Genetic Control of Susceptibility to Diethylnitrosamine Carcinogenesis in Inbred ACP (grc+) and R16 (grc) Rats

Mona F. Melhem, K. N. Rao, Heinz W. Kunz, Mary Kazanecki, and Thomas J. Gill III

ABSTRACT

The R16 strain, which carries the major histocompatibility complex-linked growth and reproduction complex (grc), and its normal counterpart, the ACP strain, were initiated at 8 wk of age with a single i.p. dose of diethylnitrosamine (DEN), and 2 wk later they were fed either a choline-deficient (CD) or a choline-supplemented (CS) diet. The rats were sacrificed 2, 4, 6, 10, and 12 mo later; complete autopsies were performed, and all of the tissues were examined histologically. Sections of the liver were also examined histochemically for \( \gamma \)-glutamyl transpeptidase activity. Shortly after the administration of DEN, the R16 strain showed a significant increase in the number and size of \( \gamma \)-glutamyl transpeptidase-positive foci and more severe histological changes (disruption of the lobular architecture, bile duct and oval cell proliferation, cellular atypia, and accumulation of fat) compared with the ACP strain. These changes occurred in animals fed either CD or CS diet, but they were much more extensive and severe in the animals on the CD diet. They did not occur in rats of either strain fed the diets alone. The first hepatocellular carcinoma appeared in the R16 rats on the CD diet at 4 mo after administration of the DEN and on the CS diet, at 10 mo. The only hepatocellular carcinoma that occurred in the ACP rats did so at 12 mo in one animal on the CD diet. Combining the data at 10 and 12 mo for the rats on the CD diet, 50% (20 of 40) of the R16 rats had hepatocellular carcinomas, whereas only 3% (3 of 30) of ACP rats did. The R16 strain (22%, 9 of 40), but not the ACP strain (0 of 30), also had a variety of other malignancies: squamous cell carcinomas (8%); renal cell carcinomas (8%); lymphomas (5%); and pheochromocytoma (3%). Similar patterns of malignancies also occurred in the R16 rats on the CS diet, and there were no malignancies in the ACP rats. These observations indicate that the grc confers unusual susceptibility to the induction of cancer by the chemical carcinogen DEN and that this genetic susceptibility to cancer of the R16 strain extends beyond the primary target organ of the carcinogen used.

INTRODUCTION

Since the observation of the French surgeon, Paul Brocca, in 1866 that a general susceptibility to cancer existed in his family (1), the concept of hereditary factors' playing an important role in susceptibility to cancer has been developed (2, 3). A number of human tumors studied cytogenetically provided evidence for specific malignant tumors, e.g., deletion of chromosomal Band Ilpl3 in Wilms' tumor (4) and of Band 13q14 in retinoblastoma (5, 6). In addition, chromosome 11 appears to carry genes involved in the regulation of growth and cell proliferation as well as being involved in the development of Wilms' tumor (7). These deletions are thought to involve normal regulatory genes—tumor suppressor genes or antioncogenes (8)—and they appear to be representative of a class of genes that act in a tissue-specific manner and may collaborate with protooncogenes in the multistep process of carcinogenesis (9, 10).

Similar evidence for a genetic basis of malignancy has also been obtained in a variety of species other than humans. Homozygous recessive genes that affect cell differentiation can lead to tumorigenesis in Drosophila melanogaster (11). Certain strains of mice are more susceptible to carcinogens than others (12), and breeding experiments suggested that a single locus may be responsible for the increased susceptibility to liver cancer (13). Genes linked to the major histocompatibility complex in the mouse (H-2) appear to play a major role in influencing tumorigenesis. In the inbred (14) and congeneric strains (15). In the rat, female COP animals are resistant to mammary gland tumors following exposure to carcinogens (16), while other strains, e.g., WF, LEW, and NSD, are highly susceptible (16–19). The WF × COP F1 hybrids are highly resistant to the development of mammary cancer, and this observation is consistent with the presence of a single autosomal dominant gene from COP that is capable of suppressing 7,12-dimethylbenz(a)anthracene-induced mammary cancer (20).

DEN3 is a potent hepatocarcinogen (21), and when it is administered to rats i.p. in a single necrogenic dose, it produces hepatocellular carcinomas in approximately 25% of the rats kept on a chow diet after 85 to 111 wk (22). When DEN is administered in the drinking water or by a gastric tube (23, 24), the stomach and esophagus may show dysplastic changes after repetitive doses due to direct contact of the DEN with the mucosa. Choline is important in the structure of lipotropic factors, and choline deficiency is a promoter for liver cancer (25). Recently, a choline-deficient diet alone was reported to cause hepatocellular carcinoma in F344 rats after continuous feeding for over 13 mo (26–28).

In order to explore the genetic basis of the susceptibility to chemical carcinogens, we chose DEN as the test carcinogen because of the extensive literature on its effects, and we chose a strain of rats carrying the MHC-linked growth and reproduction complex (grc) (the R16 strain) and its wild-type counterpart (the ACP strain) as the experimental system. The latter choice was based on our hypothesis that recessive genes affecting growth and reproduction can also affect abnormal growth, i.e., cancer (29, 30). This hypothesis is supported by our previous studies showing that grc-bearing animals (the R10 strain) developed preneoplastic foci following exposure to AAF, whereas their genetically normal counterparts (the BI strain) did not (31).

The grc-bearing animals have a deletion of at least 3.1 kilobases of DNA in the region linked to the MHC (32), and this deletion may be associated with the developmental defects in these animals and with their enhanced susceptibility to AAF. Similar deletions are involved in the pathogenesis of retinoblastoma (6) and probably of Wilms' tumor (4). Each R16 and ACP rat was given a single i.p. dose of DEN and then placed on either a CD or CS diet (33). To test the effect of diet alone, a group of R16 rats and of ACP rats was given injections of normal saline and placed on either a CD or a CS diet for 12 wk.
GENETICS OF CARCINOGENESIS IN RATS

MATERIALS AND METHODS

Animals. The rats were from our colony in the Department of Pathology (Table 1). The R16 (A'B'D'E'grc) strain was derived from an F2 hybrid obtained from the mating of a female R10 (A'B'D'E'grc) with a male ACP (A'B'D'E'grc) rat (34). The animals were always selected only for their MHC genes and for their size (determined by the grc); hence, the non-MHC genes were always randomly segregating during the derivation of the R16 strain.

The R10 recombinant was derived from a BIL intercross in which the l (grc) and n (grc) haplotypes are maintained in forced heterozygosity (35). The homozygous segregants from this intercross, the BI strain (A'B'D'E'grc*), and the R10 recombinant strain have identical genetic composition. The strain from which the non-MHC genes came.

The histochemical changes in the livers of the R16 rats were expressed as the mean ± SD of the number of foci per cm² of liver section and as the ratio of the area of the foci per section area. The liver sections were scored microscopically for bile duct and oval cell proliferation, fatty infiltrate, spongiosis, and cellular atypia. Several sections from different lobes of each liver were fixed in cold alcohol:acetic acid:water (95:4:1, v/v), dehydrated in xylene, and processed for GOT staining and hematoxylin:eosin staining. liver sections were scored microscopically for bile duct and oval cell proliferation, fatty infiltrate, spongiosis, and cellular atypia. Several sections from different lobes of each liver were fixed in cold alcohol:acetic acid:water (95:4:1, v/v), dehydrated in xylene, and processed for GOT staining (33).

Morphometric Measurements. Foci of GGT-positive hepatocytes were readily distinguished in liver sections histochemically as discrete areas of intensely orange-brown cells on a blue hematoxylin background. The areas of the sections and of the foci were measured by tracing their perimeters with the transverse arm of an electronic digitizer/planimeter interfaced with a computer by means of an acoustic coupler (Numonics Corp., Lansdale, PA). The area of the liver section was measured at X5 and that of the foci at X40. The results were expressed as the mean ± SD of the number of foci per cm² of liver section and as the ratio of the area of the foci per section area. The measurements in both strains were compared statistically at each time interval using the Student t test, and P < 0.01 was chosen as the level of significance.

RESULTS

The histochemical changes in the livers of the R16 rats were consistently and significantly much greater than those in the ACP strain is maintained by brother × sister mating and is at F50; and the R16 strain is maintained by mating a heterozygous male with a homozygous female, since the homozygous male is sterile, and it is at F20.

The animals were kept in an air-conditioned carcinogen room in a laminar flow hood with a 12-h light and dark cycle. They had free access to food and water.

Regimen. The susceptibility to cancer of the R16 and ACP strains was examined using the protocol previously published (33): 8-wk-old rats were given injections of a single i.p. dose of 200 mg/kg of body weight of DEN (Sigma Chemical Co., St. Louis, MO) followed 2 wk later by a CD or CS diet (Dyets Co., Bethlehem, PA) (37). The animals were sacrificed at 2, 4, 6, 10, and 12 mo after DEN injection, and complete autopsies were performed. Any animals that died during the course of the experiment were also autopsied. In order to assess the effect of the diets alone on liver cancer, 8 to 13 R16 or ACP rats were given injections of 0.9% saline solution and placed on either a CD or a CS diet for 12 mo, at which time they were sacrificed, and their organs were examined microscopically.

Histology. Sections from the liver, lungs, pancreas, kidneys, tumor masses, and skin lesions were fixed in Sitte's fixative and processed for paraffin sectioning and hematoxylin:eosin staining. Liver sections were scored microscopically for bile duct and oval cell proliferation, fatty infiltrate, spongiosis, and cellular atypia. Several sections from different lobes of each liver were fixed in cold alcohol:acetic acid:water (95:4:1, v/v), dehydrated in xylene, and processed for GGT staining (33).

Table 1 Genotypes of the inbred and recombinant strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>A</th>
<th>B</th>
<th>D</th>
<th>E</th>
<th>Source of the non-MHC genes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>ACP</td>
</tr>
<tr>
<td>R16</td>
<td>n</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>BIL/ACP</td>
</tr>
<tr>
<td>R10</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>+</td>
</tr>
<tr>
<td>BI</td>
<td>n</td>
<td>a</td>
<td>a</td>
<td>u</td>
<td>+</td>
</tr>
<tr>
<td>BIL</td>
<td>n/l a/l a/l u/- +/gc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The strain from which the non-MHC genes came.

a⁻, Blank allele.

mo. The grc-bearing R16 rats given DEN had a high incidence of hepatocellular carcinomas and also had tumors in many other organs, whereas the wild-type ACP rats did not. These effects occurred in rats placed on either a CD or CS diet, but they were more severe in the rats fed the CD diet; they did not occur in rats given the diets alone.

Fig. 1. Morphometric data on the liver changes in male rats of the R16 and ACP strains fed a CD or CS diet.
Table 2 Liver histology 2 mo after a single i.p. dose of DEN

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diet</th>
<th>n</th>
<th>Extent of fatty infiltrate (%)a</th>
<th>Cellular atypiaa</th>
<th>Periportal fibrosisb</th>
<th>Bile duct and oval cell proliferation</th>
<th>Mitoses/high-power field</th>
</tr>
</thead>
<tbody>
<tr>
<td>R16</td>
<td>CD</td>
<td>5</td>
<td>60 +++</td>
<td>++</td>
<td>+++</td>
<td>2-3</td>
<td></td>
</tr>
<tr>
<td>ACP</td>
<td>CD</td>
<td>5</td>
<td>15 +</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>R16</td>
<td>CS</td>
<td>5</td>
<td>0 ++</td>
<td>++</td>
<td>+++</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ACP</td>
<td>CS</td>
<td>5</td>
<td>0 0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Percentage of liver infiltrated.
* Scale: 0 to ++++ (average changes).

Table 3 Liver histology 4 mo after a single i.p. dose of DEN

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diet</th>
<th>n</th>
<th>Spongiosis*</th>
<th>Hyperplastic nodules*</th>
<th>Benign cholangioma</th>
<th>Hepatocellular carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>R16</td>
<td>CD</td>
<td>13</td>
<td>754 ++ to ++++</td>
<td>6</td>
<td>46</td>
<td>5</td>
</tr>
<tr>
<td>ACP</td>
<td>CD</td>
<td>12</td>
<td>4 33 + to ++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R16</td>
<td>CS</td>
<td>13</td>
<td>10 77 ++ to +++</td>
<td>3</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>ACP</td>
<td>CS</td>
<td>10</td>
<td>2 20 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Number and percentage of rat livers showing the change (average changes).
* Scale: 0 to ++++ (average changes).
* Some nodules are oncocytic (25 to 30%).

The histological changes in the liver followed the same pattern as the histochemical changes described above. At 2 mo (Table 2; Fig. 2), the R16 livers showed an increased number of atypical hepatocytes that had large irregular nuclei and prominent nucleoli and were often multinucleated. Periportal fibrosis and cell proliferation with numerous mitotic figures were present only in the R16 livers. Fatty infiltration was present in all animals on the CD diet but was more pronounced in the R16 strain than in the ACP strain. Bile duct and oval cell proliferation was prominent in the R16 strain on both diets, but in the ACP strain it was present minimally only in rats on the CD diet.

At 4 mo (Table 3; Fig. 3), focal spongiosis (38) was present in all rats of both strains but was more pronounced in the R16 livers. A large number of R16 rats developed cirrhosis, hyperplastic nodules, and benign cholangiomas, and these changes were more pronounced in the rats receiving the CD diet. The first hepatocellular carcinoma developed at this time in an R16 rat fed a CD diet. None of the ACP rats showed these changes.

At 6 mo (Table 4; Fig. 4), the same histological changes persisted and were more accentuated in the R16 rats with hepatocellular carcinomas being present in 14% of those rats on the CD diet. Other malignant tumors started to appear in this strain, and they were mainly soft tissue sarcomas of the pelvis and retroperitoneum with widespread metastases (Fig. 4, A and B). Cirrhosis of the liver occurred in 86% of R16 rats receiving the CD diet and in 7% of ACP rats on the CD diet. Spongiosis and cellular atypia were more pronounced in the R16 strain, and hyperplastic nodules and benign cholangiomas were seen only in the R16 strain. Oncocytic nodules consisting of large, highly eosinophilic hepatocytes that were filled with mitochondria were found in the R16 strain (Fig. 4C).

At 10 mo (Table 5; Fig. 5), many of the R16 rats developed hepatic (25 to 28%) and extrapleural (22 to 25%) malignant neoplasms. The latter were mainly large cell lymphomas (Fig.
Fig. 3. Liver of the R16 strain after injection of DEN and 4 mo on a CD diet. There are spongiosis and ballooning of the hepatocytes (A), cirrhosis with fatty infiltration (B), a benign cholangioma lined by a single layer of cuboidal bile duct epithelial cells (C and C insert), and hyperplastic nodules (D). H & E, × 100 and insert × 400.

Table 4 Liver histology and other malignancies 6 mo after a single i.p. dose of DEN

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diet</th>
<th>Spongiosis a</th>
<th>Cellular atypia a</th>
<th>Cirrhosis a</th>
<th>Hyperplastic nodules b, c</th>
<th>Benign cholangioma</th>
<th>Hepatocellular carcinoma</th>
<th>Other malignancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>R16</td>
<td>CD</td>
<td>14</td>
<td>8</td>
<td>57 ++</td>
<td><strong>12</strong> 86</td>
<td>6 43</td>
<td>4 29</td>
<td>2 14</td>
</tr>
<tr>
<td>ACP</td>
<td>CD</td>
<td>14</td>
<td>7</td>
<td>50 0</td>
<td><strong>1</strong> 7</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>R16</td>
<td>CS</td>
<td>13</td>
<td>10</td>
<td>77 ++</td>
<td><strong>6</strong> 46</td>
<td>6 46</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>ACP</td>
<td>CS</td>
<td>13</td>
<td>5</td>
<td>38</td>
<td>0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

* Number and percentage of rats showing change (average changes).
* Scale: 0 to ++++ (average changes).
* Some nodules are oncocytic (25 to 30%).

5A), renal cell carcinomas (Fig. 5B), and squamous cell carcinomas of the skin and of the maxilla (Fig. 5C). These tumors were present in the R16 rats on both the CD and CS diets, but none of the ACP rats developed any such tumors. The effect of the CD diet on the R16 livers was seen in the form of cirrhosis in 72% (17% in the CS diet group), benign cholangiomas in 56% (17% in the CS diet group), and hyperplastic nodules in 67% (33% in the CS diet group) of the rats examined. This effect was less severe in the ACP strain. Only 17% of the rats developed benign cholangiomas, and no other pathological changes occurred in either the CD or the CS groups.

At 12 mo (Table 6; Fig. 6), hepatocellular carcinomas were present in 68% of the R16 rats fed the CD diet and in 29% of the R16 rats fed the CS diet; only 6% of the ACP rats on the CD diet and none on the CS diet developed hepatocellular carcinomas. Other malignancies were present in the R16 rats only, and they consisted of renal cell and squamous cell carcinomas in 23% of the rats on the CD diet and renal cell carcinoma, adenocarcinoma of the lung, and squamous cell carcinomas in 21% of the rats on the CS diet. No extrahepatic
Fig. 4. Pathological changes in the R16 strain after the injection of DEN and 6 mo on a CD diet. Nonhepatic malignant tumors began to appear at this time: liposarcoma (A) and rhabdomyosarcoma (B) of the abdomen and pelvis. The liver showed cirrhosis with oncocytic nodules (C) that have numerous cytoplasmic mitochondria (C insert) by electron microscopy. H & E. × 250 (A, B) and × 400 (C). Electron microscopy, × 1650 (C insert).

Table 5 Liver histology and other malignancies 10 mo after a single i.p. dose of DEN

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diet</th>
<th>n</th>
<th>n %</th>
<th>n</th>
<th>n %</th>
<th>n</th>
<th>n %</th>
<th>n</th>
<th>n %</th>
<th>Type</th>
<th>% of rats with other malignancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>R16</td>
<td>CD</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 large cell lymphomas</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>with widespread metastases</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 renal cell carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 pheochromocytoma of the adrenal gland</td>
<td></td>
</tr>
<tr>
<td>R16</td>
<td>CS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>ACP</td>
<td>CD</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 squamous cell carcinoma of the maxilla</td>
<td>25</td>
</tr>
<tr>
<td>ACP</td>
<td>CS</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 renal cell carcinomas</td>
<td></td>
</tr>
</tbody>
</table>

* Number and percentage of rats showing the changes (average changes).
  * Some are oncocytic (25 to 30%).
  * Some rats had more than one type of hepatic tumor.
  * One rat developed two primary malignant tumors: hepatocellular carcinoma and renal cell carcinoma.

Tumors were seen in the ACP strain. Other changes (cirrhosis, hyperplastic nodules, and benign cholangiomas) were also more pronounced in the R16 strain. The effect of diet alone was tested by feeding the CD or the CS diet for 12 mo (Table 7). The R16 rats fed the CD diets showed some periportal fibrosis and fatty infiltration in the liver, and the ACP rats showed only minimal fatty changes. The CS diet had very little effect on either the R16 or the ACP strain. The number of GTT-positive foci did not exceed 1 to 2/cm² in the R16 or the ACP rats fed either the CD or the CS diet; Yokoyama et al. (39) reported a similar finding. An extrahepatic malignant tumor occurred in one R16 rat and in one ACP rat fed a CD diet.

Further evidence that non-MHC genes do not influence the susceptibility of the R16 strain to DEN-induced carcinogenesis comes from studies using the R16/+ heterozygote and the R16 strain.
Fig. 5. Nonhepatic pathological changes in the R16 strain after the injection of DEN and 10 mo on a CD diet. The major ones were large cell lymphoma with lung metastases (A and A insert), mesenchymal renal cell carcinoma with numerous mitoses (B and B insert), and invasive squamous cell carcinoma of the maxilla (C and C insert). H & E. × 100 (A), × 400 (A insert), × 160 (B), × 400 (B insert), × 100 (C), and × 400 (C insert).

Table 6 Liver histology and other malignancies 12 mo after a single dose of DEN

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diet</th>
<th>Cirrhosis</th>
<th>Nodules</th>
<th>Total no. of rats with hepatic tumors</th>
<th>Benign</th>
<th>Hepatocellular carcinoma</th>
<th>Other malignancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>R16</td>
<td>CD</td>
<td>22</td>
<td>18</td>
<td>82</td>
<td>18</td>
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<td>22</td>
</tr>
<tr>
<td>ACP</td>
<td>CD</td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>R16</td>
<td>CS</td>
<td>24</td>
<td>9</td>
<td>38</td>
<td>20</td>
<td>83</td>
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<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* Number and percentage of rats showing the changes (average changes).
* Some are oncogenic (25 to 30%).

× ACP F1 hybrid. Two mo after feeding DEN, neither of them showed any of the stigmata of hepatocarcinogenesis in contrast to the findings with the R16 strain (Table 2). This observation supports the conclusion that the susceptibility of the R16 strain to DEN is due to the grc alone and does not involve non-MHC genes. It also suggests that the effect of the grc is due to the loss of recessive tumor suppressor genes.

DISCUSSION

The differences in susceptibility to carcinogens in different species and in different strains of the same species have been reported (12–21, 33), but no detailed genetic analyses have been done. The results presented here show that two inbred, genetically related, and well-characterized strains of rats differ dra-
Fig. 6. Liver of the R16 strain after the injection of DEN and 10 or 12 mo on a CD diet (changes were the same at both times). There are well-differentiated hepatocellular carcinomas (A and A insert) with metastases to the lungs (B). H & E, × 250 (A), × 400 (A insert), and × 100 (B).

Table 7 Liver histology 12 mo after receiving the diets only

Animals were given a single i.p. dose of 0.9% saline solution, but no DEN.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diet</th>
<th>Spongiosis</th>
<th>Cellular atypia</th>
<th>Cirrhosis</th>
<th>Hyperplastic nodules</th>
<th>Benign cholangioma</th>
<th>Hepatocellular carcinoma</th>
<th>Other malignancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>R16</td>
<td>CD</td>
<td>13</td>
<td>7</td>
<td>54</td>
<td>+ to +++</td>
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<td>1</td>
</tr>
<tr>
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<td>11</td>
<td>1</td>
<td>9</td>
<td>+ to ++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R16</td>
<td>CS</td>
<td>8</td>
<td>7</td>
<td>87</td>
<td>+ to ++</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ACP</td>
<td>CS</td>
<td>8</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

* Number and percentage of rat livers showing change (average changes).
* Scale: 0 to +++ (average changes).
* Some nodules are oncocytic (25 to 30%).
* There was one squamous cell carcinoma in an ACP rat and one lymphoma in an R16 rat, both on a CD diet.
matically in their susceptibility to the development of cancer following exposure to one dose of DEN. The R16 strain (grc) is very sensitive to the effects of DEN with or without the additional stimulus of a CD diet, whereas its wild-type counterpart, the ACP strain (grc + ), is not. As shown in Table 1, the major genetic differences between these two strains is the presence of the grc in the R16 strain. The genes of the MHC itself are the same, and the R16 strain is heterozygous for the non-MHC genes of the ACP strain. The latter fact makes it highly unlikely that non-MHC genes are involved in the susceptibility to DEN carcinogenesis. Further support for this conclusion comes from our previous observation (31) that, following exposure to AAF, the R10 strain (grc) developed GGT*-foci in the liver but the BI strain (grc + ) did not, despite the fact that both strains have the same non-MHC genes (Table 1).

These observations implicate genes in the grc region as playing an important role in susceptibility to DEN carcinogenesis, and recent studies on the molecular analysis of the grc (32, 40) suggest that these genes may be cancer suppressor genes or antioncogenes. The grc genotype is associated with the deletion of at least 3.1 kilobases of DNA within the region of the chromosome linked to the MHC, and this deletion is associated with all of the characteristics of the grc phenotype (36, 40, 41). Its effects are not limited to the development of cancer in the liver, as in other animals exposed to DEN, but extend to the development of malignancies in a variety of other organs. This is a characteristic that would be expected of a strain that is genetically susceptible to the development of cancer, since this susceptibility would affect organs other than the major target organ of the specific carcinogen. Thus, the grc-bearing rats and their wild-type counterpart provide an experimental system in which to study the genetic susceptibility to cancer involving a mechanism similar to the development of retinoblastoma in humans in which susceptibility is due to a chromosomal deletion and in which malignancies are found in organs other than the eye, i.e., bone, breast, and lung (8, 42–45).

A choline-deficient diet can be a promoter (25, 37) and, if fed for a longer time, an inducer (26–28) of tumor formation. In the pathogenesis of DEN-induced cancer in the R16 strain, the role of the CD diet was one of accelerating the process and increasing its severity, but it was not necessary for the induction of cancer because the R16 rats on the CS diet also developed cancer. This effect of the CD diet in the R16 strain can be seen in the greater development of GGT*-foci, hepatocellular carcinomas and other malignancies (Fig. 1), and in the generally increased levels of fatty infiltration, cirrhosis, hyperplastic nodules, and benign cholangiomas in the liver (Tables 2 to 6). It had no effect on the ACP strain which remained resistant to the development of cancer. Combining the data at 10 and 12 mo, on the CD diet, 3% (one of 30) of ACP rats developed hepatocellular carcinoma but no other type of cancer, whereas 50% (20 of 40) of R16 rats developed hepatocellular carcinoma, and 22% (9 of 40) developed other malignancies. On the CS diet, no (0 of 25) ACP rats developed any type of cancer, whereas 28% (10 of 36) of R16 rats developed hepatocellular carcinoma, and 22% (8 of 36) developed other types of malignancies. Feeding the CD diet without having given DEN had no significant effect on the development of cancer.

In summary, then, the R16 strain, which carries the grc, is highly susceptible to the development of cancer following exposure to DEN whether or not a CD diet is used. These findings support the genetic formulation of the two-hit theory of carcinogenesis (8) and indicate that the MHC-linked recessive genes that affect growth and development also affect susceptibility to cancer. Genetic predisposition accounts for the first somatic hit, and it affects all of the organs. It is followed by exposure to a carcinogen leading to malignancies in the carcinogen target organ and in other organs as well.

REFERENCES

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Genetic Control of Susceptibility to DiethylNitrosamine Carcinogenesis in Inbred ACP (grc+) and R16 (grc) Rats


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