Paradoxical Effect of Three-Day Thymectomy on Sarcogenesis in the Mouse with Different Dosages of Methylcholanthrene

Luisa M. Prehn and Akinori Kojima

ABSTRACT

Past studies have shown that thymectomy in the mouse at 3 days of age but not at birth or after 7 days produces, in later life, a variety of localized autoimmune lesions. In the present work, 3-day thymectomy facilitated subsequent sarcogenesis by low dosages of 3-methylcholanthrene but inhibited oncogenesis by a high dosage. If the presence of autoimmune implies an increased antitumor immunity, it then follows that increased immunity facilitated low-dose sarcogenesis but inhibited sarcogenesis with a high dose.

INTRODUCTION

It was discovered by Kojima et al. (1) that thymectomy at 3 days of age, but not at birth or after 7 days, engendered in later life a variety of localized autoimmune lesions, thyroiditis, gastritis, oophoritis, etc. These lesions did not occur in sham thymectomized or untreated mice. The particular organ affected and the incidence were determined by the genotype (2). That the lesions were immunological was shown by the fact that disease could be transferred by splenocytes from 3-day thymectomized mice to nude mice (3), to newborn mice (4), or to animals made receptive by thymectomy at birth (5, 6). Furthermore, the lesions were infiltrated with leucocytes, predominantly lymphocytes (6). It was postulated by these investigators that, in order for lesions to occur, the thymus must reside in the mouse until immunoeffectors cells had been processed in the thymus and peripheralized; after 3 days of age the thymus would begin to produce suppressors that would prevent clonal expansion of autoreactive effector cells in the periphery, i.e., would prevent autoimmune disease.

Many of the autoimmune lesions were markedly hyperplastic, a feature that was particularly noteworthy in the stomach; the stomach lesions were often easily palpable through the abdominal wall (5). In the case of the ovary, but not as yet in other organs, the lesion occasionally progressed to overt neoplasia (7, 8).

Previous studies of the effects of thymectomy on oncogenesis were undertaken before the critical importance of the exact age at the time of surgery was understood and before it was known that thymectomy at day 3 could produce hyperplastic autoimmune lesions. This fact may account for some of the variations in the results of the past studies of the effects of thymectomy (summary in Ref. 9). In the present study we tested the hypothesis that the increased immune reactivity in the animals that were subject to an increased incidence of autoimmune lesions would facilitate low-dose but inhibit high-dose sarcogenesis.

MATERIALS AND METHODS

The general plan was to compare the tumor incidences in mice that had been thymectomized or sham thymectomized at 3 days of age and then given MCA at different dosages. The incidences of various autoimmune diseases were determined histologically (2).

The mice used were of both sexes of the C57Bl/6J x 129/J F1 or C57Bl/6J x A/J F1 genotype. The parental animals were obtained from The Jackson Laboratory and maintained in a specific-pathogen-free state. Experimental mice were thymectomized at 3 or 7 days of age under hypothermia; control animals were subjected to a vigorous sham procedure (1). The completeness of the thymectomy was, in all cases, eventually verified at autopsy. At 4 or five weeks of age both experimental and control mice were given 3-methylcholanthrene. The carcinogen was in the form of 6-mm-diameter wafers of paraffin-impregnated Millipore filter containing either 5, 0.1 or 0.01% by weight of the oncogen (10). The wafers were placed s.c., under Nembutal-alcohol anesthesia, one to a mouse in the middorsal area. Care was taken to place the wafers in as closely the same position as possible in each animal. The animals were then set aside and observed for the occurrence of tumors at the wafer sites.

RESULTS

The number of tumors and the number of animals at risk are shown in Table 1. The data of two separate experiments, both of which gave similar results, have been combined.

It is evident that the effect of thymectomy on oncogenesis depended critically upon the strength of the carcinogenic stimulus. Three-day thymectomy potentiated the response to the low concentration but inhibited the response to the high concentration (Table 1).

Since the 0.01% MCA resulted in a very low tumor incidence, we decided to investigate the effects of 3-day thymectomy on carcogenesis with 0.1% MCA. This concentration is still too low to be measurably immunosuppressive (11, 12) and the tumor latencies would be quite long. We also took the opportunity to extend the results to another mouse genotype that was known to develop a high incidence of thymectomy-induced autoimmune disease (2). A control group of the same genotype, but not subject to autoimmunity because thymectomy was delayed until the seventh day of life, was also introduced. The results are shown in Table 2; thymectomy at 3 days, but not at 7, potentiated low-dose oncogenesis.

DISCUSSION

Past studies of the effect of thymectomy on hydrocarbon oncogenesis gave rather confusing results; there were reports of potentiation of oncogenesis, but there were also a number of
low autoimmune response to these weakly immunogenic tumors may have been elevated by the 3-day thymectomy to a more nearly optimal level for tumor growth.

This explanation of the paradoxical effects of 3-day thymectomy is the one suggested by much other current work supporting the immunofacilitation theory of oncogenesis and it was this theory that inspired the present study (13). The findings of the present study support the hypothesis that there is an optimal level of host immune capacity for tumor growth, greater than zero, and reinforce the conclusion, reached by other means, that the optimal level varies inversely with the dosage of carcinogen (13).

Whatever the correct explanation of the paradoxical findings of the present study, an exciting implication is that immune mechanisms apparently play a role in the biology of spontaneous tumors and/or tumors induced by very low levels of carcinogen. Such tumors are probably better models of most human cancers than are those mouse tumors induced by large exposure. In the past, the lack of immunogenicity of these tumors and the failure to influence their appearance by immunological manipulations has caused some observers to question whether immune mechanisms play much of a role in human cancer (17). In the present work, the lowest level of carcinogen was indeed very low, as the low tumor incidence confirmed, but an immunological manipulation, 3-day thymectomy, was, nevertheless, effective in altering that incidence.

REFERENCES


Table 1 Incidence of tumors at the local site of 5% or 0.01% MCA in C5H/HeJ × 129/J F1 mice that had been thymectomized or sham thymectomized at day 3 of life

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Days after MCA</th>
<th>T/M*</th>
<th>p*</th>
<th>p^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% MCA</td>
<td>97</td>
<td>20/41</td>
<td>49</td>
<td>&lt;0.05&lt;br/&gt;0.05</td>
</tr>
<tr>
<td>0.01% MCA</td>
<td>400 days after MCA</td>
<td>9/36</td>
<td>25</td>
<td>&lt;0.1&lt;br/&gt;0.03</td>
</tr>
</tbody>
</table>

* T/M, number of mice with tumor/number of mice at risk.
^ Mann-Whitney U test, one tailed.

Table 2 Incidence of tumors at the local site of 0.1% MCA in mice that had been thymectomized or sham thymectomized at day 3 or day 7 of life

<table>
<thead>
<tr>
<th>Day 3 thymectomy</th>
<th>Day 7 thymectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/M*</td>
<td>p*</td>
</tr>
<tr>
<td>Thymectomy</td>
<td>49/69</td>
</tr>
<tr>
<td>37/72</td>
<td>51</td>
</tr>
<tr>
<td>Sham thymectomy</td>
<td>24/41</td>
</tr>
<tr>
<td>18/31</td>
<td>58</td>
</tr>
</tbody>
</table>

* T/M, number of mice with tumor/number of mice at risk; NS, not significant.
^ Mann-Whitney U test, one tailed.

Totally negative outcomes (summary in Ref. 9). The present work suggests that much of the confusion may have been caused by variations from study to study in the strengths of the oncogenic stimuli, as well as the failure to appreciate the critical importance of the exact age at the time of surgery.

In this discussion, it will be assumed, based upon the production of autoimmune lesions (1, 2), that the effect of 3-day thymectomy was to increase, not to reduce, autoimmune reactivity, including antitumor reactivity, perhaps by altering the balance between suppressor and effector cells. If 3-day thymectomy potentiated the immune reaction to tumor, the present results may, because of the relationship between carcinogen concentration and the immunogenicities of the resulting tumors, be easily rationalized (13).

Work from this laboratory has shown that spontaneous tumors and tumors of long latency that are induced by low dosages of carcinogen in solid paraffin wafers usually have little or no immunogenicity, at least as judged by classical in vivo transplantation tests; in contrast, tumors induced by high dosages are often highly immunogenic (14-16). There are data suggesting that this relationship of tumor immunogenicity to dosage of carcinogen is only partly a result of immunoselection (14). Thus, one can assume that the tumors of short latencies produced in the present study by the high concentration of MCA were usually tumors of high immunogenicity while those induced by the low concentrations had little or no immunogenicity.

Thymectomy at 3 days inhibited oncogenesis by the high dosages of MCA. This suggests that the immunodepressive action of the high dosage of MCA (12) was more than counteracted by the increased immune reaction that was engendered by both the 3-day thymectomy and the highly immunogenic tumors that high dosages tend to produce (14).

In marked contrast to the effect on high-dosage oncogenesis, 3-day thymectomy increased the incidence of the tumors induced by the low dosages of MCA. This seeming paradox can be resolved if cancers can, as has been suggested, be stimulated to grow by a weak immune reaction (13). The ordinarily very
Paradoxical Effect of Three-Day Thymectomy on Sarcogenesis in the Mouse with Different Dosages of Methylcholanthrene

Liisa M. Prehn and Akinori Kojima


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/10/4971

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, use this link
http://cancerres.aacrjournals.org/content/46/10/4971.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.