Protective Effect of Immune Sera against Transplantable Moloney Virus-induced Sarcoma and Lymphoma

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SUMMARY

Sera from mice immunized with Moloney sarcoma virus (MSV)-induced antigens were quantitated for antibodies by indirect membrane immunofluorescence (MF), cytotoxicity, and neutralization. These sera were then tested in vitro to determine whether antibody titers measured by these three methods would correlate with the effectiveness of the serum in preventing the growth of a transplantable Moloney virus-induced sarcoma (MSC) or lymphoma (LSTRA). The sources of sera were (a) BALB/c mice with progressively growing MSV-induced tumors (progressor serum); (b) BALB/c mice in which MSV-induced tumors regressed (regressor serum); and (c) BALB/c × DBA F1 mice immunized with multiple inoculations of MSV-induced allogeneic tumor cells (C57BL origin; allogeneic serum). The pooled progressor sera showed low antibody titers in all three tests. In contrast, the pooled regressor and allogeneic sera were positive in the MF and neutralization tests, although neither showed any cytotoxic activity. The MF and neutralization titers with the allogeneic serum were 2 to 4 times higher than with the regressor serum. Pretreatment of recipient mice with regressor or allogeneic sera as early as 3 days before challenge with MSC tumors resulted in (respectively) 60 and 84% survivors free of disease when the experiments were terminated at 75 days. In contrast, resistance was not noted following pretreatment with the progressor sera. The allogeneic serum was also effective against the rapidly growing transplantable Moloney leukemia virus-induced lymphoma designated LSTRA. Single injections of this serum in mice either 3 or 1 day before LSTRA challenge or 2 days after challenge resulted in significant numbers of survivors during the period of observation. Multiple injections of this serum during the latent period for tumor development resulted in up to 90% survivors, free of apparent disease, when the experiments were terminated at 60 days. These results demonstrated that the effectiveness of a serum in these systems in preventing tumor growth correlated closely with its MF and neutralization titers and suggested that measurement of antibody titers by conventional methods, particularly MF, might provide useful information on the potential therapeutic value of serum in the control of cancer.

INTRODUCTION

The cellular immune response is generally accepted as being primarily responsible for the rejection of antigenic tumors.

This has been demonstrated in animal systems by the adoptive transfer of tumor-specific immunity by lymphoid cells but rarely with serum (16, 20), and by the demonstration in vitro that lymphoid cells from appropriately immunized animals are cytotoxic to antigenic tumor cells (14). In contrast, in vitro evidence has accumulated that indicates that antibodies may act to inhibit or block the activity of immune lymphocytes, thereby allowing the tumor to circumvent the rejection mechanism of the host (15).

Recent investigations of MSV oncogenesis in mice have shed some light on the interaction of immune cells and serum in determining the outcome of virus infection. Sera from mice with progressively growing tumors induced by MSV have been reported to contain antibodies capable of blocking the cytotoxic effect of immune lymphocytes in vitro, while sera from mice with tumors that have regressed contain a factor designated "unblocking antibody" capable of abrogating the in vitro effect of blocking sera (11, 12, 14). Injection of sera containing blocking antibodies into MSV-infected mice resulted in enhanced tumor growth, whereas sera from regressor mice inhibited tumor production or induced regressions of palpable tumors (5, 13, 23). These results suggested that some sera may be effective in controlling tumor growth. Recently, Bansal and Sjögren (1) reported results in the polyoma virus tumor system that indicated that the presence of factors akin to unblocking antibodies in serum may correlate with antitumor effects of the antiserum in vivo.

These findings demonstrated the importance of characterizing each serum used in therapy experiments before reaching any conclusions on its effectiveness to control tumor growth. We decided to quantitate the antibody activities in sera from MSV-infected mice, using conventional tests, and to determine whether antibody titers measured by a method other than blocking would relate to the serum's effectiveness in influencing the growth of transplantable tumors in vivo. The tests used in these investigations (which have already been defined as useful assays for demonstrating the presence of Moloney virus-induced antigens) were MF, neutralization, and cytotoxicity (4, 8, 9). The results show that sera containing high titers of antimembrane or neutralizing antibodies inhibited the growth not only of a transplantable MSV-in-

1 The abbreviations used are: MSV, Moloney sarcoma virus; MF, indirect membrane immunofluorescence; MSVM, MSV (Moloney strain); MSC, cell line established from tumors induced in BALB/c mice by MSV; FCS, fetal calf serum; MSB, cell line established from tumors induced in C57BL mice by MSV; PBS, phosphate-buffered saline; MLV, Moloney leukemia virus; MST, median survival time.
duced sarcoma but also of a highly lethal transplantable Moloney virus-induced lymphoma when injected as early as 3 days before tumor cell challenge or during the latent period after tumor inoculation but before tumor appearance.

MATERIALS AND METHODS

Virus

MSV(M) was obtained from the Program Resources and Logistics Segment, Special Virus Cancer Program, National Cancer Institute, as Pool SVRP 215. The virus titer by focus assay on secondary mouse embryo culture was $2 \times 10^7$ focus-forming units per ml (18).

Animals

Male BALB/c x DBA F1 (hereafter called CDF1) mice and male BALB/c mice, 4 to 8 weeks of age, were obtained from the Charles River Breeding Laboratories, Wilmington, Mass. The animals were housed in plastic cages and fed Purina laboratory chow with water ad libitum.

Tumor

A Moloney lymphoid leukemia line (LSTRA), originally induced in BALB/c mice by the murine leukemia virus (Moloney), was kindly provided by Dr. John Pearson, National Cancer Institute. This line has been maintained and passaged routinely in CDF1 mice for over 300 generations. The ascitic tumor was serially transplanted i.p. at weekly intervals.

Tissue Culture

MSC Cell Line. This cell line, kindly provided by Dr. Wilna Woods, National Cancer Institute, was established from BALB/c tumors induced with MSV(M) and has previously been described (18). The cells were maintained in Roswell Park Memorial Institute medium plus 10% heat-inactivated FCS supplemented with 100 units penicillin per ml and 100 µg streptomycin per ml. The cells were passaged once a week by exposure to 0.25% trypsin. Falcon plastic flasks were then seeded with $1 \times 10^6$ cells/flask and incubated at 37°C in the presence of 10% CO2.

MSB cell line. This cell line was established from MSV(M)-induced tumors in C57BL mice. The cells were maintained and passaged as described above for the MSC cell line.

Production of Antisera to MSV-induced Antigens

Progressor Serum. Adult BALB/c mice were inoculated i.m. in the thigh of the hind leg with $10^6$ focus-forming units of MSV(M) per ml. The growth of tumors induced by this virus was monitored by palpation and measurement twice weekly. Progressively growing tumors developed in approximately 60% of the mice. This became apparent within 4 to 5 weeks after virus inoculation. These mice were separated from those in which tumors had regressed completely or were in the process of regression as determined by size measurements. The mice were bled from the brachial artery when the tumors were approximately 20 to 25 mm in diameter, which size was usually reached within 7 to 8 weeks after virus inoculation. The sera from approximately 60 mice were pooled and heat inactivated at 56°C for 30 min. This pooled serum, referred to as "progressor serum" was stored at $-20^\circ$ until used.

Regressor Serum. Approximately 40% of the tumors that developed following i.m. inoculation of MSV(M) regressed within 5 weeks of virus inoculation. These tumors usually reached a size of 5 to 7 mm in diameter before regression occurred. Following complete tumor regression, these mice were challenged 2 more times with MSV(M) at weekly intervals. The animals were bled 1 week after the last challenge. The serum samples from approximately 50 mice were pooled, heat inactivated at 56°C for 30 min, and stored at $-20^\circ$ until used. This pooled serum is referred to as regressor serum.

Allogeneic Serum. Adult CDF1 mice were immunized with multiple injections of MSB cells. The mice were inoculated i.m. at weekly or biweekly intervals with 1 to $3 \times 10^6$ MSB cells. Approximately 1 week after the last inoculation, the mice were bled from the brachial artery. The serum samples from approximately 80 mice were pooled and heat inactivated at 56°C for 30 min. The pooled serum, referred to as "allogeneic serum" was stored at $-20^\circ$ until used.

MF Test

The MF test has been described previously (21). Briefly, MSC cells were exposed to 0.25% trypsin and washed 2 times in PBS; then the cell concentration was adjusted to $1 \times 10^6$ viable cells per ml, as determined by trypan blue exclusion. The cells ($5 \times 10^4$) were then exposed to 0.1 ml of the appropriate serum dilution (2-fold) and were incubated at 37°C for 30 min. The cells were washed 3 times in PBS + 3% FCS, and then were resuspended in 0.1 ml fluorescein isothiocyanate-conjugated goat anti-mouse globulins (Hyland Laboratories, Los Angeles, Calif.). Following a 2nd incubation period at 37°C for 30 min, the cells were washed 3 times in PBS + 3% FCS and then were resuspended in 1 or 2 drops of 50% buffered glycerol. They were kept at 4°C until examined on a Reichert fluorescent microscope. Control preparations incubated with the fluorescent reagent alone or with normal serum followed by the fluorescent reagent were included in each test. Less than 1% of the MSC cells showed sector or full-ring fluorescence in these controls. A minimum of 100 cells were counted in each preparation to determine the percentage of MF-positive cells showing sector or full-ring fluorescence. The last serum detection giving approximately 10% MF-positive cells was chosen as the serum titer.

Neutralization Assay

The sera were tested for the presence of anti-MLV-neutralizing antibodies by means of the X-C assay (24).
Twofold dilutions of immune and normal sera were incubated for 60 min at room temperature with 50 plaque-forming units of MLV. These samples were then assayed for virus infectivity by the X-C assay.

$^{51}$Cr Release Cytotoxic Test

A modification of the technique described by Sanderson (25) and Wigzell (29) was used. LSTRA, MSB, or MSC cells (5 to 10 x 10^6 cells in 1 ml PBS) were incubated with 250 µCi of $^{51}$Cr (sodium chromate; specific activity, 200 to 400 µCi/ml) for 45 min at 37°C. The cells were then washed 3 times in Roswell Park Memorial Institute Medium 1640 + 10% FCS. Following the last wash, the cells were resuspended in the same medium and incubated overnight at 4°C. They were then washed 3 more times and diluted in PBS to 5 x 10^6 cells/ml. Then 5 x 10^2 cells were incubated with 0.1 ml of a 1:5 dilution of antisera and 0.1 ml of a 1:2 dilution of fresh guinea pig serum as the source of complement. Controls were included to determine the spontaneous release of $^{51}$Cr and for nonspecific toxicity due to either serum or complement alone.

Each preparation was set up in triplicate. The mixtures were incubated for 18 to 24 hr at 37°C. Following the incubation period, 0.3 ml PBS was added to each sample, and the tubes were centrifuged at 2000 rpm for 10 min. A 0.3-ml sample was removed and counted in a Packard Tri-Carb γ counter. Cytotoxicity was calculated as cpm released from cell:serum mixture minus spontaneous release divided by total initial cpm — spontaneous release X 100. The spontaneous release of $^{51}$Cr over this time interval was usually between 30 and 40%.

RESULTS

Characterization of Anti-MSV Sera. Pooled sera from BALB/c mice bearing progressively growing MSV-induced tumors (progressor serum), from BALB/c mice in which MSV-induced tumors had regressed (regressor serum), and from CDF1 mice immunized with allogeneic tumor cells (C57BL origin) induced by MSV (allogeneic serum) were titrated for antibodies by MF, neutralization, and cytotoxicity. Representative results of these tests are shown in Tables 1 to 3. The pooled progressor serum showed low antibody activity in all 3 tests. In contrast, the regressor and allogeneic sera were positive with high titers in the MF and neutralization assays. The titers with the allogeneic sera were consistently 2 to 4 times higher than those of the regressor serum. Neither serum was cytotoxic to any of the 3 cell lines tested over an 18- to 24-hr incubation period. However, the MSC and LSTRA cells were killed by a rat anti-MSV serum in the presence of guinea pig complement, whereas the MSB cells were susceptible to the allogeneic serum that contained antibodies to the C57BL histocompatibility antigens as well as to MSV-induced antigens.

Three different pools of each serum were titrated in these 3 assays during the course of these investigations. The results in each instance were similar to those shown in Tables 1 to 3.

Effect of Different Sera on the Growth of MSV-induced Tumor Cells. It had previously been reported that progressor sera caused enhancement of the growth of MSV-induced tumors, while regressor sera inhibited tumor growth or produced tumor regression (5, 13, 23). It was of interest therefore to determine whether these in vivo activities correlated with the MF and neutralization titers of the sera. BALB/c mice were inoculated i.p. with 0.2 ml of progressor or regressor serum. Three days later, these mice were challenged i.m. with 1 x 10^5 MSC cells. The mice were monitored over a 75-day period for tumor appearance, size of tumors, and progressively growing tumors. In our experience, mice that are free of disease after this time period usually remain disease free. The results are presented in Table 4. In the 2 control groups of mice, 90 and 100% of the mice developed tumors at the site of inoculation. Seventy % of the tumors in the untreated group grew progressively, while 90% of the tumors in the group treated with normal serum grew progressively over the 75-day observation period. The MST's for the 2 groups were 66 and 69 days, respectively. In the group of mice inoculated with progressor serum, resistance to tumor challenge was not observed. All 10 mice developed progressively growing tumors at the site of inoculation, and there were no survivors when the experiment was terminated.

The MST for this group was 49 days, which was not significantly different from that of the control groups (p < 0.07) but was suggestive of enhancement. In contrast, in mice pretreated with progressor serum 3 days before tumor challenge, only 5 of 10 mice developed tumors, 4 of which grew progressively. There were 60% survivors in this group, all tumor free when the experiment was terminated at 75 days.

The difference in the in vivo effect of progressor and regressor serum on the growth of MSC cells is also shown in Chart 1. The average size of the tumors in mice pretreated with progressor serum, when measured at different times after tumor challenge, was slightly increased above the control, but the differences, although suggestive of enhancement, were not significant. In contrast, tumors that developed in mice pretreated with regressor serum grew slowly over the 75-day observation period, compared with the other 2 groups.

The effect of the allogeneic immune serum preparation on the growth of MSC tumor cells was much more striking (Table 5). Only 4 of 19 mice developed tumors in the group pretreated with allogeneic serum 3 days before MSC tumor challenge. Eighty-four % of the mice were tumor free and still alive when the experiment was terminated at 75 days, as opposed to 10 and 20% in the 2 control groups. These results demonstrated that the effectiveness of the regressor and allogeneic sera and the ineffectiveness of the progressor serum in transferring resistance to a tumor cell challenge correlated closely with their MF and neutralization titers when tested in this system.

Effects of Regressor and Allogeneic Sera on the Growth of Moloney Virus-induced Leukemia (LSTRA). Since some of the tumors induced by MSV regress spontaneously, suggesting that these tumors are unusually susceptible to immune factors (5), we decided to determine the effectiveness of the different immune serum preparations on the rapidly growing MLV-induced tumor transplant designated LSTRA (7, 10). Most CDF1 mice that are inoculated s.c. with as few as 500 LSTRA cells develop a rapidly growing tumor at the site of
Table 1
Titration of progressor, regressor, and allogeneic sera on MSC cells by MF
None of the sera reacted by MF with normal BALB/c mouse embryo fibroblasts or with a number of rat cell lines producing rat type C virus particles.

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>Normal</th>
<th>Progressor</th>
<th>Regressor</th>
<th>Allogeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>&lt;1.0</td>
<td>36.0</td>
<td>61.0</td>
<td>83.0</td>
</tr>
<tr>
<td>4</td>
<td>N.T.*</td>
<td>22.0</td>
<td>52.0</td>
<td>81.0</td>
</tr>
<tr>
<td>8</td>
<td>N.T.</td>
<td>12.0</td>
<td>36.0</td>
<td>75.0</td>
</tr>
<tr>
<td>16</td>
<td>N.T.</td>
<td>3.0</td>
<td>33.0</td>
<td>58.0</td>
</tr>
<tr>
<td>32</td>
<td>N.T.</td>
<td>3.0</td>
<td>28.0</td>
<td>42.0</td>
</tr>
<tr>
<td>64</td>
<td>N.T.</td>
<td>3.0</td>
<td>13.0</td>
<td>25.0</td>
</tr>
<tr>
<td>128</td>
<td>N.T.</td>
<td>3.0</td>
<td>5.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Titer*</td>
<td>8.0</td>
<td>64.0</td>
<td>≥128.0</td>
<td></td>
</tr>
</tbody>
</table>

* N.T., not tested.  
* The serum titer end point was determined as the last dilution staining approximately 10% MSC cells.

Table 2
Neutralization titers of progressor, regressor, and allogeneic sera
Neutralization titers were determined by means of the X-C assay.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Serum dilution</th>
<th>Av. no. of plaques*</th>
<th>Titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Progressor</td>
<td>10</td>
<td>57</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Regressor</td>
<td>20</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>51</td>
<td>54</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>80</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>72</td>
<td>222</td>
</tr>
</tbody>
</table>

* Each serum dilution was tested in triplicate.  
* Reciprocal of the serum dilution that resulted in a 50% reduction in plaque-forming units was calculated as the neutralization titer.

Table 3
Lack of cytotoxic antibody activity in progressor, regressor, and allogeneic sera tested against 3 cell lines

<table>
<thead>
<tr>
<th>Serum*</th>
<th>Cytotoxicity in target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSC</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>Progressor</td>
<td>-</td>
</tr>
<tr>
<td>Regressor</td>
<td>-</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>-</td>
</tr>
<tr>
<td>Rat anti-MSV</td>
<td>-</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>Normal</td>
<td>+</td>
</tr>
<tr>
<td>Progressor</td>
<td>+</td>
</tr>
<tr>
<td>Regressor</td>
<td>+</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>+</td>
</tr>
<tr>
<td>Rat anti-MSV</td>
<td>+</td>
</tr>
</tbody>
</table>

* Sera tested at 1:10 dilution.  
* N.T., not tested.

inoculation, splenomegaly, and lymphadenopathy; they succumb to leukemia within 25 to 30 days after inoculation (J. Pearson, personal communication). When 1 X 10⁴ cells are inoculated s.c., the disease can be detected systemically within 7 days after tumor challenge, and the mice succumb to leukemia within 12 to 15 days (22). In the experiments reported here, a tumor cell challenge number of 3 X 10³ was chosen, since this was the minimum number of cells that reproducibly produced 100% tumors in all recipient mice. With this number of cells, the MST was approximately 15 days. Tumors usually started appearing at the site of injection approximately 8 days after inoculation.

Recipient CDF₁ mice were inoculated once i.p. with 0.2 ml serum at either 1 or 3 days before challenge with LSTRA cells, or 2 days after challenge. These mice were then monitored for MST and percentage of survivors over a 60-day observation period. The results of these experiments are presented in Table 6. The injection of normal or regressor sera at these 3 time intervals had no apparent effect on LSTRA disease, compared to the untreated controls. The MST in these 2 groups ranged
Effect of regressor and progressor sera on the growth of MSV-induced tumor cells

MSC cells (1 X 10⁴) were inoculated s.c. on Day 0 (Groups 1 through 4) as for tumor challenge.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum treatment</th>
<th>No. of tumors/ no. of mice inoculated</th>
<th>No. of progressors/ no. of tumors</th>
<th>MST (days)</th>
<th>% of survivors at 75 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>9/10</td>
<td>7/9</td>
<td>66</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Regressor</td>
<td>10/10</td>
<td>9/10</td>
<td>69</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Progressor</td>
<td>5/10</td>
<td>4/5</td>
<td>&gt;75</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>10/10</td>
<td>10/10</td>
<td>49</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4

a Serum (0.2 ml) was inoculated i.p. 3 days before tumor challenge.
b Survivors were free of disease when experiment was terminated at 75 days.
c The MST in Group 4 is not significantly different (p < 0.07) from the values for the control groups as determined by the 2-sample Rank sum test.

from 15 to 18 days as compared to 15 days in the control group. There were no survivors in any of these groups when the experiment was terminated. In contrast, the allogeneic serum was effective in protecting mice against LSTRA disease at all 3 time intervals. When this serum was inoculated either 3 days before or 2 days after LSTRA challenge, the MST's were increased to 26 and 25 days, respectively, with 30% of the mice still surviving, free of any detectable disease, when the experiment was terminated at 60 days. In the group inoculated with allogeneic serum 1 day before LSTRA challenge, 60% of the mice were disease free at 60 days.

A 2nd experiment was performed to determine the effectiveness of single and multiple injections of allogeneic serum when given after LSTRA challenge but before tumor development. These results are shown in Table 7. Multiple injections of the allogeneic serum given during the latent period of tumor development were highly effective in inhibiting LSTRA disease. Ninety-two % of the mice that received 3 injections of serum were still surviving, free of LSTRA disease, 60 days after inoculation of the LSTRA cells, as opposed to no survivors in the control groups.

DISCUSSION

The use of immune serum for preventing tumor growth or for the treatment of established disease has met with very little success. Enhancement of tumor growth, rather than suppression, appears to be the most frequent finding (2, 19). In some experimental systems, however, serum from specifically immune animals has prevented tumor production and in some cases has been effective against established tumors. Tumors induced by MSV have been particularly susceptible to the activity of immune serum (3, 5, 6, 17). Recent evidence suggests that the active elements in certain sera that are responsible for the protective or therapeutic effects in this system may be akin to the unblocking antibodies identified in in vitro systems (14).

It has been reported that sera from mice with MSV-induced tumors contain antibodies other than blocking or unblocking antibodies that can be demonstrated by various immunological tests. These assays include MF, cytotoxicity, and neutralization (4, 8, 9). Bubenik and Turano (3) recently reported that sera containing MF and neutralizing antibodies were effective in inhibiting tumor induction for up to 5 days after MSV infection. The purpose of these experiments was to investigate this finding further, not only against a Moloney virus-induced sarcoma but also against a rapidly growing lymphoma, and to determine whether quantitation of antibody measured by any of these 3 methods would correlate with the in vivo activity of the serum.

The sera studies in these experiments originated from the following 3 different groups of donor mice: (a) BALB/c mice with progressively growing tumors (progressor serum); (b) BALB/c mice in which tumors had regressed (regressor serum); and (c) CDF₁ mice immunized with multiple injections of allogeneic tumor cells (C57BL) induced by MSV (allogeneic serum). None of the donor mice were ever exposed to the MSC or LSTRA cells, which were used as the test tumors in these experiments. The regressor and allogeneic pooled sera contained high antibody titers directed against Moloney virus-induced antigens, as determined by MF and
neutralization, and the titers of the allogeneic serum were consistently 2- to 4-fold higher than those of the regressor serum. Interestingly, the pooled regressor serum contained low antibody titers as measured by both tests. Since regressor sera reportedly contain blocking antibodies that are measured in vitro by incubation of viable cells with antibody before reaction of the cells with immune lymphocytes (11), one might expect blocking antibodies to be directed against membrane-associated antigens. Sera that contain blocking antibodies might then be expected to contain high antibody titers as measured by the MF test. However, Skurzak et al. (27) recently reported results suggesting that the blocking phenomenon might be a quantitative and not a qualitative problem. High antibody concentrations acted synergistically with immune lymphocyte populations in vitro, while more dilute serum had an antagonistic action against the same immune cells. Our findings, which demonstrate low antibody titers in the progressor serum as measured by the MF test but high titers in the regressor and allogeneic serum preparations, would support this explanation. None of the sera contained cytotoxic antibody when measured in cytotoxic tests against MSB, MSC, and LSTRA cells. In 1 experiment, not reported...
here, rabbit complement was used instead of guinea pig complement. No cytotoxic effect was noted with this source of complement. This is in agreement with results reported by Bubenik and Turano (3) but is in disagreement with reports by others (13). It is possible that cytotoxic antibody activity would have been detected in these sera if the target cells had been incubated with antibody and complement over a longer time period, as in the colony inhibition test, or if the sera had been tested against more immunosensitive target cells.

When mice pretreated with progressor serum were challenged with MSC cells, resistance to tumor challenge was not noted. To the contrary, slightly enhanced growth was suggested. Pretreatment with regressor and allogeneic sera inhibited MSC tumor production. This was particularly striking with the allogeneic serum, which contained the highest MF and neutralizing antibody titers. Thus, in these experiments, the protective action of different immune sera correlated with their MF and neutralizing antibody titers.

More important, the most potent serum, the allogeneic serum, was also effective in preventing LSTRA disease. Inoculation of these cells into recipient mice results in a highly fatal leukemia in all mice (7, 10, 22). Regressions are never observed in this system, as opposed to the high number of spontaneous regressions in mice inoculated with MSV (5). This serum was effective against LSTRA disease when given either 1 or 3 days before LSTRA tumor cell challenge or during the latent period of tumor development. Multiple injections of regressor or regressor sera during the latent period were ineffective in influencing the course of LSTRA disease (unpublished results).

The results presented here demonstrate that quantitation of antibody activity by the MF and neutralization assays might be useful as an indicator of the potential effectiveness of sera in these systems in controlling tumor growth. The active factors responsible for the protective effects noted with the regressor and allogeneic sera, however, are unknown. A likely explanation is that the immune serum is acting synergistically with the reticuloendothelial cells of the host in rejecting the tumor grafts. However, other unknown factors might also be involved. Both the regressor and allogeneic sera were effective in preventing MSC tumor growth when inoculated as early as 3 days before tumor challenge. This was rather surprising, since mouse γ-globulin has been reported to have a half-life of 3 to 5 days (28). This suggests that antibody might not be the active factor in these sera. Whatever the mechanism, the results presented here demonstrate that well-characterized sera should be considered, as well as immune lymphocytes, in experiments investigating immunological control measures for cancer. They also indicate that quantitative measurement of antibody by more conventional methods, primarily MF, could very well provide as much information on the potential therapeutic value of serum as do the more complex blocking and unblocking tests.

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