Suppression of Moloney Sarcoma Virus Immunity following Sensitization with Attenuated Virus

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SUMMARY

Murine sarcoma virus (Moloney strain) (MSV-M)-induced tumors are unusual in that they regularly appear less than 2 weeks after virus inoculation, progress for 1 to 2 weeks, and are rejected by normal adult BALB/c mice. Rejection leaves the animals immune to tumor induction. In the present study, presensitization of normal adult BALB/c mice with attenuated MSV-M resulted in an altered pattern of tumor immunity. Injection of active MSV-M into the presensitized animals resulted in tumor induction and rejection similar to that observed in normal animals, but rejection failed to produce protection against the secondary inoculation with MSV-M. After the second inoculation with active MSV-M, tumors appeared and progressed but ultimately were rejected. Over 80% of the mice died, 25% after the primary challenge and the remainder after the secondary challenge. At death, all mice had histological evidence of leukemia which was the probable cause of death. The animals that died following the secondary challenge also had evidence of disseminated MSV-M. Solid tumor nodules were found in skeletal muscle distant from the original site of inoculation, and active MSV-M was isolated from spleen and lungs. The possibility that the results were produced by specific suppression of MSV-Moloney leukemia virus immunity is discussed.

INTRODUCTION

Rhabdomyosarcomas may be produced locally by injection of MSV-M into BALB/c mice (13, 14). Tumors became palpable within 5 to 10 days after i.m. injection of virus, progressed for 7 to 10 days, and generally regressed in adult mice (4). Since tumors that consistently regress in autochthonous hosts are rare, MSV provides a potentially valuable system for studying the role of immunological factors in controlling tumor growth. Although the mechanism that is responsible for MSV tumor rejection remains unclear, in vivo and in vitro experimentation have provided evidence for the involvement of a variety of immunological factors. MSV inoculation into newborn or adult BALB/c mice that received sublethal total-body irradiation resulted in progressive tumor growth, whereas immunologically competent adults rejected the tumor (4, 14). Also, the natural history of the tumor in adults is consistent with an immunological interpretation. The tumor progresses only until the animal is potentially capable of mounting an immune response, and after the mice have rejected the tumor they are immune to tumor induction (4).

Syngeneic, cell-mediated cytotoxicity against tumor-associated antigens has been demonstrated in several well-defined in vitro assay systems. Studies on the nature of the cellular immune reaction recently have been reviewed extensively (2, 9, 10). Pooled serum from animals in the process of rejecting tumors neutralized tumor induction with MSV, but a clear cause-effect relationship between antibodies and tumor rejection has not been demonstrated (4).

Recently, the function of immunosuppressive factors in tumor progression has become evident. Serum factors have been shown to block in vitro lymphocyte-mediated cytotoxicity in the MSV system (8), but the mechanism of action as well as the exact nature (antigen, antibody, and/or antigen-antibody complex) of the blocking factors remain obscure. Also, animals with progressing tumors have a demonstrable subpopulation of cells that is capable of both specific and nonspecific suppression of in vitro T-lymphocyte activity (6). Whether those suppressor cells are T-cells, B-cells, or macrophages remains to be resolved.

The purpose of the present paper is to describe a series of experiments wherein adult BALB/c mice were manipulated by sensitization with attenuated virus prior to exposure to active MSV-M. Basically, the presensitization resulted in a completely altered course of tumor behavior, including failure to develop complete immunity, appearance of solid tumor at various sites throughout the body, and the apparently concomitant development of leukemia. Preliminary evidence for an immunosuppressive mechanism is presented.

MATERIALS AND METHODS

Mice. Adult, male BALB/c mice, 5 to 6 weeks old, that were obtained from Laboratory Supply Co., Indianapolis, Ind., were used.

Virus. All experiments were performed with MSV-M (MSV-B-77,85,86), kindly provided by the Office of Program Resources and Logistics, Viral Oncology, National Cancer Institute, NIH, Bethesda, Md. Routinely, virus was preserved by storage at −70°C.

Virus Attenuation. Virus was inactivated by storage at...
Effect of presensitization with attenuated MSV-M. MSV-M preparations that were attenuated by storage for at least 1 month at −20° were injected s.c. into normal adult BALB/c mice. The attenuation was evidenced by the fact that no palpable tumor appeared at the site of injection, and no palpable tumor formed when the same MSV-M preparation was injected i.m. Also, when the dosage was increased 5 times, e.g., 0.5 ml of attenuated virus, there still was no evidence of either s.c. or systemic tumor. Additional evidence that the failure of the attenuated virus to produce palpable tumors was not caused by the s.c. injection was provided by the fact that active MSV-M consistently produced solid tumors in the abdominal musculature following s.c. injection. The sensitized mice were sacrificed 40 days after inoculation, and there was neither gross internal evidence of tumor nor histological alterations in the spleen or lung. However, the fact that palpable tumors did not appear does not eliminate the possibility that active MSV-M or MLV remained in the attenuated preparation.

What was the effect, if any, of presensitization with attenuated MSV-M on the natural history of the Moloney sarcoma? Was an altered immunological response to MSV developed for the present study.

Tumor Induction. The stock virus was diluted 1:10 in HBSS. Injection i.m. of 0.05 ml of the diluted stock virus routinely caused a tumor at the site of injection within 7 to 10 days in normal, untreated mice. MSV-M was injected into either hind leg. With the dose of virus used, tumors invariably were rejected within 3 to 4 weeks by normal adult mice.

Sensitization with Attenuated Virus. Normal adult male BALB/c mice received injections s.c. of 0.1 ml of diluted (1:10) MSV stock that had been attenuated by storage at −20° for at least 1 month. Four to 6 weeks after the initial sensitization, the mice received injections i.m. of 0.05 ml of appropriately diluted, active MSV-M stock.

Virus Purification. Several experiments were performed to determine whether tissue obtained from autopsied mice actually contained active virus. Cell-free homogenates of lung, spleen, or tumor were prepared by mincing the tissue into 1 to 2-mm cubes, washing 3 times with HBSS, homogenizing the tissue in a Potter-Elvehjem apparatus, centrifuging for 30 min at 2300 × g, and removing cell-free supernatant. This supernatant was either used as a source of virus or was purified further as described by Moloney for leukemia virus (12). Briefly, the tissue was homogenized as described above, centrifuged at 2300 × g for 20 min, and the supernatant was removed and centrifuged again at 2300 × g for 20 min. The supernatant was removed and centrifuged at 10,000 × g for 1 min; then, it was centrifuged for 60 min at 30,000 × g. The pellet was resuspended in Dulbecco's phosphate-buffered saline, pH 7.2 (Grand Island Biological Co., Grand Island, N. Y.), and centrifuged at 5000 × g for 8 min. The middle third of the supernatant was removed and saved while the pellet was resuspended and sedimented at 5000 × g for 8 min. The 2 supernatants were pooled and constituted the purified virus.

Preparation of Spleen Cell Suspensions. All of the following manipulations were performed with cold reagents in an ice bath. Spleens were removed, minced with iris scissors, and washed 3 times with HBSS. A single-cell suspension was prepared by passing the minced tissue through 15- and 19-gauge needles, then through sterile 4-ply gauze to remove fragments. Contaminating erythrocytes were removed by lysis with Tris-ammonium chloride buffer (1). Cells were washed 3 times with HBSS and counted, and their viability was determined by trypan blue exclusion. In passive transfer experiments, normal BALB/c mice received an i.p. injection of 3 to 4 × 10⁷ viable spleen cells either from normal or sensitized mice. At the same time the mice received an injection i.m. of the standard MSV-M inoculum.

Virus Neutralization with Serum. Mice were bled from the retroorbital sinus. Serum was obtained from normal mice, mice that had been sensitized with attenuated virus, and mice that had rejected the MSV tumor. Undiluted serum was mixed with an equal volume of the diluted virus stock, the mixture was incubated for 15 min at 37°, and 0.1 ml was injected i.m. into the hind leg of normal BALB/c mice.

RESULTS

Since there is some controversy over the frequency of occurrence of progressive MSV tumors in adult BALB/c mice, and those observations have considerable relevance to the results that will be presented here, the data that we have obtained on MSV-M tumor induction in normal BALB/c mice are presented as a background. Injection i.m. of 0.05 ml of a 1:10 dilution of stock MSV-M (standard inoculum) invariably produced a tumor that regressed (Table 1). In numerous experiments that have been performed in this laboratory with normal adult male BALB/c mice, MSV-M-induced tumors never progressed without subsequent rejection. No deaths were recorded as a result of MSV-M tumor formation in normal adult mice. Also, after having rejected the tumor, those mice generally were immune to subsequent challenge with the standard MSV-M inoculum. For example, in experiments in which 40 adult BALB/c mice that had rejected the tumor were challenged 3 weeks later with MSV-M, 3 of 40 mice developed small tumors that were only palpable for 3 to 5 days, while the remaining 37 mice developed no tumor whatsoever during the 3-month observation period (Table 1).

Effect of Presensitization with Attenuated MSV-M. MSV-M preparations that were attenuated by storage for at least 1 month at −20° were injected s.c. into normal adult BALB/c mice. The attenuation was evidenced by the fact that no palpable tumor appeared at the site of injection, and no palpable tumor formed when the same MSV-M preparation was injected i.m. Also, when the dosage was increased 5 times, e.g., 0.5 ml of attenuated virus, there still was no evidence of either s.c. or systemic tumor. Additional evidence that the failure of the attenuated virus to produce palpable tumors was not caused by the s.c. injection was provided by the fact that active MSV-M consistently produced solid tumors in the abdominal musculature following s.c. injection. The sensitized mice were sacrificed 40 days after inoculation, and there was neither gross internal evidence of tumor nor histological alterations in the spleen or lung. However, the fact that palpable tumors did not appear does not eliminate the possibility that active MSV-M or MLV remained in the attenuated preparation.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of presensitization of adult BALB/c mice with attenuated MSV-M on the development of immunity to MSV-M challenge</th>
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<tbody>
<tr>
<td>No. of mice developing solid tumor following MSV-M challenge</td>
<td>Primary²</td>
</tr>
<tr>
<td>Presensitized³</td>
<td>60/60</td>
</tr>
<tr>
<td>Normal</td>
<td>120/120</td>
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</table>

* Active MSV-M (0.05 ml) was injected i.m. into the right hind leg 30 days after presensitization.
* Active MSV-M (0.05 ml) was injected i.m. into the left hind leg 40 days after the primary challenge, 70 days after presensitization.
* Adult BALB/c mice were sensitized by s.c. injection of 0.1 ml of MSV-M that had been attenuated by storage at −20° for at least 1 month.
tumor induction produced by sensitization with the virus? The sensitized BALB/c mice were challenged with the standard MSV-M inoculum 30 days after a single injection of attenuated virus. All of the animals developed tumors in the musculature at the injection site (Table 1). The latent period prior to appearance of palpable tumors and the subsequent fate of those tumors were not significantly different from the natural history of MSV-M tumors in normal adult BALB/c mice. However, the survival of the inoculated mice was substantially altered by presensitization (Table 2). Approximately 25% of the mice died within 3 weeks after the primary virus inoculation. Similar observations were made in 4 separate experiments each involving 20 mice, 3 of which are recorded in Table 2. The cause of death in those mice has not been definitely determined. Although some of the mice had residual leg tumors when they died, they had no other evidence of solid tumor. Also, none of the tumors were progressing. In fact, all of the tumors, even in animals that died, were either totally rejected or were in the process of being rejected.

Histological examination of lymph nodes, spleen, and lungs revealed radical changes. The normal architecture of the spleen from each of the animals was totally destroyed. There was a large increase in the number of mononuclear cells, and in some cases there was considerable evidence of macrophage accumulation. That is, several spleens exhibited marked sinus histiocytosis. Lymph nodes were variably involved, and the nature of the reaction was difficult to characterize. Some nodes were completely normal in appearance, while others could only be recognized as lymph nodes by their location and size, since they were almost completely devoid of cellularity. Some of the mice had evidence of involvement of the lungs. Again, the normal architecture of that organ was broken down, and a multicellular infiltrate was observed. Substantial variation was observed from mouse to mouse, making simple characterization of the cellularity difficult and suggesting that mice were dying at different stages of the disease process. There was also evidence of tissue necrosis. There was no evidence of metastatic sarcoma in the spleen, lymph nodes, or lungs.

Sensitized mice that survived the 1st challenge with active MSV-M apparently were fully recovered, since mice that were observed for up to 90 days remained alive and tumor free. Since normal adult BALB/c mice generally became immune to tumor induction after rejecting the sarcoma, it was important to determine whether the sensitized mice also developed an effective immunity. The surviving mice received an injection of the standard MSV-M dose, this time in the opposite hind leg. Again, each of the mice developed a tumor that was rejected (Table 1). Since the size of tumors was not determined during the course of the experiments, objective quantitative data are not available. Subjectively, the tumors in those mice progressed for a substantially longer period than in normal adult mice, and the rejection period also was considerably prolonged. Nevertheless, evidence of tumor regression (decreased in tumor size) was present in all of the mice, even though some died before rejection was complete. The majority of the animals, despite evidence of rejecting their leg tumors and despite having previously rejected a rhabdomyosarcoma on the opposite leg, eventually succumbed (Table 2).

Most of the dead mice were autopsied because of the surprising finding that, not only were those presensitized mice that had rejected the MSV tumor not immune to the 2nd dose of active MSV, but the virus actually proved to be fatal. The autopsy results were striking. Grossly, all animals exhibited marked splenomegaly. Some of the spleens were at least 3 to 4 times as large as those of normal adult BALB/c mice. Some of the mice had residual leg tumor, but this was not a consistent finding. None of the animals showed evidence of unrestricted tumor growth at the injection site, but tumor nodules were found in several other sites. Those tumor nodules were small, measuring 2 to 10 mm in diameter, and were found in abdominal musculature, skeletal muscles of the back, musculature of the rib cage, and in the diaphragm. There was no evidence of invasion of the rhabdomyosarcoma nodules into any internal organs.

Histologically, there were major changes in several organs. As with animals that had died following the primary MSV challenge, there were significant alterations in the spleens of all of the animals that died following the secondary MSV challenge. Again the splenic architecture was totally broken down, replaced by mononuclear cells. Generally, 2 major cell types were apparent, those that morphologically resembled lymphocytes and those resembling macrophages. All of the animals showed evidence of involvement of the lungs. Those organs were engorged with a mixed cellular infiltrate. Generally, there was little if any evidence of normal lung tissue remaining. Lymph nodes sometimes were altered histologically. The change ranged from breakdown of normal internal structure to complete necrosis of the node. A few mice with advanced disease had foci of nonnucleated cells in the liver. None of the organs that were examined showed evidence of metastatic sarcoma.

The phenomenon described above was produced in 3 of 4 experiments by a single injection of attenuated virus. In the 4th experiment, attenuated virus was injected twice, the 2nd sensitization coming 1 month after the 1st sensitization. The results that were obtained in that experiment were substantially the same as those obtained with 1 injection. A 5th experiment was designed to determine the effect of giving multiple injection of attenuated virus. When 4 injections of attenuated virus were given at 1-week intervals, the balance was altered away from immunosuppression toward immunity. Each of the mice that had received multiple injections

<table>
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<th>Table 2</th>
<th>Effect of presensitization of adult BALB/c mice with attenuated MSV-M on survival following MSV-M challenge</th>
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<tr>
<td></td>
<td>No. of survivors following MSV-M challenge</td>
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<tr>
<td></td>
<td>Primarya</td>
</tr>
<tr>
<td>Presensitized b</td>
<td>48/60</td>
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<tr>
<td>Normal</td>
<td>120/120</td>
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a Active MSV-M (0.05 ml) was injected i.m. into the right hind leg 30 days after presensitization.

b Active MSV-M (0.05 ml) was injected i.m. into the left hind leg 40 days after the primary challenge, 70 days after presensitization.

c Adult BALB/c mice were sensitized by s.c. injection of 0.1 ml of MSV-M that had been attenuated by storage at −20° for at least 1 month.
of attenuated MSV-M developed a local tumor that was rapidly rejected (Table 3). None of the tumors reached the size observed in normal, non-immunized mice. This is subjective, since no quantitative data were obtained. All of the mice (10 of 10) survived. Thirty days later, the mice again received an injection of active MSV to determine whether they were immune. Nine of 10 mice failed to develop a tumor, indicating that they were immune. All 9 of these mice are still alive and free of tumor 3 months later. One of 10 developed a tumor that grew progressively with no evidence of rejection and which finally killed the host. That tumor was the only local MSV tumor in numerous experiments that ever progressed in adult BALB/c mice.

An experiment was performed to gain some preliminary evidence on the nature of the immunosuppressive factor. The attenuated virus preparation was separated into particulate and soluble fractions by sedimentation at 30,000 x g. The immunosuppression was retained in the sediment. Six of 6 mice that had been sensitized with supernatant developed and rejected a solid tumor in the normal manner, while 2 of 6 animals that were sensitized with the sediment died within 14 days after being injected with active virus.

**Virus Distribution in Tissue.** Since profound histological changes occurred in the spleens and lungs of animals that were presensitized and died following the 2nd injection of MSV, those organs were homogenized and injected into normal adult BALB/c mice to determine whether they contained tumorgenic virus. Both spleen and lung contained active, sarcoma-producing virus. When the tissue simply was homogenized, freed of cells and cellular material, and injected i.m. tumors appeared and were rejected. However, each of the mice that developed a local tumor later died. Since these were normal rather than presensitized mice, the finding was surprising, and 2 possible explanations immediately suggested themselves. First, alterations could have occurred in the virus causing it to be “super” oncogenic (2). Second, immunosuppressive factors could have been present in the homogenate. The choice could be made between those hypotheses by purifying the virus prior to injecting it, to determine whether mice receiving injections of purified virus would survive. When virus that was concentrated and otherwise separated from other particulate and nonparticulate factors was injected into normal mice, tumors appeared and were rejected. Those mice not only survived, but were immune to subsequent MSV challenge.

**Serum Neutralization.** Tumors appeared in 100% of normal BALB/c mice that received injections of active MSV mixed with serum either from normal BALB/c mice or from mice that were sensitized with attenuated MSV (Table 4). That is, those sera did not contain virus-neutralizing factors. However, MSV was effectively neutralized by pooled serum from mice that were immune to MSV tumorigenesis, serum from mice that had been sensitized and then exposed to active MSV once, and serum that was pooled from mice that were sensitized with attenuated virus and then given injections twice with active MSV. The mice in the last group not only were not immune, but were very close to death at the time that they were bled. Thus, pooled serum from mice that had been exposed to active MSV contained virus-neutralizing factors regardless of the ultimate fate of those animals. The mice that received injections of the MSV serum combinations received injections again of active MSV to determine: (a) whether immunity was produced and (b) whether the serum may have contained immunosuppressive factors. However, neither immunity nor immunosuppression was produced by the virus serum mixture under those experimental conditions (Table 4).

**Passive Transfer with Spleen Cells.** The results obtained thus far suggested that sensitization with attenuated virus produced a level of immunosuppression that ultimately resulted in death of the adult BALB/c mice following challenge with MSV-M. In order to gain some preliminary evidence on the source of the immunosuppression, passive transfer experiments with spleen cells were performed.

### Table 3

<table>
<thead>
<tr>
<th>Primary injection of attenuated MSV-M on immunity and survival following challenge with active MSV-M</th>
<th>Secondary injection of active MSV-M</th>
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<tbody>
<tr>
<td>No. of injections</td>
<td>Developed tumor</td>
</tr>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20/20</td>
</tr>
<tr>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20/20</td>
</tr>
<tr>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10/10</td>
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</tbody>
</table>

<sup>a</sup> Active MSV-M (0.05 ml) was injected i.m. into the right hind leg 30 days after presensitization.

<sup>b</sup> Adult BALB/c mice were sensitized by s.c. injection of 0.1 ml of MSV-M that had been attenuated by storage at −20°C for at least 1 month.

<sup>c</sup> Sensitizations were separated by 1 month.

<sup>d</sup> Sensitizations were separated by 1 week, and final sensitization was followed in 7 days by MSV-M challenge so that the time between 1st sensitization and challenge with active virus was the same for 1 and 4 injections.

<sup>e</sup> One of 10 mice developed a solid tumor, but in contrast to all other solid tumors observed in the adult BALB/c mice, this tumor progressed.

### Table 4

| Neutralization of MSV-M with serum |
|---|---|
| Treatment of serum donors | No. of adult BALB/c mice that developed tumors following injection of |
| Attenuated MSV-M<sup>a</sup> | Primary MSV-M challenge<sup>b</sup> | Secondary MSV-M challenge<sup>c</sup> | MSV-M/se-MSV-M mixture |
| — | — | — | ND<sup>d</sup> |
| + | + | + | 8/8 |
| + | + | + | 8/8 |
| + | + | + | 8/8 |

<sup>a</sup> Adult BALB/c mice were sensitized by s.c. injection of 0.1 ml of MSV-M that had been attenuated by storage at −20°C for at least 1 month. Serum was obtained 1 month later.

<sup>b</sup> Active MSV-M (0.05 ml) was injected i.m. into the right hind leg 30 days after presensitization. Serum was obtained from survivors 3 weeks later.

<sup>c</sup> Active MSV-M (0.05 ml) was injected i.m. into the left hind leg 40 days after the primary challenge, 70 days after presensitization. Serum was obtained from survivors 1 month later.

<sup>d</sup> ND, not determined.
Three to $4 \times 10^7$ viable, unfractionated spleen cells from mice that had been sensitized with attenuated MSV-M 40 days previously were injected i.p. into normal BALB/c mice. At the same time, the mice received active MSV-M i.m. In two separate but similar experiments, 2 of 9 and 2 of 10 mice died as a result (Table 5). All of the mice developed tumor at the site of injection. Two control groups were included. Normal spleen cells had no effect on the natural history of MSV-M, and test spleen cells alone had no effect at all on the mice.

**DISCUSSION**

The MSV-M-induced tumor system offers several experimental advantages over other virally or chemically induced tumors. Oncogenesis is extremely reproducible. Tumors appear very rapidly, e.g., within 5 to 10 days of virus injection (4, 13, 14). Autochthonous adult hosts generally mount an effective immune response that almost invariably results in rejection of the tumor (4).

Thus, the MSV system has served as an excellent model for studying strong antitumor immune responses. The results of the present study demonstrated that the MSV system also may be used to study immunosuppression. Presensitization of normal adult BALB/c animals with attenuated tumor virus resulted in immunosuppression that facilitated dissemination of solid tumor and allowed the development of leukemia following challenge with active virus.

The evidence for immunosuppression may be summarized as follows. While normal mice that develop and reject an MSV-induced tumor generally were immune to tumor induction following a 2nd injection of active virus, the presensitized mice were not immune to a 2nd challenge with active virus. Second, mice that died had disseminated tumorogenic virus as well as solid tumor nodules in sites distant from the original injection site. Third, the animals that had been presensitized developed leukemia and finally died following challenge with active MSV-M, although leukemia generally is not associated with MSV-induced tumors even when those tumors are progressive, as in newborn mice. Finally, the immunosuppression was transferred with lymphoid cells.

The critical experiments required to elucidate the nature of the suppression remain to be performed. At present, it is not clear whether specific or nonspecific suppression was functioning. However, the preliminary evidence presented in this paper appears to favor specific immunosuppression.

Nonspecific immunosuppression cannot be ruled out, and, at least in theory, leukemia virus could have produced such an effect. MLV has been shown to be an obligate associate (helper virus) of MSV-M (7, 11). If the attenuation process had in some way facilitated the active expression of MLV so that the sensitized animals had developed leukemia, that could have explained the immunosuppression. However, sensitized animals were observed for periods of up to 4 months and showed no signs of disease. Sensitized animals that were autopsied after 1 month (the normal time of challenge with active virus) showed no histological evidence of leukemia. If suppression were due to leukemia, then alteration of the sensitization schedule, i.e., increasing the number of doses of attenuated virus given in the same time-span from 1 to 4, might be expected to enhance the suppression. However, that altered sensitization schedule resulted instead in immunity to the tumor virus. Also, antibody responses to the MSV-M did not appear to be suppressed, since even in dying mice the antibody was present at a sufficiently high level to neutralize a standard dose of active virus. However, titrations were not performed. Finally, effective antitumor immunity was expressed at the same time as was the immunosuppression. The primary leg tumors were rejected both on primary and on secondary challenge. Considerable experimental difficulties are associated with definitive experimentation required to disprove nonspecific immunosuppression. First, other oncornaviruses could not be used because they share antigens with MLV and MSV (5). Second, if one were to use passaged, chemically induced or spontaneous tumors, it would be difficult to measure suppression because those tumors progress normally.

Nevertheless, there is strong indirect evidence in favor of specific immunosuppression from the present results. First, if the immunosuppression were nonspecific, then the primary and secondary leg tumors should have progressed as they do in animals that truly are nonspecifically immunosuppressed, e.g., newborn or X-irradiated animals. This is the key to the argument for specific suppression, and we speculate that those leg tumors were rejected by the host's immune response to neoantigens expressed on the tumor cell surface and that the specific immunosuppression was directed against viral antigens common to MSV and MLV (3, 15). Thus, the leukemia that ultimately killed the animals developed as a result of specific immunosuppression. Also, leg tumors appeared following a 2nd challenge with active virus, and tumorogenic virus was found disseminated throughout the animals, because the animals were not capable of a protective response against the viral (MSV and MLV) antigens.

Interestingly, the immunosuppression appears to have been independent of the function of humoral antibody, since animals with disseminated tumor and tumor virus also had neutralizing antibodies in their serum. Preliminary experiments demonstrating that the suppression could be passively transferred with spleen cells suggested that the suppression was mediated by cells, but this has not been proved.

**Table 5**

Passive transfer of immunosuppression with splenic lymphocytes from adult BALB/c mice sensitized with attenuated MSV-M

<table>
<thead>
<tr>
<th>Source of lymphocytes</th>
<th>No. of surviving mice following MSV-M challenge</th>
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<tr>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>Normal adult BALB/c mice</td>
<td>12/12</td>
</tr>
<tr>
<td>Sensitized adult BALB/c mice</td>
<td>15/19</td>
</tr>
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* Normal adult BALB/c mice received simultaneous injections of 3 to $4 \times 10^7$ splenic lymphocytes i.p. and 0.05 ml of active MSV-M i.m.
* Challenged i.m. 30 days later with 0.05 ml of active MSV-M i.m.
* Spleens were taken from 4 mice that had been sensitized 40 days previously by s.c. injections of 0.1 ml of attenuated MSV-M. Six control mice that received $3 \times 10^6$ splenic lymphocytes from the same source i.p. remained completely normal and healthy.
Suppression of MSV-M Immunity

We would pose the possibility that just as the MSV system is an excellent one for studying the antitumor immune response because of its magnitude, the manipulations described in the present paper create a model system where strong, possibly specific, immunosuppression may be studied.

REFERENCES

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