Transplacental Lung Tumorigenesis in the Athymic Mouse

Lučy M. Anderson, John M. Budinger, R. R. Maronpot, and Robert A. Good

ABSTRACT

Female BALB/c nu/+ mice, pregnant by nu/+ males (nu: gene for hairlessness-athymia) were given injections of urethan, a transplacental tumorigen, on Day 17 or 19 of gestation. After an average of 16 weeks under clean conventional conditions, the incidence of primary lung tumors was similar in nude and normal offspring treated with carcinogen on either gestational day, with a higher incidence after treatment on Day 19. Thus, the absence of thymus did not affect the occurrence of transplacently induced primary lung tumors or alter the well-known perinatal increase in sensitivity.

Histologically, the nu/nu tumors differed from normal in the appearance of many atypical basophilic cells and in a tendency to invade both the parenchyma and the pleural surface. These results suggested progression of the lung adenomas to a more atypical, invasive form, a progression that may have occurred prematurely in the absence of thymus-dependent immune response.

INTRODUCTION

The incidence and growth of the alveologenic primary lung tumor (adenoma) of the mouse have been found to be subject to immune system control [see the recent review by Shimkin and Stoner (13)]. However, athymic nude (nu/nu) mice responded to methylcholanthrene or urethan, administered in the first week of life, with an incidence and multiplicity of lung adenomas similar to those of phenotypically normal (nu/+ ) animals (14, 15). The incidence of adenomas induced transplacently with ethylnitrosourea was also the same in nu/nu and nu/+ offspring (16).

We have extended the study of lung tumor induction in the nude mouse to include those caused by a single transplacental exposure to urethan and have examined both the incidence and the histological characteristics of the tumors appearing in nude and normal littermates. While the incidence of tumors was similar in nude and normal progeny, there were consistent differences in their histological appearance; the nude tumors appeared to be more invasive and atypical.

MATERIALS AND METHODS

Female and male nu/+ mice (BALB/c background; 4th backcross) were obtained from the Sloan-Kettering colony. Impregnation was dated by the appearance of a vaginal plug (Day 1). Urethan (Sigma Chemical Co., St. Louis, Mo.) in sterile deionized water was injected i.p. on Day 17 (1.0 mg/g) or on Day 19 (0.1, 0.5, or 1.0 mg/g) of gestation. Controls were given injections of sterile 0.9% NaCl solution or were untreated. Mothers and progeny were housed in a conventional animal room (24 to 26°) in plastic cages with hardwood bedding and filter tops. The mice were given sterile water and nonsterile Purina mouse chow. Nude progeny were handled separately from all other mice and with special care for minimal infection.

Nude progeny were sacrificed when moribund and subjected to complete necropsy. They survived an average of 16.5 weeks (range, 10 to 33 weeks). Thirty-six of 55 nudes necropsied showed liver necrosis and enlarged spleens and lymph nodes, and presumably succumbed to viral hepatitis. The remainder appeared to have died of visceral infection or pneumonia. Some of the +/? littermates were sacrificed each time a nu/nu died to provide parallel data. All lungs and other lesions were fixed in Bouin’s solution. All surfaces of the lobes of the fixed lungs were examined with a dissecting microscope for lung tumors, which were then cut in interrupted serial sections (7 μm, every eighth section) and stained with hematoxylin and eosin. The entire lungs from all nude mice were similarly processed in interrupted serial sections.

RESULTS

Tumor Incidence. Since the nude mice died at variable times after birth, it was necessary to determine whether mice of different ages could be grouped together for comparison of tumor incidence. After treatment with urethan on Day 17 of gestation, tumor incidence in the +/? progeny as a function of age was 3 of 25 (12%) at 9 to 12 weeks, 2 of 19 (10%) at 14 to 20 weeks, and 1 of 17 (6%) at 22 to 36 weeks. Thus the percentage of mice with tumors was sufficiently constant between 10 and 36 weeks for all mice that died in this time interval to be considered together (Table 1). No tumors appeared in the control animals during this period. Only 1 tumor was observed on the pleural surface of 24 nu/nu lungs (4.2%); this incidence was somewhat lower than that in the +/? mice (6 tumors in 61 mice, 9.8%); this difference was not statistically significant.

All but 1 of the females given injections of urethan on Day 19 of gestation delivered her litter less than 24 hr later. The incidence of tumors in the nu/nu and +/? offspring was similar and showed dose responsiveness in both groups (Table 1). The incidence after urethan, 1 mg/g, was approximately 6 times that after treatment with this dose on Day 17 of gestation.
Histological Differences between nu/nu and +/- Lung Tumors. The lung tumors in the +/- offspring at 17 to 19 weeks of age were uniform throughout, were lightly basophilic, and had well-defined edges (Fig. 1). They were composed of round or cuboid cells with abundant cytoplasm and vesicular nuclei, arranged in regular, well-spaced arrays and sometimes showed papillarity (Fig. 2).

Tumors in the lungs of nu/nu offspring at 17 weeks were uniform throughout, were lightly basophilic, and had well-defined edges (Fig. 1). They were more crowded in disarray and to exhibit more cellular and nuclear atypia. Compared with the normal adenoma pattern, these cells tended to be more crowded in disarray and to exhibit more cellular and nuclear atypia.

Invasion of the Pleural Surface. Three of the 10 lung tumors in the nu/nu mice induced by treatment with urethane on Day 19 of gestation had invaded the pleural surface of the lung by 17 weeks of age and were shedding cells into the pleural cavity. Examination of these pleural lesions in serial section confirmed invasion, as opposed to accidental mechanical extrusion during dissection or fixation. For determination of whether pleural invasion is a common event, 44 tumors in progeny and 75 tumors in mothers were observed to be small, with scanty cytoplasm and basophilic nuclei (Fig. 4). Compared with the normal adenoma pattern, these cells tended to be more crowded in disarray and to exhibit more cellular and nuclear atypia.

DISCUSSION

Lung Tumor Incidence in Nude and Normal Offspring. The experiments reported here demonstrated that primary lung tumors transplacentally induced by urethane, like those resulting from transplacental treatment with ethylnitrosourea (16) and from newborn treatment (14, 15), have the same incidence in nude mice as in phenotypically normal littermates, up to about 30 weeks of age. The conditions of treatment were such as to yield a low incidence and multiplicity of tumors. Furthermore, immunosuppression of the normal animals by the carcinogen should have been low, since only a single indirect exposure to urethane occurred. Any differences in responses of the nu/nu and +/- animals to a threshold immunological challenge should therefore have been apparent. These results are in agreement with data recently obtained by O. Stutman (personal communication), showing that in nu/nu and nu/+ mice (CBA background) treated transplacentally with urethane the incidence of adenomas was similar.

Another question addressed in our transplacental study was the possible role of immunostimulation in the uniquely high sensitivity of the mouse to lung tumor induction just before birth (8). The functioning of the thymus-dependent immune system commences at around the time of birth in the mouse, with the exact time depending on the strain. At first the operation of the immune system would be expected to be quantitatively limited, giving rise to a situation in which immunostimulation of tumor growth might occur, since immunostimulation is characteristically observed when immune responses are minimal (e.g., Refs. 10, 12, and 18). If the high perinatal sensitivity to lung tumor induction were due in part to immunostimulation by the newly effective thymus-dependent immune system, then the large increase in lung tumors associated with urethane treatment less than 1 day before birth should not occur in the nude mouse. We have found that this increase does occur in the nude to the same extent as in the normal littermates, ruling out thymus-dependent immunostimulation as a causative factor. Retention of urethane in the metabolically deficient newborn, resulting in a longer exposure time, as proposed by Nomura et al. (9), remains the most convincing explanation of the perinatal sensitivity phenomenon.

Table 1

Comparison of incidence of lung adenomas in nu/nu and +/- progeny after treatment during gestation with urethane

<table>
<thead>
<tr>
<th>Day of gestation</th>
<th>Urethane dose (mg/g)</th>
<th>Total progeny</th>
<th>No. with adenomas</th>
<th>%</th>
<th>Total progeny</th>
<th>No. with adenomas</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>1</td>
<td>61</td>
<td>6</td>
<td>9.8</td>
<td>24</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>12</td>
<td>7</td>
<td>58</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>19</td>
<td>0.5</td>
<td>32</td>
<td>9</td>
<td>28</td>
<td>10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>19</td>
<td>0.1</td>
<td>12</td>
<td>2</td>
<td>16</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0*</td>
<td></td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* These groups each include 1 animal with multiple adenomas.

b Controls received 0.5 ml of 0.15 N NaCl on Day 17 or 19 or no treatment.
Histopathological comparison of lung adenomas in nu/nu and +/? littermates

Sets of serial sections of 10 nu/nu and 16 +/? adenomas from 14- to 18-week-old animals that had been exposed on Day 19 of gestation to urethan, 0.5 or 1.0 mg/g, were sorted randomly and coded. Each of 4 pathologists independently scored the tumors on the basis of 0 to 3 for the characteristics that seemed to him most descriptive of the tumors and their differences.

<table>
<thead>
<tr>
<th>Pathologist</th>
<th>Tumor Heterogeneity</th>
<th>Basophilia</th>
<th>Papillarity</th>
<th>Nuclear Heterogeneity</th>
<th>Invasiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. Budinger</td>
<td>nu/nu</td>
<td>9/10 (90)</td>
<td>12/16 (75)</td>
<td>8/10 (80)</td>
<td>8/10 (80)</td>
</tr>
<tr>
<td></td>
<td>+/?</td>
<td>12/16 (75)</td>
<td>10/16 (62)</td>
<td>10/16 (62)</td>
<td>6/16 (38)</td>
</tr>
<tr>
<td>E. Yunis</td>
<td>nu/nu</td>
<td>9/10 (90)</td>
<td>8/10 (80)</td>
<td>8/10 (80)</td>
<td>8/10 (80)</td>
</tr>
<tr>
<td></td>
<td>+/?</td>
<td>11/16 (69)</td>
<td>6/10 (60)</td>
<td>9/16 (56)</td>
<td>3/16 (19)</td>
</tr>
<tr>
<td>R. Good</td>
<td>nu/nu</td>
<td>8/10 (80)</td>
<td>6/10 (60)</td>
<td>8/10 (80)</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td></td>
<td>+/?</td>
<td>13/16 (81)</td>
<td>6/10 (60)</td>
<td>8/16 (50)</td>
<td>3/16 (19)</td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentage.

Histopathological Differences in Nude and Normal Tumors: Tumor Progression? In contrast to the similarity in incidence of the nu/nu and +/? lung tumors, their histological appearances were different, as confirmed by the scoring of the tumors by 4 pathologists. In particular, the nude tumors contained a high proportion of a cell type unusual in those from normal animals of the same age; both the cells and the nuclei in the nude tumors scored high for atypia. The nude tumors also appeared to be highly invasive. The fact that 3 of 10 nude tumors invaded the pleural surface was highly suggestive in light of the fact that no such invasion was mounted by 119 nu/+ and +/? tumors, including many that were larger and older than the nu/nu tumors.

The histological differences between the nu/nu and +/? tumors could reflect some as yet undiscovered function of the nu locus, unrelated to immune system function (for example, alteration in carcinogen metabolism) (2), or they could result from unique lung pathogen profiles and tissue responses in the nude, causing differences in tissue resistance to invasion, alterations in vascularity, etc.

Another possibility is that the atypical histological pattern and invasiveness of the nu/nu tumors reflected rapid progression of these tumors to a malignant, invasive form, permitted by the lack of thymus-dependent immunosurveillance. Progression of mouse lung adenomas was demonstrated by Kimura et al. (4, 5) in serial transplantation experiments; a percentage of the transplanted tumors eventually progressed to spindle-cell carcinomas. Coimbra et al. (1) and Ménard et al. (6, 7) studied the relationship between antigenicity and progression. They found that sensitized lymphocytes from SWR mice, immunized with extracts of lung adenomas from immunodepressed mice, reacted with adenoma cells in vitro, demonstrating the immunogenicity of the tumors that develop in the absence of immune surveillance. When the immune system was impaired by treatment with cortisone, the incidence of urethan-induced adenomas and their immunogenicity increased in direct relationship to the degree of immune system impairment, evidence that immunoselection of lung adenomas can occur. During sequential transplants of the induced adenomas, some became sarcomatous in appearance and only the immunogenic tumors from immunosuppressed hosts of origin underwent this progression (6). Thus high antigenicity and malignant progression were somehow linked in the development of the tumors, supporting the possibility that progression to a malignant form is normally suppressed by immune system function.

A direct relationship between cancer and the antigenicity of mouse lung tumors would also explain the normally low rate of metastasis. Yuhas et al. (17) found that a carcinoma of adenoma origin metastasized more readily in immunologically deficient hosts than in normal animals, although it grew more slowly. Sanford et al. (11) observed that the incidence of spontaneous adenomas was not increased in thymectomized mice but that multiplicity was greater; metastasis of the tumors permitted by absence of immunosurveillance was suggested as a possible explanation.

Additional support for the idea that tumor progression to an invasive or metastatic form can be accompanied by an increase in antigenicity can be drawn from the mouse skin
papillomas failed to regress after the termination of ATS during the promotion phase, a higher percentage of the tumors. However, in animals that were treated with ATS during the promotion phase, a higher percentage of the tumors regressed, but there was a large increase in the percentage of the remaining tumors that continued to develop into carcinomas. These results suggest that a neoplasm of initial low antigenicity (hence, no effect of ATS on incidence) tended to become simultaneously more autonomous and more antigenic, with an immune system monitoring effect at each progression step.

Aggressive malignant tumors in immunocompetent hosts are usually found to be of low antigenicity; this is the expected final result of immunoselection and tumor progression. However, the results of the lung adenoma and skin papilloma studies described above suggest that the progression-to-cancer process may be a difficult one for some tumors, due to association of increased antigenicity with cancer, so that the tumor may not succeed until a variant combining low antigenicity with cancer finally appears. Such a situation could contribute to the long latency that is characteristic of many human and animal tumors.

ACKNOWLEDGMENTS

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REFERENCES


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Fig. 1. Lung adenoma from 18-week-old +/? progeny that was exposed on Day 19 of gestation to urethan, 0.5 mg/g. H & E, × 85.

Fig. 2. Cellular composition of adenomas from 18-week-old +/? progeny that was exposed on Day 19 of gestation to urethan, 1 mg/g. H & E, × 600.

Fig. 3. Lung adenoma from 17-week-old nu/nu progeny that was exposed on Day 19 of gestation to urethan, 1.0 mg/g. H & E, × 85.

Fig. 4. Cellular composition of adenoma of 17-week-old nu/nu progeny that was exposed on Day 19 of gestation to urethan, 1 mg/g; littermate of animal whose tumor is illustrated in Fig. 2. H & E, × 600.
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