Specific and Nonspecific Stimulation of Resistance to the Growth and Metastasis of the Line 1 Lung Carcinoma

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SUMMARY

The effects of specific dead tumor cell immunization and nonspecific immunostimulation with Corynebacterium parvum on the s.c. growth of the line 1 carcinoma in syngeneic BALB/c mice have been studied. Injection of heavily irradiated line 1 carcinoma cells did not inhibit the transplantability or growth of the line 1 carcinoma, and in certain cases these treatments actually prolonged the period of rapid growth. This latter observation was traced to a mild inhibition of metastatic spread, which itself can slow the s.c. tumor growth. Treatment of the mice with 0.25 mg of C. parvum 7 days prior to transplant of the tumor had no effect on its growth by itself; but in combination with i.v.-injected tumor cells, which themselves had no effect on tumor growth, a 45% inhibition of tumor growth was induced. These data demonstrate that, in the weakly immunogenic line 1 carcinoma system, inhibition of s.c. transplants requires not only exposure to tumor antigens but also stimulation of the immunological reactivity.

INTRODUCTION

Total-body irradiation of BALB/c mice prior to the s.c. transplant of the line 1 lung carcinoma accelerates the development of spontaneous metastases but slows the growth of the transplant itself. The increased metastasis is a direct result of irradiation-induced immunosuppression (4, 5) and can be reversed by transplantation of immunocompetent cells (4, 5), especially spleen cells obtained from mice previously exposed to dead line 1 cells (4). Although the s.c. tumor inhibition can also be reversed by immunological reconstitution, its relationship to the immunosuppressive effects of the exposure is more indirect. As an example, under appropriate conditions (4, 6) artificial injection of metastases into unirradiated mice can also induce s.c. inhibition, although the degree of inhibition is less marked than that observed in irradiated mice (4). To account for these data, we argued that (4) disseminated metastases provided the stimulus for the induction of the s.c. inhibitory reactions but that the magnitude of the response was determined by the reactivity of the host immune system. This interpretation proposed that during recovery from irradiation injury a heightened antitumor response would be generated. In order to test this interpretation in a more general context, we have determined the effects of specific immunizations with dead line 1 cells and nonspecific stimulation of the immune system with Corynebacterium parvum on the growth and metastasis of the line 1 carcinoma. The data presented below have demonstrated that neither of these treatments, when given alone, can inhibit the growth of s.c. transplants of this weakly immunogenic tumor but that in combination they are able to inhibit s.c. tumor growth by more than 45%.

MATERIALS AND METHODS

Mice. The mice used in these experiments were 3-month-old BALB/c of either sex (single sex within an experiment). Unless otherwise stated, each experimental point represents the mean of at least 8 observations.

Tumor. The origin and maintenance of the line 1 alveolar cell carcinoma have been detailed elsewhere (5). Injections of the tumor. The origin and maintenance of the line 1 alveolar cell carcinoma have been detailed elsewhere (5). Injections of this tumor into syngeneic BALB/c mice result in rapid growth and metastasis. The data presented below have demonstrated that neither of these treatments, when given alone, can inhibit the growth of s.c. transplants of this weakly immunogenic tumor but that in combination they are able to inhibit s.c. tumor growth by more than 45%.

Summary of Results

Transplantation s.c. in Immunized Mice. In order to determine whether dead cell immunizations would affect the s.c. transplantation of the line 1 lung carcinoma, groups of
32 mice each were given 3 weekly i.p. injections of 0, 5 × 10³, 2.5 × 10⁴, 5 × 10⁴, or 2.5 × 10⁵ heavily irradiated line 1 cells. One week after the last immunization, each group was subdivided into 4 groups of 8 mice each and given either 10⁴, 10⁵, 10⁶, or 10⁷ viable line 1 cells s.c. Between 10⁴ and 10⁵ line 1 cells are required for 100% takes (5). All 32 of the nonimmunized (0 dead cells weekly) and all 128 of the immunized mice developed palpable tumors at the injection site, and there was no indication of an effect of immunization on the times of tumor appearance within each challenge cell dose group. Thirty-five days after transplantation, all of the mice were killed and their tumors were weighed. Again, there was no indication of s.c. tumor inhibition owing to immunization. In further studies (data not shown) the immunization route was changed (s.c.), the spacing of the immunizations was altered (as often as 3 times weekly), the size of the immunizing cell dose was increased (as high as 10⁶ cells/injection), and the interval between immunization and challenge was altered (1 day to 4 weeks). In none of these studies was inhibition of s.c. tumor growth observed, but in some of the studies it appeared that the s.c. tumors were actually growing faster in immunized mice.

This suggestion was examined more closely by comparing tumor growth as a function of time posttransplantation in mice given 3 weekly injections of 0 or 5 × 10³ dead line 1 cells and, 1 week later, a s.c. transplant of 5 × 10⁴ viable line 1 cells. Chart 1 is a plot of the mean tumor weights observed in these 2 groups of mice between the 14th and 42nd day after transplant. Rapid growth of the s.c. transplant continued for a longer time in the immunized mice, i.e., immunized mice possess significantly larger tumors at both the 35th and 42nd days after transplantation (p < 0.05).

Since metastatic spread is responsible for the slowing of the line 1 carcinoma growth under other conditions (4), we next tested the possibility that the immunizations were prolonging the period of rapid growth by inhibiting the development of spontaneous metastases. Table 1 contains the data from an experiment in which the incidence and number of spontaneous metastases were determined 35 days after s.c. transplant in nonimmunized and immunized mice. Both indices of spontaneous metastatic spread were significantly reduced by the immunization procedures (Table 1), and as a consequence the survival time of these mice was prolonged by 6 days (p < 0.05). We conclude, therefore, that dead cell immunizations have no direct effects on s.c. tumors but appear to prolong their rapid growth through a mild inhibition of metastatic spread.

**Transplantation i.v. in Immunized Mice.** In mice bearing s.c. tumors, it is not possible to determine whether relatively weak inhibition of spontaneous metastases is due to inherently weak responses or to continued release of new metastases, i.e., saturation of an otherwise effective response. In the artificial metastasis system (3), in which metastases are artificially introduced by i.v. injection, one can assess the effects of immunization against a controlled number of metastatic cells. Table 1 contains the results of a study in which mice were immunized as described above and then given i.v. injections of 2 × 10⁴ line 1 tumor cells. By the 21st day after transplant, the nonimmunized mice possessed more than 20 tumor colonies in the lungs, while in the immunized group only 1 of the 16 mice studied had a single lung tumor colony. From our past experience with mice given very few tumor cells i.v., we expected less than 20% mortality in this group and, further, that those deaths that were observed to be the result of 1 or a few large metastases. Quite the opposite, all immunized mice given i.v. tumor cells died an average of 3 weeks later than similarly treated but nonimmunized mice (Table 1), and all possessed literally hundreds of metastatic foci at autopsy. We conclude that dead cell immunizations do not eradicate metastases but merely postpone their growth in the line 1 carcinoma system.

**Transplantation s.c. and i.v. in *C. parvum*-treated Mice.** Since dead cell immunizations alone did not affect the transplantability and growth of s.c. tumors, we tested the possibility that nonspecific stimulation of the immune system, either alone or in the presence of artificially injected metastases, could result in s.c. tumor inhibition. Injection of 0.25 mg of *C. parvum* 7 days before s.c. transplant did not affect the growth of the tumor, nor did the injection of artificial metastases on the day of s.c. transplant (Table 2).
Table 1
Effects of 3 weekly immunizations with $5 \times 10^6$ dead line 1 cells on the development of artificial and spontaneous metastases in the line 1 carcinoma system

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>With lung colonies</th>
<th>Colonies/mouse</th>
<th>Mean survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay</td>
<td>Treatment</td>
<td>Injected</td>
<td></td>
</tr>
<tr>
<td>Spontaneous metastasis</td>
<td>None</td>
<td>16</td>
<td>$8.81 \pm 1.20$</td>
</tr>
<tr>
<td></td>
<td>Immunized</td>
<td>16</td>
<td>$4.28 \pm 1.50$</td>
</tr>
<tr>
<td>Artificial metastasis</td>
<td>None</td>
<td>16</td>
<td>$22.9 \pm 5.2$</td>
</tr>
<tr>
<td></td>
<td>Immunized</td>
<td>16</td>
<td>1</td>
</tr>
</tbody>
</table>

* Mean ± S.E.: based only on those mice that were metastasis positive.
* Days ± S.E.
* Seven days after last immunization, the mice were given s.c. transplants of $5 \times 10^6$ viable line 1 cells; 35 days later they were killed and the number of lung colonies was counted.
* Seven days after last immunization, the mice were given i.v. injection of $2 \times 10^6$ viable line 1 cells; 21 days later they were killed and the number of lung tumor colonies was counted.

Table 2
Effects of pretreatment with C. parvum alone or in combination with $2 \times 10^6$ artificial metastases on the growth of s.c. tumors determined 21 days after transplant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Mean tumor wt (mg)</th>
<th>Lung tumor colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>2071 ± 204*</td>
<td>0</td>
</tr>
<tr>
<td>C. parvum</td>
<td>8</td>
<td>2160 ± 392</td>
<td>0</td>
</tr>
<tr>
<td>Artificial metastases</td>
<td>7</td>
<td>2159 ± 221</td>
<td>0</td>
</tr>
<tr>
<td>C. parvum + artificial metastases</td>
<td>8</td>
<td>1133 ± 204*</td>
<td>0</td>
</tr>
</tbody>
</table>

* All mice were given an s.c. transplant of $5 \times 10^6$ line 1 cells on Day 0.
* Mean ± S.E.
* 0.15 mg of C. parvum given i.p. on Day —7.
* Two $\times 10^6$ line 1 cells given i.v. 2 hr after s.c. transplant.
* $p < 0.01$ for comparison with untreated control.

However, when the 2 treatments, immunostimulation and artificial metastatic spread, were combined, s.c. tumor growth was inhibited by more than 45%. This reduction was not due to any nonspecific effects of metastatic spread, since the s.c. cell dose was selected to suppress the development of the i.v.-injected cells (6).

DISCUSSION

The data presented above have demonstrated that the line 1 carcinoma is, at best, weakly immunogenic. The dead cell immunizations that we have used had no effect on s.c. tumor growth and induce only a minor delay in the growth of metastases. While it might be expected that live cell immunizations might produce stronger antitumor reactions (2), the fact that spontaneous metastases spread too rapidly to allow use of these methods (6) again suggests that the tumor is weakly immunogenic.

Pretreatment of mice with C. parvum had no effect on the growth of s.c. transplants, as might be expected from published reports (1) on the treatment of other weakly immunogenic tumors with similar immunostimulants. Due to the use of a rapidly growing s.c. transplant (6), the artificially injected metastases were likewise unable to affect s.c. tumor growth. Combined treatment with the immunostimulant and the artificial metastases did, however, result in marked inhibition of s.c. tumor growth. In addition to demonstrating that our original proposal (4) is a valid one (i.e., that s.c. inhibition requires both a stimulated immune response and tumor antigen dissemination), these data suggest that C. parvum is operating as an adjuvant in this system.

The irradiation-induced slowing of s.c. tumor growth is, admittedly, an unorthodox observation. Had we applied the total-body irradiation after the s.c. tumor transplant, the same observations might have been interpreted as being the product of adequate local control but accelerated metastasis. The question remains open as to whether systemic therapy studies involving immunosuppressive drugs are also affected by similar processes. Whatever the generality of these observations, it is clear that this weakly antigenic, highly malignant lung cancer offers an excellent model for the study of cancer therapy.

REFERENCES

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