CAR-3, a Monoclonal Antibody-defined Antigen Expressed on Human Carcinomas

Maria Prat, Isabella Morra, Giovanni Bussolati, and Paolo M. Comoglio

Departments of Histology [M. P., P. M. C.] and Pathological Anatomy [I. M., G. B.], University of Torino, School of Medicine, Torino, Italy

ABSTRACT

Several monoclonal antibodies were raised against the human epidermoid carcinoma line A 431. The antibody produced by clone AR-3, when tested in enzyme-linked immunosorbent assay, was found to react with the cell line used as immunogen, the human gastric carcinoma line KATO III, the colon carcinoma line HT29, and the ovarian carcinoma line SW626. This monoclonal antibody was found unreactive when tested on human peripheral blood leukocytes or on a number of normal or neoplastic cell lines. The antibody precipitated a high-molecular-weight glycosylated component.

When tested on paraffin sections by the avidin:biotin:peroxidase method, the AR-3 antibody stained pancreatic (6:7), gastric (11:14), ovarian (5:6), colon (4:8), endometrial (4:6), and cervical (4:7) carcinomas. A small minority of carcinomas of other organs was also stained. Sarcomas, lymphomas, and other tumors of nonepithelial origin were constantly negative. Staining of some normal epithelial cells was also observed. Among the fetal tissue tested, the antibody reacted with pancreatic ducts and the small intestine. The antibody recognized metastatic carcinoma cells in peritoneal effusions.

On the basis of its tissue distribution, the antigenic determinant defined by the AR-3 monoclonal antibody was called CAR-3. The monoclonal AR-3 did not cross-react with partially purified preparations of carcinoembryonic antigen, gastrointestinal carcinoma antigen, or the human milk fat globule antigen.

The AR-3 MAb appears, thus, to broaden the number of available reagents for histopathological diagnosis of carcinomas.

INTRODUCTION

Development of the MAb technique (2) enables the dissection of the phenotypic heterogeneity of normal tissues as well as that of tumor cells. Several laboratories are attempting to produce reagents identifying antigenic specificities associated with the expression of the neoplastic phenotype. Following many efforts, some antigens with associated tumor specificity have been defined (1, 3–7).

As far as antigens preferentially expressed on cancers of the gastrointestinal tract are concerned, well-known examples are the CEA (8) and the two MAb-defined GICA Ca 19.9 and 17.1A antigenic determinants (9–11). These antigens are also expressed on a significant percentage of ovarian carcinomas. None of the above antigens is strictly tumor specific and, on the other side, none is expressed on all cancer types of the gastrointestinal tract or ovary. It becomes thus evident that a panel of different MAbs will be necessary in order to precisely classify the nature of histological lesions and to trace metastatic neoplastic cells. This approach has been successfully undertaken for cancers of other organs such as bladder (12), prostate (13), and mammary carcinomas (14, 15).

In the present paper, we describe the specificity pattern of a MAb (AR-3), which was obtained by immunization with the epidermoid carcinoma cell line A 431. Such a monoclonal, when tested in ELISA on different human neoplastic and normal cell lines, reacted only with the cells used as immunogen and with lines established from human gastric, colon, and ovarian carcinomas. When tested on paraffin sections with the avidin:biotin:peroxidase method, the AR-3 stained weakly the glycolic of some epithelial cells but strongly the surface and the cytoplasm of pancreatic, gastric, colon, ovarian, endometrial, and cervical carcinoma cells. The MAb was virtually negative on all other neoplastic and normal tissue tested.

MATERIALS AND METHODS

Cells. The characteristics of the different cell lines used have been published (16–18), and their tissue origin is listed in Table 1. The BALB/c P3.X63.Ag8.653 myeloma line is a nsecrating variant. All the cell lines were maintained in RPMI 1640 medium (Gibco, NY) with 10% fetal calf serum, 2 mU glutamine, penicillin (100 IU/ml), streptomycin (100 g/ml), and fungizone (250 ng/ml). In the case of the murine myeloma, 5 × 10^-6 M 2/3-mercaptoethanol was added. Human peripheral leukocytes were prepared from heparinized peripheral blood samples. They were separated from erythrocytes on a Lymphoprep cushion. Human fetal fibroblasts were obtained from amniotic fluid and passaged in medium containing 20% fetal calf serum.

Production of Monoclonal Antibodies. BALB/c mice were immunized 3 times i.p. at 2-wk intervals with 5–10 x 10^6 paraformaldehyde-fixed A 431 cells, and they were sacrificed for the fusion experiment 4 days after the final boost. Ten-12 × 10^6 spleen leukocytes were fused with 3–5 x 10^7 P3.X63.Ag8.653 myeloma cells, using 41.7% (vol/vol) Polyethylene-glycol 4000 as fusing agent, as described elsewhere (19). Ten–15 days after the fusion, the supernatants of hybrid cell colonies were tested for antibody production in a RBIA or in an ELISA (see below). Hybrids were cloned twice by limiting dilution and adapted to grow in ascitic form.

RBIA and ELISA. Antibodies produced by the hybrids were screened for binding to cell surface antigens of glutaraldehyde-fixed cells plated on polylysine-coated 96-well polystyrene microplates. In RBIA, binding was revealed by means of affinity chromatography-purified 125I-labeled rabbit antibodies against mouse immunoglobulin as already described (20). Binding index was calculated as mean cpm of the test triplicated divided by the mean cpm of the control triplicates, where the targets were incubated with medium or an unrelated monoclonal antibody. Values equal or above 2.5 were considered positive. In the case of ELISA, the second reagent was an affinity chromatography-purified goat...
anti-mouse immunoglobulin preparation labeled with horseradish peroxidase (Kpl, Gaithersburg, MD). The reaction was visualized by adding orthophenylene diamine as chromogen (Sorin Biomedica, Saluggia, Italy) and read on the Titertek Multiscan at a 492-nm wavelength. Based on previous experience, binding of a particular antibody was considered positive when the reaction gave optical values 5 times greater than the ones developed by an unrelated antibody or medium alone. This background value was around 0.015–0.020 absorbance unit.

For inhibition experiments of ELISA, cultured cells were washed 3 times with 150 mM sodium chloride:20 mM Tris(hydroxymethyl)methylenediamine (pH 7.6) and lysed for 10 min in ice with the same solution at pH 8.0, 0.5% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, and aprotinin as protease inhibitors (lysing buffer). Extracts were then clarified at 10,000 x g for 10 min and used in scalar dilution to inhibit the binding of AR-3 MAb used at a 1:64 final dilution, which was previously found to give 75% of maximum binding on glutaraldehyde-fixed cells (21). The percentage of inhibition was calculated as follows:

\[
100 - \frac{A_{492,\text{control}}}{A_{492,\text{inhibition}}} \times 100
\]

Standard preparations of CEA or GICA antigens, partially purified in perchloric acid (CEAK, GICAK; Sorin Biomedica, Saluggia, Italy), were also used as inhibitors.

Analytical Procedures. Methionine labeling was performed by incubating cells (10^5/ml) in methionine-free medium containing [35S]methionine (25 μCi/ml; Amersham, Buckinghamshire, England; 1050 Ci/mmol) and 5% fetal calf serum.

Glycoprotein labeling was performed in complete medium containing 125 μCi of α-[3H]glucosamine per ml (Amersham; 38 mCi/mmol). Cells were incubated for 6 h, then washed 3 times with complete medium, and lysed with lysing buffer, and extracts were clarified as described. For immunoprecipitation, a two-step procedure was used, as previously described (22). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out in 5–15% acrylamide gradient slab gels, using the method of Laemmli (23). Gels were subjected to fluorography as described by Laskey and Mills (24), dried and autoradiographed. The following radiolabeled molecular weight markers were used: myosin (M, 200,000); phosphorylase b (M, 92,000); bovine serum albumin (M, 66,000); ovalbumin (M, 46,000); carbonyl anhydride (M, 30,000); and lysozyme (M, 14,000) (Amersham).

Immunoperoxidase Method (Avidin:biotin:peroxidase). The tissue sections used for the immunoperoxidase assay were derived from various biopsies which had routinely been fixed in 10% formalin and embedded in paraffin, as already described (18). Cytological preparations from ascitic sediments were prepared by means of the “celloidin bag” method and paraffin embedded, as previously described (25). Blocks stored at room temperature up to 5 yr were used. Briefly, sections were brought to phosphate-buffered saline, and the endogenous peroxidase was inhibited by treatment with hydrogen peroxide, followed or not by the periodic acid:borohydride sequence according to Heydeman and Neville (26). Slides were then incubated sequentially with normal goat and mouse antibodies directed against mouse immunoglobulin (Vector Lab., Burlingame, CA) with avidin:biotin complex (Vector Lab.; avidin:biotin complex kit) prepared 20 min before use at pH 9.4 according to Bussolati and Gugliotta (27). Controls included samples where the monoclonal was substituted by nonimmune mouse serum or immunoglobulins. The peroxidase reaction was developed according to Graham and Karnovsky (28), and then sections were counterstained with hematoxylin, dehydrated, and mounted in balsam.

RESULTS

AR-3 Reactivity with Surface Antigens of Continuous Cell Lines. From nine separate fusion experiments, about 2400 growing hybrid colonies were obtained, whose supernatants were tested in RBIA or ELISA on glutaraldehyde-fixed target cells. Fixed cells were chosen as targets, in order to select only antibodies recognizing antigenic determinants resistant to the fixation procedure, in view of the fact that these antibodies could be used on fixed human tumor sections as diagnostic tools.

Only 168 (7%) colonies were found to produce antibodies reacting against the cell line used as immunogen, and among these, only 16 did not detect antigens expressed also on a pool of human peripheral blood leukocytes (18). The most promising clone isolated so far is the AR-3. The reactivity pattern of the antibodies it produces (defined in both RBIA and ELISA) is reported in Table 1.

The greater sensitivity of ELISA, as compared to RBIA, may be explained by taking into account that, in the first case, the reaction might be amplified because of an extra incubation with the chromogen.

Molecular Characterization of the Antigen Defined by the AR-3 MAb. KATO III cells were metabolically labeled with [35S]-methionine or [3H]glucosamine and lysed with detergent, and the soluble material was precipitated with the AR-3 and analyzed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. A thick band of high molecular weight (M, > 400,000) was clearly seen in immunoprecipitates from [3H]glucosamine (Fig. 1). No bands were visible when an unrelated MAb with the same isotype was used as negative control. A band with similar electrophoretic mobility was also observed in immunoprecipitates prepared with cell extracts labeled with [35S]methionine. The minor bands of lower molecular weight present under reducing conditions. A thick band of high molecular weight (M, > 400,000) was clearly seen in immunoprecipitates from [3H]glucosamine (Fig. 1). No bands were visible when an unrelated MAb with the same isotype was used as negative control. A band with similar electrophoretic mobility was also observed in precipitates prepared with cell extracts labeled with [35S] methionine. The minor bands of lower molecular weight present in the latter case could be ascribed to contaminants precipitated specifically, since they were observed also when an unrelated antibody was used. When the same kind of experiments was performed using the A 431 cell line, the same results were

<table>
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<th>Cell line</th>
<th>Origin</th>
<th>RBIA*</th>
<th>ELISA*</th>
</tr>
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<tbody>
<tr>
<td>A 431</td>
<td>Epidermoid carcinoma</td>
<td>5.08</td>
<td>0.217</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Breast carcinoma</td>
<td>1.82</td>
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</tr>
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<td>Breast carcinoma</td>
<td>0.96</td>
<td>0.031</td>
</tr>
<tr>
<td>SK-OV-4</td>
<td>Ovarian carcinoma</td>
<td>1.82</td>
<td>0.025</td>
</tr>
<tr>
<td>SW 626</td>
<td>Ovarian carcinoma</td>
<td>1.02</td>
<td>0.111</td>
</tr>
<tr>
<td>HT 29</td>
<td>Colon carcinoma</td>
<td>3.76</td>
<td>0.157</td>
</tr>
<tr>
<td>KATO III</td>
<td>Gastric carcinoma</td>
<td>1.98</td>
<td>0.230</td>
</tr>
<tr>
<td>Hep G2</td>
<td>Hepatoma</td>
<td>NT^c</td>
<td>0.025</td>
</tr>
<tr>
<td>VM-CUB-3</td>
<td>Bladder carcinoma</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>Me 9229</td>
<td>Melanoma</td>
<td>1.01</td>
<td>0.005</td>
</tr>
<tr>
<td>Me 3819</td>
<td>Melanoma</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>HT 1080</td>
<td>Fibrosarcoma</td>
<td>0.98</td>
<td>0.031</td>
</tr>
<tr>
<td>SAOS-2</td>
<td>Osteosarcoma</td>
<td>NT</td>
<td>0.010</td>
</tr>
<tr>
<td>JU-2</td>
<td>T-lymphoma</td>
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<td>0</td>
</tr>
<tr>
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<td>T-lymphoma</td>
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<td>0</td>
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<td>WISH</td>
<td>Aminobiphenyl cells</td>
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<td>HPBL</td>
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</tr>
<tr>
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<td>Erythrocytes (ABO groups)</td>
<td>1.00</td>
<td>0.015</td>
</tr>
<tr>
<td>HFF</td>
<td>Fetal fibroblasts</td>
<td>1.15</td>
<td>0.012</td>
</tr>
</tbody>
</table>

a cpm of hybrid supernatant per cpm of control supernatant, revealed with [3H]-rabbit anti-mouse immunoglobulin. Values greater than 2.5 are considered positive.

b Absorbance units at 492 nm, revealed with horseradish peroxidase-labeled goat anti-mouse immunoglobulin. Values greater than 0.075 units are considered positive.

c NT, not tested.
extracts were reacted with the AR-3 MAb (lanes B and C) or an unrelated MAb observed (data not shown). These experiments show that the cellular precipitated from KATO III human gastric carcinoma cells labeled with [3H]-glucosamine (Lanes A and B) or [35S]methionine (Lanes C and D). Detergent cellular precipitate was assayed. Different amounts of each antigen, chosen on the basis of its localization. In fact, in the latter case, staining was restricted to the apical region of the cell surface, at the level of the glycocalyx; in the former case, a diffuse intracellular staining was also detected. In addition, in many neoplastic cells, a paranuclear focus of the antigen defined by the AR-3 MAb was frequently observed in correspondence of the Golgi area.

A positive reaction was also observed on 4 of 6 cases of endometrial carcinomas and 4 of 7 cervical carcinomas (Table 2).

A focal positivity on a minority of neoplastic cells was observed on a series of adenomas of the large intestine, areas with a higher degree of dysplasia were observed to show a higher intensity and frequency of positive staining (data not shown). Besides its stronger intensity, the staining pattern of AR-3 MAb in carcinoma cells differed clearly from that observed in normal cells also for its localization. In fact, in the latter case, staining was restricted to the apical region of the cell surface, at the level of the glycocalyx; in the former case, a diffuse intracellular staining was also detected. In addition, in many neoplastic cells, a paranuclear focus of the antigen defined by the AR-3 MAb was frequently observed in correspondence of the Golgi area.

The chemical nature of the antigen defined by the AR-3 MAb was also investigated by staining parallel sections with Alcian blue or the periodic acid-Schiff methods: no evidence was found of a mere codistribution of glycosaminoglycans and the AR-3-defined antigen. In fact, some areas that were positive with the AR-3 MAb were negative for the histochemical reactions for acidic or neutral "mucins."

To assess whether the invading carcinomatous cells could be detected by the immunoperoxidase staining with AR-3 MAb, 7 cases of early gastric cancer were studied: in 6 of these cases scattered neoplastic cells present in the lamina propria were stained. Moreover, 14 cases of neoplastic peritoneal effusions from carcinomas of the ovary (9 cases), pancreas (2 cases), and stomach (3 cases) were investigated. In 8 of the 14 cases, the atypical neoplastic cells were selectively visualized, the normal or reactive mesothelial cells being constantly negative (Fig. 3, a and b).

Nonidentity between the Antigenic Determinant Defined by the AR-3 MAb and CEA and GICA. To test the possibility that the AR-3 MAb could react with the previously described tumor-associated antigens CEA or GICA, expressed on cancers of the gastrointestinal tract or ovary, the inhibition exerted by these antigens on the binding of the AR-3 MAb of glutaraldehyde-fixed cells was assayed. Different amounts of each antigen, chosen around or above the values used for diagnostic purposes, were used. As expected, increasing amounts of detergent-soluble...
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To confirm these radioimmunometric data, the presence and distribution of the AR-3-defined antigen were compared with those of CEA and GICA on serial sections of 4 cases each of

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The AR-3 MAb recognized an antigenic determinant of obvious epithelial specificity, since it did not react with neoplastic cells from lymphomas, sarcomas, gliomas, melanomas, nor with their normal counterparts. On the basis of its tissue distribution, the antigen recognized by the AR-3 MAb was found to react with adenocarcinomas of the stomach, colon, pancreas, and ovary in the avidin:biotin:peroxidase assay. The number of positive cases for the different MAbs varied, being maximal for anti-CEA antibodies and minimal for anti-GICA antibodies (Table 3). The reactivity pattern of this antibody was established by a two techniques.

**Table 3**

| Reactivity with MAbs | AR-3 | GICA | CEA | No. observed/ no. total
<table>
<thead>
<tr>
<th></th>
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<tr>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>6/16</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>7/16</td>
</tr>
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</table>

a MAb concentration, 10 μg/ml.  
b Number of cases showing the reactivity pattern with the combination of the 3 MAbs used per number of total cases examined. The 16 cases comprised carcinomas of stomach, colon, pancreas, and ovary (4 cases each).

adeno-carcinomas of the stomach, colon, pancreas, and ovary in the anidin:biotin:peroxidase assay. The number of positive cases for the different MAbs varied, being maximal for anti-CEA antibodies and minimal for anti-GICA antibodies (Table 3). Also the number of cells positive with the three different MAbs varied; in most of the cases positive for anti-CEA and AR-3 MAbs, more than 50% of the neoplastic cells were stained, while only a minority of carcinomatous cells were positive with anti-GICA MAbs. Moreover, no strict codistribution of the three antigenic determinants was observed, since in a few cases the same area was stained by one antibody, but not the others. The nonidentity of the AR-3 MAb-defined antigen with CEA and GICA was thus established also with the immunocytochemical procedure.

**DISCUSSION**

This paper describes the production of a monoclonal antibody that reacts with carcinoma cells of the gastrointestinal tract, pancreas, ovary, and uterus. The antigenic determinant recognized was found to be expressed on a heavily glycosylated cellulosic component of high molecular weight (Mr, > 400,000).

The reactivity pattern of this antibody was established by a RBA or by an ELISA on *in vitro* tumor cell lines and, by means of an immunohistochemical method, on formalin-fixed paraffin-embedded specimens of normal and neoplastic human tissues. This step was undertaken to verify the specificity limits of the AR-3 MAb and to define its potential usefulness in histopathology. Full concordance of results was observed with the latter two techniques.

The AR-3 MAb-defined antigen was widely distributed among adenocarcinomas of the stomach, colon, pancreas, ovary, and uterus. Paradoxically, while the cell line used as immunogen was an epidermoid carcinoma, the carcinomas with the highest and frequent expression of the antigen defined by the AR-3 MAb were of entodermal origin. Occasional and focal expression was observed on some neoplastic cells in carcinomas of different organs, notably the breast. Among the many normal tissues tested, a weak positivity was observed only on the luminal surface of mucin-producing epithelial cells, in coincidence with the glycocalix area.

The AR-3 MAb recognized an antigenic determinant of obvious epithelial specificity, since it did not react with neoplastic cells from lymphomas, sarcomas, gliomas, melanomas, nor with their normal counterparts. On the basis of its tissue distribution, the antigen recognized by the AR-3 MAb will be called hereafter CAR-3.

Tumor-associated antigens displaying a distribution pattern similar to that reported here for CAR-3 have already been described. Among this class, CEA has been the first antigen to be identified as a potential tumor marker of various carcinomas (8); however, it soon appeared that antigens cross-reacting with CEA are expressed also on normal tissues (29-32). Only recently, the production of MAbs discriminating CEA antigenic determinants associated with the neoplastic phenotype has been reported (33, 34); the usefulness of these MAbs as diagnostic tools is still under evaluation. More recently, different antigenic specificities associated preferentially with carcinomas have been detected by means of MAbs. The gastrointestinal carcinoma antigen GICA was found to be expressed mainly on tumors of the gastrointestinal tract, pancreas, and ovary (9, 11); however, it was often found also on columnar epithelia of ducts of normal tissues (35). The HMFG-1 and HMFG-2 MAbs detect antigenic determinants present on some types of carcinomas and, moreover, strongly expressed on breast carcinomas (36-38). The DUT-PAN-2 antigen has been reported to be expressed mainly on pancreatic, gall bladder, or bile duct and stomach carcinomas and, at lower levels, on many types of normal glandular epithelia (39). The F36/22 MAb recognized an antigenic determinant expressed by different histotypes of adenocarcinomas and also by ductal epithelia (40-42). All of the above tumor-associated markers are typically expressed on adenocarcinomas. These findings suggest that functional and/or structural homologies between different tumor types may exist. In line with these reports, the AR-3 MAb was found to react with different types of carcinomas; however, this monoclonal seems to detect an antigenic determinant distinct from the previously described ones. In fact, competition experiments clearly showed that partially purified CEA or GICA preparations did not cross-react with the AR-3 MAb. DUT-PAN-2, in contrast to CAR-3, was found to be expressed on a mucin-like Alcian blue-stainable glycoprotein. Moreover, the pattern of reactivity of HMFG-1, HMFG-2, and F36/22 MAbs is different from that of AR-3 MAb, which barely reacted with breast carcinomas. Furthermore, the HMFG-1 and HMFG-2 MAbs detect a glycoprotein of greater than 400,000 molecular weight in human milk fat globule membranes, milk epithelial cells, and breast carcinoma cell lines (43). The AR-3 MAb, which precipitated a molecule with similar properties from gastric and epidermoid carcinoma cell lines, however, did not react with human milk fat globule membranes in a solid-phase ELISA (data not shown).

The biological significance of CAR-3 antigen is still to be elucidated; the antigen was found to be expressed on a high-molecular-weight, heavily glycosylated molecule; it is known that molecules with these properties are found in the extracellular compartment, either released in the pericellular matrix and/or in strict association with the plasma membrane. The CAR-3 antigen detected in normal tissues was localized at the apical surface of some epithelial cells. In neoplastic cells, CAR-3 was observed in huge amounts also in the intracellular compartment mainly in a paranuclear position, typical of the Golgi apparatus. Moreover, sometimes the AR-3 MAb strongly stained secretion products. This finding suggests that enhanced synthesis of the glycoconjugate bearing the CAR-3 antigenic determinant took place in neoplastic cells, leading to the overexpression of this molecule. The alternative possibility of a qualitative change of the CAR-3-bearing molecule involving the antigenic determinant itself, following neoplastic transformation, cannot be excluded. Alterna-
tively, another portion of the molecule might be affected by the change, resulting in a conformation more accessible to the MAb. The above hypotheses are in line with the many reports indicating that altered glycoconjugates are found in the majority of the tumoral cell systems examined, as compared to their normal counterparts (for reviews, see Refs. 44 and 45). Moreover, it is also known that carbohydrate moieties may contribute to the antigenic specificity of surface glycoproteins or glycolipids in different systems (44, 46, 47).

CAR-3 antigenic determinant was found to be expressed also in fetal pancreas. Its localization in the ducts, as opposed to the acinar structures, is in keeping with the origin from the ducts of most cases of pancreatic carcinomas (48). It is interesting to note that, among the different types of carcinomas which were found to be positive for CAR-3, the highest expression of this antigen, both in terms of number of positive cells and staining intensity, was found on carcinomas of the exocrine pancreas, while endocrine pancreas tumors were found mostly negative. The presence in fetal pancreas allows the inclusion of CAR-3 in the class of the oncofetal antigens. A similar conclusion has been reached also for the GICA, which was found to be expressed in various fetal tissues (49).

The preferential expression and different cellular localization of the CAR-3 antigenic determinant in carcinoma of the pancreas, stomach, colon, ovary, and uterus makes it a valuable marker for these kinds of neoplasia. Since the AR-3 MAb seems to recognize a specificity so far undetected, it could be used in association with a panel of other selected MAbs, to approach the goal of classifying the largest number of this type of carcinoma. This strategy has already been successfully used for the immunodiagnosis of carcinomas of other organs (12–15).

It is noteworthy that, in line with its specificity, the antibody could identify carcinomatous cells derived from gastric, ovarian, and pancreatic tumors and metastasizing in the peritoneal cavity. Reagents with these characteristics have been shown to be extremely useful in cases where a definite diagnosis cannot be made on the basis of cytology alone (38, 50, 51).

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Mabs AGAINST HUMAN CARCINOMAS


Fig. 2. Human tissues routinely fixed and paraffin embedded. Sections stained with AR-3 MAb and the avidin:biotin:peroxidase procedure. a, intramucosal signet-ring cell carcinoma of the stomach. The dark positive staining detects the carcinomatous cells infiltrating the tunica propria. The normal epithelial cells of the antral glands as well as the inflammatory cells, mainly lymphocytes and plasma cells, are negative; a slight apical reaction is focally present over the foveolar epithelium. × 100. b, adenocarcinoma of the stomach. The immunoperoxidase staining is mainly localized at the apical surface of pseudoglandular structures and in the cytoplasm of isolated neoplastic cells infiltrating into the stroma. × 250. c, well-differentiated adenocarcinoma of the colon infiltrating into the muscularis propria. The immunoperoxidase staining is mainly localized at the apical surface of pseudoglandular structures. A few neoplastic cells also show a diffuse cytoplasmic staining. × 250. d, mucinous cystoadenocarcinoma of the ovary. Most neoplastic cells show both an apical and a cytoplasmic staining. The stroma is negative. × 250.
Fig. 3. Human tissues routinely fixed and paraffin embedded. Sections stained with AR-3 MAb and the avidin:biotin:peroxidase procedure. 
a, adenocarcinoma of the pancreas. In the lower area, the normal pancreas appears unstained, while irregular pseudoglandular neoplastic structures show a cytoplasmic and/or apical staining with the AR-3 MAb. Secreted material present in the lumina also shows a high degree of reactivity. Surrounding inflammatory cells are completely negative. × 100. 
b, fetal pancreas. The staining with AR-3 MAb is localized in the intra- and interlobular ducts, while the acina are completely unstained. A small islet also appears unreactive. × 250. 
c, pancreatic carcinoma invasive into the omentum. Scattered carcinomatous cells, isolated or arranged in small clusters, appear heavily and diffusely reactive with AR-3 MAb. Inflammatory and connective cells are nonreactive. × 250. 
d, ascitic effusion from a patient with carcinoma of the ovary. A smear of the sediment has been stained with AR-3 MAb. Several inflammatory and mesothelial cells are present, as revealed by the nuclear counterstaining; their cytoplasm appears unreactive. Numerous large neoplastic cells show a heavy and diffuse cytoplasmic staining for CAR-3. × 400.
CAR-3, a Monoclonal Antibody-defined Antigen Expressed on Human Carcinomas

Maria Prat, Isabella Morra, Giovanni Bussolati, et al.

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