Adjuvant-Antigen Requirements for Active Specific Immunotherapy of Microscopic Metastases Remaining after Surgery

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ABSTRACT

We studied the conditions required for eradication by immunization of occult lymph node metastases which remained after surgical removal of an intradermally transplanted cavian hepatoma. Guinea pigs that received no postsurgical treatment all died with progressively growing lymph node metastases. The growth of these metastases could be prevented in a significant proportion of the animals by postsurgical treatment with vaccines containing oil-in-water emulsions of Mycobacterium bovis strain Bacillus Calmette-Guérin (BCG) cell walls admixed with live or irradiated tumor cells. Vaccines containing living tumor cells cured most of the guinea pigs but produced tumors at the vaccine sites in a few animals. Irradiated tumor cell vaccines were not tumorigenic but required more tumor cells for successful therapy. Therapy was dependent both on the dose of tumor cells and on that of BCG cell walls. Microgram doses of BCG cell walls were required for a therapeutic effect; milligram doses of BCG cell walls inhibited the therapeutic response. Animals rendered tumor free by postsurgical vaccine therapy rejected an intradermal challenge with living tumor cells.

INTRODUCTION

Active specific immunotherapy is under investigation as a method for eradication of occult metastases remaining after surgical removal of solid tumors. This form of cancer treatment has the potential of eradicating microscopic metastases without damaging normal cells. Several animal models have been developed to analyze the factors required for successful active immunotherapy (4, 5, 8, 10, 11, 16). These animal models usually consist of transplantable syngeneic rodent tumors; animals are given injections of tumor cells and subsequently immunized with tumor cells or tumor cell products. In a few instances, animals with autogenous tumors have been treated by surgical removal of primary tumors followed by immunization with tumor cells or tumor cell extracts (21, 24). Factors identified as affecting the response to active specific immunotherapy include residual tumor burden (4, 8, 10), tumor immunogenicity (4), dose and form of tumor antigen (10, 11), dose and type of adjuvant (5, 11, 12, 27), and routes and schedules of immunization (8). Generalizations that have emerged from a study of these animal models include: (a) vaccines containing adjuvants and tumor cells are generally more effective in therapy than are vaccines containing either component alone (5, 10, 11); (b) treatment is antigenically specific (5, 10, 11, 16); and (c) intact, metabolically active tumor cells are the best source of immunogen (14).

We previously reported that microscopic metastases remaining after surgical removal of an i.d. implanted cavian hepatoma could be eradicated by immunization with mycobacterial vaccines and tumor cells (27). In that report, it was established that: (a) injection of adjuvant alone adjacent to the site of tumor excision (regional injection) had a weak inhibitory effect on growth of lymph node metastases; (b) injection of adjuvant alone contralateral to the site of tumor excision (remote injection) had no inhibitory effect on growth of lymph node metastases; (c) remote or regional treatment with nonliving immunostimulants admixed with tumor cells was effective treatment against microscopic metastases; (d) BCG CW functioned effectively as adjuvant while living BCG was less effective; and (e) treatment was antigenically specific and devoid of gross systemic toxicity. In this report, we present information on the influence of dose and form of immunogen and dose of adjuvant on the success of therapy.

MATERIALS AND METHODS

Animals. Male Sewall-Wright strain 2 guinea pigs were obtained from the Laboratory Aids Branch, Division of Research Services, NIH, and from the Experimental Animal Breeding Facility of the National Cancer Institute, Frederick Cancer Research Center, Frederick, Md. They were caged in groups of 6 and given water and NIH guinea pig ration ad libitum. All animals were used when 3 to 4 months old and weighed 500 to 600 g.

Tumor Line. We used the L10 hepatocellular carcinoma which was derived from a diethylnitrosamine-induced hepatoma in a male strain 2 guinea pig. The tumor has been converted to ascites form and is maintained by i.p. passage in male weanling strain 2 guinea pigs. The in vivo passaged tumor line was restarted periodically with ascites cells stored in liquid nitrogen so that the cells used in experiments were in the 9th to the 18th i.p. passage generations. Suspensions containing greater than 90% trypan blue-excluding cells were used in experiments.

Preparations of Oil-in-Water Emulsions Containing CW. BCG CW (Lots 278 and 286) prepared as described previously (1) were obtained from Dr. Edgar Ribi (Rocky Mountain Laboratory, Hamilton, Mont.). The preparation of a mineral oil-in-water emulsion containing BCG CW in the oil phase has been described (27). Briefly, light mineral oil (Drakeol 6-VR; Penreco, Butler, Pa.) was added to BCG CW and ground to a smooth paste in a tissue grinder. This paste was then emulsified in sterile NaCl solution (8.5 g/liter) containing Tween 80 (2 μl/ml) to produce a well-dispersed oil-in-water emulsion. For doses of CW up to and including 1.88 mg per ml, emulsions

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1 The abbreviations used are: i.d., intradermal(ly); BCG, Bacillus Calmette-Guérin strain of Mycobacterium bovis; CW, cell walls; L10, tumor line 10; SDA, superficial distal axillary; EAE, experimental allergic encephalomyelitis.
contained 30 µl oil per ml; for doses of CW greater than 1.88 mg per ml, emulsions contained 100 µl oil per ml.

**Composition of Mixtures Used for Treatment.** Vaccines containing BCG CW plus L10 cells were prepared immediately before use. A suspension of living or irradiated tumor cells placed in a 17 x 100 mm polypropylene tube 2059 (Falcon Plastics, Oxnard, Calif.) was centrifuged at 140 x g; the volume of the cell pellet was noted. Tumor cells were resuspended in BCG CW emulsion by dropwise addition of the emulsion to the tumor cell slurry; after each addition of emulsion, the slurry was agitation with a Vortex mixer (Scientific Industries, Inc., Springfield, Mass.). The injection volume for an individual guinea pig was the sum of the CW emulsion volume (0.4 ml) and the volume of a single dose of live or irradiated tumor cells. Vaccines were injected i.d. on the right thoracic flank with the total volume divided equally between 4 injection sites in a vertical line down the flank.

**Irradiation.** L10 cells were irradiated with a dose of 20,000 rads. The radiation was initially delivered with an X-ray source but, in later experiments, a 137Cs γ-ray source was used. No difference was observed in immunogenicity of cells irradiated with an X-ray source or with a 137Cs γ-ray source. For X-irradiation, L10 cells (30 ml, 30 x 10^6/ml) were placed in a 250-ml No. 3023 plastic tissue culture flask (Falcon Plastics, Cockeysville, Md.). The flask was irradiated in a horizontal position between 2 vertically opposed Philips RT 250 therapeutic X-ray tubes. These were operated at 235 kV and 15 ma with 0.25 mm copper and 0.55 mm aluminum filters (half-value layer, 0.9 mm aluminum). The source to target distance was 25 cm, and the dose rate was 662 rads/min (30.2 min for 20,000 rads). For γ-irradiation, L10 cells (50 ml, 30 x 10^6/ml) were placed in a 50-ml No. 25335 polypropylene centrifuge tube (Corning Glass Works, Corning, N. Y.). During γ-irradiation, the test tube was rotated while in a vertical position in the center of the irradiation chamber of a Gammarior M 137Cs irradiator (Isomedix Inc., Parsippany, N. J.). The dose rate was 1,790 rads/min (11.2 min for 20,000 rads). After irradiation, the cell concentration and viability of the cells were determined by the trypan blue exclusion test; no decrease in viability was observed after irradiation. To test whether irradiation had eliminated tumorigenicity, tumor cells (10^7) were injected i.p. into weanling strain 2 guinea pigs. No ascites tumors formed after i.p. injection of L10 cells irradiated with 20,000 rads. One viable L10 cell is capable of initiating ascites tumor growth in this test.

**Tumor Excision.** Dermal tumors were excised with a 1-cm margin of skin under pentobarbital anesthesia supplemented with ether inhalation as previously described (28).

**Animal Model for Evaluating the Effects of Postoperative Immunotherapy.** Tumor cells (10^7) were injected i.d. into each guinea pig on Day 0. Seven days after i.d. injection, dermal tumors (9 to 10 mm in diameter) were excised. Immunotherapy was administered 2 days after surgery. Virtually all of the animals treated by tumor excision alone developed progressively growing tumors in the regional lymph nodes. These metastases developed first in the SDA lymph node which became palpable after 20 to 30 days. Death occurred after 60 to 90 days.

**Measurements.** Animals were examined at weekly intervals after surgery to detect tumor growth. Since metastases appeared first in the left SDA lymph node, this lymph node was palpated to estimate its size. For 20 to 30 days after L10 injection, it was not possible to distinguish between lymph node enlargement due to tumor growth and lymphadenopathy resulting from immunostimulation. Axillary adenopathy detected 30 days after immunostimulant treatment was a reliable indicator of tumor progression. Animals were observed for a minimum of 90 days; the usual observation period was 120 to 150 days. Recurrence of tumors after Day 90 was rare. Significance of differences between groups was determined with the Fisher exact test (1 tailed) and the χ² analysis.

**RESULTS**

Previously, we showed that a single treatment with a vaccine of live L10 cells and BCG CW could prevent the growth of microscopic lymph node metastases which remained after excision of dermal tumors (27). Vaccine therapy could prevent the growth of metastases in a high proportion of the treated animals whether administered adjacent to the site of excision or on the contralateral flank. Adjuvant alone could protect a small proportion of the animals but only if given as a regional treatment. To avoid these regional effects, vaccines were injected contralateral to the site of the primary tumor inoculation in the experiments in this study. In this way, we could investigate the influence of the conditions affecting vaccine therapy of distant occult metastases.

**Immunotherapy with Live Tumor Cell Vaccines**

The previous study showed that postoperative immunotherapy with a vaccine containing BCG CW (750 µg) and living L10 cells (6 x 10^6 cells) significantly reduced the incidence of palpable lymph node metastases. Our experience with this vaccine treatment over a series of 10 experiments including the 3 previously reported is summarized in Table 1. Vaccine treatment was highly effective in preventing the growth of metastases. When vaccines contained 6 x 10^6 living tumor cells, 60% of the treated animals were protected. The proportion of animals cured in individual experiments varied from 0 to 89% but in only 2 of the experiments was it less than 50% (data not shown). In an attempt to improve the therapeutic efficacy of the vaccine, the dose of tumor cells was increased 5-fold. Treatment with vaccines containing 30 x 10^6 living tumor cells cured 74% of the treated animals and appeared to...

<table>
<thead>
<tr>
<th>Dose of living tumor cells (x10^6)</th>
<th>No. of animals alive and tumor-free/total</th>
<th>No. of animals with tumor growth at vaccine site/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>69/115 (60)*</td>
<td>5/115 (4)</td>
</tr>
<tr>
<td>30</td>
<td>63/85 (74)</td>
<td>12/85 (14)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.
tumor cell dose. At least 50% of the animals were cured in every experiment (data not shown). However, the improvement in therapeutic activity achieved by increasing the dose of living tumor cells was counterbalanced by an increase in the tumorigenicity of the vaccine.

Occurrence of Tumor Growth at Vaccination Sites Containing Living Tumor Cells

The vaccine used in these experiments contained live tumor cells and so was potentially tumorigenic. Since the host’s reaction to BCG CW suppressed the growth of admixed tumor cells (29), tumor growth at the vaccine sites was rare. Instead, a vigorous inflammatory reaction developed at the vaccine site which eventually healed. Tumor grew out from one or more of the vaccine sites and metastasized to the draining lymph node in 4% of the animals treated with $6 \times 10^6$ live tumor cells (Table 1). When the tumor cell dose was increased to $30 \times 10^6$ cells, tumor grew at the vaccine sites in 14% of the animals. Tumor growth occurred at the vaccine sites almost exclusively in animals in which therapy failed to prevent the growth of the lymph node metastases. A higher incidence of tumor growth at vaccine sites was observed in experiments in which vaccine treatment was attempted in animals with more advanced tumors or with disseminated BCG infection.

Comparison of Vaccines Containing Live or Irradiated Tumor Cells

In order to eliminate the risks associated with live tumor cell vaccines, we tested vaccines containing L10 cells rendered nontumorigenic by irradiation. To completely eliminate the tumorigenicity of the L10 cells, we irradiated tumor cells with a dose of 20,000 rads (16). Vaccines containing irradiated tumor cells have been shown to be active against small deposits of L10 cells in the skin or lungs (10, 16). Since irradiation may reduce the immunogenicity of tumor cells (2, 6, 18, 25), we carried out a series of experiments to compare the therapeutic efficacy of vaccines containing live or irradiated L10 cells. Vaccines containing living or irradiated tumor cells were compared under 3 conditions: (a) over a range of L10 doses; (b) over a range of treatment times; and (c) in repeated experiments at a single dose level of tumor cells.

Chart 1, A and B, shows the results of 2 experiments comparing vaccines containing live or irradiated L10 cells over a range of tumor cell doses. The success of vaccine therapy differed between the 2 experiments. Nevertheless, it was clear from both experiments that the efficacy of the vaccines increased with the dose of live or irradiated tumor cells. At each tumor cell dose tested, the live L10 vaccine protected more animals than did the corresponding irradiated L10 vaccine.

Experiments were carried out to test the effect of varying the time interval between i.d. tumor implantation and treatment (Chart 1C). Dermal L10 tumors were excised 4, 7, or 9 days after implantation. Vaccines containing live or irradiated L10 cells ($30 \times 10^6$) were injected 2 days after surgery. Lymph node metastases grew in all of the animals treated by surgery alone, showing that tumor cells had reached the SDA lymph node within the first 4 days of tumor growth. At each of the times tested, postsurgical vaccine therapy prevented the growth of metastases in a significant proportion of the animals treated. The success of vaccine therapy decreased slightly as the time of treatment was delayed, although this trend was not statistically significant with either of the vaccines. The only significant difference attributable to treatment time was between the groups treated with live L10 vaccine following surgery on Day 4 and Day 7 (12 of 12 tumor-free versus 6 of 11, p < 0.05). No significant differences were observed in this experiment between vaccines containing live or irradiated tumor cells.

Table 2 summarizes our comparisons between vaccines containing live or irradiated cells (27 to $30 \times 10^6$ cells). Six experiments with direct comparisons between the vaccines are shown, together with the results from another 7 experiments. The live vaccines cured 74% (63 of 85) of the animals in these experiments and cured at least 50% in each experiment. This contrasted with the irradiated vaccines which cured the majority of the animals in only 6 of 12 experiments and cured 48% (76 of 158) overall. The superiority of the live cell vaccine was statistically significant over the series of experiments ($p < 0.001$) and in 2 of the direct comparisons.

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* M. P. Ashley, J. T. Hunter, B. Zbar, and H. J. Rapp, unpublished data.

** By the Cochran-Armitage $\chi^2$ test for significance of observed trends (13).
### Table 2

Summary of comparisons of vaccines containing live or irradiated tumor cells

<table>
<thead>
<tr>
<th>Vaccine treatment</th>
<th>None</th>
<th>CW + live L10</th>
<th>CW + irradiated L10</th>
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<tr>
<td></td>
<td>No. of animals alive and tumor free/total</td>
<td>No. of animals alive and tumor free/total</td>
<td>No. of animals with tumors at vaccine sites/total</td>
</tr>
<tr>
<td></td>
<td>Experiment</td>
<td>No. of animals alive and tumor free/total</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0/20</td>
<td>9/10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/10</td>
</tr>
<tr>
<td>2</td>
<td>0/9</td>
<td>NT</td>
<td>1/10</td>
</tr>
<tr>
<td>3i</td>
<td>0/11</td>
<td>5/10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2/10</td>
</tr>
<tr>
<td>4</td>
<td>0/15</td>
<td>NT</td>
<td>3/11</td>
</tr>
<tr>
<td>5</td>
<td>0/12</td>
<td>6/11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/11</td>
</tr>
<tr>
<td>6</td>
<td>0/19</td>
<td>NT</td>
<td>3/12</td>
</tr>
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</tr>
<tr>
<td>8</td>
<td>0/12</td>
<td>NT</td>
<td>1/15</td>
</tr>
<tr>
<td>9</td>
<td>0/11</td>
<td>9/12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/15</td>
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<tr>
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<td>0/16</td>
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<td>NT</td>
<td>28/36&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>13</td>
<td>NT</td>
<td>63/65 (74)</td>
<td>0/6</td>
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<tr>
<td>Total</td>
<td>0/156 (0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63/65 (74)</td>
<td>12/65 (14)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Vaccines containing live or irradiated L10 cells (27 to 30 x 10<sup>6</sup>) admixed with a mineral oil emulsion of BCG CW. The dose of CW was 750 μg in Experiments 3ii, 4, and 6 to 13 and 450 μg in Experiments 1, 2, 3i, and 5. Vaccine treatment was given 2 days after excision of 7-day dermal L10 tumors. Vaccines containing live or irradiated L10 cells (27 to 30 x 10<sup>6</sup>) admixed with a mineral oil emulsion of BCG CW. The dose of CW was 750 μg in Experiments 3ii, 4, and 6 to 13 and 450 μg in Experiments 1, 2, 3i, and 5. Vaccine treatment was given 2 days after excision of 7-day dermal L10 tumors.

<sup>b</sup> Significantly different from no vaccine (p < 0.05 by Fisher exact test).

<sup>c</sup> NS, not significant; NT, not tested.

<sup>d</sup> Numbers in parentheses, percentage.

<sup>e</sup> p < 0.001 whether comparing the totals from all 13 experiments (76 of 158 vs. 63 of 85), the totals from the 6 experiments in which both live and irradiated vaccines were tested (29 of 67 versus 57 of 74) or the paired comparison in these 6 experiments by the method of Cochran (13).

### Factors Affecting the Success of Vaccines Containing Irradiated Tumor Cells

Chart 2 summarizes 19 experiments undertaken to analyze some of the factors influencing the success of vaccines containing irradiated tumor cells. This chart is in effect a 3-dimensional graph showing the degree of protection that resulted from immunization with different combinations of irradiated tumor cells and BCG CW. Each of the 78 single-digit numbers in Chart 2 represents the proportion of tumor-free guinea pigs in an individual group of 9 to 12 guinea pigs. So that each group can be represented by a single-digit number, the results have been expressed as 9 x (no. tumor-free/no. tested) rounded off to a whole number. For example, 0/12, 7/12, and 12/12 are shown as 0, 5, and 9, respectively.

Certain conclusions can be drawn from the data. (a) BCG CW were a necessary component of vaccines used for eradication of metastases remaining after surgery. Vaccines containing irradiated tumor cells alone (6 to 100 x 10<sup>6</sup> cells) failed to cure any guinea pigs. (b) A supraoptimal dose of BCG CW was defined. Vaccines containing 3 mg of BCG CW were ineffective in postoperative immunotherapy. (c) BCG CW functioned effectively as adjuvants over a dose range of 47 to 750 μg. (d) A minimum dose of about 30 x 10<sup>6</sup> irradiated tumor cells was required in the presence of adjuvant to lead to destruction of lymph node metastases. The percentage of animals that were cured when treated with 30 x 10<sup>6</sup> irradiated tumor cells was required in the presence of adjuvant to lead to destruction of lymph node metastases. The percentage of animals that were cured when treated with 30 x 10<sup>6</sup> irradiated tumor cells varied from 0 to 100% with an average of 48%. Guinea pigs treated with 1 to 1.2 x 10<sup>6</sup> irradiated tumor cells were cured in 55 to 88% of cases. A plateau in the irradiated tumor cell dose-response curve was not found in individual experiments (Chart 3) but, from interpolation, appeared to be in the region of 10<sup>6</sup> irradiated tumor cells. (e) No supraoptimal dose of irradiated tumor cells was found.

Chart 2. Influence of dose of BCG CW and of irradiated tumor cells on outcome of active specific immunotherapy. The results are a summary of 19 experiments (843 animals). Each of the 78 single-digit numbers represents the proportion of tumor-free animals in an individual group of 9 to 12 guinea pigs. Each result is expressed as 9 x (no. tumor-free/no. tested) rounded off to a whole number. Numbers with an underline indicate treatment with vaccines containing 10% (v/v) oil emulsion. All other vaccines contained 3% (v/v) oil emulsion.

Five individual experiments illustrating the effects of increasing the dose of irradiated tumor cells are presented in Chart 3. Increasing the dose of irradiated tumor cells consistently im-
used to test for the presence of occult deposits of tumor cells. Guinea pigs free of palpable tumor 159 days following treatment with irradiated cell vaccines were killed, and their left SDA lymph nodes were removed. The lymph node from each guinea pig was minced with scalpels and quantitatively transferred into the peritoneal cavity of a weanling guinea pig. The development of ascites tumor in the recipient would indicate the presence of live tumor cells in the lymph node. Only one of the 22 recipients developed ascites tumor. This suggested that eradication of the tumor cells in the lymph node was complete in almost all of the animals responding to vaccine treatment since this assay reliably detects microscopic tumor cell deposits present in lymph nodes and is capable of detecting one live cell in suspensions of ascites L10 cells.

**Immunity to Rechallenge with L10 Tumor.** In 3 experiments, 82 animals rendered tumor free by postsurgical vaccine therapy were tested for persisting antitumor immunity. All of the animals rejected an i.d. challenge dose of 10^5 L10 cells given between 161 and 444 days after the primary L10 tumor implant.

**DISCUSSION**

These results indicate that dose and form of immunogen and dose of adjuvant have a major influence on the success of active specific immunotherapy. Living tumor cells were more effective as immunogens than were irradiated tumor cells, but this increased efficacy was counterbalanced by the tumorigenicity of vaccines containing living tumor cells. Growth of tumors occurred at sites of vaccination in 4% of guinea pigs treated with low-dose living tumor cell vaccines (6 x 10^6 cells) and in 14% of guinea pigs treated with high-dose vaccines (30 x 10^6 cells). In the guinea pig system, the incidence of tumors at vaccine sites was dependent on the number of tumor cells in the vaccine (Table 1) and was also influenced by the extent of the tumor burden since, at more advanced stages of tumor growth, the tumorigenicity of these vaccines was increased.

Tumor burden has also been shown to interfere with the rejection of live L10 cells admixed with live BCG (10) and with the regression of L10 tumors after intralesional injection of live BCG (15). Occurrence of tumors at sites of vaccination with living tumor cells and BCG CW was also observed by Kleinschuster et al. who immunized cattle with vaccines containing living autochthonous squamous cell carcinoma cells admixed with BCG CW. The potential tumorigenicity of vaccines containing living syngeneic or autochthonous tumor cells appears to make them unacceptable for clinical use.

Response rates comparable to those achieved with living tumor cells could be obtained with irradiated tumor cells provided that sufficient numbers of irradiated cells were injected. Dose-response studies showed an increasing proportion of guinea pigs cured as a function of an increasing dose of irradiated tumor cells. The highest doses of irradiated tumor cells tested (1 to 1.2 x 10^6 cells) led to the cure of 67% of the treated animals; this dose was at the limit of the number of cells that could be injected in 4 i.d. sites. To consistently obtain cure rates close to 100% with a single immunization, doses of

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Evidence for Complete Eradication of Lymph Node Metastases. Animals cured by active specific immunotherapy were used to test for the presence of occult deposits of tumor cells.
increased efficacy of vaccine therapy by different methods of raising the tumor cell dosage

Table 3

<table>
<thead>
<tr>
<th>Vaccine (Day 9)</th>
<th>Sites injected with vaccination</th>
<th>Boosted with (Days 16 and 23)</th>
<th>L10 cells (10^6 x dose)</th>
<th>BCG CW (μg)</th>
<th>No. of animals tumor free/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No vaccine</td>
<td>1</td>
<td></td>
<td>0/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CW + irradiated L10^a</td>
<td>2</td>
<td>4</td>
<td>30 750</td>
<td>2/12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>60 750</td>
<td>9/12^c</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>16</td>
<td>120 3000</td>
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<td>5</td>
<td>16</td>
<td>120 750</td>
<td>7/12^c</td>
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<td></td>
<td>6</td>
<td>4</td>
<td>CW + irradiated L10</td>
<td>30 (3 times)</td>
<td>11/12^c</td>
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<tr>
<td></td>
<td>7</td>
<td>4</td>
<td>Irradiated L10 only</td>
<td>30 (3 times)</td>
<td>8/12^c</td>
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<tr>
<td>CW + live L10</td>
<td>8</td>
<td>4</td>
<td>30 750</td>
<td>9/12^d</td>
<td></td>
</tr>
</tbody>
</table>

^a All vaccine injections were i.d. on the right (contralateral) thoracic flank.

^b Cells irradiated with 20,000 rads γ-irradiation.

^c Significantly different (p < 0.05) to no vaccine controls and to Group 2.

^d Tumor growth occurred at vaccine sites in 3 of 12 animals.

irradiated tumor cells greater than 10^6 may be required. Alternatively, a series of booster immunizations with lower doses of irradiated tumor cells may improve therapeutic results (Table 3). No supraoptimal dose of irradiated tumor cells was detected in dose-response studies. This observation is similar to observations of the induction of EAE in inbred guinea pigs in which the host to reject a subsequent i.d. challenge with 10^6 living tumor cells than did immunoprophylaxis. Vaccines containing 1.5 x 10^6 living tumor cells or less were sufficient to enable to the number of effector cells formed are critical factors in the success of active specific immunotherapy. A primary function of the large doses of tumor cells used in vaccines for immunotherapy may be to accelerate the formation of large numbers of specifically sensitized cells. Studies of the rate of formation and properties of these cells are in progress.

Active specific immunotherapy required larger numbers of tumor cells than did immunoprophylaxis. Vaccines containing 1.5 x 10^6 living tumor cells or less were sufficient to enable to the host to reject a subsequent i.d. challenge with 10^6 living tumor cells (26); vaccines containing a minimum of 6 x 10^6 living tumor cells were required to eradicate occult metastases remaining after surgery. One concept suggested by these results is that the rate of formation of effector cells as well as the number of effector cells formed are critical factors in the success of active specific immunotherapy. A primary function of the large doses of tumor cells used in vaccines for immunotherapy may be to accelerate the formation of large numbers of specifically sensitized cells. Studies of the rate of formation and properties of these cells are in progress.

A supraoptimal dose for CW was detected; μg doses of CW were effective immunostimulants, but mg doses of CW inhibited the ability of optimal doses of irradiated tumor cells to immunize the host. This effect was similar to the dose response for mycobacteria in the induction of EAE in guinea pigs (22). The similarity in dose requirements for adjuvant in the induction of EAE and in successful active specific immunotherapy raises the possibility that the optimal dose range for an immunostimulant may be a constant for the guinea pig and perhaps for other species as well. Doses of living BCG (7, 9, 12) and killed Corynebacterium parvum (5, 11, 25) have been found that inhibited the ability of tumor cells to immunize the host.

The mechanism for the inhibition by CW of the immunizing capacity of optimal doses of irradiated tumor cells is unknown. It is possible that the intense granulomatous reaction induced by high doses of adjuvant resulted in rapid destruction of tumor cells, thereby reducing the antigen dose (23), or resulted in functional disruption of the draining lymph nodes. Meltzer and Leonard (17) demonstrated that mg doses of mycobacteria in mineral oil impaired the ability of guinea pigs to reject tumor line 10 at the site of BCG infection and inhibited the spontaneous regression of tumor line 1. Since animals pretreated with mycobacteria in mineral oil functioned effectively as recipients of transfusions of effector cells, these authors concluded that the block in the Freund's complete adjuvant-treated animals was at the level of formation of effector cells. This block might be mediated by suppressor T-cells. Reinisch et al. (19) found that i.p. injection of C57BL/6 mice with Freund's complete adjuvant induced suppressor T-cells which inhibited in vitro responses to allogeneic tumor cells. Schrier et al. (20) found that i.v. injection of C57BL/6 mice with killed BCG on oil droplets led to the formation of macrophages that inhibited generation of delayed hypersensitivity to sheep erythrocytes.

Several animal models have been developed to study active specific immunotherapy. Tumor cell vaccines have been shown to eradicate tumor cell deposits established by prior s.c. or i.v. injection of tumor cells (11, 16). There have been few studies on the use of tumor-specific vaccines in therapy of metastases from primary tumor grafts. Bartlett and Kreider (5) treated mice with the LSTRA lymphoma with irradiated tumor cells plus C. parvum. It was not established in that study that the tumor had disseminated from the site of inoculation by the time of treatment. Baldwin and Pimm (3) found that immunization with irradiated tumor cells and BCG did not eradicate lung metastases which remained after surgical removal of a transplanted rat epithelioma. The present report differs from those discussed above since it describes successful postoperative therapy of metastases arising from a primary tumor graft by remote treatment with a tumor cell-containing vaccine. This model should permit analysis of the components of mycobacteria that are required as adjuvants in postoperative immunotherapy and should facilitate studies on the cellular basis of active specific immunotherapy.

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REFERENCES

Immunotherapy of Lymph Node Metastases


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Michael P. Ashley, Berton Zbar, James T. Hunter, et al.


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