The Adoptive Transfer of Concomitant Immunity to Murine Tumor Isografts with Spleen Cells from Tumor-bearing Animals

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SUMMARY

A methylcholanthrene-induced fibrosarcoma (MCA-10) in male and female C57BL/6 mice was used to study the presence of concomitant immunity in a syngeneic murine tumor-host system. A group of 1125 mice was inoculated s.c. with \(10^5\) viable MCA-10 cells in the right hind limb. Groups of 25 mice randomly selected from the original 1125 mice were challenged with \(5 \times 10^5\) MCA-10 cells s.c. in the left hind limb at days 1, 7, 14, 21, and 28 days after initial tumor transplantation. Untreated mice received \(5 \times 10^5\) MCA-10 cells at identical times. At 7, 14, 21, and 35 days after tumor transplantation, four additional groups of 50 MCA-10 tumor-bearing mice were sacrificed, the tumor weight to total body weight ratio was calculated, and a spleen cell suspension was prepared. Viable spleen cells from these animals (\(5 \times 10^7\) to \(5 \times 10^8\)) were injected i.p., into four groups of 25 untreated syngeneic mice. MCA-10 cells (\(5 \times 10^8\)) were inoculated s.c. into mice in each group 1, 7, 14, or 21 days after splenocyte transfer. Controls received tumor cells alone, or tumor cells and spleen cells from normal syngeneic mice.

In the groups of tumor-bearing mice, the challenge tumor cell inocula grew in 0 to 8% of animals at all intervals after initial tumor transplantation except Day 1. Tumors developed in 87 to 100% of control animals. At day 1, tumors developed similarly in the left hind limb of both control and tumor-bearing animals. Mice which received spleen cells from tumor-bearing animals manifested a significant delay in tumor development and/or decrease in tumor incidence at all initial tumor transplantation-spleen cell transfer time intervals except 1 and 7 days.

INTRODUCTION

Tumor-specific transplantation immunity can be induced experimentally in syngeneic hosts against a wide variety of chemical- and virus-induced cancers (21, 24, 30). This immunity to TSTA\(^1\) is usually demonstrated by the rejection of tumor isografts in animals immunized by excision of growing transplants of the tumor under investigation.

\(^1\)The abbreviations used are: TSTA, tumor-specific transplantation antigens; SCT, spleen cell transfer; TC, tumor challenge; MCA, methylcholanthrene; BSS, Hanks' balanced salt solution; ITT, initial tumor transplantation.

Immunity to TSTA produced by excision of tumors induced by polycyclic hydrocarbons is usually specific for the TSTA of the immunizing tumor (29, 33), whereas immunity to the TSTA of a virus-induced neoplasm usually cross-reacts with all tumors induced by the same oncogenic virus (19, 24, 29, 30). Recently, the existence of tumor-specific cell surface antigens in some human tumors has been confirmed (9, 12, 17, 22, 25, 27, 28). Eilber and Morton (9) have demonstrated a common tumor-specific sarcoma antigen in tissue culture cell lines derived from several different histological types of sarcoma and have detected specific antibodies to this common sarcoma antigen in the sera of sarcoma patients. Morton et al. (27) and Oetgen et al. (28) have also demonstrated antibodies in the sera of patients with malignant melanoma that react with autochthonous as well as allogeneic melanoma cells. These observations suggested that malignant melanomas and sarcomas contain common tumor antigens which are immunogenic in patients bearing these neoplasms, and that these patients mount a significant immune response to these antigens despite persistent growth of the primary tumor.

Recent investigations by Hellström et al. (16) have demonstrated that the growth in vitro of tumor cells from patients with neuroblastomas is inhibited by the patient's autologous lymphocytes, by lymphocytes from other patients with neuroblastomas, and by lymphocytes of close relatives of the patients whose tumors were studied. These exciting findings are clinical confirmation of earlier experimental work in which it had been demonstrated that experimental tumor-bearing animals manifested immunity to the TSTA of their tumors despite the progressive growth of the autochthonous or transplanted primary tumor (11, 23). An immune response directed against TSTA and detected in a host bearing a progressively growing tumor is referred to as concomitant immunity. Effective immunotherapy of cancer may depend upon understanding the paradox of progressive growth of an antigenic tumor in an immunologically competent host, despite the concomitant existence of immune responses against the TSTA of that tumor.

Experiments were, therefore, designed to investigate (a) the presence of concomitant immunity within a syngeneic murine tumor-host system, (b) the adoptive transfer of this immunity to untreated syngeneic mice by spleen cells from tumor-bearing mice, and (c) the magnitude of this response relative to tumor size at the time of SCT and the time interval between SCT and challenge of the spleen cell recipients with a challenge inoculum of viable tumor cells (SCT-TC interval).
MATERIALS AND METHODS

Tumor-Host System. Inbred, male and female adult C57BL/6 mice obtained from the Animal Production Unit of the NIH were used. The tumor used was a sarcoma, designated MCA-10, induced in an adult female C57BL/6 mouse by the SC. injection of 0.1 ml of a 1% solution of 20-MCA in sesame oil. The MCA-10 sarcoma was maintained by serial transplantation in female C57BL/6 mice and was used in its 8th through 10th transplant generations. The tumor was shown to contain TSTA by the method of Prehn and Main (33). Inocula of 10^6 cells, which grew to palpable tumors in 90 to 100% of untreated C57BL/6 mice, failed to grow when injected into mice that had been subjected to temporary tumor growth followed by excision of growing MCA-10 tumor transplants (6).

Preparation of Tumor Cell Preparations. Single tumor cell suspensions were prepared by a modification of the method of Hammond et al. (13). Tumor tissue was trimmed of necrotic and fibrous tissue, finely minced, and placed in trypsinizing flasks. Dulbecco's balanced salt solution containing 0.25% Pronase was then added (10 ml/g of tumor tissue). The mixture was agitated on a magnetic stirrer for 10 to 15 min at room temperature and filtered through sterile cheesecloth. DNase (0.04%) in distilled water (Sigma Chemical Co., St. Louis, Mo.) was added to the suspension (0.3 ml/g of tumor tissue). The cell suspension was then centrifuged at 250 X g for 10 min at 4°C, resuspended in BSS containing penicillin and streptomycin, and washed at least 3 times in BSS. The final cell button was resuspended in BSS, and viable cells were quantitated in a hemocytometer by determining the number of cells which excluded 0.4% trypan blue. Approximately 85 to 95% of the cells in these tumor cell suspensions were viable by this criterion.

The cumulative incidence of tumor development following transplantation of varying numbers of viable MCA-10 tumor cells in 0.1 ml of BSS s.c. in the hind limbs of untreated mice was determined (6). A s.c. inoculum of 5 X 10^5 viable tumor cells gave rise to palpable tumors in 80 to 90% of adult mice. Transplantation of 10^6 viable MCA-10 cells s.c. in 0.1 ml of BSS produced palpable tumors in all mice in 10 to 12 days. All tumors grew progressively. Spontaneous regressions were not observed.

Concomitant Immunity Studies. A group of 1125 male and female C57Bl/6 mice were inoculated s.c. in the right hind limb with 10^5 viable MCA-10 cells in 0.1 ml of BSS. One day after the ITT, 25 animals were randomly selected from this group and were inoculated s.c., in the opposite (left) hind limb with 5 X 10^3 MCA-10 cells from the same transplant generation as that used for the ITT. Twenty-five untreated C57Bl/6 mice likewise received 5 X 10^3 MCA-10 cells s.c. in the left hind limb on this day and served as challenge controls.

Additional groups of 25 MCA-10 tumor-bearing animals were randomly selected from the original 1125 MCA-10 tumor-bearing mice 7, 14, 21, and 28 days after ITT. At each of these time intervals, these mice were inoculated with 5 X 10^3 viable MCA-10 cells s.c. in the left hind limb. Again, at each time interval, 25 untreated, age-matched C57Bl/6 mice received 5 X 10^3 MCA-10 cells alone and served as challenge controls. Mice in all groups were observed 3 times weekly for tumor development in the left hind limb. The remaining animals from the original 1125 MCA-10 tumor-bearing mice were sacrificed in randomly selected groups of 50 at 7, 14, 21, 28, and 35 days after ITT. Tumors were carefully dissected from the carcasses of these mice, and the mean tumor weight to mean total body weight ratio was calculated. This allowed a correlation to be established between tumor size, time interval following initial tumor transplantation (6), and the incidence of tumor development in the left hind limbs of the experimental mice bearing progressively growing tumors in the right hind limbs.

Adoptive Transfer Studies. At 7, 14, 21, 28, and 35 days after the s.c. transplantation of 10^5 viable MCA-10 tumor cells into the right hind limbs of 1125 inbred C57Bl/6 mice, randomly selected groups of 50 tumor-bearing mice were sacrificed, and their spleens were removed. The spleens from each group of mice were minced separately with fine scissors in cold BSS and passed through 40 mesh stainless steel screens. The suspensions were then filtered through 80 mesh stainless steel screens and centrifuged at 200 X g for 10 min. The cell button from each group of 50 spleens was then resuspended in BSS to a concentration of 5 X 10^7 to 5 X 10^8 viable spleen cells/ml.

Four groups of 25 untreated, age-matched, male C57Bl/6 mice were then inoculated i.p. with 1 ml of this spleen cell suspension. One of these groups was then challenged s.c. with 5 X 10^6 viable MCA-10 tumor cells 1, 7, 14, or 21 days after "immune" SCT. At each time interval after ITT, 4 groups of 50 untreated C57Bl/6 mice were sacrificed synchronously with the groups of MCA-10 tumor-bearing mice. Cell suspensions were prepared from the spleens of these normal mice in the manner described above. Four groups of 25 untreated C57Bl/6 male mice were inoculated i.p. with 1 ml of these nonimmune spleen cells at a concentration of 5 X 10^7 to 5 X 10^8 spleen cells/ml at 1, 7, 14, or 21 days after this "normal" spleen cell transfer, 1 of these 4 groups was challenged s.c. with 5 X 10^3 viable MCA-10 tumor cells in 0.1 ml of BSS. At each time interval, 25 normal male C57Bl/6 mice were inoculated s.c. with 5 X 10^3 MCA-10 tumor cells in 0.1 ml of BSS and served as challenge controls.

Mice in all groups were then observed 3 times weekly for tumor development. Palpable tumors were also measured 3 times weekly, and tumor volumes were calculated by the formula, volume = 0.4 ab^2, where a is the major axis and b is the minor axis of the tumor measured at right angles to each other (1). x^2 statistical analyses of the cumulative incidence of tumor development in each group at the several time intervals after tumor challenge were performed with Fisher's exact test (10), and tumor volumes were analyzed with Student's 1-tailed t test (8).

RESULTS

Concomitant Immunity. As is illustrated in Chart 1, C57Bl/6 mice previously inoculated with 10^5 MCA-10 tumor cells rejected a 2nd challenge inoculum of 5 X 10^3 MCA-10 cells.
cells at all time intervals tested following ITT except Day 1. No primary tumors were as yet palpable in the right hind limbs of the experimental animals 1 or 7 days after ITT. However, tumors developed later in the right hind limb of all these animals. A significant decrease in the incidence of development of the second tumor isograft (left hind limb) was noted in those mice that received the challenge inoculum of tumor cells 7 days after the ITT, when compared to control mice that received $5 \times 10^4$ MCA-10 cells alone. No immunity to the second tumor cell inoculum was noted in mice rechallenged with $5 \times 10^3$ MCA-10 cells 1 day after ITT. The right leg primary tumors were palpable in both groups by 12 days after ITT and grew progressively.

Mice rechallenged in the opposite (left) hind limb with $5 \times 10^3$ tumor cells 14 days after ITT failed to develop any palpable tumors in the left hind limb. At 14 days, the right hind limb (primary) tumors were very small and represented approximately 0.6% of the total body weight of each animal. Again these primary tumors grew progressively in a pattern not significantly different from control animals inoculated with $10^8$ MCA-10 cells, and never rechallenged.

At 21 and 28 days after ITT, the percentages of mean tumor weight to mean total body weight were 3.9% and 9.7%, respectively. At both time intervals, MCA-10 tumor-bearing mice demonstrated a marked reduction in the incidence of development of secondary tumor isografts (left hind limb) following a 2nd challenge inoculum of $5 \times 10^3$ MCA-10 cells. Tumors developed in 95 to 100% of challenge control mice at all time intervals.

**Adoptive Transfer Studies.** Tables 1 through 5 illustrate that significant resistance to the development of MCA-10 tumor isografts could be adoptively transferred to untreated syngeneic mice by a single i.p. injection of $5 \times 10^6$ to $5 \times 10^8$ viable spleen cells from mice inoculated s.c. in the right hind limb with $10^8$ viable MCA-10 tumor cells 14, 21, 28, or 35 days prior to SCT. Table 1 demonstrates that the transfer of spleen cells from mice inoculated in the right hind limb with

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**Table 1: Adoptive transfer of immunity with spleen cells from tumor-bearing mice**

The interval between ITT and SCT was 7 days. The immune group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from MCA-10 tumor-bearing mice, and receiving inoculations 1, 7, 14, or 21 days after SCT with $5 \times 10^8$ MCA-10 cells. The normal group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from normal C57BL/6 mice, and receiving s.c. inoculations 1, 7, 14, or 21 days after SCT with $5 \times 10^3$ MCA-10 cells. The challenge group was comprised of C57BL/6 mice receiving s.c. injections of $5 \times 10^3$ MCA-10 tumor cells alone.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Interval between SCT and tumor challenge (days)</th>
<th>Total mice in group</th>
<th>10 days</th>
<th>15 days</th>
<th>25 days</th>
<th>35 days</th>
<th>Final tumor incidence at Day 40</th>
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<tbody>
<tr>
<td>Immune</td>
<td>1</td>
<td>25</td>
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<td>25 (100%)</td>
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<td>18 (100%)</td>
<td></td>
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</tr>
<tr>
<td>Challenge</td>
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<td>20</td>
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<td>24 (100%)</td>
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<td>23 (96%)</td>
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<td>22</td>
<td>22 (92%)</td>
<td></td>
</tr>
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<td>Immune</td>
<td>21</td>
<td>24</td>
<td>6</td>
<td>13</td>
<td>21</td>
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<td>21 (84%)</td>
</tr>
<tr>
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<td>24</td>
<td>8</td>
<td>16</td>
<td>24</td>
<td>24</td>
<td>24 (100%)</td>
<td></td>
</tr>
<tr>
<td>Challenge</td>
<td>24</td>
<td>10</td>
<td>21</td>
<td>24</td>
<td>24</td>
<td>24 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Cumulative tumor incidence expressed as no. of mice with tumors of the total no. of mice in the experimental group on a given day after tumor challenge at each interval between SCT and tumor challenge.

\(^b\) p < 0.05 when immune group is compared to control groups. No other statistically significant differences exist.
Adoptive Transfer of Concomitant Immunity

The interval between ITT and SCT was 14 days. The immune group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from MCA-10 tumor-bearing mice, and receiving inoculations 1, 7, 14, or 21 days after SCT with $5 \times 10^5$ MCA-10 cells. The normal group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from normal C57BL/6 mice, and receiving s.c. inoculations 1, 7, 14, or 21 days after SCT of $5 \times 10^5$ MCA-10 cells. The challenge group was comprised of C57BL/6 mice receiving s.c. $5 \times 10^5$ MCA-10 TC alone.

10$^5$ MCA-10 cells 7 days previously did not confer upon the spleen cell recipients any resistance to a challenge inoculum of $5 \times 10^5$ MCA-10 cells. Palpable tumors had not yet appeared in the original MCA-10 tumor-bearing mice 7 days after ITT, and a uniform failure to demonstrate the adoptive transfer of immunity with spleen cells from these mice was noted at all 4 SCT-TC intervals studied. Furthermore, no significant difference in mean tumor volume was noted when the size of tumors that developed in the recipients of spleen cells from tumor-bearing mice (immune) were compared with tumors developing in the recipients of spleen cells from untreated mice (normal), or with tumors arising in challenge control mice.

By 14 days after ITT (Table 2), tumors were palpable in all mice, and the mean tumor weight to body weight ratio was approximately 0.6%. Resistance to the growth of challenge tumor isografts was noted in mice that received a single i.p. inoculation of $5 \times 10^7$ to $5 \times 10^8$ spleen cells from tumor-bearing mice at this time interval after ITT. However, no resistance was noted in immune spleen cell recipients when the challenge tumor cell inoculum was administered 1 or 21 days after SCT. Resistance was apparent only at SCT-TC time intervals of 7 and 14 days. At an SCT-TC interval of 7 days, mice that received immune spleen cells evidenced a significant decrease in the incidence of tumor development at all time intervals after tumor challenge when compared to recipients of normal spleen cells or with mice that received tumor cells alone. At an SCT-TC interval of 14 days, a significant delay in the development of palpable tumors was noted in recipients of immune spleen cells from Day 10 through Day 20 after tumor challenge ($p<0.01$). No significant difference in final cumulative tumor incidence was noted between all 3 groups, however.

The mean tumor weight to mean total body weight of the MCA-10 tumor-bearing mice was 3.9%, 21 days after ITT (Table 3). Significant resistance to the development of tumor isografts was noted in mice that received spleen cells from tumor-bearing mice at SCT-TC intervals of 7 and 21 days. Again, no immunity was noted in recipients of immune spleen cells challenged 1 day after SCT. Resistance was also not apparent in recipients of immune spleen cells challenged 14 days after SCT.

At 28 and 35 days after ITT (Tables 4 and 5), the mean tumor weight to mean total body weight ratios of MCA-10 tumor-bearing mice were 9.7% and 13.9%, respectively. Again, no immunity was noted in mice inoculated with $5 \times 10^5$ MCA-10 tumor cells 1 day after SCT. However, significant inhibition of tumor development was noted in recipients of immune spleen cells at SCT-TC intervals of 7, 14, and 21 days. This resistance was most marked when recipients of immune spleen cells were challenged 21 days after SCT.

DISCUSSION

It is generally accepted that many experimental and human cancers contain tumor-specific cell surface antigens. TSTA can be detected in chemically induced and virus-specified experimental tumors by the demonstration that syngeneic animals, immunized by excision of growing transplants of the
The interval between ITT and SCT was 21 days. The immune group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from MCA-10 tumor-bearing mice, and receiving inoculations 1, 7, 14, or 21 days after SCT of 5 × 10^5 MCA-10 cells. The normal group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from normal C57BL/6 mice, and receiving s.c. inoculations 1, 7, 14, or 21 days after SCT of 5 × 10^5 MCA-10 cells. The challenge group was comprised of C57BL/6 mice receiving s.c. inoculations of 5 × 10^5 MCA-10 cells alone.

**Table 3**

Adoptive transfer of immunity with spleen cells from tumor-bearing mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Interval between SCT and tumor challenge (days)</th>
<th>Total mice in group</th>
<th>Cumulative tumor incidence&lt;sup&gt;a&lt;/sup&gt; at following intervals after tumor isograft</th>
<th>Final tumor incidence at Day 40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 days</td>
<td>15 days</td>
</tr>
<tr>
<td>Immune</td>
<td>1</td>
<td>23</td>
<td>2</td>
<td>17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
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<td>25</td>
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<td>15</td>
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<tr>
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<td></td>
<td>18</td>
<td>14</td>
<td>18</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cumulative tumor incidence expressed as no. of mice with tumors of the total no. of mice in the experimental group on a given day after tumor challenge at each interval between SCT and tumor challenge.

<sup>b</sup> p < 0.05 when immune group is compared to control groups.

<sup>c</sup> p < 0.01 when immune group is compared to control groups.

<sup>d</sup> p < 0.025 when immune group is compared to control groups.

**Table 4**

Adoptive transfer of immunity with spleen cells from tumor-bearing mice

The interval between ITT and SCT was 28 days. The immune group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from MCA-10 tumor-bearing mice, and receiving inoculations 1, 7, 14, or 21 days after SCT of 5 × 10^5 MCA-10 cells. The normal group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from normal C57BL/6 mice, and receiving s.c. inoculations 1, 7, 14, or 21 days after SCT of 5 × 10^5 MCA-10 cells. The challenge group was comprised of C57BL/6 mice receiving s.c. inoculations of 5 × 10^5 MCA-10 TC alone.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Interval between SCT and tumor challenge (days)</th>
<th>Total mice in group</th>
<th>Cumulative tumor incidence&lt;sup&gt;a&lt;/sup&gt; at following intervals after tumor isograft</th>
<th>Final tumor incidence at Day 40</th>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>15 days</td>
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<tr>
<td>Immune</td>
<td>1</td>
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<td>5</td>
<td>16</td>
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<sup>a</sup> Cumulative tumor incidence expressed as no. of mice with tumors of the total no. of mice in the experimental group on a given day after tumor challenge at each interval between SCT and tumor challenge.

<sup>b</sup> p < 0.05 when immune group is compared to normal group. p = 0.085 when immune group is compared to challenge control.

<sup>c</sup> p < 0.05 when immune group is compared to both control groups.

<sup>d</sup> p < 0.01 when immune group is compared to both control groups.

<sup>e</sup> p = 0.0000 when immune group is compared to both control groups.
Adoptive Transfer of Concomitant Immunity

The interval between ITT and SCT was 35 days. The immune group was comprised of C57BL/6 receiving i.p. injections of spleen cells from MCA-10 tumor-bearing mice, and receiving inoculations 1, 7, 14, or 21 days after SCT of $5 \times 10^3$ MCA-10 cells. The normal group was comprised of C57BL/6 mice receiving i.p. injections of spleen cells from normal C57BL/6 mice, and receiving s.c. inoculations 1, 7, 14, or 21 days after SCT of $5 \times 10^3$ MCA-10 cells. The challenge group was comprised of C57BL/6 mice receiving s.c. injections of $5 \times 10^3$ MCA-10 TC alone.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Interval between SCT and tumor challenge (days)</th>
<th>Total mice in group</th>
<th>Cumulative tumor incidence of mice with tumors of the total no. of mice in the experimental group on a given day after tumor challenge at each interval between SCT and tumor challenge.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune</td>
<td>7</td>
<td>22</td>
<td>$6^{b}$ $10^{b}$ $15^{c}$ $17^{c}$ $17^{c}$ $17^{c}$ (77%) $10^{d}$ (43%) $25$ (100%) $25$ (100%) $25$ (100%) $25$ (100%)</td>
</tr>
<tr>
<td>Normal</td>
<td>14</td>
<td>23</td>
<td>$0^{d}$ $0^{d}$ $9^{d}$ $10^{d}$ $25$ $25$ $25$ $25$ $25$ $25$ $25$ $25$</td>
</tr>
<tr>
<td>Challenge</td>
<td>21</td>
<td>22</td>
<td>$0^{e}$ $1^{d}$ $4^{d}$ $5^{d}$ $8^{d}$ $8^{d}$ (36%) $23$ (96%) $23$ (96%) $23$ (96%) $23$ (96%) $23$ (96%) $23$ (96%)</td>
</tr>
</tbody>
</table>

$^a$ Cumulative tumor incidence expressed as no. of mice with tumors of the total no. of mice in the experimental group on a given day after tumor challenge. $^b$ $p < 0.01$ when immune group is compared to control groups. $^c$ $p < 0.025$ when immune group is compared to control groups. $^d$ $p = 0.0060$ when immune group is compared to both control groups. $^e$ $p < 0.05$ when immune group is compared to control groups.

Tumor being studied, reject a subsequent isograft of the same tumor. Recently, several investigators have shown that lymphoid cells from the spleen (3, 32), regional lymph nodes (2, 32), thoracic duct lymph (7), and peritoneal surface (31, 37) of animals immunized to TSTA can adoptively transfer specific immunity to subsequent tumor isografts. However, these studies provided no direct information about the immunological response of a host to its tumor prior to experimental interference with a growing primary tumor, since immunity was produced by procedures that prevented progressive tumor growth. In fact, some evidence suggested that, in the presence of a growing tumor transplant, little or no antitumor immunoreactivity could be transferred with lymphoid cells from tumor-bearing hosts.

Bard and Pilch (3) reported that immunity to a chemically induced murine sarcoma could be adoptively transferred with spleen cells obtained from mice immunized at least 1 week earlier by excision of growing tumors. However, immune spleen cells obtained on the day of tumor excision were ineffective. A similar observation was reported by Stjernsward (36). He challenged mice, immediately after excision of primary MCA- induced sarcomas, with autochthonous tumor cells and demonstrated an increased rather than a decreased incidence of growth of the challenged inocula. Mikulska et al. (26) demonstrated that spleen cells from inbred rats bearing primary chemically induced sarcomas would not inhibit tumor growth in syngeneic recipients unless the primary tumors were resected 3 weeks before the spleen cells were obtained (26). These authors postulated that the number of sensitized lymphoid cells in animals bearing progressively growing cancers might be "limited, or exhausted by the tumor so that further injected tumor cells can grow unopposed" and that surgical extirpation of the primary tumor, followed by a time interval to allow for escape from tolerance or paralysis, was necessary to restore full immunological reactivity to the host.

Riggins and Pilch (34) reported that tumor-bearing mice given a challenge tumor inoculum without removal of the primary tumor evidenced no immunity to the 2nd tumor transplant. In a study similar to that of Stjernsward (36), they also observed no immunity to a 2nd tumor transplant when the primary tumor was resected only 20 hr prior to rechallenge (34). However, other investigators have shown that progressive growth of a primary tumor does not necessarily indicate the absence of an antitumor immune response. Rosenau and Morton (35) reported that lymphoid cells from mice bearing transplants of a MCA-induced sarcoma suppressed the growth of the explants of the immunizing tumor in vitro. Gershon et al. (11) demonstrated that inbred hamsters bearing large transplants of allogeneic lymphomas were resistant to reinoculation with cells from the same tumor, although the initial tumor allograft grew progressively. They also showed that peritoneal exudate cells from tumor-bearing hosts could "neutralize" TC's when mixed with TC's in vitro and injected into susceptible hosts. Yoshida and Southam (40), utilizing this neutralization test, showed that spleen cells from some tumor-bearing mice inhibited the growth of autochthonous TC's when TC-spleen cell mixtures were injected into untreated mice.
In our experiments, significant tumor immunity was manifested by tumor-bearing mice despite progressive and unremitting growth of a primary tumor transplant. We observed significant inhibition of 2nd tumor isografts development in mice bearing 7-, 14-, 21-, or 28-day-old tumor transplants of the same transplant generation as the challenge tumor cell inoculum. However, mice challenged 1 day after ITT failed to demonstrate immunity to a 2nd tumor isograft. Presumably, after 1 day, a primary immune response to the initial tumor transplant had not yet developed. Despite the almost uniform rejection of 2nd tumor inocula by mice bearing primary tumor transplants, no decrease in the growth rate of the primary tumors was observed. It is evident that the tumor-bearing host is capable of rejecting isografts of the same tumor growing relentlessly at another site. This concomitant immunity appears to be firmly established even prior to the appearance of a palpable primary tumor mass. The development and continued growth of the primary tumor despite evidence of an active antitumor immune response is a perplexing paradox of tumor immunology.

Spleen cells from tumor-bearing mice manifesting concomitant immunity inhibited the development of subsequent tumor isografts when transferred to untreated syngeneic hosts. We believe this to be the first demonstration of the adoptive transfer of tumor immunity with spleen cells from animals bearing progressively growing tumors. The capacity of spleen cells from tumor-bearing mice adaptively to transfer tumor immunity developed between 7 and 14 days after ITT. No immunity was noted at any SCT-TC interval in mice receiving spleen cells from animals bearing 7-day-old MCA-10 transplants. However, spleen cells obtained from tumor-bearing mice 14 days after ITT transferred significant immunity to a subsequent MCA-10 isograft. This immunity was observed when recipient mice were challenged 7 or 14 days after SCT (SCT-TC intervals 7 and 14 days) but appeared to be a transient phenomenon since no immunity was noted when recipient mice were challenged 21 days after SCT (SCT-TC interval 21 days). The degree of immunity transferred and its duration appeared to increase as the primary tumor of the spleen cell donor increased in size. At 21, 28, and 35 days after ITT, when tumor weight of total body weight ratios were 3.9, 9.7, and 13.9%, respectively, spleen cells from tumor-bearing mice produced a more marked decrease in final cumulative tumor incidence, and the immunity persisted through the longest SCT-TC interval studied (21 days). Once tumors developed, however, they grew at the same rate as tumors in control animals. Recipients of spleen cells obtained from tumor-bearing hosts at every time interval after ITT failed to evidence any immunity challenge 24 hr after SCT (SCT-TC interval 1 day). This is in agreement with the observations of Bard and Pilch (3). They transferred spleen cells to untreated mice from mice immunized by excision of growing tumors on the day of SCT. No immunity was noted when recipients were challenged 24 hr later. However, significant immunity was manifest if this "amputation-challenge interval" was at least 1 week.

Tolerance or immunological paralysis is not an adequate explanation for the failure of recipients of spleen cells from tumor-bearing hosts to manifest immunity to tumor isografts 1 day after SCT. At all time intervals after ITT except 1 day, 2nd tumor isografts were rejected in the tumor-bearing mice from which the spleen cells were obtained, suggesting that these mice were not specifically tolerant or paralyzed. Recipients of spleen cells from such mice evidenced significant tumor immunity when challenged 7, 14, or 21 days after SCT. Since the adoptive transfer of immunity has been thought to depend upon "the introduction, division and continued working of immunocompetent cells" (5), the problem may simply be one of logistics: the number of tumor cells and the tumor-doubling time versus the cytolytic capacity of a finite number of sensitized lymphocytes. When recipients were challenged 1 day after SCT, the number of living, specifically sensitized lymphoid cells and/or their capacity for proliferation may have been insufficient to cause rejection of a tumor isograft. Perhaps 7, 14, or 21 days after SCT, sufficient numbers of sensitized lymphoid cells existed within the recipients to suppress tumor growth. However, we do not exclude the possibility that a state of immunological tolerance or paralysis may develop at mean tumor weight to body weight ratios in excess of those reported here. Unpublished evidence to support this contention has been obtained by Sugarbaker et al. (E. V. Sugarbaker, J. Egan, and A. S. Ketcham, personal communication). They noted that spleen cells from mice bearing large MCA-10 tumors, 35 days or more following ITT, failed to inhibit the growth of MCA-10 isografts when spleen cells and tumor cells were mixed in vitro and inoculated s.c. into normal mice. At all earlier time intervals, spleen cells from tumor-bearing animals inhibited the growth of MCA-10 cells in their neutralization test. Barski and Youn (4) reported a similar observation in Balb/c mice bearing a tumor induced by an attenuated Rauscher virus in a syngeneic T5 cell line. Peritoneal exudate cells removed from tumor-bearing mice at the beginning of tumor growth inhibited colony formation of tumor cells in vitro. This activity had disappeared when peritoneal exudate cells from mice bearing tumors 4 weeks old or older were tested. Perhaps the development and kinetics of concomitant immunity, as well as its eventual abrogation, may vary for individual tumors depending on their relative antigenicity and/or immunogenicity.

The cells of established primary tumors may be protected from the immune lymphoid cells of the host by humoral "blocking factors" (15). These factors may suppress immunocytes directly or may cover TSTA sites on the tumor cell surface, thereby preventing recognition by immune lymphoid cells (18). Such blocking factors have been demonstrated in the serum of cancer patients by Hellström et al. (17) and can abrogate the inhibitory effect of specifically sensitized lymphocytes on colony formation in vitro. They have been characterized by Hellström and Hellström (14) as 7S immunoglobulins. IgG2 immunoglobulins have been eluted from subcellular fractions of rodent tumor cells by Witz et al. (38, 39). It remains to be shown whether immunoglobulins extracted from progressively growing tumors can mediate enhancement of tumor growth in vivo. However, the hypothesis that these may be "enhancing antibodies" is strengthened by...
the findings of Irvin et al. (20) who demonstrated that IgG2 antibodies mediated enhancement of murine tumor allografts.

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The Adoptive Transfer of Concomitant Immunity to Murine Tumor Isografts with Spleen Cells from Tumor-bearing Animals

Peter J. Deckers, Bradford W. Edgerton, Bernard S. Thomas, et al.


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