Production of Monoclonal Antibodies to Preneoplastic Liver Cell Populations Induced by Chemical Carcinogens in Rats and to Transplantable Morris Hepatomas

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ABSTRACT

Monoclonal antibodies (moabs) to neoplastic and preneoplastic liver cells in rats have been selected to follow cellular changes in the livers during chemical carcinogenesis. The moabs were induced by immunizations of BALB/c mice with four partially purified liver cell preparations: 1) oval cells induced in male Fischer rats fed 0.05% N-2-acetylaminofluorene in a choline deficient diet; 2) preneoplastic γ-glutamyltranspeptidase positive hepatocytes induced by i.p. injection of diethylaminoethylamine into male Fischer rats followed by 0.02% N-2-acetylaminofluorene and partial hepatectomy (Solt-Farber model); 3) sharply dissected neoplastic nodules induced in male Fischer rats by five 2-week cycles of 0.05% N-2-acetylaminofluorene diet; and 4) Morris hepatomas 7777 and 5123 passaged in male Buffalo rats. The hybridomas were screened by enzyme linked immunosorbent assay or by indirect immunofluorescence on composite cryostat sections of fetal and adult rat liver, liver containing neoplastic nodules, and Morris hepatoma 7777. Positive clones were limited diluted and partially characterized by indirect immunofluorescence on cryostat sections of other preneoplastic and neoplastic rat livers as well as normal rat tissues. Two moabs to oval cells, two moabs to hepatocytes, and one moab to hepatomas have been selected for further study.

INTRODUCTION

The study of the lineage of chemically induced hepatocellular carcinoma in the rat has been hampered by the absence of definitive markers for the different cell populations seen in the liver prior to the emergence of hepatomas (1–3). Conventional histochemical markers such as GGT are not specific. They stain several cell types in the liver during carcinogenesis including bile ducts, oval cells, altered hepatocytes in foci, nodular hepatocytes, and some hepatomas (4–7). The immunohistological localization of oncofetal protein products during carcinogenesis has also not been definitive. Although in some models of hepatocarcinogenesis α-fetoprotein production may be associated with developing hepatomas (8), it is also produced by proliferating hepatocytes following partial hepatectomy or cell injury (9) and proliferating oval cells early in the administration of carcinogens (10, 11).

The introduction of monoclonal antibody technology by Kohler and Milstein (12) has allowed investigators to produce monoclonal antibodies which identify specific antigens on the cell surface or within the cytoplasm of cells in a variety of organ systems. Most of the reported monoclonal antibodies have been made to highly purified antigens or to tumor cell lines. Many of the reported monoclonal antibodies have served as useful markers of tissue type and cell lineage in tumor studies of the breast (13–15), kidney (16), lung (17), colon (18), prostate (19), pancreas (20), and skin (21, 22). The high degree of specificity of the monoclonal reagents allows them to be used to define the appearance of epitopes during normal development and carcinogenesis. Several reports have appeared with moabs to liver cells and oval cells in rats. Holmes et al. (23) reported 3 moabs to hepatocytes which also stained azo dye induced tumors. Hixon and Allison (24) have produced moabs to hepatocytes and oval cells. Germain et al. (25) have also produced moabs to oval cells. Schmidt et al. (26) have produced a moab to the Novikoff hepatoma cell line which stains oval cells and azo dye induced carcinomas. Hubbard et al. (27) have produced moabs to a series of antigens which identify three specific domains of the plasma membrane in hepatocytes. Becker et al. (28) have produced monoclonal antibodies to hepatocyte plasma membrane antigens, all but one of which are lost during transformation to hepatomas. In each of these reports, the investigators used tumor cell lines, normal hepatocytes, isolated oval cells, or purified antigens to prepare the moabs.

This paper describes the production of 5 moabs to rat hepatocytes, oval cells, and hepatomas and the initial characterization of the immunohistologic localization of the epitopes recognized by these moabs on cryostat sections of fetal, normal, and carcinogen treated liver; transplantable hepatomas; and selected organs from normal rats. Our strategy was to use cell preparations isolated from the livers of carcinogen treated rats to immunize mice. Whole cell preparations from hepatomas, nodules, and enzyme digested livers containing oval cells and GGT positive hepatocytes were used. The hybridomas were screened by Elisa assay or directly on tissue sections by immunofluorescence. Screening by immunofluorescence allowed preliminary characterization and judgment of the potential usefulness of the hybridomas as part of the screening process. Five moabs which stain oval cells, hepatocytes, and hepatomas are described and compared to moabs which stain hepatocytes and oval cells reported by others.

MATERIALS AND METHODS

Animal Models

Oval Cells. Male Fischer rats (Walkersville, MD) 125 g, were fed a diet containing 0.05% AAF in a choline deficient diet (Dyets, Inc., Bethlehem, PA) for 12 days followed by normal Purina laboratory chow for 9 days. They were euthanized at 21 days, at the time of peak oval cell proliferation (11). Oval cells were also produced by feeding male Fischer rats 0.1% DL-ethionine (Aldrich Chemical Co., Milwaukee, WI) in a choline deficient diet for 28 days.

GGT Positive Hepatocytes. Male Fischer rats (200 g) were initiated with a single i.p. dose of 200 mg/kg diethylnitrosamine (Alfa, Danvers, MA), followed after 7 days by 14 days of feeding of 0.02% AAF in the middle of which they received a partial hepatectomy. They were euthanized 8 days following the termination of the 0.02% AAF diet, a time corresponding with the peak in GGT positive foci (29, 30).
Neoplastic Nodules. Male Fischer rats (150 g) were fed 0.05% AAF diet, in five 2-week cycles with 1-week rest periods on Purina laboratory chow (8). The animals were euthanized 2 weeks after the fifth cycle. Neoplastic nodules were also produced by the continuous administration of 100 ppm diethylnitrosamine in the drinking water to 150-g male Fischer rats, to 150-g ACI rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) for 12 to 16 weeks (31), or from the Solt-Farber regimen (29, 30).

Transplantable Hepatomas. Morris Hepatomas 7777, 5123, and 9098 are stored in our department (32). Sections used for this study were taken from tumors passed i.m. in ACI (9098) or Buffalo rats (7777, 5123) (Harlan Sprague Dawley).

Fetal Liver. The vaginal smears of female Fischer rats were followed daily until estrus, when the female rats were placed in a cage with a male rat. The first day with sperm in the vaginal smear was counted as day 0. The timing of the fetal development was also confirmed by measuring fetal size (33). Sections of fetal liver were examined at days 14, 16, 18, and 20 of gestation; the day of birth; neonatal days 1 and 7; and week 7.

Isolation of Cells for Immunizations

Isolation of Oval Cells. A modification of the enzyme perfusion methods of Berry and Friend (34), Sells et al. (35), and Yaswen et al. (36) was used. Livers of lightly ether anesthetized rats from the oval cell regimen were perfused at 37°C with calcium free Hank's solution through the portal vein using a Master Flex (Cole Parmer Instrument Co., Chicago, IL) at a flow rate of 50 ml/min for 5 min, followed by 0.1% collagenase, 0.1% Pronase, and 0.05% DNase (all from Sigma) in Hank's solution with 1 mM CaCl2 added at a flow rate of 7.5 ml/min for 10 min. The liver was removed, minced, and further digested with enzyme buffer with agitation. Aliquots were collected at 7- to 10-min intervals, washed, pooled, and allowed to sediment at 1 X g for 1 h on ice. The supernatant contained an enriched fraction of nonparenchymal cells. A cell count was made in a hematocytometer chamber, and viability was tested with trypan blue.

Isolation of Normal Hepatocytes and GGT Positive Hepatocytes. The livers of ether anesthetized normal Fischer rats or rats from the Solt-Farber regimen (29, 30) were perfused by a procedure similar to that for the oval cells, except the enzyme buffer contained only collagenase.

Isolation of Neoplastic Nodules. Livers were removed from ether anesthetized rats from the carcinogenic regimens used to produce nodules. The large neoplastic nodules were sharp dissected from the livers. The nodules were pooled in a Petri dish with calcium free Hank's solution on ice and finely minced to a suspension injectable through a 23-gauge needle.

Storage of Isolated Cells, Minced Nodules, and Tumors. Cells and minced tissue were placed in Nunc vials (Nunc Inter Med. Roskilde, Denmark) containing tissue culture medium with 10% fetal bovine serum and 7% dimethyl sulfoxide, slowly frozen at -70°C, and stored in liquid nitrogen.

Collection of Tissue for Histology and Immunofluorescence

All tissues were taken from rats under ether anesthesia, sliced into thin slices on dental wax, and placed in 20-ml disposable beakers (Scientific Products) containing embedding medium (OCT, Miles Scientific, Naperville, IL) and snap frozen in isopentane chilled with dry ice. This produces a round disc of OCT covered tissue which was covered with aluminum foil and placed in a labeled Kodak plastic film containers for storage at -70°C.

Production of Monoclonal Antibodies

Immunizations of mice were performed with fresh cells or tissue or with vials of frozen cells or tissue that were thawed and washed three times with cold PBS. Five X 10^6 cells in 0.5 ml PBS were used for each immunization. Minced suspensions of tumor or nodules were diluted until they would pass through a 23-gauge needle; 0.25 ml of such a suspension was used for each immunization. Female BALB/c mice, 6-8 weeks old (Timco, Houston, TX), were immunized with intact cells by i.p. injection at monthly intervals from 2 to 8 months prior to the fusion and then boosted 4 days prior to the fusion. Spleen cells from immunized mice were fused with the mouse myeloma line SP/2 (M5) (37). The hybridoma supernatants were then tested for activity, as described below. Those producing antibodies of the desired specificities were then cloned by limit dilution.

Assay of the Monoclonal Antibodies

ELISA Assay. Microtiter plates with 96 wells were prepared using intact cells as antigen. One X 10^3 oval cells, normal hepatocytes, hepaticinized rat RBC, or thymocytes (prepared by injecting cold PBS into the thymus of a normal rat) were used. The assay was performed as published (38) using the BRL hybridoma screening kit (Bethesda Research Labs, Inc., Gaithersburg, MD).

Indirect Immunofluorescence. Composite blocks were made by combining small portions of snap frozen rat fetal liver, normal adult liver, carcinogen treated liver (nodular), and Morris hepatoma 7777 in one small disposable 20-ml beaker containing OCT embedding medium and snap freezing in dry ice chilled isopentane. Cryostat sections were cut at 4 µm in an IEC cryostat, picked up on prelabeled microscope slides, and air dried for 2 h at room temperature. The slides were fixed in acetone for 5 min at room temperature and air dried. A circle was traced around the tissues using a diamond point pencil. The slides were stored in slide boxes at -20°C.

Screening of Hybridomas by Immunofluorescence. One composite slide was thawed for each supernate to be screened. The slides were rehydrated in PBS containing 0.05% Tween 20 for 10 min. Each slide was incubated with 80 µl of tissue culture supernatant at room temperature for 1 h and washed for 5 min each in 3 changes of PBS-Tween followed by incubation with 80 µl of a 1:80 dilution of fluorescein isothiocyanate conjugated goat anti-mouse IgG (Cappel, Cochranville, PA) for 1 h at room temperature. The slides were washed as before and mounted with buffered glycerol, pH 8, containing 0.1% p-phenylene-diamine to inhibit quenching (39). The slides were then examined on a Nikon fluorescent microscope. The staining patterns on the four tissues were recorded and constituted the initial characterization of each monoclonal antibody.

Characterization of the Monoclonal Antibodies

Indirect immunofluorescence was performed with undiluted tissue culture supernatant from each moab on cryostat sections of fetal, adult, and carcinogen treated rat liver; transplantable Morris hepatomas; and a series of normal rat tissues. The methods used were similar to those used for the initial screening except for the use of species specific affinity purified biotinylated sheep anti-mouse IgG as second antibody (1:100 dilution, incubated for 30 min) followed by fluorescein isothiocyanate conjugated streptavidin (1:150 dilution, incubated for 15 min) from Amersham, Arlington Heights, IL. For the moab H-4, Tween 20 was eliminated from the wash buffer because it inhibits staining.

Immunoglobulin subtyping was performed using ELISA plates containing target cells as for the screening assay or plates coated with rabbit anti-mouse immunoglobulin. Following 1-h incubations at room temperature with the moab supernatants, the additional conjugated antibodies and substrates were added as indicated following the instructions provided in the Boehringer Mannheim mouse immunoglobulin subtype identification kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) and the HyClone Lab mouse monoclonal subisotyping kit (Hyclone Labs, Logan, UT).

Histochemistry

Histochemical staining for GGT was performed on selected acetone fixed cryostat sections using a modification of the procedure of Rutenberg et al. (40) as published previously (30).

RESULTS

Table 1 summarizes the fusions performed and the panel of moabs which will be described in this report. Each fusion
MONOCLONAL ANTIBODIES TO PRENEOPLASTIC LIVER CELLS

Table 1 Summary of preparation of monoclonal antibodies

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Designation</th>
<th>Cells used for immunization</th>
<th>Tissue specificity</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>193 (2) 2</td>
<td>OV-1</td>
<td>Oval cells</td>
<td>Bile ducts, oval cells</td>
<td>IgG3</td>
</tr>
<tr>
<td>286 (2) 11</td>
<td>OV-6</td>
<td>Nodular hepatocytes</td>
<td>Bile ducts, oval cells, some nodular hepatocytes, hepatomas</td>
<td>IgG1</td>
</tr>
<tr>
<td>218 (2) 5</td>
<td>H-4</td>
<td>GGT positive hepatocytes</td>
<td>Cytoplasm of hepatocytes</td>
<td>IgM</td>
</tr>
<tr>
<td>286 (2) 1</td>
<td>H-6</td>
<td>Nodular hepatocytes</td>
<td>Plasma membrane hepatocytes</td>
<td>IgM</td>
</tr>
<tr>
<td>242 (10) T-6</td>
<td>Morris hepatoma 7777</td>
<td>Some nodular hepatocytes, hepatomas</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Summary of immunofluorescent staining of the moabs on cryostat sections of fetal, adult, and carcinogen treated liver and Morris hepatoma 7777

Indirect immunofluorescence was performed on cryostat sections of liver and tumor. The fetal liver was from day 18.

<table>
<thead>
<tr>
<th>Moab</th>
<th>Fusion</th>
<th>Cells used</th>
<th>Tissue specificity</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>OV-1</td>
<td>BD*</td>
<td>HEP</td>
<td>Oval cells</td>
<td>T-6 positive hepatocytes</td>
</tr>
<tr>
<td>OV-5</td>
<td>BD</td>
<td>HEP</td>
<td>Oval cells</td>
<td></td>
</tr>
<tr>
<td>H-4</td>
<td>BD</td>
<td>HEP</td>
<td>Oval cells</td>
<td></td>
</tr>
<tr>
<td>H-6</td>
<td>BD</td>
<td>HEP</td>
<td>Oval cells</td>
<td></td>
</tr>
<tr>
<td>T-6</td>
<td>BD</td>
<td>HEP</td>
<td>Oval cells</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Immunofluorescent staining of normal rat tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>OV-1</th>
<th>H-6</th>
<th>OV-6</th>
<th>T-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain, nerve</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pancreas</td>
<td>A, D</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stomach</td>
<td>G, S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Small bowel</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Large bowel</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bladder</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Testicle</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ovary</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endometrium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muscle</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Skin</td>
<td>A</td>
<td>B</td>
<td>E</td>
<td>K</td>
</tr>
</tbody>
</table>

Table 4 Staining of moabs on developing liver

Based on serial sections of fetal liver days 14, 16, 18, 20; birth; 1 and 7 weeks. At 14 days the liver was negatively stained except for small groups of OV-1 positive cells which were smaller than hepatocytes (on hematoxylin and eosin staining). By day 16 the hepatocytes were positively stained by H-4 but were not stained by OV-1 or OV-6. By day 18 OV-6 positive mature bile ducts appeared from within the groups of OV-1 positive cells. The hepatocytes were strongly H-4 and H-6 positive from day 18 on. T-6 stained small numbers of perportal hepatocytes only on the 20th day.

<table>
<thead>
<tr>
<th>Moab cells stained</th>
<th>Epithelial</th>
<th>Day of development*</th>
<th>Birth weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>OV-1</td>
<td>Bile ducts</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>OV-6</td>
<td>Bile ducts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-4</td>
<td>Hepatocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-6</td>
<td>Hepatocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-6</td>
<td>Periportal hepatocytes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Stage of fetal development was based on gestational age judged by observation of the cytology of vaginal smears for sperm and of fetal size (33).
MONOCLONAL ANTIBODIES TO PRENEOPLASTIC LIVER CELLS

Fig. 1. Immunofluorescent staining of normal liver by OV-1 and comparison of the immunofluorescent staining by OV-1 and OV-6 of oval cells induced by AAF and CD-ethionine. In A, OV-1 stains bile ducts in normal liver. × 400. B and C, serial sections of liver containing oval cells produced by AAF and partial hepatectomy in the Solt-Farber regimen 1 week after the cessation of AAF feeding at a time of peak oval cell proliferation. OV-1 stains more oval cells (D) than OV-6 (C). Both × 400. D and E, sections of liver containing oval cells produced by feeding ethionine in a choline deficient diet for 3 weeks. This is past the peak of oval cell proliferation. Although these are sections of the same portal area from a set of serial sections, they are about 20 µm apart (levels 1 and 6 of the set cut at 4 µm). OV-1 stains fewer oval cells (D) than OV-6 (E). OV-1 does not stain the large bile duct (BD) structure seen in E. This may be due to the absence of the bile duct in the section seen in D but is more likely due to the failure of OV-1 to stain the duct structure. PV, portal vein. Both × 200.

liver, it stains relatively few normal bile ducts in adult liver. This may account for the failure of OV-1 to stain the bile duct in Fig. 1D which was stained by OV-6 in Fig. 1E. However, since these sections were the first and sixth levels of serial sections, it is also possible that the duct structure seen in Fig. 1E is not present in Fig. 1D.

H-4 stains the fetal hepatocytes after day 16 and adult hepatocytes (Fig. 3) and does not stain hepatomas. It has a salt and pepper grainy staining. The staining is inhibited by the presence of Tween 20 in the wash buffer, which led to our earlier conclusion of negative staining of other tissues (41). It stains the fetal mesenchyme very strongly, especially around the area of developing liver, and then stains fetal hepatocytes beginning with the first week of fetal life.

Fig. 2. Immunofluorescent staining of Morris hepatoma 7777 by moab OV-6. Note the lacy cytoskeletal staining pattern (arrow). × 400.

Fig. 3. Immunofluorescent staining of normal liver by moab H-4. × 400.
around day 16. It gives strong staining of fetal hepatocytes only by the last 2 days of development. Other reactions on adult tissue include gastric mucous glands, the mucous glands of the salivary gland, nonkeratinized epidermis, and testicular interstitial cells. It gives weak staining of adult muscle and intestinal mucosa.

H-6 stains the plasma membrane of late fetal and adult hepatocytes (Fig. 4) and does not stain hepatomas. Other positive reactions include the keratinized layer of skin, gastric and intestinal mucosa, pancreatic acini, renal glomeruli, spleen, adrenal glands, surface epithelium of ovary, and endometrium.

T-6 stains the cytoskeleton of hepatomas (Fig. 5). During fetal development it stains periportal hepatocytes 1 day before birth. Other than that time point, it does not stain fetal or normal adult hepatocytes. Other positive reactions include pancreatic acini, renal tubules, and ovarian surface epithelium.

**DISCUSSION**

We were successful in producing moabs which stain at least some of the cell types seen in the livers of rats during chemical carcinogenesis. The strategy of using enriched but not purified isolated cell fractions as immunogens appears to have enhanced the number and diversity of moabs produced by each fusion. For example, fusion 286 produced moab OV-6 to oval cells, even though the immunogen was sharply dissected nodules produced by AAF. This may indicate that the nodular hepatocytes were contaminated by oval cells and possibly early tumor cells or that some of the hepatocytes included in the tissue had acquired the OV-6 epitope. Similar contamination of nodular cells with other cells is almost certainly present in preparations of nodular cells reported by others (42, 43).

Most investigators have found that moabs produced to even highly purified antigens will not only stain a specific cell or structure but will also stain other normal tissues (44). There may be developmental significance to the sharing of certain epitopes (45, 46). Several of the moabs to hepatocytes reported by Holmes et al. (44) also stained renal tubules, a pattern observed with some of our moabs. As long as the staining pattern is specific for the cell population of interest in the liver, a shared epitope in some other tissue does not invalidate the observations made in the liver. Shared epitopes are of greater significance when the moab is to be used in imaging or therapy (7). The use of moabs provides a new approach to the study of lineage during chemical hepatocarcinogenesis in the rat. The moabs produced by us and others (23-28) are able to provide specific identification of hepatocytes and oval cells by immunostaining techniques. Such specific staining had not been possible with conventional markers.

Table 5 summarizes the moabs reported by others to liver cells and oval cells of rats and compares the immunofluorescent staining of fetal and adult liver, oval cells, and tumors between the reported moabs and ours. Hixson and Allison (24) reported 6 moabs which react to oval cells, and liver cells. 258.26 and 270.26 stain the cytoplasm of hepatocytes and both primary and metastatic hepatomas (48). 270.26 stains fetal liver after day 15, and 258.26 stains fetal liver after day 19. 270.38 stains cells or that some of the hepatocytes included in the tissue had acquired the OV-6 epitope. Similar contamination of nodular cells with other cells is almost certainly present in preparations of nodular cells reported by others (42, 43).

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the cytoplasm and some membrane components of both liver cells and bile duct cells and of some tumor metastases but not primary hepatomas induced by CD ethionine (48). 270.11 stains fetal liver at 15 days and oval cells and bile duct epithelium in carcinogen treated liver. 258.7 and 258.34 are interpreted as staining primarily the cell membrane of oval cells. The staining pattern shown that 258.7 and 258.34 on CD ethionine induced oval cells in Ref. 24 is very similar to the staining with our OV-6 (Fig. 4). Our moab OV-6 stains the cytoskeleton of oval cells and bile ducts and hepatomas. The appearance of bright cytoplasmic staining by moabs to cytokeratins can appear as surface staining, especially when staining oval cells which have very scant cytoplasm. 258.34 also stained some cytoplasmic filaments. Unfortunately the staining of 258.7 and 258.34 on tumors is not reported. If 258.34 stains hepatomas then it might be similar to OV-6. Hixson and Allison (24) has concluded that oval cells may be a phenotypically complex compartment of cells composed of at least three antigenically distinct subpopulations. Several of the markers suggest that this compartment may contain a stem cell population capable of differentiating into bile duct cells or hepatocytes.

Holmes et al. (23) reported three moabs which were reactive with adult hepatocytes. Two stain the cytoplasm of hepatocytes and one stains the plasma membrane of hepatocytes. All three stain primary hepatomas. Therefore these moabs are clearly different from our moabs H-4 and H-6 which do not stain hepatomas. The moabs BDS, and HES, described by Marceau et al. (49) to oval cells and hepatocytes, respectively, may give similar staining reactions to our moabs OV-1 and H-6, respectively. However, there are insufficient data in their paper to allow more complete comparison.

The cytoskeletal moab CK55 reported by Germain et al. (25) reacts to both hepatocytes and bile duct cells. This is similar to a moab we produced (not reported here) and to another moab produced by Schmidt et al. (26) (not listed here). Since the moabs of this reaction do not offer a distinction between oval cells and hepatocytes we have not considered using them. The moab E-3 reported by Schmidt et al. (26) is very similar to ours, except that it reacts to a cytokeratin with a molecular weight of 39,000. Our moab OV-6 appears to react to a cytokeratin with a molecular weight 55,000. The comparison of its staining pattern in azo dye carcinogenesis will be the subject of a future paper. In brief, E-3 does not stain fetal bile ducts and stains fewer cells than OV-6 during azo dye carcinogenesis.

The hepatocyte membrane staining moabs developed by Hubbard et al. (27) stain different domains of the plasma membrane. HA-4 stains a M, 110,000 glycoprotein in the canaliculus, CE-9 stains a M, 39,000 glycoprotein in the sinusoidal and lateral membrane, and HA-116 stains glycoproteins with molecular weights of 90,000 and 76,000 in all three domains. Using these and similar monoclonal antibodies Feracci et al. (50) have demonstrated that developing liver cells maintain polarity during development beginning at day 14 and by day 21 have achieved an adult localization pattern. Our moab H-6 gives a staining pattern similar to HA 116. However, since no data are given on staining of tumors by HA 116, we cannot state if the reaction is identical.

Becker et al. (28) have also prepared a series of monoclonal antibodies to the hepatocyte plasma membrane. BE 8.4 and BE 11.3 are examples of the moabs which stain the plasma membrane in the sinusoidal and lateral membrane region. BE 11.3 is an example of the moabs which stain only the bile canaliculus. Most of these moabs stained normal liver but not tumors. The authors describe these epitopes as transformation sensitive, because they are not present in hepatomas but are present in normal liver. The moab BE 11.3 is the only one of these moabs to stain hepatomas. None of these are similar to H-6, because they are all limited to specific plasma membrane domains.

On the basis of the tissue staining patterns of the monoclonal antibodies reported by other investigators, we are able to conclude that our moabs most probably stain different epitopes.

The two monoclonal antibodies OV-1 and OV-6 appear to stain different populations of oval cells and bile ducts at different stages of the Solt-Farber regimen. OV-1 stains more oval cells than OV-6 during the early proliferation, and OV-6 stains more oval cells than OV-1 during the involution or disappearance of oval cells during the Solt-Farber regimen. It is probable that some of the oval cells express both epitopes stained by OV-1 and OV-6. Studies with double staining are in progress to confirm this. The day 21 of feeding of the CD-ethionine regimen demonstrated in Fig. 1, D and E is slightly past the peak oval cell proliferation, and OV-6 stains more oval cells than OV-1. The earlier time periods of CD-ethionine have not been examined; thus it is not possible to be sure if the change in phenotype of the oval cell population seen in the Solt-Farber regimen also occurs with the CD-ethionine regimen. These observations support the findings of Hixson and Allison (24) and others that the oval cell population is phenotypically diverse.

We are currently using a panel of our monoclonal antibodies to study sections of rat liver taken at weekly intervals during the continuous administration of diethylnitrosamine (51, 52) and monthly intervals during the later stages of the Solt-Farber model (29). Three of the monoclonal antibodies appear to identify preneoplastic cell populations in the mid to late stages of these models, which will be described in the accompanying paper (53).

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Production of Monoclonal Antibodies to Preneoplastic Liver Cell Populations Induced by Chemical Carcinogens in Rats and to Transplantable Morris Hepatomas

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