Regression by Active Specific Immunotherapy of Established Dermal Tumor Transplants and Lymph Node Metastases in Guinea Pigs

ELIYAHU YARKONI* AND HERBERT J. RAPP
Laboratory of Immunobiology, National Cancer Institute, Bethesda, Maryland 20205

Guinea pigs, each with an established, syngeneic dermal tumor (line-10) and microscopic lymph node metastasis, were treated by intradermal inoculation of living line-10 tumor cells admixed with emulsified heat-killed Mycobacterium bovis BCG cells. This treatment caused complete regression of established dermal tumors (about 10 mm in diameter) and prevented the growth of microscopic lymph node metastases in 25 of 39 treated animals (64%). All control animals treated by intradermal inoculation with heat-killed M. bovis BCG cells attached to oil droplets died with progressive dermal and lymphatic tumor growth.

The guinea pig line-10 (L10) tumor model has been used in studies of immunotherapy. Vaccines containing L10 cells and mycobacterial preparations were used to treat guinea pigs challenged intradermally with L10 cells immediately before vaccination (3–6), or 2 days after the excision of 7-day-old dermal tumors (1, 16). In this report we tested the capability of vaccination to cause regression of established dermal tumors and lymph node metastases without surgical intervention.

All experiments were done with an ascitic variant of tumor L10 derived from a hepatocarcinoma induced by diethylnitrosamine in a strain 2 male guinea pig. Intradermal inoculation of 10^6 L10 cells resulted in progressive intradermal tumor growth, and by 1 week, tumors cells were present in the draining axillary lymph nodes; guinea pigs usually died 2 to 3 months later (10). Adult male or female syngeneic guinea pigs, Sewall Wright strain 2, each received an intradermal injection of 10^6 L10 cells on the left side about 2.5 cm posterior to the superficial distal axillary lymph node. After 7 days the animals were treated by intradermal injections containing heat-killed Mycobacterium bovis BCG bacilli and oil in emulsified form (KCE) (13, 14) and living L10 cells. The final concentrations of vaccine components were: L10 cells, 1.5 \times 10^7 to 0.5 \times 10^7; killed cells, 5 to 0.17 mg/ml; oil (squalane), 3%; and Tween 80, 0.1%. To test the effect of vaccines on remote tumors and lymphatic metastases, vaccines were inoculated intradermally into sites drained by lymph nodes other than those nodes draining the established tumor (see footnotes to Table 1). Animals in control groups received no treatment or injections of KCE alone.

An experiment was conducted to determine the doses of L10 cells and KCE and the method of vaccination needed to bring about complete regression of the dermal tumor and its lymphatic metastases (Table 1, experiment 1). The results of this experiment were as follows. (i) The established tumor transplant did not regress in animals exhibiting tumor growth at the vaccinated site(s). (ii) When a mixture of 9 \times 10^6 tumor cells and 3 mg of KCE (group 1), or 3 \times 10^6 tumor cells and 1 mg of KCE (group 2), was given at one site, tumor cells grew at those sites in seven of eight and in eight of eight guinea pigs, respectively. The established tumor on the contralateral side may have a depressive effect on the immunologic response at the vaccinated site. Tumor growth at the vaccinated sites occurred in 14% of the animals when vaccines containing 30 \times 10^6 L10 cells and 0.75 mg of M. bovis BCG cell walls in emulsified oil were inoculated intradermally into guinea pigs 2 days after the excision of 7-day-old dermal L10 (1). (iii) The growth of tumor at the vaccinated sites in tumor-bearing animals was significantly reduced when a mixture of 9 \times 10^6 tumor cells and 3 mg of KCE was divided equally among three sites such that three regional lymph node chains were affected by the vaccine (group 3); in six of eight animals the tumor cells did not grow at the vaccinated sites (P < 0.05; in comparison with group 1 or 2), and in four of these animals, the established tumors were eradicated (P < 0.05, in comparison with group 1 or 2). (iv) When the dose of KCE was reduced to 0.3 mg per site, tumor cells grew at the vaccinated sites, whether the mixture contained 1 \times 10^6 or 3 \times 10^6 tumor cells per site (groups 4 and 6). (v) When the dose of vaccine was 1 \times 10^6 tumor cells and 1 mg of KCE per site, regression of the tumor cells at the vaccinated sites occurred in six of eight...
TABLE 1. Eradication of dermal tumor and lymph node metastases after intradermal vaccination with living tumor cells and KCE

<table>
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<tr>
<th>Expt</th>
<th>Group</th>
<th>L10 cells/site (× 10⁶)</th>
<th>KCE/site (mg)</th>
<th>No. of sites</th>
<th>No. of tumor-free animals¹/ no. tested</th>
<th>No. of animals with tumor growth at vaccination site(s)/no. of vaccinated animals</th>
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¹ Guinea pigs, each with a 7-day-old intradermal tumor on their left side (about 2.5 cm posterior to and on line with the superficial distal axillary lymph node), were vaccinated intradermally with mixtures of living L10 cells plus KCE. Animals which were vaccinated on one site received six, 0.1-ml injections of vaccine, on the right side about 2 cm posterior to and on line with the superficial axillary lymph node. Animals which were vaccinated on three sites received two, 0.1-ml injections of vaccine per site. The three vaccination sites were on the right and left flanks about 2 cm anterior to and on line with the superficial inguinal lymph nodes and on the right side about 2.5 cm posterior to and on line with the superficial distal axillary lymph node.

² Tumor-free animals means complete disappearance of the dermal tumor and no clinical evidence of metastatic disease at least 90 days after vaccination. No growth of tumor occurred at vaccination sites in these animals.

³ P values were obtained from 2 × 2 contingency tables of the Fisher exact test; test groups were compared with control groups (no treatment or KCE alone).

₄ Emulsions of killed cells were prepared by grinding. All other emulsions were prepared by ultrasonication.

animals (group 5; P < 0.005, in comparison with group 6). However, this vaccine did not cause eradication of the established tumor. (vi) KCE given intradermally on the side contralateral to the tumor transplant cured no animals.

Vaccination at three sites was utilized in another experiment (Table 1, experiment 2). In this experiment, 86% of the animals treated by vaccination with tumor cells and adjuvant were cured, and no tumor cells grew at the vaccinated sites.

*M. bovis* BCG cell walls in emulsified oils, prepared by a grinding method, were used as an adjuvant in previous active specific immunotherapy studies (1, 5, 6, 16). Therefore, in the third experiment we compared the adjuvant activity of KCE prepared by grinding with KCE prepared ultrasonically. The results of this experiment (Table 1, experiment 3) showed that both methods of preparation produced active adjuvants; 8 of 12 animals were tumor free when ultrasonically prepared KCE was used, and 7 of 12 animals were cured when KCE prepared by grinding was used. No growth of tumor cells occurred at the vaccinated sites.

In the studies reported here, we were able to cure 25 of 39 animals, each with a relatively large dermal tumor (about 10 mm in diameter) and microscopic lymph node metastases. For about 2 weeks after vaccination, it was not possible to distinguish between the growth of the tumor in the control group and that in the group treated with the specific vaccine. However, 7 days later (21 days after vaccination), regression of the dermal tumor was apparent. Previous studies (15) have shown that 14-day-old intradermal tumors are sixfold heavier than 7-day-old tumors. In the studies reported here, the dermal tumor regressed in some animals, whereas the lymph node metastases continued to grow in others. This phenomenon might reflect a greater efficacy of tumor suppressive activity in the skin than in the lymph nodes. Similar observations have been reported previously (8, 11). Smith et al. (11) demonstrated that systemic transfer of immune cells from syngeneic
donors could effectively eliminate 7-day-old intradermal L10 tumors. The adoptive transfer of the immune cells was not effective against 14-day-old tumors.

Clinical use of untreated tumor cells for active specific immunotherapy poses ethical problems; nevertheless, the use of untreated tumor cells may have a rational basis. Several investigators reported that irradiated tumor cells are markedly less immunogenic than untreated cells (1, 2, 7, 9, 12; G. L. Bartlett, J. W. Kreider, and D. M. Purnell, Fed. Proc. 35:225, 1976). Therefore, optimal immunization may require the use of untreated tumor cells.

In conclusion, vaccination against cancer in guinea pigs has a potential to eradicate not only microscopic lymph node metastases, but also a relatively large tumor burden in the skin. The number of sites at which the vaccine is given and the quantitative composition of the vaccine have a substantial effect on the efficacy of active specific immunotherapy.

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LITERATURE CITED