Full Expression of Blood Group-related, Transplantation-related, and Carcinoembryonic Antigens in Human Colorectal Cancer Cells with Different Degrees of Phenotypic Differentiation

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ABSTRACT

Seven established human colon carcinoma cell lines with distinct degrees of phenotypic differentiation were evaluated for the presence of blood group-related and transplantation-related antigens in relation to their production of carcinoembryonic antigen (CEA). All lines presented A and B antigens regardless of the patients' original red blood cell type. However, tumor cells from patients originally classified as O-type had lower expression of both A and B antigens and high production of CEA. Cells from patients with an original A type had low to undetectable CEA production and high expression of both A and B antigens. There was no particular segregation of transplantation-related antigens with respect to phenotypic expression. All lines presented HLA-A, -B, and -C, as well as -DR antigens. These results demonstrate that colon carcinoma cells have the ability to fully express both blood group-related and transplantation-related antigens, even if discordant with the donor's red blood cell phenotype. Furthermore, it appears that expression of A antigen is intimately related to synthesis of CEA.

INTRODUCTION

The expression or deletion of BGA, such as ABO, P, MN, T, and Le as well as TRA HLA-A, -B, -C, and -DR by human neoplasms has been well documented in the last 30 years (1, 5, 7, 8, 10, 15, 22, 26, 30, 31, 35, 38, 47, 49). Although many attempts have been made to correlate the expression or deletion of such antigens with clinical prognosis, their primary functional role and the mechanisms responsible for their synthesis or deletion are presently poorly understood. However, documentation of their expression and evaluation of their distribution in neoplastic tissues may provide further insights into the mechanism of tumor growth and metastasis and of the host immune recognition processes that enhance or impair tumor proliferation.

Colorectal cancer cells have been the particular subject of intensive studies concerning the deletion or expression of BGA, their relation to CEA production, and the clinical significance of those findings (3, 6, 7, 9-12, 16-23, 27, 41-44, 48). Also, Daar et al. (8) and Rognum et al. (39) have demonstrated the anomalous expression of HLA-DR antigens in tissue sections of human colorectal cancer, a manifestation not found in the normal epithelium surrounding the tumor tissue. In contrast, McLean et al. (32) who studied several human colorectal cell lines with a panel of monoclonal antibodies produced in their laboratories failed to detect HLA-A, -B, -C, or -DR antigen expression on such cells.

In this paper, we document the presence of ABO, Le, T, Tn, HLA-A, -B, -C, and -DR antigens in 7 well-characterized established human colorectal carcinoma cell lines. These cell lines have distinct degrees of phenotypic expression including their morphological differentiation and their ability to synthesize CEA (14).

MATERIALS AND METHODS

Cell Lines. Our studies were performed on 7 established human colorectal carcinoma cell lines representing the proposed 3 biological groups of the classification of Leibovitz et al. (29). Leibovitz and coworkers have suggested that cultured human colon carcinoma cells could be separated into at least 3 groups on the basis of morphological differentiation, chromosome number, and rate of CEA production. We have validated this hypothesis and have included the characteristics of cell proliferation and heterotransplantation into athymic animals as an additional marker of phenotypic segregation (14). In the present studies, Group 1 consisted of 2 lines, designated LoVo and SW48; Group 2 comprised 2 lines, called SW480 and SW620; and Group 3 was represented by lines SW403 and SW1116. A seventh line, SW742, originally classified as Group 3 by Leibovitz et al., was shown in our laboratory to correspond to Group 2.

LoVo cells are propagated as monolayer cultures in Ham's F-10 medium supplemented by 20% fetal calf serum, vitamins, glutamine, and antibiotics. Some of the biological properties of LoVo and the SW cells have been described previously (13, 14, 29).

BGA. BGA-A and -B antigens were demonstrated by indirect immunofluorescence. Stock cultured colon cancer cells were harvested by the appropriate technique (14) and washed 3 times with PBS. Viability was tested with the trypan blue exclusion test (37) yielding greater than 90% viable cells. Aliquots of 100 μl (5 x 10⁶ cells) were centrifuged and resuspended in 500 μl of decomplemented and undiluted anti-A and anti-B sera derived from human donors (Gamma Biologicals, Houston, TX). Quality control of serum specificity is routinely performed in our blood bank using reagent erythrocytes of known antigenic specificity (Gamma Biologicals, Houston, TX, and BCA, Westchester, PA). After gentle mixing on a vortex (Scientific Industries, Inc., Bohemia, NY), samples were incubated at 37 °C for 30 min. Cells were then washed three times with PBS and reincubated for 30 min with a 1:20 dilution of goat anti-human -,-globulin (broad spectrum) labeled with FITC (Kallestadt, Biologics, Houston, TX). Following several washes with PBS, the cells were resuspended in 50% buffered glycerol, deposited onto slides, coverslipped, and examined with a fluorescent microscope by 2 independent observers who counted 200 cells each and determined the number and character.

1 Supported in part by Grant CA23272 from the National Cancer Institute.

2 To whom requests for reprints should be addressed, at Section of Laboratory Hematology, Department of Laboratory Medicine, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, 6723 Bertner Avenue, Box 73, Houston, TX 77030.

3 The abbreviations used are: BGA, blood group-related antigens; TRA, transplantation-related antigens; PBS, phosphate-buffered saline; CEA, carcinoembryonic antigen; FITC, fluorescein isothiocyanate; T-antigen, Thomsen-Friedenreich antigen.

* B. Drewinko and B. Lichtiger, unpublished data.
ABO AND HLA ANTIGENS IN COLON CANCER CELLS

M. T. van de Vondervoort, A. W. G. C. van Helden, C. W. Wiggers, and J. W. J. van der Kwast

Institute for Tumor Biology, University of Amsterdam, The Netherlands.

ABSTRACT

Cross-reacting tumor antigens were studied in 15 colon cancer cells by serological and immunofluorescent methods. All cell lines expressed A and B antigens. The A and B expression in the tumor cells of the other 2 groups generally displayed a lower content of these BGA (Table 1). All cells in every line expressed HLA antigens with equal distribution, except for LoVo cells (Group 1), which expressed only HLA-B antigens. All cell lines expressed intense T and Tn antigens with the exception of the reaction mediated by S. scirera (Tn), which was only 2+ for Groups 1 and 2 and 1+ for Group 3. With the exception of LoVo cells (Group 1), all cell lines expressed modest amounts (1 to 2+) of Lea antibody (Table 1).

RESULTS

Segregation of patients according to their original RBC-ABO group revealed a most interesting relation. All tumors of Group 3 had been derived from O-type patients, while all tumors of Group 2 were from A-type patients. The only B-types were found in Group 1, one patient being AB and the other being only B (Table 1).

The expression of BGA-A and -B by the tumor cells did not correlate with the patient's blood group type. Both BGA-A and -B were expressed in all cell lines regardless of the patients' RBC grouping. Yet, both patients with type O RBC had only 1+ to 2+ expression of A and B antigens on their tumor cells, while the tumor cells of the other 2 groups generally displayed a greater content of these BGA (Table 1). All cells in every line evidenced distinct and uniform immunofluorescence in a granular, pericellular membrane fashion with no capping and occasional patches of intracytoplasmic material.

Ethanol treatment decreased the overall fluorescence, but none of the cell lines became negative. Fluorescence (and binding) was enhanced at 4°C returning the fluorescence of all of the ethanoltreated cells to that of the nontreated counterparts. All of the cell lines expressed intense Tn and T antigens with the exception of the reaction mediated by S. scirera (Tn), which was only 2+ for Groups 1 and 2 and 1+ for Group 3. With the exception of LoVo cells (Group 1), all cell lines expressed modest amounts (1 to 2+) of Lea (Table 1).

DISCUSSION

The expression or deletion of BGA by gastrointestinal tumors, the significance of this phenomenon with respect to gut cell differentiation, and the immunochemical relation of such antigens to CEA have been subjects of intense scrutiny. The antigenic determinant of each BGA is exquisitely unique, and its specificity is conferred by a single sugar attached terminally to oligosaccharide acceptors by characteristic sugar transferases (34). BGA-H, -A, and -B specificity are, respectively, conferred by either an A/-acetyl-A, and -B have a common branched terminal sequence consisting of fucose, galactose, and N-acetylgalcosamine. The -A and -B specificity are, respectively, conferred by either an N-acetyl-galactosamine or a galactose attached to this terminal sequence. The oligosaccharide is in turn attached to either a proteic or a membranous-bound dots) to 4+ (large patches of fluorescent materials covering the cells). In other experiments, cell aliquots of each line were seeded into Petri dishes containing sterile slides and allowed to grow to confluence. Slides were then rinsed and stained briefly in PBS. Half the replicate slides were air dried, while the other half were treated with 95% ethanol for 1 h before air drying. Labeling with anti-A and anti-B serum was accomplished as described above, but incubation was conducted at both 37 °C and at 4 °C to enhance binding (24). After a rinsing in PBS, cells were exposed to goat anti-human γ-globulin at room temperature. The cells were then rinsed, coverslipped, and examined as described above.

The presence of T, Tk, Th, and Tn antigens was tested by RBC polyagglutination (33) using lectin Arachis hypogaea for T antigen; Glycine soja for T and Tn antigens; and Dolichos biflorus, Salvia sciera, and Salvia horminum for Tn antigen. Aliquots of colon carcinoma cells (5 x 10^6) were washed 3 times with PBS, spun to form a dry button at 2000 x g for 2 min, and incubated for 1 h at room temperature with 10 μl of the appropriate lectin. Controls were neuraminidase-treated RBC prepared as described elsewhere (33, 36, 46). Agglutination was scored macroscopically by 2 independent observers from 0 (no agglutination) to 4+, where the lectins induced a solid clump of cells.

To test for Le antigen, aliquots of 5 x 10^6 colon carcinoma cells were washed 3 times with PBS and then centrifuged to a dry button to which 50 μl of goat anti-human Lea antibody (Ortho Diagnostics, Raritan, NJ) were added. The cell button was resuspended and incubated for 30 min at room temperature; the cells were then washed 3 times with PBS, resuspended in a 1:20 dilution of rabbit anti-goat IgG labeled with FITC (Behring Diagnostics, Somerville, NJ), and incubated for 30 min at 37 °C. The cells were then washed 3 times with PBS, resuspended in 50% glycerol/PBS buffer, deposited on a slide, coverslipped, and read with a fluorescent microscope.

TRA. HLA-A, -B, -C, and -DR typing was performed with standard techniques (50). Briefly, cells were harvested and washed as described above and suspended in serum-free McCoy's medium at a titer of 2000 cells/μl. One-μl aliquots were delivered with Hamilton dispensing pipets (Hamilton Co., Reno, NV) to Terasaki microcytotoxicity trays preloaded with 1 ml of anti-HLA-A, -B, -C, and -DR sera (One Lambda, Inc., Los Angeles, CA) obtained from multiparous females. The cells were mixed with a sonic oscillator (Electro-Technic Products, Chicago, IL) for 15 s and then incubated at room temperature for 30 min, after which time, 5 μl of undiluted rabbit complement were dispersed in each well with a 6-needle repeating dispenser (Hamilton Co., Reno, NV). The trays were incubated at room temperature for 60 min before receiving 3 μl of a 5% aqueous eosin solution added to each well. After incubation for 2 min, 8 μl of a 37% formaldehyde-buffered solution were added to each well and mixed. Trays were washed with petrolatum and examined by 2 independent observers with an inverted phase-contrast microscope (Olympus, Tokyo, Japan) at ×120 magnification scoring 500 cells in each well. Negative controls consisted of tumor cells incubated only with 0.9% NaCl solution and counterstained with eosin. Positive controls were tumor cells treated with HLA sera of broad spectrum specificity plus complement and counterstained with eosin. Staining intensity was quantified from 0 (nonreactive, live cells: small and refractile) to 4+ (reactive, dead cells: large with dull brown color) (50).

CEA Content. Aliquots of 5 x 10^6 cells were seeded into 16-oz Owens bottles containing 35 ml of medium and allowed to grow unrefed. Both the supernatant and the cells were harvested, washed, and counted at approximately the same time the cells were submitted for BGA and TRA assays. Supernatants and cell extracts were assayed for CEA activity by the method of Chu and Reynoso (4) using commercial CEA kits (Hoffmann-La Roche Inc., Nutley, NJ). All reagents were standardized against the First British Standard for CEA (73/601) obtained from the National Institute for Biological Standards and Control, Medical Research Council, London, England (28). To extract cell-bound CEA, cells were harvested, washed with 0.9% NaCl solution, resuspended in 2 ml of deionized H2O, and disrupted in a sonicator (Heat Systems Ultrasonics, Plainview, NY) operating at full power for 16 min. The precipitate was removed by centrifugation for 20 min at 8000 x g at 4 °C in a Sorvall RC-5 refrigerated centrifuge. The supernatant was dialyzed against 3 changes of deionized water and a final change of 0.01 M ammonium acetate buffer (pH 6.8) for at least 3 h.

RESULTS

Segregation of patients according to their original RBC-ABO group revealed a most interesting relation. All tumors of Group 3 had been derived from O-type patients, while all tumors of Group 2 were from A-type patients. The only B-types were found in Group 1, one patient being AB and the other being only B (Table 1).

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In other experiments, cell aliquots of each line were seeded into Retri-65 bottles containing 35 ml of medium and allowed to grow unrefed. Both the supernatant and the cells were harvested, washed, and counted at approximately the same time the cells were submitted for BGA and TRA assays. Supernatants and cell extracts were assayed for CEA activity by the method of Chu and Reynoso (4) using commercial CEA kits (Hoffmann-La Roche Inc., Nutley, NJ). All reagents were standardized against the First British Standard for CEA (73/601) obtained from the National Institute for Biological Standards and Control, Medical Research Council, London, England (28). To extract cell-bound CEA, cells were harvested, washed with 0.9% NaCl solution, resuspended in 2 ml of deionized H2O, and disrupted in a sonicator (Heat Systems Ultrasonics, Plainview, NY) operating at full power for 16 min. The precipitate was removed by centrifugation for 20 min at 8000 x g at 4 °C in a Sorvall RC-5 refrigerated centrifuge. The supernatant was dialyzed against 3 changes of deionized water and a final change of 0.01 M ammonium acetate buffer (pH 6.8) for at least 3 h.
explain the diversity of findings concerning the expression of
embryo with production of both water-soluble BGA and CEA
after birth but is suppressed in the distal colon (45). Thus,
Secretion of water-soluble BGA is retained by the right colon
tion, a situation that coincides with the appearance of CEA.
to secrete water-soluble BGA when they begin mucus produc
tion in the very early stage of embryo development with the production of
neoplastic transformation of the colorectal mucosa may mimic the
connection between the synthetic pathways for both products.
Ontogenically, the gastrointestinal tract produces both water-
and alcohol-soluble BGA. In the very early embryo, the mucosal
cells contain alcohol-soluble antigens as part of the cell wall
moiety attached to the oligosaccharide epitope was not lipid as
expected from very undifferentiated cells.
Thus, a series of investigators who showed an immunochemical
relation between CEA and BGA-A (2, 12, 17-19, 21, 23, 26, 33,
44, 57, 2, 25, 13, 4) have reported the persistence of
hypogae4+4+4+4+4+4+4+Glycine soja4+4+4+3+3+4+4+Dolichos biflorus3+4+3+4+4+2+3+2+Salvia sclarea2+2+2+2+1
expression by colorectal cancer cells had no relation to degree
of aggressiveness or other phenotypic properties.
Thus, in contrast to tumors of the urinary bladder (5), T-antigen
expression by colorectal cancer cells had no relation to degree
of aggressiveness or other phenotypic properties.
Expression of TRA by colorectal cancer cells has been docu-
menced previously by Daar et al. (8), who studied 15 samples of
fresh tumor tissue and found that 8 of 15 tumors expressed
HLA-DR antigens, while 14 of 15 expressed HLA-ABC antigens.
Similarly, Rognum et al. (39) evaluated 33 specimens of large
bowel carcinomas and observed 90% positivity, while Thompson
et al. (47) found positive staining in 7 of 9 colon carcinomas. In
contrast, McLean et al. (32) used monoclonal antibodies devel-
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Note: a ND, not detectable.
b ng of CEA per 10⁶ cells (intracellular only).

Table 2 | Distribution of T-antigen in 7 established colorectal carcinoma cell lines |
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be removed by alcohol treatment, suggesting that the structural
moiety attached to the oligosaccharide epitope was not lipid as
expected from very undifferentiated cells.

The results described previously by Cowan (7), Sheahan et al. (42), and
Cowan (7), Sheahan et al. (42), and
Davidsohn et al. (11) restricted their observations to the presence
of water-soluble BGA and reported absent or greatly
diminished BGA reactivity, especially that of BGA-A, but failed to demonstrate
connection between the synthetic pathways for both products.

Our data, obtained on established colorectal cancer cell lines,
support the contention that BGA expression is independent of
cell differentiation. Thus, the undifferentiated cells from Group 3
expressed both BGA-A and -B in a manner qualitatively similar
right colon after birth but is suppressed in the distal colon (45). Thus,
neoplastic transformation of the colonic mucosa may mimic the
very early stage of embryo development with the production of
alcohol-soluble BGA or, with more differentiation, that of the later
baby embryo with production of both water-soluble BGA and CEA
(12).

These ontogenic-related changes in mucosal activity help to
explain the diversity of findings concerning the expression of
BGA-A and -B in gastrointestinal neoplasms when different fixation techniques were used. Cowan (7), Sheahan et al. (42), and
Davidsohn et al. (11) restricted their observations to the presence
of water-soluble blood groups and reported absent or greatly diminished BGA reactivity lost in the alcohol-fixation step of their preparations. Davidsohn (9) and Kuhns (26) further considered
that the loss of BGA was directly related to the degree of tumor
anaplasia. In contrast, Glynn and Holborow (15), Denk et al. (12),
and Cooper and Haesler (6) evaluated both water- and alcohol-
soluble BGA of tumor samples and observed the persistence of
BGA activity, especially that of BGA-A, but failed to demonstrate
a correlation between BGA expression and morphologic differ-
entiation.

Interestingly, the tumor cells of both patients with O-type RBC
segregated to Group 3, the category of high CEA-producing
colon carcinoma cells, while all A-type RBC patients clustered
into Group 2, the very low CEA-producing category. This associa-
tion may not be fortuitous and could indicate a dominant
connection between the synthetic pathways for both products.
In accord-
ance with this mechanism, all of our colorectal cancer cell lines
expressed both BGA-A and -B regardless of the patient’s RBC
phenotype; however, the cells obtained from O-type patients
expressed quantitatively lower BGA-A and -B than those derived
from patients who were either A, B, or AB.

All of the colorectal cancer cell lines clearly expressed T
antigens, regardless of grouping, with no cryptic transfiguration.
Thus, in contrast to tumors of the urinary bladder (5), T-antigen
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-C, and -DR antigens in 15 colon carcinoma cell lines utilizing several clones of the same lines that we used in our experiments. These investigators showed that the line LoVo and 12 of its subclones failed to react with the monoclonal antibodies for HLA-A, -B, -C, and -DR antigens and postulated that these cells might contain HLA antigens at undetectable levels. In our study, we used antisera of proven reactivity, which have been thoroughly standardized and greatly controlled, and demonstrated full expression of all TRA by all cell lines. Thus, it is possible that the monoclonal antibodies used in the experiments of McLean et al. did not have the potency or specificity to detect such antigens. It also appears that cells may lose, acquire, or otherwise modify their TRA profile in long-term culture. Thus, our TRA profile for line SW480 (and its related cell line SW620) coincides with that published by the American Type Culture Collection. However, the profile determined by us for both LoVo and SW48 cells differs substantially from that reported by that agency. Whether this discrepancy is artifactual (reagent related) or due to longitudinal changes in TRA expression remains unknown.

Although we noted a weak association of colorectal cancer cell group with particular HLA-C and -DR loci, no distinct and reliable relation can be proposed at the present time. Similarly, and in contrast to the findings of Rognum et al. (39), histological differentiation was not associated with a particular TRA staining pattern.

In summary, colorectal cancer cells have the ability to express both BGA and TRA, and this expression may be both concordant or illegitimate with respect to the patient’s phenotypic make-up. It is possible that inappropriate expression of such antigens may in turn induce the formation of antibodies directed against RBC antigens unrelated to the transfusion history of the host (25) and that these antibodies might be used as markers of tumor growth and spread.

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