Immunological Approaches to the Purification of Putative Premalignant Hepatocytes from Genotypic Mosaic Rat Livers

John M. Hunt, Mark T. Buckley, Brian A. Laishes, and Harold A. Dunsford

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Surface-localized rat RT1 alloantigens on isolated hepatocytes have been used to achieve partial purification of putative premalignant liver cells from rats undergoing chemically induced hepatocarcinogenesis. Genotypic mosaic rat livers were constructed by transplantation of carcinogen-altered F-344 (RT1A) or WF (RT1u) donor liver cells into livers of WF × F-344 F1 host rats, whose liver cells bear alloantigens of both parental strains: WF and F-344, RT1u and RT1A, respectively (J. M. Hunt et al., Cancer Res., 42: 227-236, 1982). Donor and host origin hepatocytes were thus distinguishable immunologically with anti-RT1u or anti-RT1A alloantisera. Donor rats were treated with diethylamino-2-chloroethylamine (200 mg/kg i.p.) followed by an experimental regimen of dietary 2-acetylaminofluorene and partial hepatectomy. Standard host rats received only the 2-acetylaminofluorene-partial hepatectomy regimen. At 10 to 21 days after transplantation, mosaic host rat livers typically contained 7% donor origin hepatocytes, 96% of which were positive histochemically for y-glutamyl transpeptidase.

Host origin hepatocytes could be effectively purified by affinity chromatography (“panning”) of isolated hepatocytes. To obtain donor origin hepatocytes, the putative progenitor cells of liver carcinomas in these mosaic livers, two approaches were used. Alloantibody-mediated rosette formation followed by sedimentation through Ficoll/metrizamide resulted in a 4- to 10-fold enrichment for donor origin hepatocytes isolated from mosaic livers. Similarly, a 5- to 11-fold enrichment for donor origin hepatocytes was achieved by specific alloantibody-mediated cytology of host hepatocytes with rabbit complement followed by purification of viable donor origin cells by sedimentation on metrizamide cushions.

Hepatocellular carcinomas which developed in the genotypic mosaic host rat livers were excised 17 to 21 months after donor liver cell transplantation and passed s.c. or i.m. in incompatable rats. The transplantable tumors were typed for strain of origin by indirect immunofluorescence using rat alloantisera, and expression of dietary 2-acetylaminofluorene-partial hepatectomy regimen. At 10 to 21 days after transplantation, mosaic host rat livers typically contained 7% donor origin hepatocytes, 96% of which were positive histochemically for y-glutamyl transpeptidase.

Host origin hepatocytes could be effectively purified by affinity chromatography (“panning”) of isolated hepatocytes. To obtain donor origin hepatocytes, the putative progenitor cells of liver carcinomas in these mosaic livers, two approaches were used. Alloantibody-mediated rosette formation followed by sedimentation through Ficoll/metrizamide resulted in a 4- to 10-fold enrichment for donor origin hepatocytes isolated from mosaic livers. Similar 5- to 11-fold enrichment for donor origin hepatocytes was achieved by specific alloantibody-mediated cytology of host hepatocytes with rabbit complement followed by purification of viable donor origin cells by sedimentation on metrizamide cushions.

Hepatocellular carcinomas which developed in the genotypic mosaic host rat livers were excised 17 to 21 months after donor liver cell transplantation and passed s.c. or i.m. in incompatable rats. The transplantable tumors were typed for strain of origin by indirect immunofluorescence using rat alloantisera, and five of six tumors displayed antigenicity reflecting donor strain origin. We conclude, therefore, that the transplanted donor liver cell populations contain cellular precursors of hepatocellular carcinomas which may be isolable using combinations of the purification strategies described.

INTRODUCTION

The genotypic mosaic liver model for chemically induced rat hepatocarcinogenesis is being developed to facilitate the purification and characterization of viable premalignant liver cells which are members of a cellular lineage leading to hepatocellular carcinomas (10). The experimental system for this model involves the i.v. transplantation of carcinogen-altered premalignant parent-strain donor liver cells into livers of F1 hybrid host rats. The hepatocellular carcinomas in these mosaic livers, as has been demonstrated previously in cryostat histological sections by indirect immunofluorescence using polyvalent alloantisera (10).

1 Supported by NIH Grants CA-24818, CA-07175, CA-09020, and CA-37150 and by American Cancer Society Institutional Research Grant IN-35U-2. Presented in part at the 74th Annual Meeting of the American Association for Cancer Research, San Diego, CA (7).
2 Present address: Department of Pathology and Laboratory Medicine, University of Texas Medical School, P. O. Box 20708, Houston, TX 77225.
3 To whom requests for reprints should be addressed.
4 Present address: Genesis Laboratories, Inc., Edina, MN 55435.
5 The abbreviations used are: AAF, 2-acetylaminofluorene; DEN, diethylnitrosamine; FA, fluorescent antibody-staining reaction by indirect immunofluorescence (× or ÷); GT, histochemical enzyme phenotype (× or ÷) for y-glutamyl transpeptidase; PAA, buffer containing bovine serum albumin (0.2%) and sodium azide (0.1%) in phosphate-buffered saline; PBS, phosphate-buffered saline containing (per liter) 8.0 g NaCl, 0.2 g KCl, 2.16 g Na2HPO4, 1.2 g KH2PO4, and 2.0 g KH2PO4; PH, two-thirds partial hepatectomy.
6 J. M. Hunt, M. T. Buckley, and B. A. Laishes, manuscript in preparation.
In the present study, these polyvalent alloantisera have been used (a) to identify donor origin liver cells at early stages of liver carcinogenesis in suspensions of liver cells prepared from host rats, (b) to effect partial purification of donor origin and host used (a) to identify donor origin liver cells at early stages of liver carcinogenesis, and of hepatocellular carcinomas. The as yet unanswered question is, "Does the early-lesion GT+ liver cell population contain the progenitor cells of the carcinomas (19)?" The novel application of cell purification strategies described here, rosetting (17), complement-mediated cytolysis (4), and cell "panning" (9), should make possible preparative purification of viable donor origin liver cells during the period of carcinoma development as an initial step toward answering this question.

**MATERIALS AND METHODS**

**Rat Strains**

F-344 (RT1*) and WF (RT1*) rats were obtained from the Harlan Sprague-Dawley, Walkersville, MD, colony. WF x F-344 F1, rats (RT1*/RT1*) were bred in a colony at the McArdle Laboratory. Rats were maintained as described (10).

**DEN-AAF-PH Donor Rat Regimen**

Donor rats received DEN (200 mg/kg i.p.; Eastman Chemical Co, Rochester, NY) followed by a selective regimen of dietary 0.02% AAF (Aldrich Chemical Co., Milwaukee, WI) and PH (Chart 1). Control Diet 101 and diet (No. 121) containing 0.02% AAF were purchased from Dyets, Inc., Bethlehem, PA. Donor rat livers were dissociated with type I collagenase (Sigma Chemical Co., St. Louis, MO) as described (10), and suspensions of liver cells were prepared in PBS for i.v. transplantation into host rat livers via mesenteric vein tributaries of the hepatic portal vein. Liver cell suspensions typically contained hepatocytes, approximately 10 to 20 µm in diameter, with viabilities of 69 ± 12% (SD, n = 27 donor rats) as determined by trypan blue dye exclusion. The hepatocyte population contained typically 31 ± 13% (n = 8 donor rats) cells histochemically positive for the enzyme marker γ-glutamyl transpeptidase (GT*). The donor liver cells had been shown previously to stain by indirect immunofluorescence using rat alloantisera independently of GT* or GT× phenotype in agreement with the donor rat strain. Thus, F-344 anti-WF antiserum reacted positively with WF and WF x F-344 F1 donor liver cells but negatively with F-344 donor liver cells (Ref. 10, Table 4). Similarly, anti-F-344 antiserum reacted positively with F-344 and WF x F-344 F1 donor liver cells but negatively with liver cells from WF donor rats.7

**AAF-PH Host Rat Regimen**

AAF-PH host rats received the same selective AAF-PH regimen used for DEN-AAF-PH donor rats (Chart 1) (10).

**Histochemical Staining for GT× Phenotype**

The staining reaction solution was made fresh by dissolving 5 mg glycylglycine free base (Sigma) and 5 mg Fast Blue BB salt (Sigma) in 9.5 ml Tris-saline, pH 7.4. Tris-saline was made by mixing 20 ml 0.1 M Tris-HCl (pH 7.4) with 56 ml 0.15 M NaCl. To 9.5 ml of this solution was added 0.5 ml of a stock solution containing 5 mg γ-glutamyl-4-methoxy-β-naphthylamide (Vega, Tucson, AZ), 0.1 ml dimethyl sulfoxide (Sigma), 0.1 ml 10 M NaOH, and 3.8 ml H2O.

Suspensions of isolated liver cells were stained histochemically for GT× phenotype by incubation for 15 min at room temperature with the staining solution, followed by one rinse in 0.15 M NaCl and incubation for 2 min in 0.1 M CuSO4. The percentage of GT× liver cells was determined microscopically in a hemacytometer.

**Indirect Immunofluorescent Staining of Isolated Liver Cells**

Alloantisera (F-344 anti-WF and WF anti-F-344) were prepared as described (10). Isolated liver cells were stained for immunofluorescence in suspension by incubating 2 x 10⁶ cells for 30 min at 4 °C in 0.2 ml PBS (10) containing a 1/10 (for anti-WF) or 1/5 (for anti-F-344) dilution of alloantisera. Following 2 PBS rinses by centrifugation (100 x g, 4 min), the cells were incubated for 20 min at 4 °C with a 1/10 dilution in PBS of fluorescein isothiocyanate-conjugated rabbit anti-rat IgG (Miles Laboratories, Elkhart, IN). Following 2 more PBS rinses, the cells were scored as FA* or FA" in wet mounts with a fluorescence microscope as described (10).

**Simultaneous Localization of GT× Phenotype and Cell Surface Immunofluorescence in Isolated Hepatocytes from F-344-to-F, Genotypic Mosaic Host Rat Livers**

Viable F-344 donor liver cells (2 x 10⁶) were transplanted into WF x F-344 F1, host rats. At the time indicated, host rat livers were dissociated with collagenase (see below), and the hepatocytes were stained histochemically for GT× phenotype as the last step in the indirect immunofluorescence staining procedure using anti-WF alloantisera as described above. Thus, FA" cells were judged to be of F-344 donor origin in this staining system.

**Histology of Host Rat Liver Tumors**

Sections of primary and passaged tumors were fixed in 10% buffered formalin, dehydrated, embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin and with mucicarmine. Mucicarmine-stained sections were counterstained with metanil yellow. Photomicrographs were taken with a Zeiss microscope using Kodak Technical Pan film.

**Indirect Immunofluorescence of Tumor Cells**

Hepatocellular carcinomas arising in parent-to-F1, genotypic mosaic livers 17 months after F-344 or WF donor liver cell transplantation were

---

7 J. M. Hunt, unpublished results.
excised and passaged s.c. in parental-strain or F1 rats. At passage 2 or
3, the tumor issue was dissociated by mincing with scissors and stirring
with 0.05% type I collagenase (Sigma) in Hanks' balanced salt solution
(Grand Island Biological Co., Grand Island, NY) for 10 min at 37 °C. The
cells were then rinsed and stained with anti-F-344 or with anti-WF
alloantisera in suspension as described above to determine the strain of
origin of the tumor cells by indirect immunofluorescence. Control staining
was typically <2% FA* with normal rat serum instead of alloantisera. As
previously demonstrated for donor rat liver cells (see above), alloantise-
rum FA staining was equally effective with GT* and GT- cells.

Alloantigen-specific Hepatocyte Purification

Standard ratios of cells and reagents for each procedure are given,
which were scaled up or down as necessary.

Rosette Formation by Isolated Host Rat Hepatocytes (Chart 2). Isolated F-344-to-F1 host rat hepatocytes (2 x 106) were incubated for
30 min at 4 °C in 1.3 ml PBS containing a 1/10 dilution of F-344 anti-WF
alloantiserum. Following 2 rinses of the cells by centrifugation in PAA,
the hepatocytes were mixed in a 3-ml volume with Staphylococcus
aureus Protein A-coupled sheep erythrocytes in PAA (1 x 108 sheep
RBC/ml) prepared as described (17) from Protein A (Sigma) and pre-
served sheep erythrocytes. The cell mixture was centrifuged for 5 min
at 200 x g and incubated as a cell pellet for 30 min at 20 °C. The pellet
was gently resuspended and chilled on ice, and the resulting suspension
was layered onto cushions of 10% Ficoll (Pharmacia, Piscataway, NJ)/
10% metrizamide (Sigma) in PAA, using 1 ml cell suspension and 3 ml
cushion in 15-ml tubes. The tubes were centrifuged for 15 min at 200 x
g and the cells localized at the top of the cushion were removed and
stained histochemically for GT* phenotype as described above.

Complement-mediated Cytotoxicity of Isolated Host Hepatocytes
(Chart 3). To 2.0 ml of a 1/10 dilution of F-344 anti-WF alloantisera in
Medium 199 (Grand Island Biological Co.) were added 0.5 ml of a 1/2
dilution of rabbit complement in Medium 199 and 0.5 ml of F-344-to-F1,
host hepatocytes at 5 x 106 cells/ml in Medium 199. The incubation
vials were stoppered and incubated for 45 min at 37 °C in a rotary shaker at
100 rpm. The cell suspension was then chilled on ice and slowly mixed
with 6.0 ml of a 30% metrizamide solution in buffer (14) containing, per
liter, 2.4 g N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Sigma),
0.18 g CaCl2, 0.5 g KCl, and 0.22 g NaOH (pH 7.6). The resulting cell
suspension was overlaid with 1 ml Medium 199 and centrifuged for 10
min at 200 x g. Cells at the top of the metrizamide cushion were removed
and stained for GT* phenotype as described above.

Affinity Chromatography (Panning) of Host Rat Hepatocytes on
Antibody-coated Polystyrene Dishes (Chart 4). Isolated F-344-to-F1,
host rat hepatocytes (2 x 106) were incubated for 30 min at 4 °C in 1.0
ml of a 1/10 dilution of F-344 anti-WF alloantisera in PBS. The cells
Decant non-adherent cells

RESULTS

The histopathology of the liver tumors arising in F1 host rats 17 to 21 months after parental donor liver cell transplantation (Figs. 1 to 9) was similar to that observed for syngeneic liver cell transplantation (13). The incidences of hepatocellular carcinomas in F1 host rats receiving 2 x 10^6 viable donor liver cells 17 to 21 months earlier were 12 of 26 (46%) for F344 donors, 3 of 4 (75%) for a WF donor, 8 of 9 (89%) for F1 donors, and 1 of 3 (33%) for PBS controls in which PBS was substituted for donor liver cells. These incidence data are derived from 14 separate transplantation experiments.

Tumors typically numbered 2 or more per host rat liver and occurred in the presence of accompanying neoplastic nodules, enzyme-altered foci (eosinophilic and basophilic), cystic change, sinusoidal dilation, fatty change, and occasional cholangiocarcinoma (Fig. 8) (23). Four of the tumors (JH3-5L, JH3-6N, 171B-3N, and 17X-1L) were of the well-differentiated trabecular or mixed type (23). Figs. 1 and 2 show the trabecular pattern of the original primary and third passage of Tumor JH3-5L, respectively. Fig. 3 and 4 show the trabecular pattern of the original primary and second passage of tumor JH3-6N, respectively. Three successive passages of Tumor 171B-3N also showed persistence of the trabecular pattern of hepatocellular carcinoma observed in the primary site. Thus, the growth patterns of these transplantable hepatocellular carcinomas were relatively stable on successive passage. The s.c.-passaged tumor from Host Rat 17X-1L also showed a trabecular pattern of growth. However, the primary tumors in Host Rat 17X-1L included poorly and well-differentiated trabecular patterns, as well as the mixed trabecular and glandular pattern shown in Fig. 6.

An anaplastic hepatocellular carcinoma from Host Rat 171H-1R is shown in Fig. 5. The liver of this rat showed vascular invasion by anaplastic carcinoma as well as a pulmonary metastasis of anaplastic carcinoma. The s.c. passaged tumor derived from this host rat liver showed a growth pattern of anaplastic carcinoma.

Primary liver carcinomas in Host Rat 171H-1L included poorly and well-differentiated trabecular as well as glandular (Fig. 7) hepatocellular carcinomas and a small area of cholangiocarcinoma (Fig. 8). The only occurrence of mucin, detected as mucicarmine-positive material, in any of the liver tumors in this study was in the glandular lumina of the cholangiocarcinoma, as indicated by the arrows in Fig. 8. The only tumor from Host Rat 171H-1L which grew in s.c. passage showed a glandular pattern (Fig. 9) and was negative for mucin. This tumor thus appeared to be derived from a glandular-pattern hepatocellular carcinoma.

Liver tumors were selected for s.c. transplantation into histocompatible (parental donor strain or F1) rats on the basis of tumor size (>3 mm diameter) at the time of autopsy. The 6 tumors described in this report (Table 1) represent the only liver tumors of 28 transplanted at autopsy which grew s.c. for at least 2 passages. The ability of the 6 tumors described here to be passaged and to maintain their basic histological growth patterns

<table>
<thead>
<tr>
<th>Carcinoma</th>
<th>Source of carcinoma</th>
<th>% of positive (FA*) cells with Anti-F344</th>
<th>Anti-WF</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-5L</td>
<td>F-344-to-F1</td>
<td>96</td>
<td>9</td>
</tr>
<tr>
<td>3-6N</td>
<td>F-344-to-F1</td>
<td>94</td>
<td>2</td>
</tr>
<tr>
<td>171H-1R</td>
<td>WF-to-F1</td>
<td>8</td>
<td>85</td>
</tr>
<tr>
<td>171B-3N</td>
<td>F-344-to-F1</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>17X-1L</td>
<td>F-344-to-F1</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>171H-1L</td>
<td>F-344-to-F1</td>
<td>94</td>
<td>6</td>
</tr>
</tbody>
</table>
with only minor changes in atypia is strong indication that the lesions transplanted were hepatocellular carcinomas. Table 1 summarizes indirect immunofluorescent staining results for these 6 s.c.-transplantable hepatocellular carcinomas. Typing of dissociated tumor cells with alloantisera specific for the parental rat strains indicated that at least 5 of these 6 carcinomas are of donor origin, i.e., originated from a subpopulation of the donor rat liver cells transplanted 17 to 21 months earlier into the host rat livers (Chart 1).

Detection of Donor Origin Liver Cells in Genotypic Mosaic Livers at Early Times after Transplantation. Genotypic mosaic host rat livers constructed by transplantation of F-344 DEN-AAF-PH donor liver cells into AAF-PH WF × F-344 F, host rats (Chart 1) were dissociated by collagenase perfusion at Days 10 to 13 after transplantation to yield suspensions of liver cells. Previous indirect immunofluorescence staining of such liver cell preparations with anti-WF alloantisem demonstrated therein a population of FA− (i.e., of F-344 donor origin) hepatocytes. This FA− population percentage, typically 10 to 20% of all hepatocytes present, correlated numerically with the percentage of GT+ hepatocytes in the host liver cell suspensions (8). These results were consistent with cryostat liver section-staining results for identically treated host rats (10). Simultaneous staining for GT+ phenotype and by indirect immunofluorescence of genotypic mosaic liver cells isolated at Day 13 to 21 after transplantation now directly demonstrates the close correspondence of GT+ phenotype with the FA− donor origin identity of this subpopulation (Table 2). Fig. 10 illustrates typical simultaneous staining results. Two GT+ hepatocytes which are FA− and 5 GT− hepatocytes which are FA+ are shown in the center of the photographic field. The GT+ cells in these mixed populations could be stained FA+ by using WF anti-F-344 instead of F-344 anti-WF alloantisem by indirect immunofluorescence, indicating that they are of F-344 donor origin (Fig. 10, inset) and that staining with alloantisem to determine strain of origin is independent of GT+ enzyme phenotype.

Partial Purification of Donor Origin Hepatocytes Isolated from Genotypic Mosaic Host Rat Livers at Early Stages of Carcinogenesis. Previous results based on indirect immunofluorescent staining of cryostat sections from genotypic mosaic host rat livers indicated that 92% of liver colonies were of donor origin and GT+, 3% were of host origin and GT+, and 5% were of host origin and GT− (10). In the present study, the genotypic mosaic host rat livers were dissociated by collagenase digestion and analyzed in suspension using indirect immunofluorescence with alloantisem to determine strain of origin in conjunction with reagents for demonstrating GT+ phenotype histochemically. These liver cell suspensions thus represented mixed populations of F, host rat liver cells and parental donor strain (F-344 or WF) liver cells differing alloantigenically from the host liver cells. Simultaneous FA/GT staining of cells isolated by in situ collagenase perfusion of genotypic mosaic host rat livers on Days 13 to 21 after transplantation of F-344 donor cells showed that 94 to 97% of the GT+ hepatocytes stained FA+ with anti-WF alloantisem and were judged to be of F-344 donor origin by this criterion (Table 2). Typically, 3 to 6% of GT+ hepatocytes were FA+, indicating a small endogenous population of host rat-derived GT+ hepatocytes. These results corroborated the earlier data from cryostat sections of host rat livers and made it possible to justify the use of the easily scored GT+ histochemical phenotype as a marker for rapid identification of donor origin liver cells isolated from host rat livers up to at least Day 21 posttransplantation, a relatively early stage of hepatocarcinogenesis in this experimental system.

Three immunological approaches were applied to effect partial purification of the alloantigenically distinguishable donor and host origin hepatocytes isolated from genotypic mosaic host rat livers (Charts 2 to 4). Rosette formation (Chart 2) utilized alloantisem as a strain specific reagent and S. aureus Protein A-coupled sheep erythrocytes as a nonspecific agent to bind to antibody-coated hepatocytes and alter their sedimentation characteristics in a Ficoll/metrizamide cushion. This technique yielded 4- to 10-fold enrichment for donor origin GT+ hepatocytes from suspensions of genotypic mosaic host rat host liver cells (Table 3). In a typical isolation, 5.6 × 10⁶ hepatocytes (viability, 91%) were recovered at the top of the Ficoll/metrizamide cushion from a starting mixed population of 3.2 × 10⁹ host rat liver cells (viability, 88%). Complement-mediated cytolysis of host origin hepatocytes was effected using an alloantisem which reacted specifically with the F, host liver cells but not with the parental donor origin liver cells isolated from host rat livers (Chart 3). Sedimentation of antiserum- and complement-treated host liver cell suspensions

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Simultaneous staining by indirect immunofluorescence (FA) and for GT phenotype of genotypic mosaic host rat liver cells in suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cells (2 × 10⁶) from F-344 DEN-AAF-PH donor rats were transplanted into WF × F-344 F, AAF-PH host rats. Livers of host rats were dissociated with collagenase at the times indicated, and the liver cells were stained simultaneously for GT phenotype and by indirect immunofluorescence with F-344 anti-WF alloantisem. Percentages of GT+ and GT− hepatocytes which were FA− (i.e., of F-344 donor origin) were quantitated with a fluorescence microscope.</td>
<td></td>
</tr>
<tr>
<td>Days posttransplantation</td>
<td>% of GT+ hepatocytes that are FA−</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>13</td>
<td>95 (129/137)</td>
</tr>
<tr>
<td>15</td>
<td>97 (221/228)</td>
</tr>
<tr>
<td>16</td>
<td>96 (158/175)</td>
</tr>
<tr>
<td>21</td>
<td>94 (104/111)</td>
</tr>
<tr>
<td>28</td>
<td>91 (170/173)</td>
</tr>
</tbody>
</table>

¹ Numbers in parentheses, number of FA− cells/total number of cells counted. ND, not done.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Alloantigen-based enrichment for donor origin GT+ hepatocytes from F-344-to-Ft genotypic mosaic livers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cells (2 × 10⁶) from F-344 DEN-AAF-PH donor rats were transplanted into WF × F-344 F, AAF-PH host rats. Livers of host rats were dissociated with collagenase on Days 13 to 22 after transplantation and were enriched for either donor origin or host origin cells as described in &quot;Materials and Methods.&quot; Factors of enrichment are given only for donor origin cells, since a single hepatocyte-panning step yielded 94 to 99% pure host liver cells in a less-than-2-fold enrichment.</td>
<td></td>
</tr>
<tr>
<td>% of donor origin GT+ hepatocytes</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Enrichment technique</td>
<td>Experiment Initial</td>
</tr>
<tr>
<td>Rosette formation</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
</tr>
<tr>
<td>Complement-mediated cytolysis</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
</tr>
<tr>
<td>Hepatocyte &quot;panning&quot;</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
</tr>
</tbody>
</table>
yielded cell preparations enriched 5- to 11-fold for donor origin (GT*) liver cells. Since in this separation strategy the donor origin cells were spared cytotoxicity, their viability was relatively high. The cell viability on a 20% metrizamide cushion was 78% in a representative experiment in which 2.5 × 10^6 host liver cells (viability, 91%) initially were antibody and complement treated, and 1.6 × 10^6 cells were recovered at the top of the cushion.

Highly purified populations of host origin hepatocytes were obtained from suspensions of genotypic mosaic liver cells by alloantibody-mediated adherence of the host origin cells to polystyrene dishes coated with rabbit anti-rat IgG antibody (9) (Chart 4). Hepatocytes adhering to the dishes were 94% to 99% of host origin (GT~) (Table 3). In a typical experiment starting with 3.2 × 10^6 host liver cells (viability, 88%), 2.3 × 10^4 cells were adsorbed and recovered from the dishes with a viability of 25%.

**DISCUSSION**

The development of hepatocellular carcinomas in the liver cell transplantation protocol described by Laishes and Rolfe (13) requires about 17 months (510 days). The alloantiserum-typing results of tumors arising in parental-to-F1, genotypic mosaic rat livers (10) presented in Table 1 underscore the potential utility of this experimental system for detecting, isolating, and characterizing populations of liver cells containing cellular precursors of hepatocellular carcinomas. The rat RT1 Class I alloantigens provide a heritable marker system for detecting donor origin premalignant hepatocytes throughout the time course of carcinoma development. Identification and characterization of subpopulations of donor origin hepatocytes during this time course may eventually be possible so that novel early and late biochemical changes can be identified which are indicative of a genetic commitment of some donor origin cells to lineages leading to hepatocellular carcinomas. A combination of the cell purification strategies described in this report (Charts 2 to 4) will be used to achieve the preparative-scale isolation of viable donor origin cells in which such subpopulations must evolve.

The basis for the liver cell separation techniques described in this report is the presence or absence of rat alloantigenic determinants on donor- and host-origin liver cells. These separation techniques are independent of enzymatic phenotypes expressed by the liver cells. The GT+ phenotype of putative premalignant liver cells has been exploited in a panning procedure to purify GT+ liver cells (5). Preliminary results of analysis of origin liver cells isolated from genotypic mosaic host rat livers 4.5 and 9 months post-donor liver cell transplantation indicate that some donor origin cells at these times do not possess the GT+ phenotype. These results are consistent with the phenomenon of phenotypic reversion of GT+ liver cells observed histologically (1). The cellular origin of late-appearing GT+ donor origin liver cells still remains unclear, as does their relationship to liver carcinomas, some of which are GT~ histochemically (13). Thus, the utility of the GT+ enzyme marker in the genotypic mosaic liver system appears to be limited to the early stages of carcinoma development. Future experiments must be now directed at elucidating the subpopulations of donor origin liver cells evolving with time in the host rat livers which are cellular precursors of hepatocellular carcinomas. The alloantigen marker system will provide a means of identifying donor origin cells during carcinoma development.

**ACKNOWLEDGMENTS**

We are grateful to Drs. Henry Pilot and Yuan-Ding Xu for histopathological diagnosis. We thank Chris Ann Neuhauser and Michael Böttcher for excellent technical assistance; Mary Erbs-Folz, Jane Weeks, Marjorie Brown, and Girigi Vanguri for histology; and Barbara Miller for word processing.
REFERENCES


Fig. 1. Primary well-differentiated trabecular hepatocellular carcinoma in caudate lobe of the liver of Host Rat JH3-5L. H&E, × 250.

Fig. 2. Transplanted trabecular hepatocellular carcinoma JH3-5L at the third s.c. passage in a WF x F-344 F, rat. H&E, × 250.

Fig. 3. Primary well-differentiated trabecular hepatocellular carcinoma in fused caudate and triangular lobe of Host Rat JH3-6N H&E, × 250.

Fig. 4. Transplanted moderately well-differentiated hepatocellular carcinoma JH3-6N at the second s.c. passage in a F-344 rat. H&E, × 250.

Fig. 5. Primary anaplastic hepatocellular carcinoma from liver of Host Rat 17H-1R. H&E, × 250.

Fig. 6. Primary hepatocellular carcinoma of mixed trabecular and glandular pattern from triangular lobe of Host Rat 17X-1L. H&E, × 250.

Fig. 7. Primary hepatocellular carcinoma of glandular pattern from caudate lobe of Host Rat 17H-1L. H&E, × 250.

Fig. 8. Primary cholangiocarcinoma in caudate lobe of Host Rat 17H-1L, showing 2 lumina positive for mucin (arrows). Mucicarmine staining with metanil yellow counterstaining, × 250.

Fig. 9. Transplanted hepatocellular carcinoma 17H-1L showing glandular pattern at the second s.c. passage in a F-344 rat. H&E, × 250.

CANCER RESEARCH VOL. 45 MAY 1985

2232

Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 1985 American Association for Cancer Research.
Immunological Approaches to the Purification of Putative Premalignant Hepatocytes from Genotypic Mosaic Rat Livers

John M. Hunt, Mark T. Buckley, Brian A. Laishes, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/45/5/2226

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.