Detection of an Altered Form of Cell-CAM 105 on Rat Transplantable and Primary Hepatocellular Carcinomas

Douglas C. Hixson2 and Kerry D. McEntire

Department of Medical Oncology, Rhode Island Hospital-Brown University, Providence, Rhode Island [D. C. H.]; and the University of Texas System Cancer Center, Science Park Research Division, Smithville, Texas [K. D. M.]

ABSTRACT

Monoclonal (MAb 362.50) and polyclonal (anti-gp105-2) antibodies have been used to examine the expression by transplantable (THC) and primary (PHC) hepatocellular carcinomas of a 105 kd rat hepatocyte cell adhesion molecule designated cell-CAM 105. Two-dimensional gel analysis of components immunoprecipitated with MAb 362.50 or anti-gp105-2 antibodies from detergent extracts of cell-CAM 105 from three different THC exhibited a more basic pi than its counterpart from normal rat hepatocytes. Immunoprecipitation of detergent extracts from radioiodinated hepatocytes with anti-THC antisera raised in rabbits by four immunizations with THC cells showed that six THC lines which were negative when stained by indirect immunofluorescence with MAb 362.50 expressed sufficient levels of cell-CAM 105 to induce precipitating antibodies. In contrast, antisera collected after eight immunizations with THC 253.1 and THC 252.2, showed no detectable reactivity with cell-CAM 105 suggesting that these THC lines had completely lost the expression of this molecule. Immunofluorescence analysis of normal rat tissues indicated that cell-CAM 105 was also present in the brush border of the small intestine and a subset of tubules in the kidney, raising the possibility that THC cells were expressing an isofrom normally found in nonhepatic tissues. However, cell-CAM 105 isolated from kidney showed a mobility on two-dimensional gels that was distinct from both the THC and hepatocyte forms of this molecule. Indirect immunofluorescence analysis of PHC induced by ethionine in a choline-deficient diet or by the Solt/Farber protocol showed that 52% and 65% of the persistent hepatic nodules induced by ethionine in a choline-deficient diet and by the Solt/Farber protocol, respectively, were reactive with MAb 362.50. Immunoprecipitation analysis of PHC induced by ethionine or diethylstilbestrol and choline-deficient diet showed that one of four PHC was expressing an altered form of cell-CAM 105 with the more basic pi characteristic of the THC form of this molecule. Taken together, these results suggest that quantitative and qualitative changes in the expression of cell-CAM 105 may constitute an important step in the acquisition of the malignant phenotype.

INTRODUCTION

The availability of specific antisera and monoclonal antibodies against cell adhesion molecules has made it possible to critically evaluate the role of cell adhesion in the interactions of normal epithelial cells and in the aberrant behavior characteristic of malignant cells. In a previous report (1), we presented data demonstrating that the two most acidic glycoproteins in the family of eight M, 105,000-110,000 wheat germ agglutinin-binding glycoproteins from normal hepatocytes were responsible for the adhesion-blocking activity of anti-cell-CAM2, and anti-gp105 antisera. These two components, which were shown by peptide mapping to be closely related in structure, were thus identical to cell-CAM 105, the M, 105,000 cell-adhesion molecule described by Ocklind and Obrink (2). We also demonstrated major changes in the expression of cell-CAM 105 on 13 different THC lines (1). This altered expression was evidenced by the lack of reactivity of THC cells stained by indirect immunofluorescence with polyclonal antisera possessing reactivity with cell-CAM 105 and by the absence of cell-CAM 105 in two-dimensional gel profiles of components immunoprecipitated with these polyclonal antisera from detergent extracts of radioiodinated THC cells. Although our previous results were consistent with the loss of cell-CAM 105 on the 13 THC lines tested, they did not rule out the possibility that cell-CAM 105 was still expressed at levels not detectable by the immunochromatic and immunocytochemical techniques employed or alternatively that cell-CAM 105 had undergone structural changes which altered its mobility on two-dimensional gels. In this report, we have used immunochromatic and immunocytochemical techniques in combination with monoclonal and polyclonal antisera to investigate these alternative possibilities. Our results confirm that a few THC lines no longer express cell-CAM 105 while others express low levels of a structurally altered form of this molecule. The presence of similar changes on PHC suggest that these alterations might be associated with the acquisition of the malignant phenotype.

MATERIALS AND METHODS

Isolation and Maintenance of Normal Hepatocytes and THC Lines. Normal hepatocytes were obtained by a modification (3) of the collagengene perfusion technique of Bonney et al. (4). THC 1682c, THC 1682a, and THC 1677 were derived from primary hepatocellular carcinomas induced in male ACI rats maintained on a choline-deficient diet containing 0.2% ethionine (5). Novikoff (6) and AS-30D (7) THC lines were derived from azo dye-induced hepatocellular carcinomas. THC 252, THC 311c, and THC 253 (8, 9) were obtained from Dr. Frederick Becker (The University of Texas-M.D. Anderson Hospital and Tumor Institute, Houston, Texas). THC 253.1 and THC 252.2 were culture lines derived from THC 253 and THC 252, respectively (1). Procedures for maintenance of THC lines in vivo by s.c., i.p., or intraperitoneal transplantation or in vitro in Waymouths 752/1 medium (GIBCO, Grand Island, NY) have been described previously (3).

Primary tumors were induced in male ACI rats by the following protocols: (a) feeding for 17 weeks of a choline-deficient diet containing 0.1% ethionine followed by a choline sufficient diet for 32 weeks; (b) i.p. injection with 200 mg/kg DENA followed by CS diet for 1 week and CD diet for 51 weeks; (c) the resistant hepatocyte protocol of Solt and Farber (10). For radiolabeling, primary tumors were excised, minced in PBS containing 100 KIU aprotinin/ml, gently dissociated by passage through a 3-ml disposable syringe, filtered through a 200-mesh stainless steel screen and washed two times in PBS containing 100 KIU aprotinin/ml and 10−6 M KI.

Isolation of Kidney Cells. The abdominal cavity was opened with a v- and the abdominal flap reflected onto the chest wall. Following ligation of the aorta and posterior vena cava above the origin of the
renal vein and artery, a 22-gauge needle connected to a peristaltic pump was inserted into the dorsal aorta inferior to the renal vein and artery and held in place with a ligature. The vena cava was then cut just above the origin of the ilioiliac vein and the kidney was perfused at 10 ml/min with 20-ml HBSS. Perfusion was continued with 40-$\mu$l HBSS containing 0.05% collagenase (Worthington type III, Freehold, NJ) and 10-$\mu$M CaCl$_2$. The kidneys were then removed, minced in HBSS, mechanically dissociated by repeated passage through the large end of a Pasteur pipet, and filtered through 200-$\mu$m nylon mesh. After 2 washes in PBS containing 10^{-4} M KI, 5 × 10^6 cells were radiodinated and subsequently extracted in NP-40 lysis buffer (10 mM Tris, 0.15-M NaCl, 0.5% NP-40, pH 7.8).

Hybridoma Construction. To construct hybridomas secreting antibodies specific for cell-CAM 105, mice were injected i.p. with 1 × 10^7 hepatocytes. After 10 days, mice were re-injected i.v. with 1 × 10^8 hepatocytes. Three days after the second injection, spleen cells were harvested and fused with 8653 myeloma cells as previously described by Allison et al. (11). Hybridomas showing growth in selective medium (12) were screened for reactivity with normal hepatocytes using the radiometric binding assay described below. Positive cultures were re-screened by indirect immunofluorescence on frozen sections of ACI rat liver and by SDS-PAGE analysis of components immunoprecipitated from 50-ml of radiodinated hepatocyte extract with antibodies from 50-$\mu$l culture fluid adsorbed to protein A bearing Staphylococcus aureus (10% suspension, Enzyme Center Inc., Boston, MA) coated with rabbit anti-mouse IgG antibodies. Hybridomas secreting antibodies immunoprecipitating components whose mobility on SDS-PAGE and distribution on frozen sections were identical to cell-CAM 105 were cloned twice by limiting dilution and recloned in soft agar.

Polyclonal Antiserum against Cell-CAM 105. Rabbit antisera specific for cell-CAM 105 was prepared by taking advantage of our previous finding that cell-CAM 105 is the most immunogenic of the components bound to wheat germ agglutinin. The wheat germ agglutinin binding fraction isolated from normal hepatocyte lysate as previously described (3) was immunoprecipitated with monoclonal antibodies against components 7.8, and 9.3 and incorporated into liposomes (3). Rabbits were immunized twice at 10-day intervals by i.m. injection with liposomes containing components from the equivalent of 2 × 10^6 hepatocytes. Serum collected after the first and second immunizations was designated anti-gp105-2.

Rabbit Antiserum against Normal Hepocytes and THC Cells. Rabbit antisera against normal hepatocytes and THC cells were raised by i.p. injection at 10-day intervals with 5 × 10^6 cells suspended in 1 ml of PBS. Serum used in the present studies was collected 10 days after the fourth injection.

Purification of IgG and Fab Fragments. The IgG fraction from anti-gp105-2 antisemur and normal rabbit serum was isolated by precipitation in sodium sulfate as described by Weir (13). Fab fragments were produced by digestion with papain according to the procedure of Porter (14). Digested IgG (50 mg in 0.01-M borate buffer, pH 8.0, Worthington, Freehold, NJ) was fractionated by size-exclusion chromatography on a 2- × 55-cm column of Biogel A-0.5 M (BioRad, Richmond, CA) equilibrated in borate buffer. Fractions (1 ml) collected at a flow rate of 25 ml/h were assayed for absorbance at 280 nm. Fractions demonstrated by SDS-PAGE to contain Fab free from contamination by undigested IgG were pooled, dialyzed extensively against 0.1-M ammonium bicarbonate, lyophilized, and stored at -20°C.

Radiometric Binding Assay. Normal ACI rat hepatocytes isolated by collagenase perfusion were suspended in aggregation buffer which consisted of Waymouth MB 721/1 medium (GIBCO, Grand Island, NY) supplemented with 0.5-µg insulin/ml (Elanco, Indianapolis, IN), 2 µg bovine serum albumin/ml (Sigma, St. Louis, MO), 50 µg Garamycin/ml (Shering Corp., Kenilworth, NJ), 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma), and 20 µg DNAse/ml (Sigma), filtered sequentially through cheese cloth (six layers), 200-$\mu$m nylon mesh, and 60-$\mu$m nylon mesh. Cells were resuspended in glass scintillation vials to a concentration of 2 × 10^6/ml in aggregation buffer without Fab or in aggregation buffer containing Fab fragments purified from anti-gp105-2 antisemur or normal rabbit serum. After a 20-min incubation at 4°C on a gyroratory shaker rotating at 120 rpm, 0.5-ml aliquots of cell suspension were placed into wells of a 24-well tissue culture plate and rotated at 37°C at 70 rpm for 120 min. At 20-min intervals, cell suspensions from each well were diluted to 10 ml in PBS and counted using a 200-µm aperture in a Coulter Model ZBI electronic particle counter at 1/amplification and 1/current settings of 8 and 1/4, respectively, and lower and upper threshold settings of 20 and 60. The degree of aggregation was expressed as the percentage decrease in single cells. The percentage inhibition of aggregation by Fab was calculated as follows:

\[ \text{Single cells with immune Fab at 120 min} \times 100 \]
\[ \text{Single cells with nonimmune Fab at 120 min} \]

Immunochemical and Immunocytochemical Protocols. Methods for immunoprecipitation analysis of detergent extracts prepared from cells surface-labeled with 125I (500 mCi/ml; Amersham, Arlington Heights, IL) by the method of Keski-Oja et al. (15) have been described (3, 4). The procedure for comparison of the reactivity of two different antisera by immunodepletion analysis has also been described (3, 13). One-dimensional SDS-PAGE gels were run as described by Laemmli (16). Two-dimensional gel analysis was performed as described by O’Farrell (17). Staining, destaining, drying, and autoradiography of gels were carried out as described previously (1, 3, 18). Apparent molecular weights were calculated from protein standards (β-galactosidase, M, 116,000; phosphorylase B, M, 97,000; bovine serum albumin, M, 68,000; ovalbumin, M, 43,000) run concurrently with radioactively labeled samples. Isolation of wheat germ agglutinin binding glycoproteins on Sepharose bound wheat germ agglutinin was accomplished by procedures described previously (3). Wheat germ agglutinin (Vector Laboratories Inc., Burlingame, CA) was coupled to Sepharose 4B (Pharmacia, Piscataway, NJ) using the cyanogen bromide coupling method of Cuatrecasas (12). Iodination with chloramine T was performed as previously described by Allison et al. (11). Methods for staining frozen sections by indirect immunofluorescence have been described previously (3). Fluorescein-conjugated, affinity purified, rabbit anti-mouse IgG was obtained from Sigma. Antigens separated by SDS-PAGE and transferred to nitrocellulose were identified by the indirect immunoperoxidase protocol described by Johnson et al. (19).

FACS. Hepatocytes (2 × 10^6) or THC cells (5 × 10^5) were stained with MAb 362.50 or MAb 324.5 (21) and fluorescein-conjugated goat anti-mouse IgG (Sigma) diluted 1:2000 in PBS and plates were incubated for 1 h. Following three washes in PBS, wells were filled with 200 µl of PBS containing 4 mg/ml bovine serum albumin. Plates were centrifuged at 150 × g for 2 min, and the liquid removed by flicking the inverted plates with a single rapid vertical motion. Wells were then filled with 200 µl of PBS containing 1 mg/ml bovine serum albumin. Plates were centrifuged, emptied, and filled with PBS. After an additional wash in PBS using this three-step procedure, wells were filled with 50 µl of rabbit anti-mouse IgG antiserum (Sigma) diluted 1:2000 in PBS and plates were incubated for 1 h. Following three washes in PBS, wells were filled with 50 µl of protein A (Sigma) (10° cpm/50 µl, 25 µg/ml) which had been previously labeled with 125I using chloramine T (20). After a 1-h incubation at 25°C, plates were washed three times in PBS, dried, and placed on X-ray film with an intensifying screen for 12–24 h.

FACS. Hepatocytes (2 × 10^6) or THC cells (5 × 10^5) were stained with MAB 362.50 or MAB 324.5 (21) and fluorescein-conjugated goat anti-mouse IgG and fixed in 2% paraformaldehyde essentially as described by Allison and McIntyre (11). Cells were analyzed using a BD FACS 440 fluorescence-activated cell sorter fitted with a 70-µm orifice and a standard fluorescein filter pack. Analysis was performed using a 488-nm argon ion laser operating at a power setting of 200 mW. Appropriate gates were set by forward scatter analysis to exclude debris from the fluorescence analysis. Ten thousand cells from each sample were accumulated for each analysis.
RESULTS

Production of Polyclonal and Monoclonal Antibodies Specific for Cell-CAM 105. Anti-gp105-2, a rabbit antiserum specific for cell-CAM 105 was prepared by taking advantage of the fact that cell-CAM 105 was one of the most immunogenic of the components bound by wheat germ agglutinin. This antiserum was very specific and showed no reactivity with other labeled components in extracts of radioiodinated hepatocytes (Fig. 1, A and B) or with chloramine T labeled wheat germ agglutinin binding glycoproteins (data not shown). As shown in Fig. 2, Fab fragments prepared from anti-gp105-2 antiserum strongly inhibited the aggregation of hepatocytes in suspension, producing at a concentration of 50 µg/3 x 10⁵ cells, a 77% decrease in aggregation relative to that observed in the presence of Fab fragments produced from preimmune serum.

A similar immunization protocol was used in the production of 362.50, a hybridoma secreting MAb which in immunoprecipitation assays were specific for cell-CAM 105 (Fig. 1C).

Cell-CAM 105 was also the only component detected when Western blots of 100 µg of wheat germ agglutinin-binding glycoproteins were stained with MAb 362.50 and peroxidase conjugated goat anti-mouse IgG (data not shown). The identity of the antigen recognized by MAb 362.50 and anti-gp105-2 antiserum was confirmed by demonstrating the ability of MAb 362.50 to deplete extracts of the antigen reactive with anti-gp105-2 and by the identical pattern of V-8 peptides produced by digestion of antigens reactive with the two antibodies (data not shown).

Two-Dimensional Gel Analysis of Cell-CAM 105 from THC Cells. In our previous studies (1), we had been unable to isolate reactive antigens from extracts of radioiodinated THC 1682A (maintained either in vivo or in vitro) and THC 252.2 cells by immunoprecipitation with anti-cell-CAM₈ antiserum. In the present investigation, similar results were obtained with both anti-gp105-2 antiserum and MAb 362.50. We were also unable to isolate detectable amounts of reactive antigen from THC 253.1, THC 253, and THC 311c. In contrast reactive antigen was readily isolated from THC 1677, THC 1682c, THC AS-30D, and Novikoff THC. In Fig. 3 it can be seen that cell-CAM 105 from each of the positive THC lines showed a more basic pi (4.4-4.8) than cell-CAM 105 from normal hepatocytes (pi 4.1-4.3). The upper and lower forms were also less well resolved on these THC lines and in the case of THC 1682c and THC AS-30D, showed increased microheterogeneity.

Immunoprecipitation Analysis with Anti-THC Antisera. A sensitive xenoinmunization assay was used to determine if cell-CAM 105 was completely absent from the cell surface of THC.
cells or was present at concentrations insufficient for detection by either direct immunoprecipitation of THC extracts or by immunofluorescence. This assay involved the assessment of the ability of each THC line in question to induce the production of antibodies in rabbits capable of immunoprecipitating cell-CAM 105 from extracts of radioiodinated hepatocytes. Fig. 4 shows the 2-D PAGE profiles of components immunoprecipitated with 5 μl of antiserum from 50 μl of radioiodinated hepatocyte extract with a specific activity of approximately 3000 cpm/μl. As shown in Fig. 4, A–C, immunization with THC 253.1 and THC 252.2 did not produce any detectable reactivity with cell-CAM 105. Rabbits immunized four times with THC 253 and THC 311C cells also showed negative reactivity but did display weak anti cell-CAM 105 activity after an additional four immunizations (Fig. 4, E and F). In agreement with our previous results using mouse antiserum (1), immunization with THC 1682A cells maintained in vitro did not produce detectable titers of anti-cell-CAM 105 antibodies (data not shown). In contrast, immunization with THC 1682A cells maintained by s.c. transplantation produced significant reactivity against cell-CAM 105 (Fig. 4D). The highest levels of anti-cell-CAM 105 activity were induced by immunization with THC 1677, THC AS-30D, THC 1682c, and Novikoff THC (Fig. 4, G–J), the four THC lines which were also positive in immunoprecipitation assays with MAb 362.50 and anti-gp105-2 antiserum.

Indirect Immunofluorescence Analysis of Normal Tissues and THC with MAb 362.50. Immunofluorescence analysis of acetone-fixed frozen sections of normal rat tissues indicated that antigens reactive with MAb 362.50 were present in kidney tubules and small intestine brush border. No reactivity was detected with sections from muscle, brain, skin, heart, lungs, stomach, spleen, trachea, large intestine, bladder, tongue muscosa, adrenal gland, and cornea. When frozen sections of liver-containing nodules of THC 252, THC 253, THC 1682A, THC 1682B, THC 1683, and THC 1682c (Fig. 5D) were stained by indirect immunofluorescence with MAb 362.50 and examined by fluorescence microscopy, none of the THC nodules showed...
reactivity significantly above background fluorescence observed in control section stained with P3x63Ag8 myeloma culture supernatant. Although THC 1683 and THC 252 were negative when analyzed by FACS (data not shown), a low level of reactivity with MAb 362.50 significantly above background was detected on THC 1682c cells (Fig. 6).

Expression of Cell-CAM 105 by PHC. Indirect immunofluorescence analysis of persistent hepatic nodules present 8 months after removal from CDE diet showed that three of 25 nodules had a reduced reactivity and 13 of 25 were unreactive with MAb 362.50. A high percentage of persistent nodules present 8–12 months after initiation of the Solt/Farber protocol (Fig. 5C) also displayed an altered expression of cell-CAM 105 with two of 17 showing a significant reduction and 11 of 17 showing no observable reactivity with MAb 362.50.

Immunoprecipitation of MAb 362.50 Reactive Antigens from Kidney Tubules and Persistent Nodules. Fig. 7 shows the 2-D PAGE analysis of cell-CAM 105 isolated by immunoprecipitation from extracts of collagenase dissociated, radioiodinated PHC induced by DENA and CD diet (Fig. 7B) or by CDE diet (Fig. 7, C and D). Histologically, the PHC used for Fig. 7, B–D, were moderately well differentiated. Fig. 7E shows cell-CAM 105 from THC cells isolated from the second s.c. passage of a PHC induced by the Solt/Farber protocol. This THC was extremely slow growing and required up to 6 weeks to produce a visible nodule. This is in contrast to THC 1682c and THC 1683 which formed 2–3-cm² tumors in 7–10 days. Cell-CAM 105 from the CDE-induced tumors (Fig. 7, C and D) and the slow growing THC (Fig. 7E) showed a mobility identical to that of cell-CAM 105 from normal hepatocytes. However, cell-CAM 105 from the DENA/CD induced PHC (Fig. 7B) displayed the more basic pI characteristic of THC forms of this molecule.

Immunoprecipitation analysis of extracts prepared from radioiodinated kidney cells isolated by collagenase perfusion indicated that there were also two forms of cell-CAM 105 in kidney (Fig. 7A), both of which showed a more basic pI than their counterpart from normal hepatocytes.

DISCUSSION

In a previous investigation (3), we demonstrated the apparent loss by eight different THC lines of two acidic components recognized by antiserum (anti-gp105) raised against the wheat germ agglutinin-binding glycoproteins from normal rat hepatocytes. These two acidic components were subsequently shown to be identical to cell-CAM 105, a cell surface glycoprotein involved in the intercellular adhesion of rat hepatocytes (1). In the present study, we have investigated further the expression of cell-CAM 105 by THC using both monoclonal (MAb 362.50) and polyclonal antibodies (anti-gp105-2) demonstrated to be specific for cell-CAM 105 by immunoprecipitation and by their ability to inhibit hepatocyte reaggregation. Results from immunofluorescence analysis indicate that all of the THC lines examined were either negative or were expressing cell-CAM 105 at levels not readily detected by immunofluorescence microscopy. This latter possibility was subsequently verified by FACS analysis showing a low level of expression by THC 1682c. Results from immunochemical analysis with MAB 362.50 demonstrated further that several apparently negative THC lines were in actuality expressing low levels of a structurally altered form of cell-CAM 105 that was not apparent in
two-dimensional gel profiles of anti-gp105 immunoprecipitates (1) because of a close similarity in size and pi to what was designated in our previous study as component 3 (3).

Using a sensitive assay based on the ability of anti-THC antiserum to immunoprecipitate cell-CAM 105 from extracts of radioiodinated hepatocytes, we were able to confirm the complete loss of this molecule on some THC lines and the expression by others at levels not readily detectable by immunoprecipitation or immunofluorescence assays. The decrease or loss of cell-CAM 105 did not show any consistent relationship to the length of transplantation since Novikoff THC and THC AS-30D, two THC with the longest history of transplantation, had the highest levels and THC 1682A, a THC maintained for less than 50 generations, had one of the lowest levels of expression. It was of interest to note that four of five THC showing the lowest level of expression or complete loss of cell-CAM 105 were established from PHC induced by 2-acetylaminofluroene. Whether or not PHC induced by 2-acetylaminofluroene display a similar decrease remains to be determined.

Because most of the THC lines examined had been maintained for extended periods by serial transplantation, there was concern that the quantitative and qualitative changes in cell-CAM 105 were not primary events in the development of hepatocellular carcinomas but instead, were secondary changes resulting from selection or adaptation to growth as s.c. tumors. However, immunofluorescence analysis of PHC induced by the Solt/Farber and CDE protocols indicated that a high percentage of primary hepatocellular carcinomas also displayed a marked decrease in the expression of cell-CAM 105. Further, it was shown by immunocytological analysis that one of four PHC was expressing low levels of the more basic THC-associated form of this molecule. The fact that not all primary tumors displayed this alteration suggested that there might be a relationship between the expression of the THC form of cell-CAM 105 and the biological characteristics of persistent nodules and PHC. Experiments are currently in progress to address this possibility by correlating cell-CAM 105 expression with various biological parameters such as transplantability, metastatic potential, adhesion/aggregation to hepatocytes in vitro, and bile canalicular formation both in situ and in vitro.

In the present manuscript, consideration was given to the possibility that THC cells were inappropriately expressing an isoform normally found on one of the other cell-CAM 105 positive tissues. Cell-CAM 105 isolated from kidney, however, was quite different from both THC and hepatocyte forms of this molecule. It also seems unlikely that the more basic polypeptide of cell-CAM 105 on THC cells represents an alteration associated with proliferation or reversion to a fetal form since the mobility by 2-D PAGE of radioiodinated cell-CAM 105 isolated from 24-h regenerating and 20-day fetal hepatocytes was identical to cell-CAM 105 from adult hepatocytes.

A number of other laboratories have recently reported on the characterization of liver cell glycoproteins showing a close similarity in structure to cell-CAM 105. These include gp110, a M, 110,000 bile canalicular glycoprotein which has been extensively characterized by Petelle et al. (22) and Diamond et al. (23); HA-4, a MAb-defined bile canalicular antigen described by Hubbard et al. (24) and Bartles et al. (25); a MAb-defined transformation-sensitive glycoprotein identified by Becker et al. (26); and a M, 110,000 bile transport protein isolated by Landmann et al. (27). All of these glycoproteins show similarities in size before and after enzymatic or chemical deglycosylation. Antibodies raised against each of these molecules react primarily with the bile canalicular membrane domain of hepatocytes and display a similar reactivity with other epithelial tissues. We have recently confirmed using polyclonal antibodies and MAb prepared by Petelle et al. (22) and Becker et al. (26), respectively, that both gp110 and the transformation-sensitive antigen described by Becker et al. (26) are identical structurally and antigenically to cell-CAM 105. In addition, Feracci et al. (28) have recently reported that antibodies to HA-4 can block the reaggregation of rat hepatocytes, an observation which together with the close structural similarities establishes a close identity between HA-4 and cell-CAM 105.

Gaining insight into the relationship between the structure and function of cell-CAM 105 will be an essential step in understanding the possible consequences of or causes for the dramatic decrease or loss of this glycoprotein on a high percentage of primary and transplantable hepatocellular carcinomas. To this aim, we have focused in the accompanying report on identifying the structural features which distinguish THC and hepatocyte cell-CAM 105.

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