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SUPPRESSED ANTIBODY RESPONSE IN MICE TREATED WITH 3-METHYLCHOLANTHRENE AT BIRTH

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INTRODUCTION

In a previous study\(^1\), remarkable regression of lymphoid tissues was observed in mice after a single application of 3-methylcholanthrene (MC) at birth or early in life. In many of the treated animals, the lymphoid tissue appeared to be seriously damaged through loss of germinal centers in the spleen and the lymph nodes and through loss of thymocytes in the cortex of the thymus. The thymus was invisible in many such animals at autopsy, and hence the treatment of newborn mice with MC might be regarded as "chemical thymectomy," by analogy with the term "chemical bursectomy" as used by Meyer et al.\(^2\) in reference to the effects of administering testosterone to chickens.

Recently, striking effects of neonatal thymectomy on antibody responses in mice were reported by Miller\(^3\) and others\(^4\). On the other hand, MacLean et al.\(^5\) called attention to simultaneous occurrence of thymoma and agammaglobulinemia in man. As was reported previously\(^6\), many thymomas developed in mice after delayed recovery from damage produced in lymphoid tissue with MC. It was thus of interest to test antibody response after neonatal treatment of mice with the drug in order to determine what relationship immunological unresponsiveness might have to the occurrence of thymomas.

MATERIALS AND METHODS

CF 1 Swiss mice were used. This stock has been bred for many years in the Department of Medical Microbiology, Stanford University. Thymomas were produced in a high percentage of these mice after neonatal treatment with a single injection of MC\(^7\).

A 0.3% solution of MC in heavy mineral oil was prepared, and was injected intraperitoneally into newborn or suckling mice according to the schedule indicated in Table 1. The treated mice were subjected to histological examination and to tests of antibody response.
Table 1. Antibody Response in CF1 Mice Previously Treated with MC.

<table>
<thead>
<tr>
<th>Category</th>
<th>MC Treatment</th>
<th>Age at first antigen inocul. (days)</th>
<th>Average body weight at inocul. (g)</th>
<th>No. of inoculs</th>
<th>Interval from last inocul. (days)</th>
<th>Individual precipitin titers</th>
<th>Average weight (mg) at titration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Dosage (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>14</td>
<td>2</td>
<td>9</td>
<td>2,0,0</td>
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<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>12</td>
<td>2</td>
<td>15</td>
<td>64,4,2,2,1</td>
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<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>11</td>
<td>2</td>
<td>15</td>
<td>32,16,8,2</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>12</td>
<td>3</td>
<td>15</td>
<td>32,16,8,4</td>
</tr>
<tr>
<td>Experimental</td>
<td>1</td>
<td>0.3</td>
<td>4</td>
<td>13</td>
<td>2</td>
<td>15</td>
<td>0,0,0,0,0,0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.3</td>
<td>5</td>
<td>17</td>
<td>2</td>
<td>15</td>
<td>0,0,0,0</td>
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<tr>
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<td>0.3</td>
<td>4</td>
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<tr>
<td></td>
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<td>0.6</td>
<td>6</td>
<td>18</td>
<td>2</td>
<td>21</td>
<td>16,4,0,0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.0</td>
<td>6</td>
<td>19</td>
<td>2</td>
<td>15</td>
<td>2,0,0</td>
</tr>
</tbody>
</table>

For histological examination, suitable organs were removed, and were fixed in formalin. The tissues were sectioned in paraffin blocks, and were stained with hematoxylin and eosin.

For tests of antibody response, the antigen was egg albumin (EA), dispersed in Freund's adjuvant; i.e., 2 ml. of a 2% solution of EA in saline was added to a mixture of 6 ml. of heavy mineral oil, 2 ml. of Aquaphor, and 100 mg of dry heat-killed tubercle bacilli (H37Rv). MC-pretreated and control animals each received weekly intraperitoneal injections of 0.1 ml. of the antigen mixture the fourth and fifth weeks after birth, and some animals also received an injection the sixth week. The animals were bled from the carotid artery on the 15th day after the last inoculation or on an alternative day (Table 1).

The sera, separated in a refrigerator, were tested for precipitating antibodies to EA and tuberculoprotein (TP) on the day after the bleeding. Titrations for precipitins were carried out according to a modification of Ouchterlony's double diffusion method in agar; i.e., a 1:50,000 solution of EA and a 1:200 solution of TP were tested against serial dilutions of the sera in 0.01-ml. cavities in Fleck's agar containing Merthiolate in 1:10,000 dilution. The reactions were observed 24 and 96 hours later, and again after immersing the plates in 10% aqueous acetic acid.

**RESULTS**

Some of the mice died of the running syndrome approximately 2 weeks after MC application, and others died after one or more inoculations with the antigen mixture. The MC-treated mice were more sensitive to the irritation produced by
the mixture than were the control animals.

At autopsy, inoculated mice showed significant adhesions in the peritoneal cavity. The spleen appeared markedly enlarged 15 days after the last of 3 inoculations or 21 days after the second of 2 inoculations. Mesenteric lymph nodes were also enlarged in the latter situation.

In histological examination, copious infiltration and proliferation of myeloblasts were found to have completely destroyed the normal cellular pattern of the spleen, causing enlargement of the organ. Leukocytes, infiltrating from the spleen, were abundant in the preitoneal cavity, suggesting that the antigen mixture had intensive leukopoietic and leukotaxis-promoting ability, presumably due to the tubercle bacillus in the Freund's adjuvant.

Myeloblasts, occurring in the spleen and infiltrating into the other lymphoid tissues, as well as into the lungs, liver and kidneys, seemed to be quite leukemic in appearance, but lacked evident signs of malignancy; development of the myeloblasts into polymorphonulears, however, was easily demonstrated in tissue sections. These findings can be regarded as a kind of leukemoid reaction, even though later stages have not yet been thoroughly investigated.

Histological findings were closely paralleled by antibody response. The results of the titrations for precipitins are indicated in Table 1. Control mice showed precipitins for EA to a marked extent 15 days after the last antigen inoculation. Mice previously treated with MC had negligible antibodies on the 15th day after the second inoculation. Each dosage of MC was more effective in depressing antibody production in newborn mice than in sucklings. No reaction to TP was demonstrable in any of either the pretreated mice or the controls.

**DISCUSSION**

It has been suggested that suppressed antibody response may play a role in carcinogenesis. In fact, the depression of antibody production was pointed out in relation to hemolysin titration, and to delayed survival of skin homografts, after treatment of animals with MC. The present study was carried out in order to determine antibody response in mice pretreated with MC at birth, as such treatment resulted in serious lymphoid damage.

The expected depression in antibody production appeared in the MC-pretreated mice. At present, however, it is not clear whether this depression was due to the thymectomizing effect of MC or to a direct disturbance of metabolism, since some carcinogenic hydrocarbons are known to have an inhibitory effect on animal growth or on the growth of cells in tissue culture. In any case, the suppressed response in antibody production appeared to be limited in duration to a rather early stage of sensitization, as the tubercle bacilli injected together with the antigen eliminated the immunological unresponsiveness induced by treating the mice with MC. In a previous paper, it was reported that myeloblasts appeared in the spleen
after treatment of mice with MC at birth, and the presence of these myeloblasts was regarded as being due to the preservation of cells resistant to the effects of the drug. The injection of Freund's adjuvant with the antigen induced marked proliferation of the myeloblasts, probably because of irritation set up by the tubercle bacilli in the adjuvant, and the result was enlargement of the spleen and other lymphoid tissues, leading to subsequent antibody response.

The attempt to demonstrate suppressed antibody production in the present study was limited by the side-reactions caused by the tubercle bacillus in the Freund's adjuvant. Nevertheless, the results obtained suggest the possibility that antibody suppression plays a role in carcinogenesis, as tumor immunity seems not to involve any such intense antigenic irritations as does complete Freund's adjuvant. The leukemoid reaction induced by Freund's adjuvant in MC-pretreated animals, however, is an additional problem.

**SUMMARY**

3-Methylcholanthrene was shown to have a suppressive effect on antibody response in mice pretreated with this drug at birth. In this experiment, CF1 Swiss mice were neonatally treated with a single injection of 0.3 to 1.0 mg of methylcholanthrene suspended in heavy mineral oil. Four to six weeks after treatment, the mice were subjected to tests of antibody response to repeated subcutaneous applications of egg albumine dispersed in Freund's adjuvant. In titration of precipitins in sera, suppressed antibody production was seen in the experimental animals. In addition, leukemoid infiltration of myeloblasts was seen in the spleen and peritoneal cavity of these animals, being attributed to Freund's adjuvant in which the antigen was dispersed for the study.

**ACKNOWLEDGMENT**

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**REFERENCES**