Observations on Thymectomy and Carcinogenesis

A. C. ALLISON AND R. B. TAYLOR

National Institute for Medical Research, Mill Hill, London, N. W. 7, England

SUMMARY

Neonatally thymectomized rats of two strains inoculated with SV40 developed more tumors after a shorter latent period than control rats. About 17% of CBA mice thymectomized at 3 weeks of age developed tumors after room infection with polyoma virus. No significant difference was found between the incidence of skin tumors of neonatally thymectomized and control rats treated with 7,12-dimethylbenz(a)anthracene. From these and other observations, it is concluded that neonatal thymectomy does not consistently increase the incidence of chemically induced tumors but does increase the incidence of tumors after exposure to polyoma and SV40 viruses and adenovirus type 12. Oncogenesis by leukemia viruses and the Bittner agent is not increased by thymectomy. The latter viruses, but not the former, induce tolerance after neonatal exposure; hence thymectomy can have no potentiating effect.

INTRODUCTION

The discovery (25, 26) that neonatal thymectomy reduces the capacity of mammals to respond immunologically to several different kinds of stimuli opened up a new approach to analysis of the role of immunity oncogenesis. Several investigations have shown that neonatally thymectomized mice develop more tumors after a shorter latent period than do control mice infected with polyoma virus (18, 21, 23, 28, 30, 37). Some increase in frequency of tumors induced by adenovirus type 12 has also been reported in mice thymectomized neonatally (16) and in hamsters thymectomized when 3 weeks of age (39) as compared with sham-operated animals.

However, not all virus-induced tumors are increased by thymectomy. It has long been known that thymectomy diminishes the incidence of lymphoid leukemia in mice infected with Gross and Moloney viruses (14, 29). In mice carrying the Bittner agent, thymectomy reduces rather than increases the incidence of mammary tumors (19, 24).

With regard to tumors induced by chemical carcinogens, contradictory results have been reported. Some investigators (13, 27) have reported that neonatal thymectomy shortens the latent period and increases the frequency of skin tumor formation and fibrosarcoma induction by 3,4-benzpyrene and MCA in mice. However, other investigators have been unable to observe any significant effect of thymectomy on the latent period, frequency of induction, or growth of neoplasms induced by MCA in mice (5, 18, 19, 32).

Hence the effects of thymectomy are not uniform, but vary from system to system, and further analysis may shed light on the role of tolerance and other immunologic mechanisms in oncogenesis. Before general conclusions can be drawn, more observations on other systems are required, and we are taking this opportunity to present the results of some experiments and incidental observations made in our laboratory during the last few years.

MATERIALS AND METHODS

Mice. CBA mice maintained as an inbred strain in our laboratory were used. These are bred in an isolated breeding unit and kept for experimental work in non-isolated rooms.

Rats. Albino rats, randomly bred in a closed colony, and August rats, maintained as an inbred strain at the Chester Beatty Research Institute, were used.

Thymectomy. Surgical thymectomy was carried out under anesthesia by cooling in the case of newborn animals while Avertin anesthesia was used for mice aged 3 weeks or older.

SV40 Virus. Strain 776, kindly provided by Dr. Bernice Eddy, was propagated and titrated in green-monkey-kidney cells (10). CF tests on sera of tumor-bearing animals were carried out after inactivation at 56°C for 20 min with the use of a checkerboard system and two units of complement against a standard passaged August rat tumor homogenate (6). Tumors were homogenized in 5 volumes of Medium 199, centrifuged, and stored at -68°C before testing.

Polyoma Virus. The SE strain of polyoma virus was propagated and titrated in mouse embryo cell cultures. HI antibodies were determined by standard procedures (11).

OBSERVATIONS

Tumor Induction by SV40 Virus in Rats

Rats of two strains were thymectomized within 48 hours of birth and inoculated when 5 days old with 10⁶-8 TCD₅₀ of SV40 virus subcutaneously. Most of the rats survived thymectomy, although some died with severe pulmonary infection similar to that described by Azar (4) in thymectomized rats. In survivors of both strains, the incidence of SV40 tumors was significantly higher and the latent period was significantly shorter than in control animals (Table 1). Tumors all appeared subcutaneously at the sites of inoculation and were rather fibrous sarcomas. About one-third of animals coming to autopsy had pulmonary metastases. Two tumors in August rats were successfully trans-
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TABLE 1
Development of Tumors in Rats Inoculated with SV40

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>% tumors</th>
<th>Probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albino</td>
<td>None</td>
<td>18</td>
<td>0</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>Thymectomy</td>
<td>10</td>
<td>30</td>
<td>0.0012</td>
</tr>
<tr>
<td>August</td>
<td>None</td>
<td>17</td>
<td>0</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>Thymectomy</td>
<td>8</td>
<td>37.5</td>
<td>0.023</td>
</tr>
<tr>
<td>Total</td>
<td>None</td>
<td>35</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Thymectomy</td>
<td>18</td>
<td>33.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Calculated by Fisher's exact method.

planted by trocar subcutaneously in other rats and used as antigens for CF tests. Six sera from thymectomized and six from control rats bearing large SV40-induced tumors were tested for CF antibodies. Five out of the six control rats and four out of the six thymectomized rats showed significant CF antibody against a standard SV40 tumor antigen. There was no reaction with polyoma- or adenovirus-12-induced tumors.

Incidence of Tumors Induced by 7,12-Dimethylbenz(a)anthracene in Skins of Rats

Albino rats were thymectomized within 48 hours of birth, and 6 weeks later the hair of the back removed by close clipping with electric clippers. Once weekly for 5 months, 0.1 ml of a freshly prepared 1% acetone solution of 7,12-dimethylbenzene(a)anthracene was applied to the dorsal skin. Animals were observed at weekly intervals and the number of tumors recorded (Table 2). Tumors appeared earlier and more frequently in males than in females, but at no time was the difference between thymectomized and control animals significant. The majority of tumors were papillomas, but at 11 months about 30% showed the gross and microscopic appearance of carcinomas, invading muscle and occasionally metastasizing to lymph nodes. The incidence of carcinomas was of the same order in thymectomized and control rats, and the number of regressing papillomas was not significantly different in the two groups.

Occurrence of Tumors in Mice Thymectomized at 3 Weeks of Age and Spontaneously Infected with Polyoma Virus

These observations were made in various groups of mice used for experiments unrelated to carcinogenesis. Most had received neonatal injections of bovine serum albumin and/or immunization in adult life with various antigens. The appearance of tumors was not associated with any of these treatments. As shown in Table 3, most of the tumors developed in mice thymectomized at 3 weeks. The first tumor was seen at 6 months and the remainder at 9–10 months. Between 6 and 9 months 6 mice died but were not examined and may have had tumors. Thus of 64 mice in this group, at least 11 (17%) developed tumors. Two of the salivary gland tumors were transplanted in CBA mice through four transfer generations. Of the other mice present in the same room over the same period, 1 out of 20 thymectomized at 2 months developed a tumor, but none of 200 nonthymectomized controls had a tumor.

It seems clear that the tumors are due to room infection with polyoma virus. Stock CBA mice tested about the time that the experiments started did not show polyoma HI antibodies, while the majority of the mice tested at the time when the tumors appeared had HI titers of greater than 1:100, irrespective of whether they had tumors or not. Hence at some time in the intervening period, a polyoma virus infection had spread through the colony in the manner described by Rowe et al. (34). The pathology of the tumors was also characteristic of polyoma (35). The majority were relatively undifferentiated tumors of the salivary or mammary glands, but two subcutaneous sarcomas and one kidney carcinoma were also observed.

DISCUSSION

Thymectomy and Polyoma Tumor Formation

It is now widely accepted that polyoma-induced tumors have a specific antigen capable of eliciting a homograft-type response. Moreover, neonatal thymectomy consistently depresses cell-mediated immune responses, although levels of different immunoglobulins are not lower than usual and some humoral immune responses are not significantly impaired (19). Hence it might be expected that neonatal thymectomy would increase the number of tumors and shorten the latent period for tumor induction with polyoma virus. This is consistently found (18, 19, 21, 23, 28, 30, 37); even strains such as C57BL, which are as a rule very resistant to polyoma oncogenesis, become susceptible after neonatal thymectomy. Law and Ting (21) have shown that in C57BL mice thymectomized at 3 days infection with polyoma virus as late as 6 months of age induces tumors in nearly 50% of animals. In 3-day thymectomized female C3H mice subsequently acquiring a room infection with polyoma virus, Law (18, 19) found that about two-thirds developed salivary gland tumors.

Our own results show that CBA mice, thymectomized as late as 3 weeks after birth and subsequently acquiring room infec-
tions with polyoma virus, can develop polyoma-type tumors. At least 17% of thymectomized mice kept under observation for 1 year developed tumors; none were seen in 200 control non-thymectomized mice kept under the same conditions. Only 1 tumor was seen in mice thymectomized at 8 weeks. In interpreting these observations, it is worth recalling our previous finding (36) that even mice thymectomized at 8 weeks showed a sudden decrease in capacity to produce graft-versus-host reactions about 30 weeks after the operation. This suggests that some delayed deficit in cell-mediated immunity may occur, although there is no immediate defect in homograft rejection unless animals are also irradiated. Presumably, the deficit is less delayed and greater when thymectomy is performed at 3 weeks. Thymectomy in hamsters at 3 weeks has been reported to increase the incidence of tumors by adenovirus type 12 (39). Since there is no acute running in mice if thymectomy is performed at 1 to 3 weeks, this might be a good time to choose for experiments on oncogenesis.

**Thymectomy and Oncogenesis by Other Viruses**

The results presented in this paper show that neonatal thymectomy increases the incidence and shortens the latent period for tumor formation with SV40 virus in rats. The sera of thymectomized animals contained CF antibodies against tumor antigens nearly as frequently as did nonthymectomized animals. Hence thymectomy fails to prevent the formation of antibodies against tumor antigens as well as polyoma antibodies which are directed against virus.

Our own results and those of Kirschstein et al. (16) on mice and observations of Denys and Pereira (unpublished) on rats show unambiguous enhancement of adenovirus type 12 tumor formation by neonatal thymectomy, and Yohn et al. (39) have suggestive evidence of enhancement by thymectomy at 3 weeks in hamsters.

Thus there is clear evidence that tumor inductions by polyoma, SV40 and adenovirus type 12 are all enhanced by thymectomy whereas tumor inductions by murine leukemia viruses and the Bittner agent are not. In the case of leukemia, it is often thought that thymectomy removes the most vulnerable target cells. However, in thymectomized animals bearing genetically identifiable thymic grafts the leukemias which develop are often of host origin and do not originate from cells of the thymic graft (7, 20). Hence the thymus may alter cells in such a way that they are susceptible to neoplastic conversion, and it does not seem likely that immune processes play a major part in leukemogenesis (19).

In any case, neonatal infection results in tolerance, so that there is no resistance against transplants containing specific antigen (3). In the case of the Bittner agent, there is now good evidence that mice infected shortly after birth also become tolerant to mammary tumor antigens that are detectable by transplantation (17, 31). In these circumstances thymectomy would not be expected to increase tumor formation. The finding of fewer tumors in thymectomized animals suggests intervention of some other factor. Among the possibilities to be borne in mind are the presence of some thymus-dependent humoral factor enhancing tumor formation or the higher concentration in thymectomized animals of another agent interfering with the Bittner agent. The interpretation that tolerance is responsible for the failure of thymectomy to enhance mammary tumor formation is open to experimental verification. If infection is delayed, then the mice are no longer tolerant; hence thymectomy should enhance tumor formation in such mice.

It seems that a useful generalization is beginning to emerge (2). Neonatal exposure to leukemia viruses and the Bittner agent results in tolerance, and neonatal thymectomy does not potentiate tumor induction. In contrast, neonatal exposure to polyoma or SV40 viruses or adenovirus type 12 does not result in tolerance to tumor antigens, and neonatal thymectomy, by depressing the cell-mediated immune response, increases tumor formation. Elsewhere (1, 2) we have summarized reasons for concluding that animals neonatally exposed to polyoma, SV40, and adenovirus type 12 do not become tolerant. Such animals show after some delay resistance against transplants of tumor cells containing the corresponding antigens (9, 15, 37), and repeated injections of virus during the latent period decrease the yield of tumors (1, 9, 10). This type of immunization would not be possible in tolerant animals.

**Effect of Thymectomy on Chemically Induced Tumors**

Miller et al. (27) investigated the incidence of tumors in neonatally thymectomized and sham-operated, randomly bred albino mice to the skins of which 2 mg of 3,4-benzoypyrene were applied over 20 weeks. It was concluded that tumors arose earlier and tended more frequently to become malignant in the thymectomized mice, that the regression rate was higher in controls, and tended more frequently to become malignant in the thy

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**TABLE 2**

**Incidence of Skin Tumors in Control and Neonatally Thymectomized Albino Rats after Application of 7,12-Dimethylbenz(a)anthracene as Described in the Text**

<table>
<thead>
<tr>
<th>Months after first application of DMBA*</th>
<th>Control Males</th>
<th>Thymectomized Males</th>
<th>Control Females</th>
<th>Thymectomized Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>4/15* (0.3)*</td>
<td>3/8 (0.4)</td>
<td>1/17 (0.1)</td>
<td>1/9 (0.1)</td>
</tr>
<tr>
<td>9</td>
<td>12/14 (1.8)</td>
<td>7/8 (1.3)</td>
<td>14/17 (0.9)</td>
<td>7/8 (1.1)</td>
</tr>
<tr>
<td>11</td>
<td>13/13 (6.2)</td>
<td>7/7 (6.8)</td>
<td>17/17 (3.2)</td>
<td>8/8 (3.4)</td>
</tr>
</tbody>
</table>

* DMBA, 7,12-dimethylbenz(a)anthracene.
* Number of animals with tumors/number surviving.
* Mean number of tumors per animal.

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**TABLE 3**

**Incidence of Tumors in CBA Mice after Room Infection with Polyoma Virus**

<table>
<thead>
<tr>
<th>Age at thymectomy (weeks)</th>
<th>Period of observation (months)</th>
<th>Age when tumors appeared (months)</th>
<th>Total No. of mice</th>
<th>No. with tumors</th>
<th>% with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>12</td>
<td>6-10</td>
<td>64</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

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**TABLE 4**

**Incidence of Skin Tumors in Control and Neonatally Thymectomized Albino Rats after Application of 7,12-Dimethylbenz(a)anthracene as Described in the Text**

<table>
<thead>
<tr>
<th>Period of Observation (weeks)</th>
<th>Control Males</th>
<th>Thymectomized Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>4/15* (0.3)*</td>
<td>3/8 (0.4)</td>
</tr>
<tr>
<td>9</td>
<td>12/14 (1.8)</td>
<td>7/8 (1.3)</td>
</tr>
<tr>
<td>11</td>
<td>13/13 (6.2)</td>
<td>7/7 (6.8)</td>
</tr>
</tbody>
</table>
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More work has been done on induction of sarcomas MCA in mice. Grant and Miller (14) and Defendi and Roossa (8) obtained suggestive evidence of an effect of neonatal thymectomy on shortening of the latent period and influencing of the frequency of MCA-induced fibrosarcomas. However, Law (18, 19), in extensive experiments with C57BL and C3H mice with two doses of MCA, was unable to find any differences in the latent periods, frequency of induced neoplasms, or growth potential of the induced neoplasms in thymectomized and sham-thymectomized litters. Neither Nishizuka et al. (32) nor Balner and Dersjant (5) were able to find any difference in the latent period or total number of MCA-induced sarcomas in mice, though the former reported that the incidence of hepatomas was increased in thymectomized male mice after neonatal exposure to MCA.

Balner and Dersjant (5) attempted to test the hypothesis that an increased incidence of antigenic tumors is related to the impairment of immune reactivity after early thymectomy. The immune reactivity of each thymectomized mouse was determined by grafting homologous skin before administering the carcinogen. Surprisingly, the incidence of tumors was, if anything, lower in mice with depressed homograft reactivity as compared with the incidence in those showing normal homograft reactivity. In rats thymectomized as adults (which show no impairment of homograft reactivity), Fumarola and Giordano (12) found a decreased incidence of 3,4-benzopyrene-induced sarcomas as compared with controls.

Hence it seems that an enhancing effect of neonatal thymectomy on chemical carcinogenesis cannot be consistently demonstrated, as it can with certain virus-induced tumors. In considering explanations, several points must be borne in mind.

(a) The antigenicity of chemically induced tumors is very weak by comparison with strong histocompatibility antigens. Even the polyoma tumor antigen is much weaker than H-2 antigens, and the antigens of chemically induced tumors are likely to be weaker still. (b) MCA has itself an immunosuppressive effect (22, 23), which would tend to reduce any difference in strength of cell-mediated immune response between intact and thymectomized animals. (c) There may be other factors, including persistent infection, in thymectomized animals that are unfavorable for tumor growth. Hence the results of thymectomy might be expected to show variability from system to system, depending upon the particular conditions. The simplest explanation, that neonatally thymectomized animals would show an increased incidence of many different types of tumors because the tumors are frequently antigenic and thymectomy depresses cell-mediated immunity, is no longer tenable.

REFERENCES

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