Analysis of Hepatocyte Plasma Membrane Polarity during Rat Azo Dye Hepatocarcinogenesis Using Monoclonal Antibodies Directed against Domain-associated Antigens

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ABSTRACT

While carcinogenesis is known to induce various alterations in epithelial cell polarity, little information is available about the fate of plasma membrane polarity during the neoplastic process. Using three monoclonal antibody-defined antigens as markers of the three plasma membrane domains of rat hepatocytes, the sinusoidal, the lateral, and the canalicular, we demonstrated by immunohistochemical techniques that changes in hepatocyte plasma membrane polarity occur at every stage of 3'-methyldimethylaminoazobenzene-induced hepatocarcinogenesis. At the preneoplastic stage, the division of hepatocyte plasma membrane into three distinct domains was retained despite rearrangements in hepatocytic architecture, characterized by the disorganization of hepatocytic plates and the formation of pseudocirrnicular structures. The most striking change was the distribution of the canalicular-associated antigen over the entire plasma membrane in disorganized plates. At the neoplastic stage, changes in plasma membrane polarity depended on the degree of morphological differentiation of neoplastic cells. Poorly differentiated cells constantly expressed the monoclonal antibody-defined antigens and showed no evidence of plasma membrane polarization. Differentiated cells constantly expressed the three antigens, and their plasma membrane was divided into antigenically distinct domains. The changes in hepatocyte plasma membrane polarity demonstrated in situ during 3'-methyldimethylaminoazobenzene hepatocarcinogenesis may therefore be compared with situations known to occur in vitro, in cultured epithelial cells presenting varying degrees of polarization. Our observations suggest that these alterations are of relevance to the in vivo biology of cancer.

INTRODUCTION

Most normal epithelial cells display a polarized organization. One of the aspects of this asymmetry is the division of the plasma membrane into morphologically, functionally, and biochemically distinct domains (1, 2). Carcinogenesis is known to induce various alterations in epithelial polarity, which may affect, according to the case, the overall cellular shape, the intracellular distribution of organelles, or the secretory functions (3). However, little information is available about the fate of plasma membrane polarity during the neoplastic process. Most current knowledge about maintenance of plasma membrane domains in cells with altered polarity derives from in vitro models using epithelial cell lines, such as MDCK3 epithelial cells (4) and HT29 cells (5). While alterations in cell polarity induced in these in vitro models share some similarities with those observed in the neoplastic process, their relevance to in vivo biology of cancer is not assessed. Difficulties in the study of plasma membrane polarity in vivo mainly arise from the fact that only a limited number of domain-specific markers was available before the development of hybridoma technology. A number of domain-specific antigens can now be defined using monoclonal antibodies. A previous report from this laboratory (6) has described the production of a set of monoclonal antibodies characteristic of the three morphological and functional domains of hepatocyte plasma membrane (7), the sinusoidal, the lateral, and the canalicular. The sinusoidal domain, which corresponds to the basal domain of simple epithelial cells, is involved in exchanges with blood. The lateral domain is committed in cell-cell adhesion and communication. The canalicular domain, which corresponds to the apical domain of simple epithelial cells, is specialized for bile secretion. The availability of monoclonal antibodies against domain-associated antigens of hepatocyte plasma membrane makes it possible to search for alterations of plasma membrane polarity in a widely used model of epithelial carcinogenesis, chemical hepatocarcinogenesis in the rat. The present study was designed to investigate, by light and electron microscopic immunohistochemistry, the fates of hepatocyte plasma membrane domains at the various stages of hepatocarcinogenesis induced by a chemical carcinogen, 3'-methyldimethylaminoazobenzene, in order (a) to search for alterations of hepatocyte plasma membrane polarity during the neoplastic process and (b) to correlate those changes with the alterations in cell polarity occurring in this in vivo model.

MATERIALS AND METHODS

Study Design. Male Fischer 344 rats (Animalerie de l'IRSC, Villejuif, France) were used throughout the study. Animals were housed in an air-conditioned room at controlled temperature and humidity, with a 12-h light-dark cycle. They received tap water ad libitum. The study group, 38 rats, was fed a diet containing 0.06% 3MeDAB (Koch Light Laboratories, London, England) from the age of 4 weeks to their sacrifice, as described previously (8). Twelve rats, used as controls, were fed a standard commercial diet (UAR, Villacoublay, France). The animals were sacrificed at the following time points after initiation of the intoxication: 4, 6, 10, 12, 14, 16, 18, and 22 weeks. At each time point, 4 animals of the study group were sacrificed except at weeks 16 and 22 at which 7 animals were sacrificed. At least one rat from the control group was sacrificed at each time point.

Histological typing of 3MeDAB-induced lesions was performed according to previously published classifications and descriptions of preneoplastic (9–12) and neoplastic (9, 12, 13) lesions.

Monoclonal Antibodies. Three monoclonal antibodies, termed A39, B1, and B10, were used. Their mode of production has been detailed in a previous report (6). Briefly, BALB/c mice were immunized i.p. with plasma membranes of liver cells prepared according to the technique of Neville (14). Their splenic cells were fused with Sp2-0Ag14 mouse myeloma cells. Hybridomas were screened by indirect immunofluorescence on liver cryostat sections. The antigens defined by A39, B1, and B10 have been characterized by immunoblotting (6) and immunoprecipitation (15) in Sprague-Dawley rats. A39 reacted with two main bands, corresponding to antigens with apparent molecular weights between 30,000 and 36,000. B1 recognized a single band with molecular weights between 30,000 and 36,000. B10 reacted with two bands with molecular weights of 150,000 and >300,000. The three antigens have been shown to behave as integral membrane proteins by alkaline extraction of a purified hepatocyte plasma membrane preparation (15).

Received 12/30/87; revised 8/2/88; accepted 8/18/88.

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1 This work was supported in part by grants from the Fondation pour la Recherche Médicale and the Conseil Scientifique, Faculté Xavier Bichat, Université Paris VII.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MDCK, Madin-Darby canine kidney; 3MeDAB, 3'-methyldimethylaminoazobenzene; endo F, endo-β-N-acetylglucosaminidase F; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline.
The specificity of the monoclonal antibodies A39, B1, and B10, first documented in Sprague-Dawley rats, was reinvestigated in the Fischer 344 strain and is presented below.

Enzymatic Deglycosylation. Plasma membrane fractions were prepared according to the technique of Neville (14) from Fischer 344 rat livers. Enzymatic deglycosylation was carried out with neuraminidase from *Vibrio cholerae* (Calbiotech-Behring, La Jolla, CA) and with endo-β-N-acetylglucosaminidase F (endo-glycosidase F; Boehringer Mannheim Biochemica, Mannheim, Federal Republic of Germany). For neuraminidase treatment, plasma membrane samples containing 100 μg protein were incubated for 18 h at 37°C with 0.1 unit neuraminidase in sodium acetate buffer, pH 5.5, containing 154 mM NaCl and 2 mM CaCl₂, as described previously (16) with slight modifications. Endo F digestion was performed according to the manufacturer's recommendations, essentially as described previously (17). Plasma membrane proteins were solubilized in 0.5% SDS and boiled for 3 min. The following additions were then made for 100 μg of protein: (a) 75 μl of a solution containing 100 mM sodium acetate (pH 6), 45 mM EDTA, 1% Trition X-100, and 0.1% SDS; (b) 0.2 unit endo F. The final solution was incubated for 18 h at 37°C. Plasma membrane proteins incubated in corresponding buffers in the absence of enzyme were used as controls. After incubation, samples were subjected to SDS-polyacrylamide gel electrophoresis on 5–15% gradient gels and immunoblotting as already described (15). For calibration, standard molecular weights (Bio-Rad, Richmond, CA; Pharmacia, Uppsala, Sweden) were run in parallel.

Immunoperoxidase Procedure. Immunoperoxidase was performed on fixed liver samples, obtained from 3 rats at each time point, except at the 16th and 22nd weeks at which 5 rats were studied. Rats were anesthetized with sodium pentobarbital (Cln-Midy, Saint-Jean, France). Through the portal vein, livers were washed for 4 min with 0.1 M PBS, bubbled with 95% O₂ and 5% CO₂, and then fixed for 10 min with 4% paraformaldehyde (Merck, Darmstadt, Federal Republic of Germany) diluted in phosphate buffer, pH 7.4. Liver slices, 1 to 2 mm thick, were immersed in the same fixative for 2 to 4 h. For each liver, 3 or 4 blocks from different slices were processed. Blocks were snap frozen in isopentane, prechilled with liquid nitrogen. Sections 8 μm thick were cut with a cryostat. Liver sections were incubated overnight at 4°C in undiluted hybridoma culture supernatant. For control reactions, primary monoclonal antibody was omitted and replaced by either PBS or normal mouse serum diluted 1:500 in PBS. Normal liver surrounding 3MeDAB-induced lesions was used as a positive control.

Immunoblotting Procedure for Liver Tissue Homogenates. Immunoblotting was performed on frozen liver tissue obtained from 6 animals presenting with preneoplastic lesions and 5 animals with hepatocarcinomas. After anesthesia, the liver was immediately removed. Slices 1 mm thick were snap frozen and stored in liquid nitrogen until use. Tissue samples used for homogenization corresponded either to whole liver slices for rats with preneoplastic lesions or to carcinomas excised from the surrounding liver parenchyma for rats with neoplastic lesions. Tissue samples were homogenized with a Potter homogenizer in ice cold 1 mM sodium bicarbonate, pH 8.2, containing 0.5 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged for 30 min at 10,000 × g at 4°C. The pellet was dissolved in 2% SDS-0.03 M N-ethylmaleimide (Sigma, Saint Louis, MO)-10% glycerol-50 mM Tris-HCl buffer, pH 6.8. Samples containing 100 to 150 μg of protein were run through a 7.5 or a 9% polyacrylamide slab gel according to the method of Laemmli (19). Electrophoreses were performed at room temperature with water cooling, at 30 mA and for 5–7 h. Proteins were then electrophoretically blotted onto a nitrocellulose sheet (Schleicher and Schuell, Dassel, Federal Republic of Germany) as described by Towbin et al. (20). Blotting was performed overnight at 0.15 mA. The nitrocellulose filters were quenched for 1 h at 37°C in 20% newborn calf serum (IBF, Paris, France) in PBS-0.1% Tween 20 (Merck, Darmstadt, Federal Republic of Germany) and then incubated for 3 h at room temperature in hybridoma culture supernatants diluted 1:2 in PBS-0.1% Tween 20. After a washing, filters were incubated for 3 h at room temperature with gold-labeled anti-mouse immunoglobulin antibodies (Janssen Biotech NV, Olen, Belgium), diluted 1:100 in PBS-0.1% Tween 20 containing 20% newborn calf serum. Silver enhancement of gold staining was performed according to the manufacturer's recommendations (Intense kit; Janssen Biotech NV). For calibration, human erythrocyte membranes (kindly provided by Dr. Dhermy, Inserm U160, Clichy, France) were run in parallel (21). Liver slices from control rats were processed in the same way and run on the same gels.

### RESULTS

#### Specificity of the Monoclonal Antibodies

**Immunolocalization in Normal Fischer 344 Rat Liver (Table 1).** As described previously in Sprague-Dawley rats (6), A39 labeled mainly the sinusoidal domain and occasionally the canalicular membrane of hepatocyte plasma membrane (Fig. 1, a and d). B10 labeling was restricted to the canalicular domain (Fig. 1, e and f). The only significant differences between Sprague-Dawley and Fischer 344 strains were observed for B1. While B1 mainly labeled the lateral domain in Sprague-Dawley rats, this monoclonal antibody constantly labeled both the lateral and the sinusoidal domains in Fischer 344 rats (Fig. 1, c and d). The basolateral domain of the biliary cells reacted with A39 and B1, and their apical membrane stained with B10 and occasionally with A39.

#### Table 1. Results of immunolocalization

<table>
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<th>Expression of antigens</th>
<th>Presence of distinct domains</th>
<th>Distribution of antigens defined by</th>
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<tr>
<td><strong>A39</strong></td>
<td><strong>B1</strong></td>
<td><strong>B10</strong></td>
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<tr>
<td>Normal liver cells</td>
<td>+</td>
<td>sin⁺ (can)</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>+</td>
<td>sin, lat</td>
</tr>
<tr>
<td>Biliary cells</td>
<td>+</td>
<td>can</td>
</tr>
<tr>
<td>Prenecplastic lesions</td>
<td>+</td>
<td>basolat</td>
</tr>
<tr>
<td>Disorganized plates</td>
<td>+</td>
<td>basolat</td>
</tr>
<tr>
<td>Pseudoacinar str</td>
<td>+</td>
<td>can, lat</td>
</tr>
<tr>
<td>Poorly diff carcinomas</td>
<td>−</td>
<td>can, lat, sin</td>
</tr>
<tr>
<td>Adenocarcinomas</td>
<td>+</td>
<td>pericell</td>
</tr>
<tr>
<td>Well-diff carcinomas</td>
<td>+</td>
<td>basolat</td>
</tr>
</tbody>
</table>

⁺ sin, sinusoidal; lat, lateral; can, canalicular; basolat, basolateral; apic, apical; pericell, pericellular; str, structure; diff, differentiated; +, present; −, absent.
Characterization of the Corresponding Plasma Membrane Antigens (Fig. 2). By immunoblotting, A39 was shown to react with two main bands, corresponding to antigens with an apparent molecular weight of, respectively, 30,000 and 34,000. B1 recognized a single band corresponding to an antigen with an apparent molecular weight of 100,000. B10 reacted with a main band with a molecular weight of 150,000.

Enzymatic deglycosylation with either neuraminidase or endo F did not affect the immune detection of the three antigens studied (Fig. 2). The mild alterations observed in reactivity pattern and/or staining intensity for A39- and B10-defined antigens might be attributed to the differences existing between buffer and incubation conditions used for enzymatic deglycosylation and those used in standard electrophoretic procedures (22). In any case, our results strongly suggest that the epitopes to which the three monoclonal antibodies bind are of peptidic rather than of carbohydrate nature.

All three antigens were glycoproteins. As indicated by the decrease in apparent molecular weight induced by neuraminidase treatment, all three contained sialic acid residues (Fig. 2). As shown by the results of endo F digestion, all three antigens contained N-linked oligosaccharide chains. The decrease in apparent molecular weight observed for A39-, B1-, and B10-defined antigens respectively accounted for approximately 20, 25, and 38% of the initial molecular weights calculated under the same electrophoretic conditions (Fig. 2).

Immunolocalization in 3MeDAB-induced Lesions (Table 1)

Early Lesions (4–16 Weeks). The early lesions observed in 3MeDAB-induced hepatocarcinogenesis were characterized (a) by a marked proliferation of oval cells and (b) by the subsequent development of preneoplastic foci and nodules constituted by morphologically altered hepatocytes.

Oval Cell Proliferation. This was marked at 4 weeks and maximal at 6 weeks. Subsequently, oval cells decreased in number but remained present in significant amounts until 16 weeks. Typical oval cells presented a distinctive phenotype. They constantly reacted with B1, which labeled their whole...
Fig. 2. Characterization of A39-, Bl-, and B10-defined antigens. SDS-polyacrylamide gel electrophoresis analyses and immunoblotting of plasma membrane preparations. Lanes N, nontreated plasma membrane fractions; Lanes 1, neuraminidase-treated plasma membrane fractions; Lanes 2, plasma membrane fractions incubated in neuraminidase buffer in the absence of enzyme; Lanes 3, endo F-treated plasma membrane fractions; Lanes 4, plasma membrane fractions incubated in endo F buffer in the absence of enzyme. Preparation of samples, SDS-polyacrylamide gel electrophoresis, and immunoblotting were performed as described under "Materials and Methods." Samples were run on 5-15% gradient polyacrylamide slab gels. Standard molecular weights are indicated by arrows and correspond to myosin (M, 250,000), β-galactosidase (M, 130,000), phosphorylase b (M, 94,000), bovine serum albumin (M, 67,000), ovalbumin (M, 43,000) and carbonic anhydrase (M, 30,000).

In the normal state, A39 reacts with two main bands with molecular weights of 30,000 and 34,000, Bl reacts with a M, 100,000 band, and B10 reacts with a M, 150,000 band. In the three cases, while reactivity pattern may be modified by the incubation conditions, immune detection of the antigens is not affected either by neuraminidase or by endo F digestions. The efficiency of enzymatic deglycosylation is assessed by the decrease in apparent molecular weight of the antigens.

Fig. 3. Immunolabeling of oval cells by Bl and B10. Light microscopy (a, × 180; b, × 160; immunoperoxidase staining). Tracts of oval cells (arrowheads) divide the liver lobules. Intense Bl labeling is detected into areas corresponding to oval cell proliferation (a). The same areas are unreactive for B10 (b). Surrounding hepatocytes display a sinusoidal and lateral Bl labeling reminiscent of that of normal hepatocytes (a). B10 labeling on hepatocytes is either canalicular or canalicular and lateral (b). Electron microscopy (c, × 6000; d, × 7000; immunoperoxidase staining without countercoloration). The entire plasma membrane of oval cells (O) is labeled by Bl (double arrows) (c). On d, several oval cells (O) are visible between two hepatocytes (H). The isolated oval cell is not decorated by B10, while the oval cells organized in a duct-like structure display an apical labeling. H, hepatocyte; O, oval cell. Bars: a, b, 50 μm; c, d, 2 μm.

plasma membrane (Fig. 3, a and c). A39 labeling, distributed all along the plasma membrane, was faint and inconstant. No labeling was observed for B10 (Fig. 3, b and d). Numerous duct-like structures resulting from oval cell maturation were also observed. The cells lining these ductular structures were polarized and their antigenic phenotype was reminiscent of that of normal biliary cells. These cells possessed a basolateral domain reactive with B1 and A39 and an apical domain faintly reactive with A39. B10 labeling was rarely observed; when present, it was restricted to the apical membrane (Fig. 3d).

Preneoplastic Foci and Nodules. These, consisting of a mixture of cytologically abnormal hepatocytes, were observed from 10 to 16 weeks. An average of 10 foci and/or nodules were analyzed for each animal at each time point. As defined previously (9), foci were smaller than a hepatic lobule, whereas nodules had a diameter equivalent to or greater than the diameter of a lobule. No correlation was attempted between the staining patterns and the cytological types usually described in these lesions (9). Indeed, certain of the morphological characteristics used to define the various cell types observed within preneoplastic lesions cannot be demonstrated in the frozen sections used in this study. However, the technique allowed a
good correlation between staining patterns and rearrangements in hepatocytic architecture. Two main architectural patterns could be recognized, the disorganized plates and the pseudo-acinar structures (11). In disorganized plates, the trabecular disposition of hepatocytes was retained, but plates were irregularly branched and of variable thickness. In pseudoacinar structures, hepatocytes were organized in a tubular manner around a stellate bile canaliculus. In both patterns, expression of the three monoclonal antibody-defined antigens was constant. Hepatocyte plasma membrane retained a division into three antigenically distinct plasma membrane domains. The differences between the two architectural types lay in the pattern of distribution of the domain-associated antigens. In disorganized plates, the main abnormality was the labeling of sinusoidal and lateral domains by B10 (Fig. 4, a and c). There were no significant modifications of distribution patterns of B1 and A39 (Fig. 4b and d). In pseudoacinar structures, B1 labeling was modified. It was strong all along the sinusoidal domain but was inconstant and faint along the lateral membrane (Fig. 5, a and c). Distributions of the two other monoclonal antibodies, B10 (Fig. 5, b and d) and A39, were unchanged.

Neoplastic Lesions (16–22 Weeks). Hepatocarcinomas were found in 13 animals from the 16th to the 22nd weeks of intoxication. These malignant tumors displayed a wide range of morphological features. Three types of hepatocarcinomas were recognized, poorly differentiated carcinomas, adenocarcinomas, and well-differentiated hepatocarcinomas (12, 13). In the same tumor, poorly differentiated areas usually coexisted with areas presenting varying degrees of differentiation.

In poorly differentiated areas, neoplastic cells displayed no evidence of morphological polarization. Expression of the monoclonal antibody-defined antigens was inconstant. The loss of reactivity for B10 was the most frequently observed and was usually complete. For A39 and B1, reactivity was always present on a variable proportion of neoplastic cells (Fig. 6a). The labeling, when present, revealed no antigenically distinct domain and was distributed all along the plasma membrane (Fig. 6c). In certain neoplastic cells, intracellular lumina with nu-
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Fig. 5. Preneoplastic lesions, pseudoacinilar structures. Light microscopy (a, ×550; b, ×400; immunoperoxidase staining): hepatocytes are organized in tubular structures centered by dilated bile canaliculi. B1 labeling (a) is mainly sinusoidal (arrow) and faintly lateral (arrowhead). B10 labeling (b) decorates only bile canaliculi. Electron microscopy (c, ×7000; d, ×4500; immunoperoxidase staining without countercoloration): B1 labeling (c) is mainly distributed on the sinusoidal or basal domain (arrow). The lateral domain is constantly labeled (arrowhead). B10 labeling (d) remains restricted to the canalicular domain; no labeling of the lateral (arrowhead) and sinusoidal (arrow) domains is visible. S, sinusoidal lumen; C, bile canaliculus; H, hepatocyte.

Bars: a, b, 25 μm; c, d, 2 μm.

Numerous microvilli having features of the so-called vacuolar apical compartment (23), have been observed. The membrane of these organelles was labeled by B10 (Fig. 6e), but not by A39 and B1.

In adenocarcinomas, expression of the three monoclonal antibody-defined antigens was constant. Neoplastic cells were morphologically polarized. Their organization was reminiscent of that of simple epithelial cells, exemplified in the liver by biliary cells. They presented an apical pole facing the glandular lumen and a basolateral pole in contact with adjacent neoplastic cells or with the extracellular matrix. These morphological domains were antigenically distinct. Distribution of the three monoclonal antibody-defined antigens on neoplastic cells was reminiscent of the normal distribution of these antigens on simple epithelial cells. A39 and B1 labeled the basolateral domain of neoplastic cells (Fig. 6b). B10 labeled only the apical domain (Fig. 6d).

In well-differentiated hepatocarcinomas, the three monoclonal antibody-defined antigens were constantly expressed. Neoplastic cells were morphologically polarized and presented an organization reminiscent of that of normal hepatocytes, except that no distinction between a sinusoidal and a lateral domain was usually possible because of the changes in microvascularization occurring within the neoplastic tissue. Two antigenically distinct domains could be recognized. As in normal hepatocytes, B10 labeling was restricted to the canalicular membrane (Fig. 6f). A39 and B1 labeled the remaining plasma membrane.

Control Reactions. All control reactions were negative.

Immunoblotting

In all cases, tissue samples from carcinogen-treated rats (6 with preneoplastic lesions and 5 with hepatocarcinomas) displayed the same pattern of reactivity as did normal liver tissue homogenates (Fig. 7). A39 reacted with two main bands, corresponding to antigens with apparent molecular weights of, respectively, 30,000 and 34,000. B1 recognized a single band corresponding to an antigen with an apparent molecular weight
Fig. 6. Neoplastic lesions. Poorly differentiated carcinomas: light microscopy (a, × 180; immunoperoxidase staining). Neoplastic cells are heterogeneous as regards the expression of A39-defined antigen; numerous negative cells coexist with positive cells. Electron microscopy (c, × 8,500; e, × 14,000; immunoperoxidase staining without countercoloration): B1 labeling (c) is distributed all along the plasma membrane of these unpolarized neoplastic cells (arrow). B10 labels the membrane of an intracellular vacuole lined by microvilli, resembling the so-called vascular apical compartment (e). Adenocarcinomas: light microscopy (b, × 450 and d, × 450; immunoperoxidase staining): polarized neoplastic cells line glandular structures. Their basolateral domain reacts with B1 (b) and their apical membrane (arrow) is labeled by B10 (d). Well differentiated hepatocarcinomas: electron microscopy (f, × 9,500; immunoperoxidase staining without countercoloration): B10 labeling of neoplastic cells is restricted to a structure resembling a normal bile canaliculus, limited by well formed tight junctions (short arrowheads). N, nucleus. Bars: a, 50 μm; b, d, 25 μm; c, e, f, 1 μm.

of 100,000. B10 reacted with one $M_r$ 150,000 band and an additional $M_r$ 210,000 band.

DISCUSSION

In this work, monoclonal antibodies against domain-associated antigens were used to demonstrate, by an in vivo study using immunohistochemical techniques, that changes in hepatocyte plasma membrane polarity occur during the course of chemically induced hepatocarcinogenesis and that these changes correlate with rearrangements in hepatocyte architecture and with alterations in cell polarity.

Although the altered staining patterns observed for the three monoclonal antibodies used in this study are likely to result from an inappropriate distribution of the corresponding antigens, a cross-reactivity of the monoclonal antibodies with abnormal membrane-associated proteins synthesized by transformed hepatocytes must be considered. Immunoblotting results make this possibility unlikely. Indeed, the number of reactive bands and the apparent molecular weights of the antigens detected in homogenates from preneoplastic and neoplastic lesions are similar to those detected in normal liver. Furthermore, the apparent molecular weights determined in this study for both normal and carcinogen-treated Fischer 344 rats compare well with previous results obtained by immunoblotting (6) and immunoprecipitation (15) for normal Sprague-Dawley rats. In addition, that the monoclonal antibodies used in this study react with the protein core rather than with the carbohydrate moieties of the corresponding plasma membrane antigens contributes to reduce the probability of a cross-reactivity between unrelated glycoproteins, which is most often due to shared carbohydrate determinants (24).

Changes observed in 3MeDAB-induced preneoplastic and neoplastic lesions are of a different nature. In preneoplastic lesions, the division of hepatocyte plasma membrane into three antigenically distinct domains (7, 25), is retained. However, two of these domains, the lateral and the sinusoidal, are affected by antigenic changes. The pattern of these changes is correlated with the pattern of rearrangement in hepatocytic architecture (11). The most striking antigenic changes involve both the
lateral and the sinusoidal domains in disorganized hepatocytic plates. They are mainly due to the spreading over on the entire plasma membrane of the canalicular-associated antigen defined by B10. These results are reminiscent of those obtained by histoenzymological studies for two other canalicular-associated proteins, alkaline phosphatase and an ATPase, which have been shown to spread over on the entire hepatocyte plasma membrane in preneoplastic lesions induced by 3MeDAB or by various other chemical carcinogens (26-28).

Our study, confirming previous reports (28), shows that alterations of membrane protein processing by hepatocytes. In neoplastic lesions, changes in plasma membrane polarity occur early in the course of carcinogenesis. Therefore, these changes cannot be considered markers of an imminent neoplastic transformation. Furthermore, these antigenic changes may not be specific for the putative preneoplastic lesions. A similar pattern of distribution for B10 labeling has been observed in our laboratory in unrelated physiological and pathological conditions, i.e., a limited stage of rat liver development, from day 19 of gestation to day 5 postpartum (29), and experimentally induced cholestasis in the rat (30). These concurrent observations suggest that different cellular changes may result in similar alterations of membrane protein processing by hepatocytes.

In preneoplastic lesions, changes in plasma membrane polarity depend on the degree of differentiation of neoplastic cells. Poorly differentiated neoplastic cells, which are morphologically unipolarized and exhibit reduced intercellular junctions, constantly express the monoclonal antibody-defined antigens and display no evidence of membrane polarization. In contrast, differentiated neoplastic cells, which are morphologically polarized and possess well-developed intercellular junctions, constantly express the monoclonal antibody-defined antigens and display division of their plasma membrane into antigenically distinct domains. These in vivo observations share similarities with certain situations observed in cultured epithelial cells.

That the basolateral antigens defined in our study by the monoclonal antibodies A39 and B1 remain unpolarized in the absence of well-formed cell-cell interactions is in keeping with observations performed in various in vitro models using primary cell cultures, such as primary hepatocyte monolayers (15), and cultured cell lines, such as MDCK and HT29 cells (23, 31–35). The inconstant detection of those antigens on the surface of poorly differentiated neoplastic cells, as observed in our study, may correspond to variations in the amount of protein actually inserted in the plasma membrane or may be a further example of the phenotypic heterogeneity which is known to occur in many malignant clones (36). A lack of reactivity due to modifications in glycosylation, which are frequently observed for glycoproteins synthesized by neoplastic cells (37), is unlikely because the monoclonal antibodies used in this study react with epitopes of peptidic rather than carbohydrate nature.

The apical marker defined in our study by the monoclonal antibody B10 is also frequently undetected on the surface of poorly differentiated neoplastic cells. This is in keeping with its inconstant expression by certain hepatoma cell lines, as shown by a previous work from our laboratory (15). These results concur with numerous observations made on hepatoma cell lines and human hepatocarcinomas for a variety of canalicular-associated antigens (38–43) to suggest that the expression of canalicular proteins is highly sensitive to transformation. The absence of detection of canalicular antigens may correspond either to an actual lack of insertion or to a decreased amount of the corresponding proteins in plasma membrane. The two mechanisms have been demonstrated for apical membrane proteins in in vitro models using cultured epithelial cell lines (23, 44). It is of interest that in unpolarized MDCK cells, abnormal targeting of apical markers is associated with appearance of a cytoplasmic storage organelle, the so-called vacuolar apical compartment, in which apically directed proteins may accumulate (23). Structures with comparable morphological features have been described in a variety of epithelial neoplasms (45–47) and cancer cell lines (48). Our ultrastructural observations show that such structures are actually present in chemically induced poorly differentiated liver carcinomas. As for vacuolar apical compartment in MDCK cells (23), the membrane of these organelles was found to be labeled by the monoclonal antibody directed against an apical protein but was unreactive with the antibodies binding to antigens associated with the basolateral domain. Abnormal processing of apically targeted proteins in a vacuolar apical compartment may therefore be of relevance to explain impaired in vivo biogenesis of apical membrane in neoplastic cells.

In conclusion, changes in hepatocyte plasma membrane polarity can be demonstrated by in situ analysis at every stage of the neoplastic process. They correlate well with the alterations in cell polarity and in cell-cell interactions induced by the neoplastic process. To a large extent, they may be compared with the situations observed in vitro in cultured epithelial cells with varying degrees of polarization. In situ demonstration of changes in plasma membrane polarity during chemical hepatocarcinogenesis in the rat suggests that these alterations are of relevance to the in vivo biology of cancer.

ACKNOWLEDGMENTS

The authors thank Paulette Echinard-Garin for her expert assistance. They also thank José Uriel for his helpful advice in the preparation of the manuscript.

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