Biotecnología Aplicada, Vol. 8, No. 2, 206-215 (1991)

Colon cancer associated antigen expression on normal and neoplastic human tissues detected by IORC2: A novel monoclonal antibody

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Received in June 1990
Approved in September 1990

SUMMARY

Established human colon cancer cell line SW1116 (poorly differentiated) was used to raise IORC2 monoclonal antibody (Mab) by standard hybridoma technique.

IORC2 Mab appeared to show restricted epithelial specificity when tested on routine paraffin wax-embedded and cryostat histological sections by the biotin-streptavidin-peroxidase-complex (Amersham, U.K.) technique. The reaction was intense on SW1116 and SW948 colon cancer cell lines, glandular epithelial derived tumors especially colon adenocarcinomas and normal colon mucosa. Reaction in a low percent of cells is also observed in the small intestine and the bronchus epithelial cells. There was no reaction with nerve, muscle or hematopoietic tissues.

The potential applications of Mab IORC2 in the management of human colorectal cancer are discussed.

RESUMEN

Inmunizando con la línea de cáncer del colon SW1116 (pobremente diferenciado), se obtuvo el anticuerpo monoclonal IORC2 empleando la técnica convencional de generación de hibridomas.

El anticuerpo monoclonal IORC2 muestra un reconocimiento preferencial por el tejido epitelial en cortes frescos y en cortes fijados e incluidos en parafina, según se evidencia, empleando el sistema de detección avidina-biotina-peroxidasa-complejo (Amersham, U.K.). Se observa una reacción intensa en las líneas de cáncer del colon SW1116 y SW948 y en los tumores derivados del epitelio glandular, especialmente los adenocarcinomas del colon y la mucosa normal del colon. También se observa reacción en un bajo por ciento de células del intestino delgado y del epitelio bronquial. No hubo reacción en los tejidos de origen nervioso, muscular y hematopoyético.

Las aplicaciones potenciales del anticuerpo monoclonal IORC2 en el manejo del cáncer colorrectal humano se discuten.

INTRODUCTION

Colorectal cancer is currently regarded as one of the most common death causing malignancies particularly in North America and Europe (Siddiqui and Chopra, 1984). The prognosis of colorectal cancer has remained unchanged over the last two decades (Hardcastle *et al.*, 1986) mainly due to the advanced stage of the majority of tumors at the time of diagnosis.

Advent of the monoclonal antibody (Mab) technology (Kohler and Milstein, 1975) renewed hopes of finding tumor associated

antigens bearing the promise of developing methods for tumor diagnosis and therapy (Stahli *et al.*, 1985; Poels *et al.*, 1986).

In the present study we describe immunohistochemically the reactivity of IORC2 Mab to different epithelia of the colon, including mucosa remote and adjacent to carcinoma, and normal colon mucosa, as well as other epithelial and non-epithelial derived tumors and normal organs. The potential applications of IORC2 Mab as a diagnostic and therapeutic tool are evaluated.

MATERIALS AND METHODS

Monoclonal Antibody

The murine monoclonal antibody IORC2 was obtained from the fusion of the SP/2O AG 14 myeloma with spleen cells of a BALB/c mouse immunized with the SW1116 colon cancer cell line. This IgG1 Mab was purified from mouse ascitic fluid by protein A sepharose chromatography.

Specimens

Surgically resected human tissue biopsies contained in paraffin blocks and from fresh tissue were selected for this study. These specimens included carcinomas of the colon, lung, breast, ovary, endometrium as well as normal colon mucosa, mucosa remote and adjacent to colon carcinomas, some benign lesions and normal tissues obtained from autopsy material from organ donors. In addition, some tumors of neural, hematopoietic and sarcomatous origin were also examined.

Cell lines

Cell suspension was adjusted to 1 million cells/ml in TBS (Tris buffered saline) containing 1% bovine serum albumin. Two hundred microliters of the cell suspension were applied to each slide. Slides were then air-dried overnight and after fixation in acetone:methanol for 90 seconds they were transferred directly to TBS. Finally slides were processed by the APAAP technique as previously described by Cordell *et al.*, 1984.

Tissue processing

Paraffin embedded tissues were fixed in 10% buffered formalin solution, dehydrated, cleared and paraffin embedded in a routine manner. Fresh tissues were snap frozen in liquid nitrogen and stored at -20°C. Histopathology was evaluated on H&E stained frozen sections and rehydrated formalin fixed tissue sections. Consecutive sections made from histologically evaluated blocks were used for immunostaining by the immunoperoxidase technique.

Immunoperoxidase staining

Mab binding to tissue sections was demonstrated by the biotin-streptavidin-peroxidase-complex system (Amersham, U.K.). Briefly, deparaffinized and rehydrated sections were treated with 3% H_2O_2 (methanol solution) for 30 minutes to block endogenous peroxidase activity. These and air-dried cryostat sections were incubated in a moist chamber with the IORC2 Mab for one hour at room temperature, followed by biotin conjugated sheep anti-mouse Ig and biotin-streptavidin-peroxidase-complex each for 30 minutes at room temperature. Between incubations the sections were washed with TBS. The site of peroxidase localization was shown using a solution containing 3 mg of 3-3 diaminobenzidine, 5 ml of TBS and 5 microliters of 30% H_2O_2. Slides were then rinsed in tap water, counterstained with Mayer's Hematoxiline, covered with Balsam mounting media and coverslipped. Positive and negative control sections were included in each staining. The brown staining reaction was scored as follows: - no reaction; + weak; + + moderate; + + + marked staining.

RESULTS

Histological studies demonstrated no difference in staining intensity between cryostat and formalin-fixed paraffin-embedded tissues so IORC2 Mab specificity was assayed mainly on paraffin sections.

Table 1 shows IORC2 Mab staining pattern in lymphoma, melanoma lung, breast and colon tumor cell lines. This Mab was negative for lines tested except for two of the colon cancer cell lines that showed strong positive staining.

Table 1
IORC2 MAB STAINING PATTERN IN TUMOR CELL LINES

Cell Line	Tissue Origin	Reaction Intensity
U 1752	Lung	–
U 2020	Lung	–
A 231	Breast	–
MDA-MB-157	Breast	–
MDA-MB-134	Breast	–
MDA-MB-435	Breast	–
CEM-T	Leukemia (T-ALL)	–
Raji	Burkitt Lymphoma	–
A 375	Melanoma	–
FEMX	Melanoma	–
M-14	Melanoma	–
HT 29	Colon	–
SW 948	Colon	+ + +
SW 1116	Colon	+ + +

The IORC2 Mab reacted with most malignant tumors of glandular epithelia studied (table 2), i.e.: colon, breast, lung, ovarian and endometrium as well as the non-malignant lesions of glandular epithelia studied.

IORC2 Mab staining of colon adenocarcinoma tissue was intense but with a heterogeneous pattern of cellular reactivity (fig. 1). Positive tumor areas showed a cytoplasmic staining with an apical accentuation. Intraluminally secreted material was also commonly positive. On the contrary, normal colon mucosa was generally homogeneously stained. This homogeneous staining pattern was observed in most transitional mucosa (adjacent to tumor) (fig. 2), mucosa remote from carcinomas and normal colon mucosa (patients without tumor lesions) (fig. 3).

Staining of breast and lung cancer tissues also showed a heterogeneous pattern of cellular reactivity with a variable number of positive cells (figs. 4 and 5). IORC2 Mab stained mucinous but not serous component of ovarian tumors (fig. 6). As summarized in table 3, IORC2 Mab expression in normal tissues was noted in very few cell types from the organs and tissue types evaluated. An intense reaction was observed only in a limited percent of normal cells of the small intestine and one of the lungs studied. IORC2 Mab was negative in all normal tissues studied as well as in all tumors of neural, hematopoietic or sarcomatous derivation and squamous epithelial derived lesions tested (table 2).

Table 2
IORC2 MAB STAINING PATTERN IN TUMOR DISEASES

Tumor site	Histology	Intensity				Total of cases
		–	+	++	+++	
A. Glandular epithelial derived tumors						
Colon	Adenocarcinomas	0	0	3	21*	24
Breast	Canalicular Ca	0	1	2	2	5
	Lobular Ca	1	0	0	0	1
Lung	Large Cell Ca	0	0	0	2	2
Ovary	Serous Cisto ADC	1	0	0	0	1
	Mucinous Cisto ADC	0	0	0	1	1
	Mucinous Cisto Adenoma	0	0	0	1	1
Endometria	ADC	0	0	0	1	1
Prostate	Fibroadenomatous hyperplasia	0	0	0	1	1
B. Other tumors with other origins						
Skin	Epidermoid Ca	2	0	0	0	2
Non-HDG Lymphoma		1	0	0	0	1
Rabdomyosarcoma		1	0	0	0	1
Ganglioneuroblastoma		1	0	0	0	1
Osteoclastoma (Giant cells tumor)		1	0	0	0	1
Neurofibroma		1	0	0	0	1
Osteosarcoma		1	0	0	0	1
C. Colon mucosa						
Transitional mucosa		0	0	0	9*	9
Remote mucosa		0	0	1	8**	9
Normal mucosa		0	0	0	2	2

* 2 cases selective staining/low percent of glands.
** 3 cases selective staining/low percent of glands.

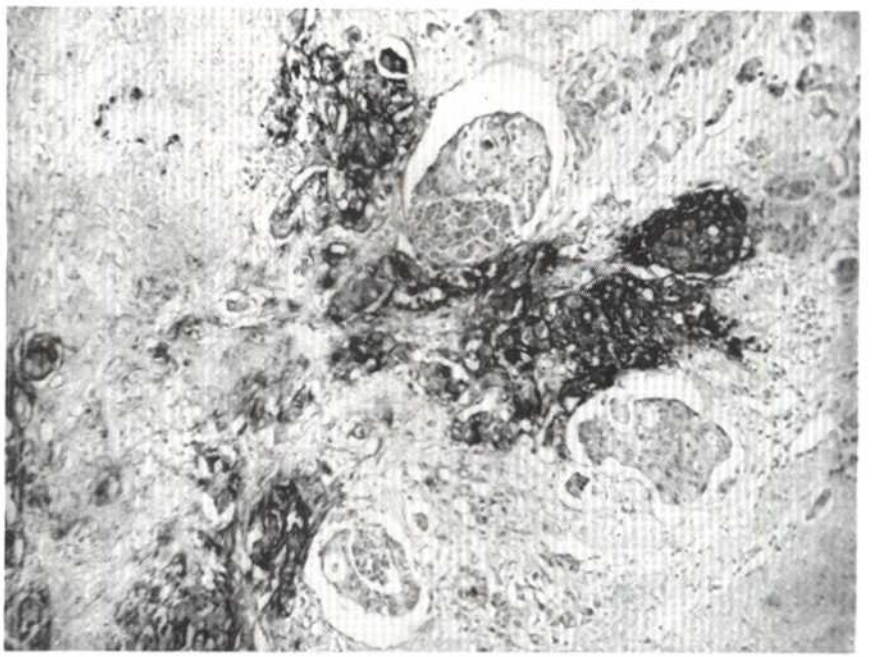

FIG. 1. Colonic adenocarcinoma with intense heterogeneous pattern of cellular reactivity for IORC2 Mab. (X80).

FIG. 2. Colon mucosa adjacent to tumor. An intense homogeneous staining pattern is observed. (X125).

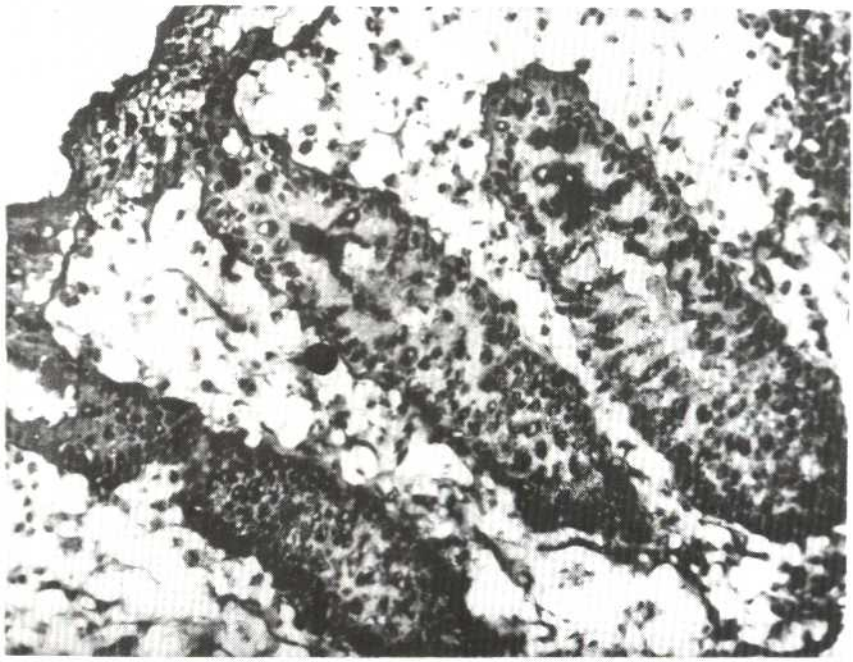

FIG. 3. Normal colon mucosa. Note homogeneous pattern of cellular reactivity for IORC2 Mab. (X250).

Table 3
IORC2 MAB STAINING PATTERN IN NORMAL TISSUES

Tissue	Total number of cases positive/negative
Skin	0 / 2
Kidney	0 / 2
Brain	0 / 1
Thyroid gland	0 / 2
Heart	0 / 2
Lymph node	0 / 1
Adrenal gland	0 / 1
Hypophysis	0 / 2
Stomach	0 / 1
Spleen	0 / 2
Pancreas	0 / 2
Liver	0 / 2
Lung	1 / 2 (5% cells)
Small intestine	1 / 0 (1-2% cells)

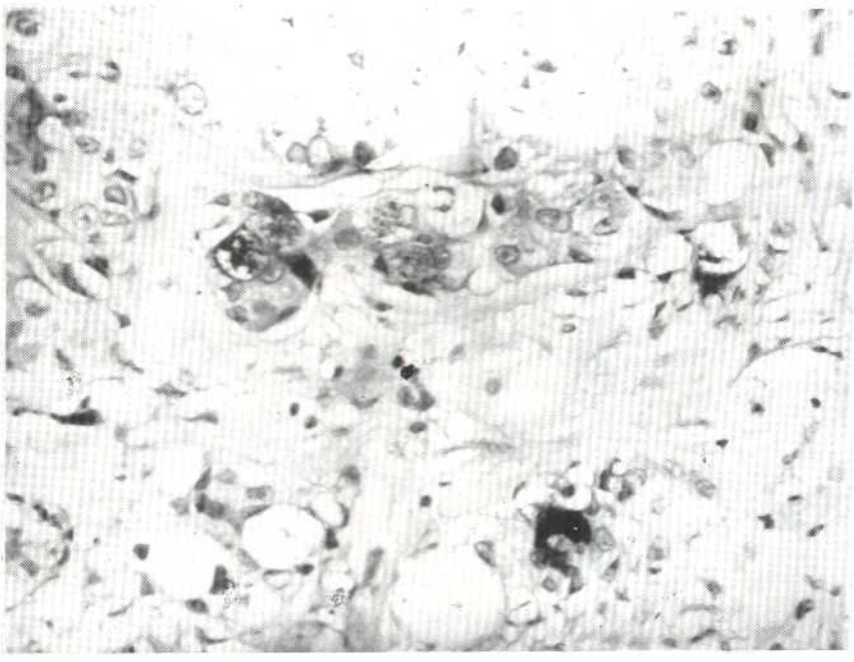

FIG. 4. Breast canalicular carcinoma. Scattered posite cells a few of which show a very intense reaction (arrow). (X250).

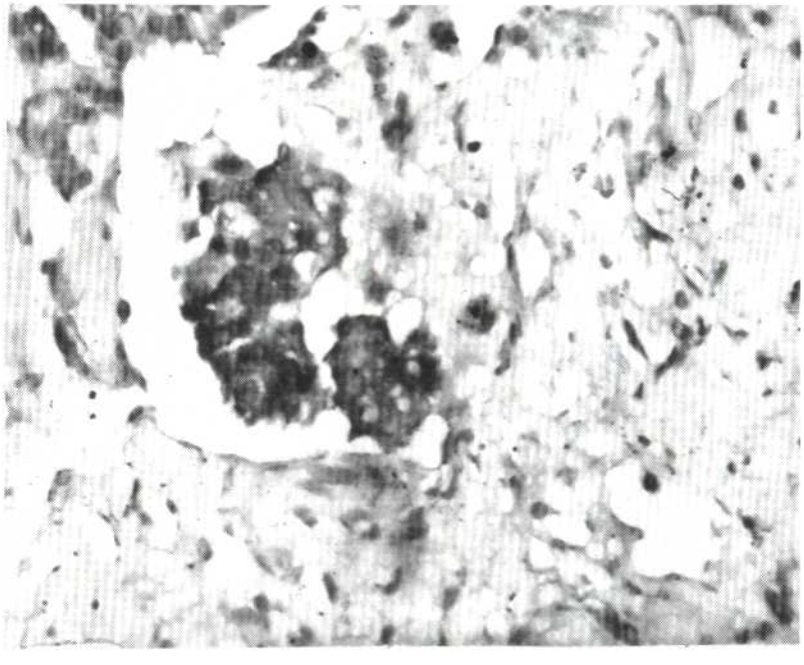

FIG. 5. Lung large cell carcinoma. Small groups of positive cells are observed among tumor areas. (X250).

FIG. 6. Ovary mucinous cystoadenocarcinoma. Strong posite staining of mucinous component. (X80).

In general IORC2 Mab staining was strongly detected in the mucus of globlet cells and luminal secretion products.

DISCUSSION

Malignant transformation of colonic mucosa is accompanied by cellular alterations such as the appearance of new antigens, the expression of fetal antigens, and/or structural/functional changes in existing cell surface macromolecules (Drewinko *et al.*, 1986). These changes can be detected by the use of Mabs generated by hybridoma technology.

This study describes a monoclonal antibody that reacts with an antigen associated with human colon cells. IORC2 Mab bound to glandular epithelial derived tumors especially colon adenocarcinomas and to normal colon mucosa. The monoclonal defined antigen was not expressed on tumors of neural, hemaotopoietic or sarcomatous derivation. The human colorectal carcinoma cell lines SW1116 and SW948 showed a strong expression of the IORC2 antigen on the cell surface and cytoplasm.

The presence of IORC2 antigen in breast, lung, and some endometrial and ovarian adenocarcinomas may be of use in the immunodiagnosis and immunotherapy of some non-colorectal tumors.

Changes in antigenic structures are often observed in the mucosa adjacent to cancer (Shetye *et al.*, 1989). However, in our study the expression of the antigen recognized by IORC2 Mab was not altered in the mucosa adjacent to cancer. Moreover, the normal colon mucosa and the mucosa remote from tumor were always similarly stained with the

IORC2 Mab as the transitional mucosa. Thus this antigen may be a normal component associated with colonic mucosa.

Antigen expression was essentially homogeneous in all parts of normal colonic mucosa cripts while IORC2 Mab staining pattern was heterogeneous in the colorectal tumors tested. Heterogeneity of expression of tumor-associated antigens has been previously documented, not only between tumors from different persons, but among tumors from a single patient (Goodwin *et al.*, 1987).

This suggests that the tumor selective recognition of IORC2 Mab as well as the expression of IORC2 antigen in normal colon mucosa may be a limiting factor for the use of this monoclonal antibody in radioimmunolocalization of tumors and in immunoconjugated therapy trials, although well known colorectal carcinoma associated antigens such as GA 73-3 (reacts with same antigen as 17-1A) and GICA 19-9 have been shown to be expressed in normal colonic mucosa (Goodwin *et al.*, 1987; Shetye *et al.*, 1989) and have been widely used in immunotherapy (Goodwin *et al.*, 1987; Shetye *et al.*, 1988) and radioimmunolocalization (Baun *et al.*, 1985; Chatal *et al.*, 1982) respectively with favorable results.

A promising approach to overcome the handicap of the heterogeneity of the expression of tumor associated antigens is the simultaneous assay of several markers based on the premise that cancer cells are biochemically heterogeneous and may synthesize a broad spectrum of possible tumor markers (Mercer and Talamo, 1985). The use of IORC2 Mab with complementary Mabs as a battery of markers should be considered when planning radioimmunodetection, monitoring and immunotherapy with Mabs in colorectal cancer.

Blocking experiments have demonstrated that IORC2 Mab recognizes a different antigen than the well known colorectal tumor associated antigens GICA 19-9 and 17-1A (manuscript in preparation). In our Institute a battery of 6 other Mabs that react with human colon cells has been generated. Characterization experiments are ongoing.

Our battery of anti-colon Mabs and GICA 19-9 and 17-1A could be adequate for simultaneous assays using several markers.

The predominantly apical staining of the tumor cells with IORC2 Mab and its presence in secreted material is in accordance with the notion that it is actively secreted into the serum. Preliminary trials by ELISA on serum from patients with colorectal cancers seem to corroborate this statement (unpublished data).

Considering the former and the fact that although normal tissues may produce an antigen, this does not warrant that they secrete it and, if so, not necessarily at the same levels as the corresponding tumor cells. Current studies are in progress in our Institute for evaluating the potential usefulness of IORC2 Mab as a serodiagnostic marker.

ACKNOWLEDGMENTS

We would like to thank Dr. C. Domínguez for his contribution of biopsy material. We are also grateful to E. Fernández, H. Moreno, O. Hernández and J. Delgado for their technical assistance and to M. Nuñez and E. Espinosa for performing photomicrographs.

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