the presence of sialic acid residues.

A range of saturating and subsaturating concentrations of YPan1, YPan2, and 19-9 antibodies were also incubated with up to 100 μM N-acetylneuraminic acid (sialic acid) overnight at 4°C prior to incubation with SW1116 cells. Preincubation with, and the presence of, sialic acid had no effect on the reactivities of any of the three monoclonal antibodies with SW1116 cells.

Effect of Trypsin Digestion on Cell and Tissue Reactivity. Chart 4 shows that whereas a low concentration of trypsin (0.002 mg/ml) had no effect on YPan1 and 19-9 reactivities (Charts 4A and 4B), YPan2 reactivity was increased at short incubation times (Chart 4C). Increasing the concentration of trypsin decreased the duration of incubation required to reach maximum reactivity until, at 0.25 mg/ml, trypsin maximal reactivity was seen at the earliest sampling time (10 min). A trypsin concentration of 0.01 mg/ml produced the greatest increase in reactivity (approximately 75% increase over control after 60 min of incubation). At the highest concentration (0.25 mg/ml), light microscopic examination of the cells in the wells indicated that loss of reactivity between 30 and 90 min of incubation was probably due to loss of cells from the plate. In the case of YPan1 and 19-9 no significant increase in reactivities was seen at any concentration of trypsin or duration of incubation examined (Chart 4A and B). Loss of 19-9 and YPan1 reactivity was observed beginning with 0.01 mg/ml trypsin (Chart 4A and B). At that concentration of trypsin no loss of reactivity was seen with YPan2 even after 90 min of incubation (Chart 4C).

Competition Studies with Capan-2 and SW1116 Fixed Cells. Friguet et al. (25) have described an ELISA double antibody binding system which they termed an "ELISA additivity test" to determine whether different monoclonal antibodies recognize the same epitopes on antigen molecules. The principle is to determine the maximal absorbance at saturating levels of one
antibody when it is added by itself and then to determine if there is an increase in total absorbance when both antibodies are added together at their respective saturating concentrations. Additivity indices are then calculated from these data (25). Values of 25% or less indicate that two antibodies probably recognize the same antigenic region, whereas values of 40% or greater indicate that two antibodies are probably reacting with different regions (25). In their studies a soluble antigen was used. We applied the same principles to antigens on the surface of fixed whole cells. Chart 1 shows results of determinations of saturating concentrations for YPan1, YPan2, and 19-9 against Capan-2 and SW1116 cells. Table 4 shows an example of the data used to calculate the additivity indices according to the formula shown in that table (25). Table 5 summarizes the additivity indices of the pairs of antibodies. The additivity indices of all three pairs of monoclonal antibodies obtained with SW1116 cells were similar to those obtained with Capan-2 cells. With both SW1116 and Capan-2 fixed cells the additivity indices of YPan1 vs 19-9 were less than 25%, and according to Friguet et al. (25) this would suggest competition between the two antibodies for the same antigenic region. On the other hand, the additivity indices for YPan2 versus YPan1 and YPan2 versus 19-9 were all greater than 40%, so that YPan2 probably reacts with a region which is distinctly different from that of either YPan1 or 19-9.

Comparison of Maximum Reactivities with Different Cell Lines. Chart 5 shows the saturation curves of the three monoclonal antibodies against three other cell lines, RWP-1, RWP-2, and SW1990. If indeed 19-9 antigen and YPan1 antigen were identical, it would be expected that the ratio of maximum absorbances of 19-9 to YPan1 should be the same from one cell line to another if no other factors preferentially affected binding of one antibody over another. Table 6 shows that although 19-9/YPan1 is very similar (close to one) in four cell lines, Capan-2, SW1990, SW1116, and RWP-1, the ratio for RWP-2 is not. In the case of RWP-2 the ratio is well over two. There were greater differences in the YPan1/YPan2 ratios. For example, the difference between the SW1990 ratio and that of RWP-2 is a factor of 10. Whereas YPan1/YPan2 and 19-9/YPan2 may equal 1, may be greater than 1, or may be much less than 1, the 19-9/YPan1 ratio was not found to be significantly less than 1 in any
fixed cell lines, as determined by an ELISA (Charts 1 and 5). On the other hand, the presence of a 1:100 dilution of unlabeled 19-9 monoclonal antibody greatly decreased binding of radiolabeled 19-9 at all three levels of CA 19-9-soluble antigen.

**DISCUSSION**

In this article we report on the production of two monoclonal antibodies using a human pancreatic cancer cell line as the immunogen. Both of these antibodies react with pancreatic cancer tissues and only weakly, if at all, with a large number of normal tissues. YPan2, in particular, has a preference for cancerous over normal pancreatic tissue. Several other monoclonal antibodies specific for cancerous gastrointestinal tissues have been reported. A murine monoclonal antibody (19-9) originally directed against human colon adenocarcinoma cell line SW1116 reacts with an antigen in sera of patients with pancreatic cancer (11). 19-9 recognizes a monosialoganglioside (i.e., sialylated Lea antigen) in human pancreatic carcinoma (26). Tissue reactivity with 19-9 is abolished by pretreatment of the tissue with neuraminidase (9), which suggests that sialic acid residues are important in determining the degree of binding. Although the antigen isolated from the colon cell line used for the elicitation of the antibody is a ganglioside (27, 28), the antigen in fetal organs that is reactive with YPanl is probably a mucin (11). The antigen in fetal tissues, as well as certain colon and lung carcinoma cell lines (12) and which may be a mucin-like molecule (30). Treatment of HPAF cells with neuraminidase resulted in loss of reactivity with DU-PAN-2. DU-PAN-2 did not show any reactivity with one pancreatic cancer cell line, MIA-PaCa-2 (12), with which YPans 1 and 2 reacted. Thus it is very likely that the YPans are reacting with antigen(s) on pancreatic cancer cells which are distinct from those recognized by DU-PAN-2. Parsa (15) has reported recently on a monoclonal antibody which reacts with normal pancreatic duct cells but not with acinar cells. This antibody reacts with pancreatic tumor tissue sections, as well as with the pancreatic cancer cell lines Capan-1 and Capan-2 (15). Further work will be necessary to determine if the antigen(s) recognized by this antibody is related to those recognized by YPans 1 and 2.

In conclusion, we have reported on the production of two monoclonal antibodies which recognize pancreatic cancer-associated antigens that appear to be different from several which have been reported previously (7, 12). The results described in this report suggest that YPan1 and YPan2 may be of potential use in the diagnosis of pancreatic cancer. Isolation and characterization of the antigens defined by these two monoclonal antibodies are currently in progress.

**ACKNOWLEDGMENTS**

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**REFERENCES**

7. Koprowski, H., Stepanski, Z., Mitchell, K., Herlyn, M., Herlyn, D., and Fuhrer, P. Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic gen was used. In this latter experiment, neither YPan1 nor YPan2 inhibited binding of radiolabeled 19-9 with its antigen, which indicated that they probably were not reacting with the same epitope as 19-9 on the soluble antigen. The discrepancy in these two sets of results may be attributable to large degree of steric hindrance possible on the surface of whole cells, especially as YPan1 is an IgM with a molecular weight of about 900,000. Thus, the YPan1 and 19-9 epitopes, although not identical, may normally be in proximity to each other on the cell surface, whereas the epitope of YPan2 is not. The absence of competition in the soluble antigen RIA assay is consistent with results in which saturation curves were constructed against several different cell lines. The ratio of maximum absorbances for YPan1 and 19-9 was not constant from one cell line to another. Thus the relative levels of their respective antigens may differ in different cells, and so the antigens are not identical.

Among the pancreas-associated monoclonal antibodies produced by Metzgar et al. (12, 14), antibody DU-PAN-2 recognizes an antigen which is present in cancerous and normal pancreatic tissues, as well as certain colon and lung carcinoma cell lines (12) and which may be a mucin-like molecule (30). Treatment of HPAF cells with neuraminidase resulted in loss of reactivity with DU-PAN-2. DU-PAN-2 did not show any reactivity with one pancreatic cancer cell line, MIA-PaCa-2 (12), with which YPans 1 and 2 reacted. Thus it is very likely that the YPans are reacting with antigen(s) on pancreatic cancer cells which are distinct from those recognized by DU-PAN-2. Parsa (15) has reported recently on a monoclonal antibody which reacts with normal pancreatic duct cells but not with acinar cells. This antibody reacts with pancreatic tumor tissue sections, as well as with the pancreatic cancer cell lines Capan-1 and Capan-2 (15). Further work will be necessary to determine if the antigen(s) recognized by this antibody is related to those recognized by YPans 1 and 2.
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Fig. 1. Immunoperoxidase staining of well-differentiated pancreatic adenocarcinoma reacted with YPan1. Both luminal contents and border are intensely stained but not the cytoplasm of the cancer cells. × 100.

Fig. 2. Immunoperoxidase staining of moderately differentiated pancreatic adenocarcinoma by YPan1. Staining of luminal contents and border is intense. Cytoplasm of some cancer cells were also intensely stained. However, most cancer cells showed weak cytoplasmic staining only. × 260.

Fig. 3. Immunoperoxidase staining of well-differentiated pancreatic adenocarcinoma reacted with YPan2. There is diffuse strong staining of cytoplasm, in contrast to the staining of YPan1 (see Fig. 1). × 250.

Fig. 4. Immunoperoxidase staining of moderately differentiated pancreatic adenocarcinoma by YPan2 showing intense staining of cytoplasm. × 380.

Fig. 5. Immunoperoxidase staining of normal pancreas by YPan1. Staining of normal pancreas is characterized by reactivity with the luminal border of duct cells. × 435.

Fig. 6. Effect of neuraminidase on the immunoperoxidase staining of well-differentiated pancreatic adenocarcinoma by YPan1. There is strong staining of the luminal contents (A) which does not occur if a serial section is preincubated with neuraminidase (B). × 225.

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