Effect of Cortisone on Oncogenesis by Murine Sarcoma Virus (Moloney)

D. A. Shachat, A. Fefer, and J. B. Moloney

Introduction

Corticosteroids have been reported to render animals more susceptible to viral carcinogenesis. Mice naturally infected with polyoma virus, which normally did not develop neoplasms, developed tumors after cortisone administration. Similarly, tumors induced by Shope fibroma virus in normal adult rabbits regressed, whereas those induced by the same virus in cortisone-treated adult rabbits were progressively growing, generalized, invasive lesions. The ability of cortisone to increase host susceptibility to oncogenesis has been generally attributed to its immunodepressive activity. However, studies performed in tissue culture systems, in which a host response is precluded, suggest that cortisone may enhance the susceptibility of cells to viral infection and increase virus release.

To further investigate the effect of cortisone on viral oncogenesis, a tumor model was selected, which permitted the measurement of specific antibody as well as the virus content of induced tumors. The murine sarcoma virus (MSV) (Moloney) rapidly induces sarcomas in newborn, weanling, and adult mice. The tumors induced in adult mice usually regress. The sarcomas contain and release virus, as demonstrated by electron microscopy, the ability of cell-free virus extracts to induce tumors in vivo, and focus formation in vitro. Animals inoculated with MSV produce antibody which reacts with Moloney lymphoma cells by the indirect fluorescent antibody test. This report concerns the effect of cortisone on host susceptibility to tumor by MSV, regression of autochthonous tumors, formation of specific antibody, and virus content of induced tumors.

Materials and Methods

Mice. Five- to six-week-old BALB/c and C57BL/6 mice of both sexes were obtained from the production colonies of Microbiological Associates, Inc.

Virus. A single pool of Moloney sarcoma virus, prepared by differential centrifugation (RP #72) and passaged in weanling BALB/c mice for 72 generations, was used for all induction and regression studies. A second pool of MSV (RP #90), similarly prepared and passaged in the same strain of mice, was used for virus recovery studies.

Sera. Mice were bled from the retro-orbital sinus. Individual sera, recovered by centrifugation, were stored at the temperature of frozen CO₂.

Indirect Fluorescent Antibody Test. The technique developed by Möller, using suspensions of viable cells, was employed. In the present studies, 0.05 ml of undiluted antisera was added to a pellet containing 2 x 10⁶ trypan blue-unstained Moloney lymphoma cells. Following a 20-minute incubation at 37°C, the cells were washed 4 times. The pellet was then incubated at 37°C for 20 minutes with 0.05 ml of fluorescein-conjugated, goat antiserum globulin (Hyland) diluted 1:5. After 4 more washings, the cells were examined under the fluorescence microscope. Samples were read blind. Cells manifesting diffuse fluorescence, of the type that Möller demonstrated to be indicative of dead cells, were omitted from consideration.

This was not a significant problem as the viability of our cell culture systems is high. However, studies performed in tissue culture systems, in which a host response is precluded, suggest that cortisone may enhance the susceptibility of cells to viral infection and increase virus release.

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Summary

The role of cortisone in viral carcinogenesis was investigated by inoculating murine sarcoma virus (Moloney) into normal BALB/c and C57BL/6 weanling mice and into mice of the same strains pretreated with 2.5 mg of cortisone acetate. The results obtained from both assay systems indicated that more tumors were induced in cortisone-treated mice. A tumor model was selected, which permitted the measurement of specific antibody as well as the virus content of induced tumors. The murine sarcoma virus (MSV) (Moloney) rapidly induces sarcomas in newborn, weanling, and adult mice. The tumors induced in adult mice usually regress. The sarcomas contain and release virus, as demonstrated by electron microscopy, the ability of cell-free virus extracts to induce tumors in vivo, and focus formation in vitro. Animals inoculated with MSV produce antibody which reacts with Moloney lymphoma cells by the indirect fluorescent antibody test. This report concerns the effect of cortisone on host susceptibility to tumor by MSV, regression of autochthonous tumors, formation of specific antibody, and virus content of induced tumors.

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Summary

The role of cortisone in viral carcinogenesis was investigated by inoculating murine sarcoma virus (Moloney) into normal BALB/c and C57BL/6 weanling mice and into mice of the same strains pretreated with 2.5 mg of cortisone acetate. The two strains were equally susceptible to tumor induction. Pretreatment with cortisone increased their susceptibility to oncogenesis. Fifty-four out of eighty normal mice developed tumors. All tumors induced in normal mice ultimately regressed. However, only 26/78 tumors induced in cortisone-treated mice regressed. All mice were bled 21 days after virus inoculation and their sera were tested against Moloney lymphoma cells by the indirect fluorescent antibody test. Cortisone did not significantly alter the production of the antibody. Tumors induced in normal and cortisone-treated BALB/c mice were assayed for viral activity by tumor induction in vivo and focus formation in vitro. The results obtained from both assay systems indicated that more virus was present in tumors induced in cortisone-treated animals.
cells was estimated. Each sample was therefore graded as follows: negative, less than 40% of cells positive; 1+, 40–70% of cells positive; 2+, 70–100% of cells positive. Specific sera staining more than 40% of cells were considered “positive” for antibody. Each test included a negative control (normal C57BL/6 serum) and a positive control (specific antiserum with known activity). The negative control was always examined for 120–180 viable cells, and the precise frequency of staining determined. Normal serum usually stained less than 10% of the cells.

**Virus Recovery.** Cell-free extracts were prepared from tumors induced in intact and cortisone-treated BALB/c mice by differential ultracentrifugation (14). The concentration was adjusted so that 1 gm of tumor tissue provided 1 ml of extract. Serial ten-fold dilutions of the extract were inoculated into newborn BALB/c mice; thereafter, these animals were examined daily for tumor development. The same extracts were assayed for in vitro viral activity, as measured by focus formation in a mouse fibroblast tissue culture system previously described (7). All extracts were tested at the same time.

**RESULTS**

**Effect of Cortisone on Host Susceptibility to Tumor Induction.** Graded dilutions of MSV were inoculated i.m. (0.1 ml/mouse) into intact weanling BALB/c and C57BL/6 mice, and into mice pretreated with 2.5 mg cortisone acetate (Upjohn), i.m., 24 hours earlier. All mice were palpated daily for tumor development. The results, presented in Table 1, indicate that the two strains of normal mice were equally susceptible to tumor induction. Pretreatment with cortisone increased the incidence of tumors and decreased the latency period to tumor detection in mice inoculated with the lower dose of virus.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cortisone (mg)</th>
<th>Dilution of Moloney sarcoma virus 10⁻¹</th>
<th>10⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>0</td>
<td>17/20 (9)</td>
<td>9/20 (14)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>20/20 (6)</td>
<td>18/19 (10)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0</td>
<td>19/20 (7)</td>
<td>9/20 (14)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>20/20 (6)</td>
<td>20/20 (10)</td>
</tr>
</tbody>
</table>

Induction of tumors in normal and cortisone-treated mice inoculated with Moloney sarcoma virus. The mean latency period (in days) to tumor detection is given in parentheses.

a No. of mice developing tumor

No. of mice inoculated

**Effect of Cortisone on Regression of Primary Tumors.** Table 2 reveals that all tumors induced in normal mice ultimately regressed. By contrast, most tumors induced in cortisone-treated mice failed to regress. The effect of cortisone on regression was most evident in mice inoculated with the higher dose of virus.

**Antibody Production by Intact and Cortisone-treated Mice Inoculated with MSV.** The virus-inoculated normal and cortisone-treated mice were bled 21 days after MSV injection and their sera were tested individually for antibody by the immunofluorescence test. Attempts to prepare a suspension of viable sarcoma cells adequate for use as target cells were unsuccessful. However, transplantation and serologic studies have shown that Moloney sarcoma cells and Moloney lymphoma cells (or their causative agents) are antigenically similar (3). In the absence of an assay for antibody directed specifically against Moloney sarcoma cells, sera from mice inoculated with MSV were tested against Moloney lymphoma cells. The target cell employed was LSTRA—a Moloney virus-induced ascitic lymphoma of BALB/c origin, in its 320th transplant generation. Sera which stained 40% or more of the lymphoma cells were considered positive.

The results, presented in Table 3, reveal that only 3/21 normal BALB/c mice and 12/22 normal C57BL/6 mice exhibited detectable antibody. Pretreatment with cortisone did not significantly affect the number of mice with detectable antibody.

**Virus Recovery from Tumors Induced in Normal and Cortisone-treated Mice.** Weanling BALB/c mice were inoculated with 0.1 ml (i.m.) of undiluted MSV. Half of the mice were pretreated with 2.5 mg of cortisone acetate (i.m.) 24 hours prior to virus inoculation. All mice developed tumors within 4 days. Seven days after virus inoculation, cell-free extracts were prepared from equal weights of tumors induced in normal and cortisone-treated mice, and were tested for viral activity.
Strain BALB/c C57BL/6 Dilution of Moloney sarcoma virus in vivo and in vitro. The results are presented in Table 4. 

Extract BALB/c Moloney lymphoma cells, by the fluorescence test. virus.

weanling mice inoculated with graded doses of Moloney sarcoma virus.

Active in both assay systems. A 10^4 dilution of extract from tumors of untreated mice. Similarly, extracts from cortisone-treated mice contained three times as many focus forming units/ml as did extracts from tumors induced in untreated mice.

DISCUSSION

Cortisone has been reported to increase host susceptibility to viral oncogenesis (6, 18). The enhanced susceptibility has been ascribed to the immunodepressive activity of the corticosteroids (1, 6). However, in the Moloney sarcoma system, it has been suggested that immunologic competence is not the prime determinant of host susceptibility to tumor induction (4). This suggestion is supported by the ability of MSV to readily induce tumors in both weanling and adult C57BL/6 mice (4)—an immunologically reactive strain well known for its resistance to oncogenesis by other viruses (10–12, 14). It is also supported by the observation that mice immunologically depressed by sublethal X-irradiation are no more susceptible to tumor induction by MSV (4) than are normal adult mice.

The increased susceptibility of cortisone-treated mice to tumor induction by MSV, as manifested by increased incidence of tumor formation and decreased latency period to tumor detection, may not, therefore, be attributable to an immunodepressive effect of the dose of cortisone employed. The increase in recoverable virus from tumors induced in cortisone-treated mice suggests that cortisone may increase susceptibility to oncogenesis by exerting an effect on the relationship between the virus and the target cell. Cortisone has been reported to enhance viral infectivity and viral multiplication in various tissue culture systems. Cells grown in media containing cortisone were more susceptible to cytopathic changes induced by poliovirus (16) and vaccinia virus (8). Furthermore, more vaccinia and psittacosis viruses were recovered from L cells grown in media containing cortisone than from control cultures (8, 17).

Similarly, the addition of cortisone to isolated sections of chicken chorioallantoic membrane maintained in culture media and infected with influenza B virus resulted in a significant increase in the concentration of recoverable virus (9).

Finally, preliminary studies suggest that mouse embryo cells grown in media containing cortisone, and subsequently infected with MSV, yield more MSV than do cells from control cultures not exposed to cortisone (J. W. Hartley, unpublished data). These studies suggest that cortisone may exert a nonimmunologic effect on target cells; this is manifested by an increase in virus production or release. This effect is consistent with the increase in MSV recovery from tumors induced in cortisone-treated mice and may explain their increased susceptibility to oncogenesis by MSV. However, the possibility should be considered that cortisone may have induced a nondetectable depression of immunologic responsiveness in vivo, resulting in increased virus release and increased susceptibility to tumor induction.

Tumors induced by MSV have been reported to be antigenic in histocompatible hosts (3) and to regress spontaneously in autochthonous hosts (3, 4). Lymphoid cells, but not serum, from mice whose primary MSV-induced tumors had regressed, neutralized transplantable Moloney sarcoma cells in vitro, preventing their outgrowth in vivo (5). These observations are consistent with the view that regression of autochthonous tumors and rejection of transplanted tumors may be largely mediated by sensitized lymphoid cells. Sublethal preirradiation of the host delayed or prevented the regression of both autochthonous (4) and transplanted (5) Moloney sarcoma cells. The immunologic competence of the host was, therefore, postulated to be a critical factor in determining whether an autochthonous tumor will grow or regress.

The data presented demonstrate that cortisone, like X-irradiation, prevents or delays autochthonous tumor regression. Whether the explanation resides in the effect of cortisone on virus release from target cells or in its immunodepressive action, or both, cannot yet be determined. Although cortisone

Table 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dilution of Moloney sarcoma virus</th>
<th>Cortisone (mg)</th>
<th>No. of mice with detectable antibody</th>
<th>No. of mice tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>10−1</td>
<td>0</td>
<td>1/10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10−2</td>
<td>2.5</td>
<td>1/12</td>
<td>10</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>10−1</td>
<td>0</td>
<td>7/10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10−2</td>
<td>2.5</td>
<td>3/9</td>
<td>10</td>
</tr>
</tbody>
</table>

Specific antibody production by normal and cortisone-treated weanling mice inoculated with graded doses of Moloney sarcoma virus.

* Serum was considered positive if it reacted with 40% or more BALB/c Moloney lymphoma cells, by the fluorescence test.

Table 4

<table>
<thead>
<tr>
<th>Dilution of extract</th>
<th>In vivo assay of extracts from tumors induced in:</th>
<th>In vitro assay of extracts from tumors induced in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated BALB/c mice</td>
<td>Cortisone-treated BALB/c mice</td>
</tr>
<tr>
<td>10−1</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>10−2</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>10−3.5</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>10−4</td>
<td>5/20</td>
<td>17/20</td>
</tr>
</tbody>
</table>

Assays of cell-free extracts from tumors induced by Moloney sarcoma virus in untreated and cortisone-treated BALB/c mice.

* Number of mice inoculated

b Foci too numerous to count.

c Number of foci present in duplicate plates per dilution of extract.

in vivo and in vitro. The results are presented in Table 4. Extracts from tumors induced in cortisone-treated mice were more active in both assay systems. A 10^4 dilution of extract from tumors of cortisone-treated mice induced tumors in 17/20 newborn mice, in contrast to only 5/20 tumors induced by an equivalent dose of extract from tumors of untreated mice. Similarly, extracts from cortisone-treated mice contained three times as many focus forming units/ml as did extracts from tumors induced in untreated mice.

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did not measurably decrease the number of mice producing antibody detectable by immunofluorescence, it is possible that cellular reactivity was sufficiently depressed to prevent autochthonous tumor regression.

ACKNOWLEDGMENTS

We thank Dr. J. W. Hartley for performing the in vitro assay for viral activity.

REFERENCES

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