Identification and Localization of Human Pancreatic Tumor-associated
Antigens by Monoclonal Antibodies to RWP-1 and RWP-2 Cells

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ABSTRACT

Human pancreatic adenocarcinoma cell lines, RWP-1 and RWP-2 (Dexter, D. L., Matook, G. M., Meitner, P. A., Bogaars, H. A., Jolly, G. A., Turner, M. D., and Calabresi, P. Cancer Res., 42: 2705-2714, 1982), were used as immunogens for the production of monoclonal antibodies to tumor-associated membrane antigens. BALB/c mice were immunized by i.p. injection of viable cells and hybridomas resulting from the fusion of splenocytes to myeloma cell line P3x63Ag8.653 were screened by enzyme-linked immunosorbent assay for antibodies which reacted with both RWP-1 and RWP-2 cells. Hybridomas AR2-20 and AR1-28, both IgG1 antibody-producing cell lines, demonstrated membrane staining by immunofluorescence cytochemistry on three of seven pancreatic tumor cell lines but not on six human tumor cell lines of nonpancreatic origin, or on normal human fibroblasts. The antibodies stained frozen sections of RWP xenografts, propagated s.c. in nude mice, and tumor cells in paraffin sections of seven of seven cases of pancreatic ductal adenocarcinoma, using indirect immunofluorescence and immunoperoxidase histochemistry, but not normal adult or fetal pancreas, or a number of other normal adult tissues.

Immunoprecipitation of 125I-labeled RWP-2 cells resulted in a single band with a molecular weight of 190,000 under reducing conditions. Sequential immunoprecipitation demonstrated that both AR2-20 and AR1-28 bind to the same molecule.

INTRODUCTION

Tumor-associated antigens of human pancreatic adenocarcinoma have been identified by means of polyclonal heteroantisera (3, 6) and more recently by monoclonal antibodies (1, 13, 16, 17), but none of the antigens recognized are found exclusively or universally on pancreatic cancers. Monoclonal antibody technology provides a useful tool for the examination of malignant cell surface phenotypes. Appropriate screening procedures can be utilized to select antibodies which react with determinants that are present on malignant cells, but which are not found on normal cells or on tumor cells of different origins. Identification of the distribution of such antigens and their biochemical characterization are important for studying factors which distinguish tumor cells from their normal counterparts.

This report describes 2 murine hybridomas, AR1-28 and AR2-20, which produce IgG1 monoclonal antibodies directed against human pancreatic adenocarcinoma cell lines, RWP-1 and RWP-2 (4). These 2 antibodies bind to 3 of 7 pancreatic carcinoma cell lines and do not react with normal adult or fetal pancreas, as assessed by indirect immunofluorescence. RWP-1 and RWP-2 xenografts and 7 of 7 clinical specimens of pancreatic ductal adenocarcinoma were also stained to varying intensities with both antibodies, but 9 other cell lines and normal tissues were not.

Radioimmunoprecipitation established a molecular weight of 190,000 for the antigen(s) recognized by these 2 antibodies. Sequential radioimmunoprecipitation showed that a single molecule was recognized by the 2 antibodies.

MATERIALS AND METHODS

Animals. BALB/c mice were housed in plastic cages lined with cedar chip litter and were allowed access to water and Purina rodent chow ad libitum.

Cell Lines. Cell lines used in these studies were obtained from the following sources: human pancreatic adenocarcinoma cell lines RWP-1 and RWP-2 (4) (Dr. D. L. Dexter, Providence, RI); SW-1990 (10) (Dr. A. Kyriazis, Newark, NJ); AsPC-1 (2) and BxPC-3 (2), human fibroblasts, (Dr. L. Sokoloff, Stony Brook, NY); BALB/c mouse myeloma line P3x63/Ag8.653 (8) from the Salk Institute, La Jolla, CA; human lymphoblastic leukemia lines RPMI 8392 (B-cell) and RPMI 8402 (T-cell) from Bethesda Research Laboratories (Gaithersburg, MD); human cell lines CRL 1500 (breast adenocarcinoma), CRL 1572 (ovarian teratocarcinoma), CCL 218 (colon carcinoma), CCL 2 (cervical carcinoma), CRL 1435 (prostatic carcinoma), CRL 1420 (pancreatic carcinoma), and CRL 1469 (epithelioid pancreatic carcinoma) were obtained from the American Type Culture Collection (Rockville, MD).

Cell lines were maintained in a humidified 5% CO2 incubator in the following media: 90% RPMI 1640, 10% FBS (RWP-1, RWP-2, AsPC-1, BxPC-3, P3x63Ag8.653, RPMI 8392, RPMI 8402, and CRL 1500); 90% minimum essential medium: 10% FBS (CRL 1572, CCL 218, and CCL 2); 90% DMEM: 10% FBS (CRL 1469, human fibroblasts); 87.5% DMEM: 10% FBS: 2.5% horse serum (CRL 1500); 90% RPMI 1640, 10% NCTC 109 (M.A. Bioproducts, Walkersville, MD); RPMI 8402, and CRL 1500); 90% DMEM: 10% FBS: 2.5% horse serum (CRL 1420); 93% Ham's F-12 medium: 7% FBS (CRL 1435); 86% L15 medium: 20% FBS (SW-1990); HAT selection medium for the hybridomas consisted of RPMI 1640, 10% NCTC 109 (M.A. Bioproducts, Walkersville, MD), 20% FBS, 1% mouse spleen-conditioned RPMI 1640, and 1% of stock solutions of 100 μg/ml of hygromycin, 40 μg/ml of aminopectin, and 160 μg/ml of thymidine. All media were supplemented with penicillin/streptomycin and glucose; all sera were used heat inactivated at 56°C for 30 min. The medium used for RWP-1 and RWP-2 cells was also supplemented with 20 μg gentamicin sulfate/ml. CRL 1420, CRL 1500, CCI 2, and CRL 1572 cell lines were subcultured after treatment with 0.25% trypsin. RPMI-1640, RPMI-1640, SW-1990, AsPC-1, BxPC-3, CRL 1469, CCL 218, and CRL 1435 cell lines were passaged after treatment with 0.25% trypsin:0.05% EDTA.

Fusions. Fusions were performed according to the method of Galfre et al. (5), with slight modifications. Three BALB/c mice (2 to 4 months

1 Supported in part by NIH Grant 431-1373.
2 M. H. Tan and T. M. Chu, unpublished results.
3 The abbreviations used are: FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; DPBS, Dulbecco's phosphate-buffered saline (0.15 M sodium chloride: 0.001 M dibasic sodium phosphate: 0.008 M potassium chloride: 0.003 M monobasic potassium phosphate), pH 7.2 to 7.4; DPBS-Tween, DPBS with 0.05% Tween 20; TBS, Tris-buffered saline (10 mM Tris: 0.15 M sodium chloride, pH 7.6); TBS-TX, TBS with 0.1% Triton X-100, 0.02% sodium azide, 0.05 M sodium EDTA, 10 μg aprotinin/ml, and 10 μg leupeptin/ml; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; HAT, hypoxanthine, aminopterin, and thymidine; PVC, polyvinyl chloride.
old) were used for each set of fusions and were immunized by i.p. injection of either the RWP-1 or RWP-2 cell line (approximately 10^6 unfixed cells) which had been harvested from monolayer culture with cell scrapers (Costar; Belco Glass, Inc., Vineland, NJ), then washed twice with DPBS. After 4 weeks, mice were boosted with a second i.p. injection of 10^6 cells of the same line as that used for the initial injection and were sacrificed 3 to 5 days later. Their spleens were collected aseptically and dissociated into single-cell suspensions. Approximately 10^6 splenocytes were obtained per immune spleen and were fused to P3x63Ag8.653 myeloma cells at spleenocyte/myeloma cell ratios between 10:1 and 1:1 in 0.5 ml of 40% polyethylene glycol 1000 (Koch-Light, Colnbrook, England) diluted in serum-free RPMI 1640. A feeder population of normal mouse splenocytes was added and cells were cultured overnight in T-75 flasks in hybridoma medium without HAT. HAT stock solutions were added to give the appropriate concentrations, and cells were plated out in 96-well tissue culture plates at ~10^3 spleen cells/well. Cultures were fed twice weekly with HAT medium for the first week, medium with hypoxanthine and thymidine for the second week, and medium without HAT thereafter. Fusions were assayed by ELISA after the appearance of macroscopically visible colonies (3 to 6 weeks after the fusion).

ELISA. Tissue-cultured cells were attached to 96-well PVC plates according to the method of Kennett (9). Briefly, tissue-cultured tumor cells were harvested mechanically, washed twice in DPBS, and 10^4 cells/well were added to 96-well PVC microtiter plates (Costar, Belco Glass). Plates were loaded on centrifuge adapters and centrifuged for 5 min at 800 x g in a Sorvall Model RT 6000 at 4 °C. Cells were fixed for 15 min at room temperature by the addition of an equal volume of cold 0.5% glutaraldehyde in DPBS. Plates were washed with DPBS, and the remaining glutaraldehyde was neutralized with 100 mM glycine in RPMI 1640 or DMEM containing 1% bovine serum albumin. Wells were filled with 1% bovine serum albumin in RPMI 1640 or DMEM and plates were stored at -20 °C until use.

Fifty μl of culture supernatant were sterilely removed from each well of the plates containing the hybridomas, and were placed into sterile 96-well PVC transfer plates (Cooke, Dynatech Laboratories, Inc., Alexandria, VA), which have a small pinhole at the bottom of each well. Frozen ELISA plates were thawed at 37 °C and washed twice with DPBS-Tween. Twenty-five μl of DPBS-Tween were added to each well to draw the culture supernatants from the transfer plates into the ELISA plates. Goat anti-mouse IgG antiserum specifically reactive with heavy and light chain (Cappel Laboratories, Cochranville, PA) was conjugated to horse-radish peroxidase by periodation (15), and was used to detect the presence of antibody. All incubations were performed for one of the following lengths of time at indicated temperatures, and were followed by two 5-min washes with DPBS-Tween: 1 h at 37 °C, overnight at 4 °C, or 4 h at room temperature. Development of the colorimetric substrate, o-phenylenediamine (Fisher Scientific Co., Fair Lawn, NJ) in 0.1 M, pH 5.0, sodium phosphate-citrate buffer containing 0.012% hydrogen peroxide, was stopped after 15 to 30 min by the addition of 1 N sulfuric acid, and A_{405} values were read on a manual Dynatech ELISA reader. Readings >0.099, using DPBS-Tween or non-antibody-containing spent medium from the parent myeloma line as the blanks, were regarded as positive.

Cloning and Growth of Hybridomas in Mouse. Hybridomas of interest were expanded and subcloned by limiting dilution. Hybridomas were propagated as i.p. tumors in mice. Mice were primed 2 to 4 weeks prior to the inoculation of the hybridomas with an i.p. injection of 0.5 ml of Pristane (2,6,10,14-tetramethylpentadecane; Aldrich Chemical Co., Milwaukee, WI). Ascites fluids were centrifuged to remove debris and were stored at -20 °C.

Determination of Antibody Isotypes. Monoclonal antibodies were concentrated from spent medium by precipitation overnight in cold 2 M ammonium sulfate. Precipitates were collected by centrifugation, redissolved in DPBS to ~10% of their initial volume, and dialyzed against DPBS. Protein concentrations ranged from 1 to 1.5 mg/ml. Single preparations of concentrated supernatants were used for these experiments. Isotypes were determined by double immunodiffusion in 1% agarose with rabbit anti-mouse isotype-specific antiserum previously prepared in this laboratory (14).

Immunohistochemistry. Formalin-fixed paraffin sections were deparaffinized in 3 changes of toluene, and rehydrated through a graded series of ethanol washes to DPBS immediately prior to use. Rehydrated sections were treated by a 1-h wash in cold 10% sucrose in DPBS and were washed in DPBS again before incubation with the monoclonal antibodies. Unfixed tissues were embedded in O.C.T. compound (VWR Scientific, Inc., South Plainville, NJ) and frozen in 2-methyl butane cooled by liquid nitrogen. Blocks were either immediately sectioned (4 to 10 μm) on a cryostat (International Equipment Co., Needham Heights, MA) or wrapped in aluminum foil and stored at -70 °C. Mechanically harvested cells and frozen sections were air dried onto gelatin-coated slides and were fixed by immersion in ice-cold acetone for 30 s, unless otherwise stated.

Tissue-cultured cells were evaluated by immunofluorescence; tissue sections were evaluated by immunofluorescence or by immunoperoxidase. Dilutions for secondary antisera utilized were determined by checkerboard titrations. Spent culture media were used undiluted; ammonium sulfate-precipitated antibodies (generally, 1 to 1.5 mg total protein/ml) were diluted 1:10 in DPBS; rabbit anti-mouse isotypic antisera were used at 1:100; fluorescein-isothiocyanate-conjugated goat anti-rabbit IgG (fluorescein:protein ratio of 4) were used at 1:40 or 1:80; and horseradish peroxidase-goat and mouse IgG was used at 1:100. Spent medium from cultures of parent myeloma cell line P3x63Ag8.653 was used as negative controls for all secondary antisera. 3,3'-Diaminobenzidine (Sigma Chemical Co., St. Louis, MO) at 0.5 mg/ml in 0.05 M Tris-HCl buffer, pH 7.6, with 0.001% hydrogen peroxide, was utilized as the substrate for the horseradish peroxidase-goat anti-mouse IgG. Immunoperoxidase slides were counterstained with Gi's hematoxylin and mounted with Permount (Fisher Scientific Co.). Immunofluorescence slides were mounted with Immuno-mount (Shandon Southern Instruments, Inc., Sewickley, PA), and were examined on a Zeiss Axiomat fluorescence microscope equipped with epillumination.

Collection of Tissues. Human specimens were obtained through the courtesy of the Departments of Pathology at University Hospital, State University of New York, Stony Brook, NY, and at St. Charles Hospital, Port Jefferson, NY. Fetal tissues were obtained from prostaglandin-induced abortions of fetuses ranging from 15 to 17 weeks gestational age (courtesy of Dr. C. Kaplan, Department of Pathology, State University of New York, Stony Brook). All material was obtained incidental to diagnostic or therapeutic procedures not related to these studies.

RWP xenografts were kindly provided by Dr. D. L. Dexter and G. Mattock, Providence, RI.

Radioimmunoprecipitation. RWP cells were radiolabeled with 125I using the iodo-Bead method (18) (Pierce Chemical Co., Rockford, IL). RWP-2 cells were washed with DPBS and were harvested with cell scrapers, then washed twice again with DPBS. Five x 10^6 to 10^7 cells were placed in 0.5 ml DPBS in a 15-ml conical centrifuge tube. 125I RWP-2 cells were washed with DPBS and were harvested with cell scrapers, then washed twice again with DPBS. Five x 10^6 to 10^7 cells were placed in 0.5 ml DPBS in a 15-ml conical centrifuge tube. 125I (Research Products International Corp., Mt. Prospect, IL) was diluted to a concentration of 1 mCi/ml. Two Iodo-Beads were washed with DPBS by filtration and were added to 0.25 to 0.5 μCi of 125I and left at 22 °C for 5 min. Cells were added to the reaction mixture and were allowed to react for 7 min at 22 °C with shaking. Cells were pipetted from the reaction vessel into 50-ml polypropylene tubes, leaving the Iodo-Beads in the 15-ml tube, and were then centrifuged and washed 3 times (50 ml each) to remove free iodide. Cells were solubilized for 30 min on ice in 1 ml TBS-TX, centrifuged at 2000 x g for 30 min, or at 12,000 x g for 2 min to remove insoluble matter. TBS-TX-solubilized cell extracts were precleared by incubation for 30 min on ice with 50 μl of a 10% Staphylococcus aureus (Sigma) suspension in DPBS with 0.02% sodium azide and 0.02 mM sodium EDTA, followed by centrifugation at 12,000 x g for 1 min in a Beckman microfuge to remove proteins which would bind nonspecifically to the staphylococci. An aliquot of this suspension was counted in a γ counter, and the extract was stored frozen in 0.5-mL
Twenty-μl volumes of concentrated monoclonal supernatant were incubated with 100 μl (5 x 10⁶ cpm) of S. aureus-cleared-radioabeled cell lysate for 30 min on ice in 1.5-ml microfuge tubes. Fifty μl rabbit anti-mouse IgG1 or rabbit anti-mouse IgM, and 100 μl of a 10% Staph A solution were added and were incubated sequentially for an additional 15 to 30 min each on ice. The solutions were then centrifuged in a Beckman microfuge at 12,000 x g for 1 min in order to pellet the staphylococci. Supernatants were used for second incubation with other monoclonal antibodies. Staphylococcal pellets were washed twice with TBS containing 0.5 M NaCl, and twice with TBS containing 0.25 M NaCl, then once with TBS-TX. Protein was eluted by boiling in sodium dodecyl sulfate sample buffer containing 1% dithiothreitol for 3 to 10 min and loaded onto sodium dodecyl sulfate-polyacrylamide gradient (5 to 20%) gels (11). Gels were dried onto filter paper and were exposed to X-AR film (Eastman Kodak Co., Rochester, NY) at -70 °C for 7 days.

RESULTS

Selection of Hybridomas. Hybridomas which were obtained from the fusions of splenocytes from 6 immune mouse spleens to myeloma cells were screened for reactivity with both the RWP-1 and the RWP-2 cell lines, by using glutaraldehyde-fixed cells as the target antigens in ELISAs. Thirty-eight different toants on a panel of human tumors of epithelial origin, which

Localiation of Antigens. Immunofluorescence staining was performed on aceton-fixed RWP tumor cells obtained from tissue culture in order to localize the antigens. Cells were mechanically harvested from monolayers to avoid possible effects of trypsinization on antigen structure and reactivity with the antibodies. Hybridomas secreting antibodies directed against cell surface antigens were evaluated using spent culture supernatants on a panel of human tumors of epithelial origin, which included 5 human pancreatic carcinoma cell lines other then AR2-20 and AR1-28, respectively, on paraffin sections from tumors obtained at autopsy were studied by immunohistochemistry. Fig. 1d is a fluorescent photomicrograph of the staining pattern obtained with AR2-20 on a paraffin section of one of the clinical cases examined. The apical surfaces of the ductal adenocarcinoma cells are prominently stained, whereas the fibroblasts and connective tissue surrounding the tumor are negative. Similar staining was seen using AR1-28. The apparent discrepancy may be due to the diffusion of the diaminobenzidine from the site of reaction, another example of a well-differentiated ductal adenocarcinoma. Mucin droplets are not stained, and the reaction product of diaminobenzidine is located in the basal portion of the tumor cell. It was therefore of interest to determine the ability of AR2-20 and AR1-28 to stain tumor cells in formalin-fixed, paraffin-embedded sections of clinical cases of pancreatic adenocarcinoma by immunohistochemistry. Fig. 1d is a fluorescence photomicrograph of the staining pattern obtained with AR2-20 on a paraffin section of one of the clinical cases examined. The apical surfaces of the ductal adenocarcinoma cells are prominently stained, whereas the fibroblasts and connective tissue surrounding the tumor are negative. Similar staining was seen using AR1-28. Fig. 2a and 2b, respectively, on paraffin sections from another example of a well-differentiated ductal adenocarcinoma. Mucin droplets are not stained, and the reaction product of diaminobenzidine is located in the basal portion of the tumor cells. It is difficult to distinguish whether the membrane is also stained by this method. The apparent discrepancy may be due to the diffusion of the diaminobenzidine from the site of reaction, and retention of the dye by surrounding cellular proteins. All 7 cases of ductal adenocarcinoma examined thus far have been reactive with these antibodies. Three of the cases demonstrated heavy apical membrane staining, while staining was moderate in one case, and faint in the remaining 3 cases.

RWP cells were maintained in nude mice as s.c. solid tumors which formed ductal structures similar in morphology to that of the original tumors from which they had been established. Frozen sections of nude mouse RWP xenografts, stained with AR2-20

The following normal tissues (number of cases) failed to stain with AR1-28 and AR2-20: adrenal gland, 3; aorta, 3; bladder, 1; bronchus, 2; cecum, 1; cervix, 1; colon, 5; duodenum, 6; epididymus, 2; epiglottis, 2; esophagus, 4; gallbladder, 2; heart, 3; ileum, 6; jejunum, 6; kidney, 3; liver, 4; lung, 1; lymph node, 2; skeletal muscle, 2; ovary, 1; pancreas, 7 adult, 2 infant; parotid gland, 1; prostate gland, 2; rectum, 3; seminal vesicle, 1; spleen, 4; stomach, 6; submandibular gland, 1; testis, 2; newborn thymus, 2; trachea, 3; uterus, 1; and vagus nerve, 1.

Immunofluorescence staining of tissue-cultured cells

Table 1

<table>
<thead>
<tr>
<th>Staining with following monoclonal antibodies</th>
<th>AR2-20</th>
<th>AR1-28</th>
<th>AR1-33</th>
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<tbody>
<tr>
<td>Human pancreatic tumor cell lines</td>
<td></td>
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<tr>
<td>RWP-1</td>
<td>M</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>RWP-2</td>
<td>M</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>AsPC-1</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>BxPC-3</td>
<td>M</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>CRL 1420</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>CRL 1469</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>SW-1990</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>Nonpancreatic human cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>CCL 2 (cervical carcinoma)</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>CCL 1435 (prostatic carcinoma)</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>CCL 218 (colonic cancer)</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>CCL 1500 (teratocarcinoma)</td>
<td>0</td>
<td>0</td>
<td>C</td>
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<tr>
<td>RPMI 8402 (T-cell)</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>RPMI 8392 (B-cell)</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
</tbody>
</table>

* M, membrane staining; C, cytoplasmic staining; 0, lack of staining.
AR 1-28 also reacted with nude mouse xenografts of these 2 cell lines, and AR2-20 and AR1-28 antibodies capable of distinguishing human pancreatic ductal adenocarcinoma cells from normal pancreatic cells and tumor tissues. AR1-33 showed a filamentous cytoplasmic pattern which was particularly discernible in larger cells and displayed a greater intensity around the nuclei.

The purpose of this study was to produce murine monoclonal antibodies capable of distinguishing human pancreatic ductal adenocarcinoma cells from normal pancreatic cells and tumor cells of other tissue origins. Two IgG1 antibodies, AR2-20 and AR1-28, were selected for their limited range of reactivity against pancreatic tumor cell lines, RWP-1 and RWP-2. AR2-20 and AR1-28 also reacted with nude mouse xenografts of these 2 cell lines and 7 of 7 clinical specimens of pancreatic adenocarcinoma, but not with normal pancreas or with several types of other normal human epithelial cells. In addition, one other pancreatic adenocarcinoma cell line, BxPC-3, but not 4 human pancreatic cell lines (CRL 1420, CRL 1469, AsPC-1, and SW-1990) express this antigen. The presence of these antigens in clinical material obtained from patients indicates that they are not artifacts of tissue-cultured cells or of the RWP's in particular. The RWP cell lines continue to express these antigens after being routinely cultured for more than 2 years.

Two possible explanations could account for these observations: (a) these monoclonal antibodies are recognizing a subpopulation of pancreatic adenocarcinomas, or (b) all pancreatic tumors express these determinants in vivo and they are no longer expressed after establishing the cells in tissue culture. However, the number of cases examined thus far is very small (7 pancreatic cell lines and 7 clinical cases), and we must await acquisition of larger numbers of cases to test for the presence of antigens recognized by these antibodies to clarify the distribution and applicability.

Since these antibodies retain their reactivity with formalin-fixed, paraffin-embedded material, they may be useful for both diagnostic purposes and retrospective studies. The peroxidase technique offers the advantage of better examination of the tissue morphology surrounding the tumor cells. However, fluorescent-labeled immunoglobulins being conjugates are incapable of the diffusion seen with the peroxidase substrate allow for clearer localization. A recent study on monoclonal antibodies to cell surface immunoglobulin also indicates that the properties of monoclonal antibodies are dependent on the subclass of the antibody and on the type of assay utilized (7), which may affect staining patterns.

These antibodies are not directed against carcinoembryonic antigen or α-fetoprotein, which are cytoplasmic antigens. They are also not directed against histocompatibility or blood group antigens, since they failed to stain human lymphoblastoid cell lines or normal autologous tissue present on the sections.

**DISCUSSION**

The purpose of this study was to produce murine monoclonal antibodies capable of distinguishing human pancreatic ductal adenocarcinoma cells from normal pancreatic cells and tumor cells of other tissue origins. Two IgG1 antibodies, AR2-20 and AR1-28, were selected for their limited range of reactivity against pancreatic tumor cell lines, RWP-1 and RWP-2. AR2-20 and AR1-28 also reacted with nude mouse xenografts of these 2 cell lines and 7 of 7 clinical specimens of pancreatic adenocarcinoma, but not with normal pancreas or with several types of other normal human epithelial cells. In addition, one other pancreatic adenocarcinoma cell line, BxPC-3, but not 4 human pancreatic cell lines (CRL 1420, CRL 1469, AsPC-1, and SW-1990) express this antigen. The presence of these antigens in clinical material obtained from patients indicates that they are not artifacts of tissue-cultured cells or of the RWP's in particular. The RWP cell lines continue to express these antigens after being routinely cultured for more than 2 years.

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dence from immunoprecipitation indicates that the antigen(s) recognized by AR2-20 and AR1-28 have molecular weights of 190,000 ± 10,000 and are identical, but that the determinants recognized may or may not be congruent. Based on the molecular size and staining properties displayed by the antibodies, AR1-28 and AR2-20, this antigen does not appear to be similar to those which have been identified by other groups using monoclonal antibodies against pancreatic carcinomas (1, 17).

The distinctive staining patterns obtained with the 2 monoclonal antibodies strongly suggest that 2 separate determinants are being recognized. Such a possibility is highly likely, considering that the 2 monoclonal antibodies resulted from separate fusions against different pancreatic tumor cell lines. The coincidence of obtaining 2 monoclonal antibodies to a single molecule by chance is unusual and seems to indicate that either the molecule is quite immunogenic in mice, or that the selection procedure which was used enhanced the probability of discerning such a molecule. It also implies that at least 2 molecular populations containing the antigenic determinants exist on the surface of the pancreatic cell membranes. Populations of antigenic molecules which express one or both of the determinants may arise by one of the following mechanisms: (a) partial proteolytic degradation of the antigen, resulting in the loss or unmasking of a determinant; (b) masking of one of the determinants by another molecule; or (c) the formation of aggregates leading to the expression of a new determinant (see Chart 1). Further characterization of the antigens recognized by these 2 antibodies is currently in progress.

REFERENCES

Fig. 1. RWP-1 cells stained by indirect immunofluorescence with AR1-28 (a); AR2-20 (b); and AR1-33 (c); × 320. Granular membrane staining is apparent over the nuclei in a, whereas a more uniform staining pattern is seen in b. c, linear, filamentous cytoplasmic pattern with no intensification at the cell edges. d, paraffin section showing apical staining of ductal adenocarcinoma cells in a well-differentiated pancreatic adenocarcinoma stained by immunofluorescence with AR2-20. The tumor cells comprising the duct and a group of tumor cells in the duct show prominent edge staining. × 128.
Fig. 2.  a, frozen section of a pancreatic adenocarcinoma stained by indirect immunoperoxidase with AR2-20 and counterstained with hematoxylin. Heaviest peroxidase staining is seen at the margins of the tumor cells (arrow), x 250.  b, frozen section of a normal human adult pancreas stained with AR2-20, x 100. Acini and ductal cells are unstained.  c, paraffin sections of a well-differentiated human pancreatic ductal adenocarcinoma stained by immunoperoxidase with AR2-20.  d, paraffin section from the same case as seen in c, stained with AR1-28. Hematoxylin counterstain, x 100. Note the absence of staining in the mucin droplets (c), and the heavy staining of the clusters of tumor cells (d) (arrows).
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