Quantitative Analysis of Genetic Susceptibility to Liver and Lung Carcinogenesis in Mice

Tommaso A. Dragani, Giacomo Manenti, and Giuseppe Della Porta

Division of Experimental Oncology A, Istituto Nazionale Tumori, Milan 20133, Italy

ABSTRACT

One-week-old male and female mice of the A, BALB/c (C), and C3H/He (C3) strains and of the AC3 and CC3 F1 hybrids were treated with a single dose (300 mg/kg s.c.) of urethan and then kept without further treatment until 30 and 40 weeks (males) or 30 and 65 weeks (females). The degree of difference in susceptibility to hepatocarcinogenesis between the susceptible C3 and the resistant A and C mice, in the different age and sex groups, was 4–12-fold by the analysis of the number of nodules/cm² (N/cm²), which represents an estimation of tumor frequency and was more than 400-fold as indicated by the percentage of liver volume occupied by nodules (%), an estimation of tumor size. With regard to lung carcinogenesis, mice of the A strain proved 5–10- and 20–70-fold more susceptible than the C and the C3 mice, respectively, as indicated by the number of microscopically identified lung tumors/mouse (N), an estimation of tumor frequency. The lung tumor size, as estimated by the mean tumor volume (V), was similar in A and C mice but much higher in the A than in the C3 groups (13–1000-fold difference). The AC3 hybrid was highly susceptible to both liver and lung carcinogenesis. The CC3 hybrid was as susceptible to lung carcinogenesis as its C parent and had an intermediate susceptibility to hepatocarcinogenesis. Our results indicate that a major determinant in the genetic susceptibility to hepatocarcinogenesis, and perhaps to lung carcinogenesis too, is tumor growth, as evidenced by a much greater tumor size in genetically susceptible than resistant mice.

INTRODUCTION

The genetics of liver and lung tumor susceptibility has been extensively studied in the mouse, taking advantage of differences observed in the many available inbred strains. Tumor susceptibility refers to the development of tumors either without a specific exogenous stimulus or upon treatment with a known carcinogen. In both instances, susceptibility is not absolute and refers to relative quantitative values. Strains genetically susceptible to hepatocarcinogenesis comprise C3H/He, CBA, and CF-1, and resistant strains include C57BL/6J and BALB/c (1–10). Strains genetically susceptible to lung tumorigenesis comprise A, SWR, and BALB/c mice, and among resistant ones are C57BL/6J and C3H/He (3, 4, 11). Susceptibility to liver and lung carcinogenesis is a dominant or a semidominant genetic trait, because F1 hybrids between susceptible and resistant mice are susceptible (3, 9, 11, 12).

Quantitative estimations of the degree of difference in susceptibility to hepatocarcinogenesis among strains showed wide variations, depending on the examined parameters, e.g., tumor incidence, tumor multiplicity, and tumor size (3, 10, 13–17), whereas for lung carcinogenesis analyses were almost exclusively based on the number of macroscopically counted lung tumors/mouse and indicated a 30–90-fold difference between genetically susceptible and resistant mice (11). Drinkwater and Ginsler (15) found that a single locus (named Hcs) accounted for about 80% of the difference in susceptibility to diethylnitrosamine- or ethylnitrosourea-induced liver carcinogenesis between the C3H/He and C57BL/6J strains and that another locus may play a minor role. They used as an index of susceptibility the number of liver tumors >2 mm in diameter/animal (tumor multiplicity). Malkinson et al. (18) using urethan-treated recombinant inbred strains between A/J and C57BL/6J mice indicated that three loci are involved in the control of genetic susceptibility to lung tumorigenesis.

However, an analysis of the number of genes involved in the control of genetic susceptibility to liver and lung carcinogenesis should include microscopic nodules to distinguish between animals carrying no tumors and animals with hundreds of microscopic neoplastic lesions that in a few weeks or months can grow to large tumors (19–23). Quantitative parameters should allow estimation of tumor frequency and size, because the genetically susceptible phenotype may result from a high susceptibility either to initiation (high tumor number) or to progression (high tumor size). We and others have previously suggested that the gene or the genes responsible for the genetic susceptibility to murine hepatocarcinogenesis may control nodule growth, but not tumor frequency (16, 17).

To further analyze susceptibility to liver and lung carcinogenesis we have treated preweanling mice with urethan, a carcinogen which induces multiple types of tumors in rodents, including liver and lung tumors (24, 25). In mice, a correlation has been reported between the susceptibility to the development of neoplasms at specific sites after urethan treatment and the degree of spontaneous tumor incidence in that particular organ (3, 13, 26, 27).

As quantitative indexes of susceptibility to liver carcinogenesis, we used the number of nodules/cm² (N/cm²) at different cutoffs for radii and the percentage of liver volume occupied by nodules (%). For the analysis of lung carcinogenesis, we tested the number of microscopically identified tumors/mouse (N), the mean tumor volume (V), and the total tumor volume (N x V).

MATERIALS AND METHODS

Animals and Treatments. Male and female C3H/He (C3) and BALB/c (C) mice were purchased from Bantin and Kingman (Hull, United Kingdom), A/J/Ola (A) mice were purchased from Olac Ltd. (Oxon, United Kingdom). Mice were then bred in our laboratory, and male C3 mice were crossed with A and C females to obtain A/J/Ola × C3H/He F1 (AC3) and BALB/c × C3H/He F1 (CC3) mice, respectively. Groups of 72–93 mice from each strain/hybrid were treated at 1 week of age with a single s.c. dose of 300 mg/kg body weight urethan (BDH, Poole, United Kingdom; diluted in 9.9% NaCl solution). After weaning, the mice were kept under observation. About 50% of male and female mice of each strain/hybrid were killed at 30 weeks of age, whereas the remaining males were killed at 40 weeks of age and the remaining females at 65 weeks of age. They were fed a standard pellet diet (Piccioni, Gessate, MI, Italy) and acidified tap water ad libitum and were housed in plastic cages in a temperature- and humidity-controlled room.

Received 5/1/91; accepted 9/19/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by grants from PF CNR "Oncologia" and Associazione Italiana Ricerca Cancro.

2 To whom requests for reprints should be addressed, at Istituto Nazionale Tumori, Via G. Venezian 1, 20133, Milan, Italy.

6299
Liver and Lung Carcinogenesis

Hepatocellular Nodules and Tumors. When the mice were killed, livers were removed, examined for gross lesions, fixed in Bouin’s solution, embedded in paraffin, cut, and stained with hematoxylin and eosin for histopathological examination. For each mouse, hepatocellular nodules equal or larger than 115 μm in diameter were counted in two sections, one from the left and one from the median liver lobe, and their size was measured (16, 28). The nodules with diameters larger than 5 mm observed in the whole liver were microscopically classified as adenomas and carcinomas (29).

The size distribution of nodules was calculated using the smoothing procedure and increasing values for the cutoffs for radii (30, 31). The cutoff of 120 μm allowed estimation of almost the total N/cm² value, whereas larger cutoff values provided estimation of the N/cm² of nodules with size larger than the cutoff. In the size distribution analysis, the percentages of lesions larger than the indicated cutoff values were calculated by dividing the mean N/cm² value at that cutoff by the N/cm² value at 120 μm, considered as the 100% fraction. The percentage of liver volume occupied by nodules (V%) was calculated as described previously (28, 32).

Lung Tumors. When the animals were killed, lungs were filled with 0.5–1 ml Bouin’s fixative by intratracheal injection, removed, and immersed in Bouin’s solution. Then, they were embedded in paraffin, cut, and stained with hematoxylin and eosin for histopathological examination.

For each mouse we counted the number and measured the diameter of lung tumors in one section including all the lung lobes. Since most of the tumors appeared roughly circular in tissue sections, it was decided to calculate their volume by assuming that the tumors were spherical. The quantitative analysis of lung tumor susceptibility cannot be based on the stereological analysis of lung tumors, because the lung is not a solid organ and measurement of lung section areas is unreliable since it depends on the degree of distension of the lung alveoli. Therefore, we used the number of microscopically identified lung tumors/mouse (N). This parameter underestimated lung tumor frequency in mice carrying small tumors, because profiles of large tumors appear more frequently in tissue sections than do small ones (30, 32). We estimated lung tumor size by measuring mean lung tumor volume (V), sum of the individual tumor volumes, divided by the number of tumors and total lung tumor volume (N × V).

RESULTS

The single 300-mg/kg body weight dose of urethan administered to 1-week-old mice was well tolerated, since a total of 391 of 401 (97.5%) mice survived the treatment to weaning. The mice were kept under observation without further treatment. Mice dying before the end of the experiments were excluded from calculations. They included: among the males 4 C3, 2 A, 1 C, and 1 AC3; and among the females 2 C3, 6 A, 2 C, 3 AC3, and 1 CC3. One C3 and one C male mouse killed at 40 weeks of age bore a diffuse lymphoma and were excluded from calculations.

The incidence of liver nodules of any size, the N/cm² at cutoff for radii of 120 μm, the V% of liver nodules, the number of hepatocellular adenomas/mouse and that of carcinomas/mouse, and the incidence, N, V, and (N × V) of lung tumors are reported in Table 1 for the different strains/hybrids and sexes.

Liver

Males, 30 Weeks. All C3, AC3, and CC3 mice had liver nodules, whereas C mice showed a 26% incidence, and no nodules were observed in the A strain. The N/cm² was similar in C3 and AC3 mice (63 and 51, respectively), slightly lower in CC3 mice (29), and much lower in C mice (0.7). The V% showed wide variations among the strains/hybrids, with the C3 mice showing the highest value (24.8), the AC3 an intermediate value (4.18), and the CC3 and the C mice the lowest values (0.26 and 0.03, respectively). Adenomas larger than 5 mm in diameter were frequent in C3 mice (1.4/mouse) but rare in the other strains. A few carcinomas were seen only in the C3 mice.

Males, 40 Weeks. The 100% incidence of nodules was maintained in C3, AC3, and CC3 mice; the C mice showed a 40% incidence; and the A mice, which had no detectable nodules at 30 weeks, showed a 74% incidence. The N/cm² was similar in C3, AC3, and CC3 mice, i.e., from 44 to 45, but it was lower in C3 mice (1.4/mouse) than in C mice (1.0). The V% of AC3 mice almost reached the same value observed in the C3 strain (35 and 47).

Table 1 Liver and lung tumor susceptibility in male and female A, C, C3, AC3, and CC3 mice treated once with urethan (300 mg/kg s.c.) at 1 week of age and sacrificed at 30, 40, and 65 weeks of age

<table>
<thead>
<tr>
<th>Strain/hybrid</th>
<th>Sex</th>
<th>Age (wk)</th>
<th>No. of mice</th>
<th>Incidence</th>
<th>N/cm²</th>
<th>V%</th>
<th>Adenomas/ mouse</th>
<th>Carcinomas/ mouse</th>
<th>N × V</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>30</td>
<td>18</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>C</td>
<td>30</td>
<td>19</td>
<td>26</td>
<td>0.7</td>
<td>0.03</td>
<td>± 0.02</td>
<td>± 0.02</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>M</td>
<td>30</td>
<td>22</td>
<td>100</td>
<td>63.0</td>
<td>24.77</td>
<td>± 0.04</td>
<td>24.77 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>AC3</td>
<td>M</td>
<td>30</td>
<td>19</td>
<td>100</td>
<td>51.0</td>
<td>4.18</td>
<td>± 0.11</td>
<td>4.18 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>CC3</td>
<td>M</td>
<td>30</td>
<td>16</td>
<td>100</td>
<td>28.9</td>
<td>4.26</td>
<td>± 0.07</td>
<td>4.26 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>M</td>
<td>40</td>
<td>19</td>
<td>74</td>
<td>11.8</td>
<td>3.06</td>
<td>± 0.02</td>
<td>3.06 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>40</td>
<td>20</td>
<td>40</td>
<td>3.7</td>
<td>0.10</td>
<td>± 0.06</td>
<td>0.10 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>M</td>
<td>40</td>
<td>17</td>
<td>100</td>
<td>44.1</td>
<td>4.70</td>
<td>± 0.03</td>
<td>4.70 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>AC3</td>
<td>M</td>
<td>40</td>
<td>18</td>
<td>100</td>
<td>45.0</td>
<td>3.54</td>
<td>± 0.02</td>
<td>3.54 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>CC3</td>
<td>M</td>
<td>40</td>
<td>13</td>
<td>100</td>
<td>43.8</td>
<td>2.52</td>
<td>± 0.74</td>
<td>2.52 ± 0.74</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>F</td>
<td>30</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>C</td>
<td>30</td>
<td>20</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>F</td>
<td>30</td>
<td>15</td>
<td>93</td>
<td>29.1</td>
<td>0.29</td>
<td>± 0.09</td>
<td>± 0.09</td>
<td></td>
</tr>
<tr>
<td>AC3</td>
<td>F</td>
<td>30</td>
<td>22</td>
<td>86</td>
<td>26.5</td>
<td>0.05</td>
<td>± 0.01</td>
<td>± 0.01</td>
<td></td>
</tr>
<tr>
<td>CC3</td>
<td>F</td>
<td>30</td>
<td>22</td>
<td>9</td>
<td>1.7</td>
<td>0.7</td>
<td>± 0.04</td>
<td>± 0.04</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>F</td>
<td>65</td>
<td>12</td>
<td>25</td>
<td>5.7</td>
<td>0.07</td>
<td>± 0.04</td>
<td>± 0.04</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>F</td>
<td>65</td>
<td>18</td>
<td>22</td>
<td>2.2</td>
<td>0.01</td>
<td>± 0.00</td>
<td>± 0.00</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>F</td>
<td>65</td>
<td>19</td>
<td>100</td>
<td>24.3</td>
<td>4.58</td>
<td>± 0.04</td>
<td>4.58 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>AC3</td>
<td>F</td>
<td>65</td>
<td>21</td>
<td>100</td>
<td>58.7</td>
<td>1.63</td>
<td>± 0.02</td>
<td>1.63 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>CC3</td>
<td>F</td>
<td>65</td>
<td>22</td>
<td>100</td>
<td>67.2</td>
<td>1.02</td>
<td>± 0.04</td>
<td>1.02 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE.

N/cm² and V% evaluated at cutoff for radii of 120 μm.
respectively), whereas at 30 weeks it was \( \approx 6 \) -fold lower in AC3 than in C3 mice. The CC3 hybrid showed a 11-fold lower \( V\% \) value (2.8) when compared to the AC3 hybrid (33) but, compared with its C parental strain, CC3 mice showed a \( \approx 29 \)-fold higher \( V\% \) value. Adenomas were seen at a higher frequency in C3 (0.8/mouse), followed by AC3 (0.3/mouse) and by CC3 (0.1/mouse) mice, but they were not seen in A and C mice. Carcinomas were relatively frequent in C3 (2.5/mouse) and AC3 (1.8/mouse) mice but were absent in the other mice. The frequency of carcinomas resulted higher than that of adenomas in the C3 and AC3 mice.

As a means of comparing the relative growth rates of hepatic lesions, the percentages of lesions larger than the indicated size cutoff are plotted in Fig. 1A. The 100% fraction represents the \( N/cm^3 \) value at 120 \( \mu \)m cutoff. In A and C mice, the \( N/cm^3 \) values rapidly decreased with increasing size cutoff. At a cutoff of 360 \( \mu \)m only 15–22% of A and C nodules, respectively, were present. At a cutoff of 600 \( \mu \)m, 0–5% of A and C nodules were seen. On the other hand, nodules with radii \( >720 \mu \)m represented more than 30% of the total number of C3 and AC3 nodules. The CC3 mice, compared with the C3 and AC3 ones, showed a more rapid decrease in \( N/cm^3 \) values, but their fraction of lesions remained higher than those of the A and C mice at any cutoff.

**Females, 30 Weeks.** Mice with detectable nodules of any size comprised 86 and 93% of AC3 and C3 mice, respectively, but only 9% (2 mice) of the CC3 hybrid and none of the A and C mice. The \( N/cm^3 \) was similar in C3 and AC3 mice (29 and 26, respectively), with half the values of those observed in the corresponding males at 30 weeks. The \( N/cm^3 \) in CC3 mice was much lower (1.7) than that in the C3 and AC3 females. The \( V\% \) was 0.29 in C3 and 0.05 in AC3 mice and 78–97-fold lower than in their corresponding males at 30 weeks. No adenomas or carcinomas were seen in any strain/hybrid.

**Females, 65 Weeks.** The incidence of nodules reached 100% in C3, AC3, and CC3 mice and 25 and 22% in A and in C mice, respectively. The \( N/cm^3 \) values among C3, AC3, and CC3 mice were similar, i.e., 24, 59, and 67, respectively. These values were also similar to, or even higher than, those seen in the corresponding male groups at 40 weeks. Again, A and C mice showed the lowest \( N/cm^3 \) values: 5.7 and 2.2, respectively. The \( V\% \) values showed wide variations among C3, AC3, and CC3 mice, 53.6, 16.13, and 1.66, respectively. A and C mice showed very low \( V\% \) values, i.e., 0.07 and 0.01, respectively. Comparing the A and C3 strains, the \( N/cm^3 \) was \( \approx 4 \)-fold lower but the \( V\% \) was 800-fold lower in the A than in the C3 mice. Adenomas were seen at about the same frequency in C3 and AC3 mouse, but not in the other strains. Carcinomas were seen only in C3 and AC3. The number of carcinomas/mouse was higher in the C3 (2.8) than in the AC3 (0.3) group.

The percentages of lesions larger than the indicated size cutoff are shown in Fig. 1B. A small percentage of A and C lesions appertained to the large size classes. In fact, only 14 and 0% of A and C nodules, respectively, had radii larger than 240 \( \mu \)m. Most of the C3 nodules were large, since nodules with radii \( >720 \mu \)m represented 63% of the C3 nodules. The fraction of AC3 lesions larger than 720 \( \mu \)m was 19%, i.e., lower than that of C3 mice, but higher than that of the other strains. In analogy with the male group, the \( N/cm^3 \) values in CC3 mice decreased more rapidly than in C3 and in AC3 mice, over the increasing cutoffs.

Lung

**Males, 30 Weeks.** The incidence of lung tumors ranged from 9% (C3) to 94–95% (A, AC3) mice, with intermediate values in C and in CC3 mice (37–38%). The number of tumors/mouse (\( N \)) was higher in A and AC3 mice (4.3 and 1.7, respectively) than in the C, CC3, and C3 mice (0.5, 0.6, and 0.1, respectively). The mean tumor volume (\( V \)) was also higher in A and AC3 mice (0.54 and 0.62, respectively) than in C, CC3, and C3 mice (0.43, 0.18, and 0.01, respectively). The total tumor volume (\( N \times V \)) followed the same pattern of the other parameters, with values of 2.14, 1.14, 0.5, 0.24, and 0.01 in A, AC3, C, CC3, and C3 mice, respectively.

**Males, 40 Weeks.** The picture among the different strains/hybrids was not much different from that seen at 30 weeks, with a slight increase in all parameters. The \( N \times V \) values were 6.89 and 8.73 in A and AC3 mice, respectively; 1.0 and 1.3 in C and CC3 mice, respectively; and 0.0004 in C3 mice.

**Females, 30 Weeks.** The incidence as well as the \( N \), \( V \), and \( N \times V \) values were quite similar to those observed in the male groups at 30 weeks of age for each strain/hybrid, without exceptions. The \( V \) and \( N \times V \) values were slightly lower in the female groups than in the corresponding male groups.

**Females, 65 Weeks.** All the parameters evaluating lung tumor response showed increased values with respect to those seen at 30 weeks, in all strains/hybrids. A and AC3 mice had a 100%
lung tumor incidence; C and CC3 had 78% and 91%, respectively; and C3 mice had a 37% incidence. The N value was high in A and AC3 mice (8.8 and 4.8, respectively), intermediate in C and in CC3 mice (1.9 and 2.6, respectively), and low in C3 mice (0.4). The tumor volume was also higher in A and in AC3 mice (5.37 and 5.19, respectively), than in C and CC3 mice (2.19 and 1.82, respectively) and in C3 mice (0.41). The \((N \times V)\) values showed the highest change with respect to 30 weeks. In A and AC3 mice we observed \((N \times V)\) values of 47.75 and 31.49, respectively, i.e., a \(\approx\)40 fold increase with respect to 30 weeks. \(N \times V\) was 4.95 and 4.43 in C and in CC3 mice, respectively but only 0.42 in C3 mice.

DISCUSSION

We found, according to our expectation, that the C3 mice were highly susceptible to hepatocarcinogenesis but resistant to lung tumorigenesis and that the A and C mice were resistant to hepatocarcinogenesis but susceptible to lung carcinogenesis. The AC3 hybrid resembled the parental strains for the high susceptibility to both liver and lung tumor development. The susceptibility of the CC3 hybrid to lung tumorigenesis resembled that of the C parent, but its susceptibility to hepatocarcinogenesis, although higher than that of C mice, was much lower than that of the C3 parent and of the AC3 hybrid.

When C3 mice were compared with A and C mice, the degree of difference in susceptibility was only 4–12-fold as estimated by the \(N/cm^3\) values but was more than 400-fold by the \(V\%\) values (males, 40 weeks; females, 65 weeks). The present observation in urethan-treated mice confirms previous findings indicating that a major determinant in the genetic susceptibility to hepatocarcinogenesis is tumour growth (16, 17). Size distribution analysis demonstrated that nodules of large size represented a much higher fraction of C3 and AC3 than of A, C, and CC3 lesions (Fig. 1). Therefore, we should conclude that all nodules of C3 and AC3 mice had a predisposition to grow faster.

Interestingly, the male AC3 mice killed at 30 weeks showed a \(\approx\)6-fold lower \(V\%\) value than the C3 mice, but at 40 weeks the same male AC3 mice resembled their C3 parent in the \(V\%\) value (34.55 and 47.4 in AC3 and in C3, respectively). This observation implies that AC3 liver nodules grew at a slower rate than C3 nodules from initiation to 30 weeks but that they grew at a much faster rate from 30 to 40 weeks, suggesting that their growth depended on the achievement of a critical size. As genetic changes that are responsible for the size-dependent growth of AC3 nodules, we can hypothesize the production of angiogenic factors or the loss of genes associated with tumor progression, as demonstrated in other systems (33, 34).

The much lower susceptibility to hepatocarcinogenesis of the CC3 hybrid than that of its C3 parent, as estimated by the nodule size (\(V\%\) values) and by size distribution analysis, indicates that the expression of the genes responsible for the genetic susceptibility to hepatocarcinogenesis is somehow restricted in the F1 progeny by the C cross. Many possible genetic mechanisms can explain these findings. Therefore, the genetics of susceptibility to murine hepatocarcinogenesis appears to be more complex than that in which only one gene would play a major role (15).

Female mice are far less susceptible to liver carcinogenesis than male mice, due to a differential sex-related endogenous promotion of the initiated lesions, and our results are in agreement with previous findings (35–39). Herein we show that the overall frequency of detectable nodules (minimal cutoff value) was not much different between male and female mice. In fact, at 30 weeks of age, the \(N/cm^3\) values in female mice of the C3 and AC3 strains were about one-half of those observed in male mice of the same strains. However, liver nodules grew faster and resulted of greater size in males than in females, as shown by the \(\approx\)80-fold higher \(V\%\) values in males than in females of both strains. This is in favor of an hormonal control of nodule growth but not of initiation frequency.

Hanigan et al. (17) suggested that the Hcs locus, responsible for the difference in susceptibility between C3H/HeJ and C57BL/6J mice, is preferentially expressed in male mice relative to female mice. In fact, they have seen minor or no differences in the liver tumor multiplicity and growth rate of glucose-6-phosphatase-deficient hepatic foci among ethylnitrosourea-treated female C3H/He and C57BL/6J mice. However, their conclusions are based on observations carried out at early time points (12 to 20 weeks of age). Our results are at variance because we have shown that genetic susceptibility to liver carcinogenesis is not sex dependent, i.e., both males and females of a genetic susceptible strain/hybrid (C3, AC3) have a much higher susceptibility to hepatocarcinogenesis than the corresponding males or females of a genetic resistant strain, provided that the observation period is sufficiently long (65 weeks) to allow expression of the susceptible phenotype.

As expected, A mice were more susceptible to lung carcinogenesis than C mice and both A and C strains were more susceptible than the C3 strain (11). The quantitative degree of difference was 5–10-fold between A and C strains and 20–70-fold between A and C3 strains, as estimated by the \(N\) values. The lung tumor size, as estimated by the mean tumor volume (\(V\)), was similar in A and C mice (1.1–2.5-fold difference), but much higher in A than in C3 mice (13 to \(\approx\)1000-fold difference). Therefore, the difference in susceptibility to lung carcinogenesis between A and C mice appeared to reside in the lung tumor frequency, not in the lung tumor size. On the other hand, differences between A strain and the resistant C3 strain were in both lung tumor frequency \(N\) and size \(V\). We cannot rule out the possibility that urethan initiation frequency was similar in the lungs of the two strains and that initiated lung cells of the C3 strain cannot express their transformed phenotype, due to C3-specific lung tumor suppressor genetic factors. Additional studies are needed to clarify whether the genetic difference in lung carcinogenesis between A and C3 mice resides in the initiation or in the progression phase.

The total lung tumor volume \((N \times V)\), a parameter which is a function of both tumor frequency and size, gave a good estimation of tumor progression, because it showed an age-dependent increase. The increase was particularly apparent from 30 to 65 weeks (female groups) as evidenced by a \(\approx\)40-fold increase in A, AC3, and CC3 mice and by an 18-fold increase in the C mice. In the same time period, \(N\) increased by only 3–6-fold in the same strains.

The susceptibility to lung carcinogenesis was dominant in the F1 hybrids, and quantitatively it was very similar to that of their susceptible parental strain (A or C), with minor differences in the various parameters estimating tumor response, i.e., with variations from 0.4- to 3-fold. However, our observations refer to a single dose. Comparison of susceptibility between F1 hybrids and parental strains may differ with dosage. In fact, other reports showed that F1 mice were of intermediate lung tumor sensitivity (11, 40).

In conclusion, we have examined different quantitative pa-
rameters to evaluate susceptibility to liver and lung carcinogenesis in murine strains and in their F1 hybrids. Our results showed that for the analysis of susceptibility to both liver and lung carcinogenesis, the most sensitive estimation is given by the parameters taking into account both tumor frequency and size, i.e., the V% and the (N × V) in liver and lung carcinogenesis, respectively. A quantitative estimation of tumor response is a requisite for building hypotheses on the pathogenesis of susceptibility and also for the analysis of the genes involved in susceptibility.

ACKNOWLEDGMENTS

The authors wish to thank Graziella Pasquini, F. Stefania Falabella, and Umberto Rubertelli for their valuable technical assistance and Sergio Dragani for the gift of the computer programs for the stereological analysis of liver nodules and for the calculations of lung tumor parameters.

REFERENCES


Quantitative Analysis of Genetic Susceptibility to Liver and Lung Carcinogenesis in Mice

Tommaso A. Dragani, Giacomo Manenti and Giuseppe Della Porta


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/23_Part_1/6299

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.