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MYCOBACTERIUM BOVIS (STRAIN BCG) EFFECTS ON THE GROWTH AND METASTASIS OF A TRANSPLANTABLE HAMSTER LUNG ADENOCARCINOMA

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Abstract. The effect of Bacillus-Calmette-Guerin (BCG) on the growth of an adenocarcinoma of the lung induced in a Syrian hamster was evaluated. It inhibited intradermal growth of the tumor when the tumor cells were mixed with high doses of BCG prior to intradermal injection. The development of lung metastasis after excision of the primary intradermal tumor was inhibited by BCG at doses 100 times lower than those used to inhibit the growth of intradermal tumors. The results suggest that effective immunotherapy may depend on the number of viable BCG cells, the route of administration, and the anatomic location of the tumor.

Syrian golden hamsters when treated intratracheally with benzo(a)pyrene and iron oxide develop respiratory tract malignancies which for the most part resemble the majority of human lung cancers both in location and morphologic characteristics (Saffiotti et al 1968). We have been assessing the effects of BCG preparations on the development of primary lung tumors (Zwilling et al 1977) and have sought in this study to assess the effect of BCG on the growth and metastasis of a transplantable hamster lung adenocarcinoma induced by the intratracheal application of the carcinogenic agent benzo(a)pyrene.

MATERIALS AND METHODS

Animals. Inbred male Syrian golden hamsters, strain LSH/LAK, were obtained from Charles River Lakeview (Lakeview, N.J.). Animals were housed 5 per cage under standard laboratory conditions and were used when 7-12 weeks of age.

Tumor cells. The tumor cell line used in this study was a bronchogenic adenocarcinoma, designated LG 1002. It was induced in an outbred Syrian golden hamster by the intratracheal instillation of benzo(a)pyrene adsorbed to ferric oxide particles and suspended in saline (Saffiotti et al 1968). The tumor was maintained by serial passage in the hamster cheek pouch. Tumor cell inocula were prepared from the 2nd to 10th transplant generations by digesting small tumor pieces with trypsin and suspending the cells at the desired concentration in Hank's balanced salt solution (HBSS).

Mycobacterial antigen. The Phipps strain of Mycobacterium bovis, strain Bacillus-Calmette-Guerin (BCG), TMC#1029, Lot #NC734A was obtained from the Trudeau Institute (Saranac Lake, N.Y.). Vials containing 1.2 x 10^8 colony forming units (cfu) were stored frozen at—70 °C. Dilutions of the BCG for injection were made in Hank's BSS. Concentration of the BCG suspension was accomplished by centrifugation at 200Xg for 20 minutes.

Immunizations. Tumor cell inoculations were given intradermally in the intrascapular region of the back or into the hind footpad of animals anesthetized with Brevitol Sodium. Locally administered BCG was admixed with the tumor cell inoculum prior to injection. Intratracheal injections of BCG were performed by the method described by Saffiotti et al (1968) for carcinogen instillation.

Monitoring of tumor growth. Growth of the tumor cell inocula given intradermally in the back was monitored by measuring the greatest and the least diameters of the tumor nodule with calipers. Tumor size was expressed as the product of the two diameters. Tumor growth in animals receiving footpad injections was monitored by measuring the footpad thickness with calipers. Footpad tumor growth was expressed as a tumor index which was calculated as the ratio of the thickness of the injected footpad to the thickness of the un.injected footpad.

Intradermal tumors were excised along with sufficient surrounding skin to insure the complete removal of the primary tumor mass. The
wound was inspected for the presence of subcutaneous tumor growth and the edges were apposed and fastened with sterile skin clips, which were removed 7–10 days later. Animals were checked daily for regrowth of the tumor at the excision site. To enumerate the number of metastases, the lungs were cleared of blood by severing the dorsal aorta and injecting 3–5 ml of saline into the right ventricle. They were then removed, fixed as described by Williams and Nettersheim (1973) and strained and cleared according to the method of Yuhas (1973). Mean tumor sizes and number of metastases were compared using the Student's t-test. Tumor incidence was compared using the Fisher's exact test.

**RESULTS**

**BCG on the growth of intradermal tumors.** Animals received either $6 \times 10^6$, $1 \times 10^7$ or $6 \times 10^7$ cfu BCG admixed with either $1 \times 10^4$ or $1 \times 10^5$ tumor cells. The growth rate of tumors in animals injected with $10^5$ tumor cells is shown in figure 1. The growth of tumors in all 3 BCG-treated groups was significantly inhibited after ($P<0.025$) compared to the control group. One of 5 animals in the $6 \times 10^6$ BCG group and 1 of 3 in the $10^7$ BCG group failed to develop tumors. Tumors which did develop in these groups grew at a slower rate than did the control tumors. All animals receiving $6 \times 10^6$ BCG developed tumors; tumor appearance was delayed and growth rate decreased. Similar results were obtained when BCG was admixed with $1 \times 10^5$ tumor cells.

**BCG on the growth of tumors inoculated into the footpad.** Since the skin of most rodents does not lend itself to a vigorous delayed-type inflammatory response, the footpad was studied as an alternate site of inoculation. In this experiment, $10^4$ tumor cells were admixed with $10^5$ to $10^7$ cfu BCG and injected into the hind footpad of the hamsters. Injection of $10^6$ tumor cells alone and $10^7$ cfu BCG alone served as controls. Tumor growth was measured as the ratio of the thickness of the tumor injected footpad to the uninjected footpad. The results, expressed as a tumor index, are shown in figure 2. At days 30 and 37, the mean tumor indices for the groups receiving $10^5$ and $10^6$ cfu BCG were significantly smaller ($P<0.05$) when compared to the tumor control group. By day 42, all of the BCG-treated groups had significantly smaller

![Figure 1](image_url)  
**Figure 1.** Effect of BCG on the growth of LG1002 tumors. Varying numbers of BCG were admixed with $1 \times 10^6$ LG1002 tumor cells and inoculated intradermally and tumor growth was monitored for 45 days.
Figure 2. Growth of LG1002 tumor cells following footpad inoculation of BCG-tumor cell mixtures. Numbers in parenthesis denote number of animals with tumor/number inoculated. 1x10^6 LG1002 cells were admixed with varying numbers of BCG and tumor growth was monitored for 42 days.

(P<0.01) tumors compared to the controls. Footpad swelling in animals receiving 10^7 BCG mixed with 10^4 tumor cells was observed to be comparable in size to that seen in animals receiving 10^7 BCG alone. All animals receiving 10^7 BCG failed to develop tumors and the tumor incidence in animals receiving other doses of BCG varied from 20% to 60%.

BCG on the development of pulmonary metastasis. Studies of the effect of BCG on lung metastasis were initiated because of our observation that intradermal injection of LG1002 tumor cells resulted in numerous metastases to the lung. In preliminary experiments we determined that lower doses of BCG were more effective at reducing the number of metastases than higher doses and that BCG treatment was more effective when administered after excision of the primary tumor rather than at the same time or prior to excision.

Table 1 shows the results of an experiment in which 1 x 10^6 cfu BCG was injected intratracheally on the same day as tumor excision or 1 or 2 weeks following excision. Three weeks after excision animals were sacrificed and the mean number of metastases per lung in animals not receiving BCG was 106.7. When animals were treated with BCG at the time of excision or one week following tumor excision, the number of metastases per lung was reduced to 85.2 and 86.7 respectively. When treatment was delayed until 2 weeks following excision of the primary tumor, the mean number of
metastases per lung was significantly reduced to 39.3 \( (P<0.05) \).

**DISCUSSION**

Suppression of tumor growth by BCG was observed to be dose related, higher doses of BCG being more effective. The minimum dose of BCG that significantly suppressed intradermal tumors was 6 \( \times 10^6 \) cfu. This finding is consistent with those of others (Baldwin and Pimm 1973, Harmel et al 1973, Kreider et al 1976, Zbar et al 1971). Barlett et al (1976) have shown that the ratio of BCG to tumor cells may be as important as the absolute number of BCG; high BCG to tumor cell ratios were inhibitory while lower ratios gave little protection.

An increase in the effectiveness of BCG-mediated inhibition of tumor growth was noted when the BCG-tumor cell mixture was inoculated into the footpad of the hamster. Significant tumor suppression was observed in the footpad at doses of BCG less than that required for suppression of intradermal growth. An intense inflammatory reaction to the injection of BCG alone was also observed.

BCG was generally more effective at inhibiting the development of lung metastases when given after excision of the primary tumor. These findings are in agreement with those of Sparks et al (1973) in which postoperative immunotherapy with subcutaneous injections of BCG or BCG tumor cell vaccine resulted in a significant increase in the median survival time. Proctor et al (1976), however, have reported that BCG given 10 days prior to subcutaneous tumor inoculation was generally more effective in inhibiting the development of lung metastases than BCG given on the same day or 10 days after tumor. In our studies the dose of BCG found to be most effective in inhibiting metastases was at least 100 fold less than the effective dose found to inhibit the growth of intradermal tumors.

The mechanism by which BCG affects tumor growth appears to be complex in nature. It has been suggested that lymphocytes reacting to BCG antigens may nonspecifically destroy tumor cells (Meltzer et al 1976). During the course of this reaction, lymphocytes may also produce a variety of lymphokines that result in activation of macrophages (Fidler et al 1976, Kripke et al 1977). It has been reported by Zwilling and Campolito (1977) and Hibbs et al (1972) that macrophages activated as a result of BCG infection acquire the capacity to destroy a variety of tumor cells. The results of our investigation indicate that the inflammatory response induced by BCG may differ depending on anatomical location. Effective immunotherapy using agents such as BCG may depend on the number of viable organisms and the route of administration.

**LITERATURE CITED**


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