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BCG-Induced Enhancement of Tumor Growth in Experimental Bladder Tumor Model

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Transitional-cell carcinoma of the bladder has been induced by chronic oral administration of N-(4-(5-nitro-2-furyl)-2 thiazolyl) formamide (FANFT) in C3H/He mice, and successfully transplanted in syngeneic animals. Treatment was attempted under 2 major categories, namely, by preimmunization with tumor antigens or BCG, and by BCG administration through different routes. Classic tumor challenge experiments showed the presence of tumor associated antigens on FANFT tumor cells. In this present study, BCG treatment promoted rather than inhibited the growth of FANFT tumor. Some proposed explanations for mechanism (s) of tumor enhancement following BCG immunostimulation were discussed.

(Key Words: BCG, Immunotherapy, FANFT Tumor)

INTRODUCTION

It is now well established that Bacillus Calmette Guerin (BCG) has preventive and therapeutic activities on numerous experimental tumors and certain types of human tumors. However, the efficacy of BCG immunotherapy varies, depending upon the time and mode of injection as well as the type of malignancy. Furthermore, under certain conditions BCG was found to be without any beneficial effects, and it was even shown to cause tumor enhancement (7, 8, 13). To provide data on the stimulating effects of BCG, an animal model was used. The chemical N-(4-(5-nitro-2-furyl)-2-thiazolyl) formamide (FANFT) is a carcinogen for the urinary bladder and has a high incidence of tumor induction in many species, including mice. The tumors formed are primarily transitional cell and resemble their human counterpart both grossly and histologically. The tumors are malignant, fulfilling the criteria of invasiveness, metastasis, and transplantability (12). In this paper, the following was shown; BCG-induced enhancement in tumor reccurence and rapid tumor growth when FANFT bladder tumor-bearing mice were pre-immunized with BCG alone or injected with BCG and whole tumor cells. The results supported anecdotal reports of accelerated tumor growth in patients who received BCG. Therefore, caution must be exercised in clinical trials using repeated large doses of BCG.

MATERIALS AND METHODS

The FANFT murine bladder tumors propagated by transplant into the

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legs of C3H/He syngeneic mice were used throughout this study. Single-cell suspensions of tumor were prepared by a modification of the method of Madden and Burk as previously described (4). Briefly, tumor tissue was aseptically excised, finely minced with scissors, and placed in a flask where enzymatic dissociation was carried out in Hank's balanced salt solution (HBSS) containing 0.25% trypsin. The mixture was agitated on a magnetic stirrer for 20 minutes at 37°C and then filtered through a fine wire mesh. The resulting cell suspension was centrifuged at 1,500 rpm for 10 minutes at 20°C, resuspended, and washed several times with HBSS. The cells were resuspended and the number of viable cells was determined in a hemocytometer by counting the number of cells that excluded 0.25% trypan blue. The final concentration of viable cells adjusted to 1×10^5 per 0.1 milliliter. BCG (Galaxo strain) was obtained from the University of Illionis (904 West Adams Street, Chicago, Ill 606071). The role of immunization with tumor antigens or with BCG was studied. Also, the effect of the site of BCG injection was explored. After tumor inoculation (1×10^5 cells) animals were randomized into control groups of 10 mice and treatment groups with 10 mice per group. In experiment 1, controls received 0.1 ml saline in the groin. BCG was administered once in a dose of 5×10^5 viable units. There were 4 experimental groups: BCG plus tumor cells, tumor cell alone, BCG alone, and a control group. Experiment 2 was studied in 5 groups: BCG intraperitoneal (i.p). BCG subcutaneous (s.c) groin, ipsilateral, and a control group. BCG was administered twice weekly in the same dose for six doses, or until tumors measured 10 mm in diameter. When animals developed palpable tumors (4-6 mm in diameter in Experiment 1) all mice had the tumor-bearing limb amputated and after recovery were challenged with tumor inoculates of 1×10^5 viable cells in the left flank. Growth of the challenge tumor inoculate was then measured.

The data presented in Table 1 and 2 were statistically evaluated using Z-test for significance of proportions.

Table 1 Effect of BCG treatment on tumor promotion

Treatment before tumor Challenge	No. with tumor/total no of animals on Day 21
Normal saline solution[a)]	2/7 (28%)
Tumor cells alone[b)]	0/7 (0%)
Tumor cells plus BCG[c)]	4/6 (66%)
BCG alone[d)]	6/9 (66%)

a) Non-tumor-bearing amputees
b) Tumor-bearing amputees, no BCG
c) Tumor-bearing amputees, BCG
d) Non-tumor-bearing amputees, BCG
The differences between group immunized with tumor cells alone and groups receiving BCG were significant to $p < 0.05$

Table 2 Effect of the site of BCG injection

Treatment before tumor Challenge	No. with tumor/total no of animals on Day 21
Normal saline solution	4/10 (40%)
BCG i.p.	6/8 (75%)
BCG s.c. groin ipsilateral to site of tumor	5/7 (71%)
BCG s.c. groin contralateral to site of tumor	5/8 (62%)
BCG intralesional	4/7 (57%)

There was no significantly different incidence of tumor takes between groups receiving BCG and the control group.

RESULTS

We have recently established the immunogenicity of a tumor by classic tumor challenge experiments. Animals bearing 6 and 8mm tumors rejected subsequent tumor challenge after amputation of the tumor-bearing limb. All animals inoculated with 10^6 cells developed tumors with an average diameter of 14mm by day 30. Only 50% of previously immunized animals (tumors amputated when 6 and 8mm in diameter) develop tumors, with an average diameter of 7mm at day 30. Four of 14 amputated host animals challenged with 10^5 cells developed small tumors. When tumor size was allowed to reach a diameter greater than 13mm before amputation of the limb, all animals accepted subsequent tumor challenge with growth of challenge tumors paralleling that of controls. Implantation and grwoth of a different murine tumor were not affected by immunization with the FANFT tumor (data not shown).

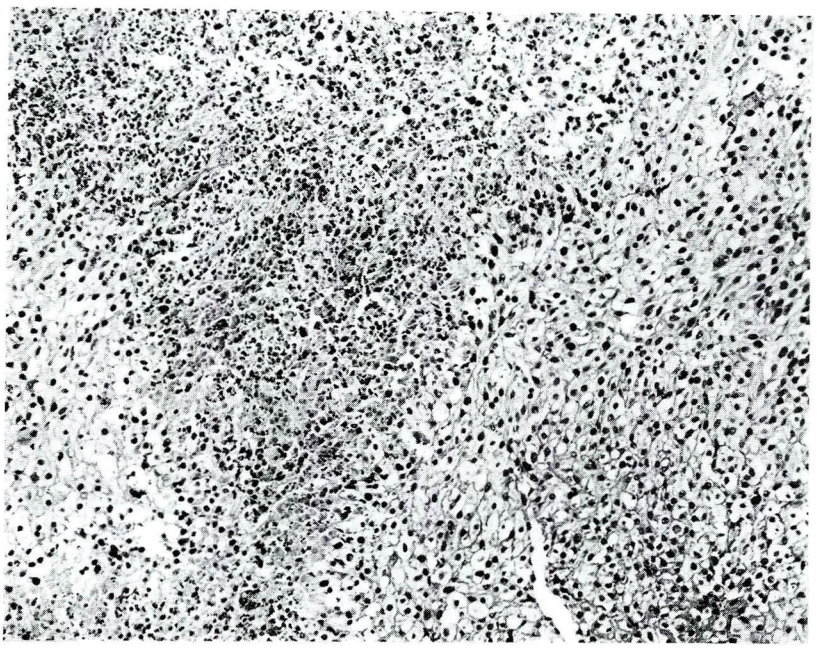

Fig. 1 Low power view of transplantable tumor in mice shows that the bulk of the tumor is made up of broad sheets of cells. ×150

Tumor growth occurred in 2 of 7 control mice and the tumor did not grow in any of the mice immunized with whole tumor cells. Six out of 9 mice immunized with only BCG grew tumors in the flank area, while 4 out of 6 mice immunized with BCG plus tumor cells had tumors on day 21 (see Table 1). Thus, immunization with BCG alone or BCG in conjunction with tumor cells produced no effect on the incidence of tumor takes, whereas there was an enhancement of tumor growth in the BCG-immunized mice ($P < 0.05$ compared to the group immunized with only tumor cells). Moreover as shown in Table 2, immunization with BCG failed to induce protection against FANFT tumor. Tumor growth rate in the groups receiving BCG was higher than those of control groups, although there was no significant difference. Many tumors used in these studies were a solid mass of transitional cell carcinoma serially transplanted in syngeneic mice. (Figs. 1 and 2).

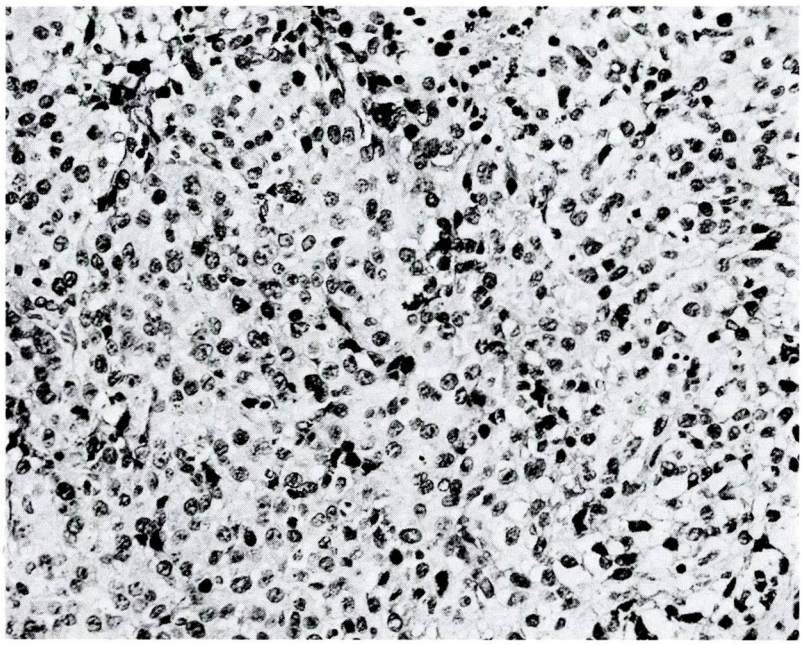

Fig. 2 This high power view of figure 1 demonstrates the tumor details, notably small cells formed by nuclear abnormalities and clear cytoplasm. × 300

DISCCUSION

Our amputation challenge experiments showed the presence of tumor associated antigens on FANFT tumor cells. The results support the study of Javadpour *et al.* (5) who found that FANFT tumor cells were immunogenic and cross-reactivity with syngeneic fetal antigens did not exist. Morales *et al.* (9) reported that immunization with irradiated FANFT tumor cells (MBT-2) or hypotonic membrane preparations from this tumor protected the animals against challenge with viable MBT-2 cells.

More interesting, however, was the finding that immunization with BCG failed to induce protection against FANFT tumor. BCG has been widely used in systemic adjuvant therapy in the hope that it may in some way selectively stimulate the cellular immune response and thereby facilitate an antitumor response. A serious potential hazard of BCG administration is immunological enhancement of tumor growth. The enhancement or rapid tumor growth induced by BCG has been throughly demonstrated in some experimental models. In these models BCG was given in large doses prior to or shortly after the transplantation of tumors.

Repeated administration of BCG has also been reported to depress cellular immunity in animals as measured by delayed cutaneous hypersensitivity reactions to certain common skin antigens (7). Wepsic *et al.* (15) reported the enhancement of tumor growth following immunization with BCG cw using transplantable Morris Hepatoma 3924a. Turcotte *et al.* (14) discussed the influence of culture conditions on the antitumor BCG phenotypes in experimental tumors. Experimental immunotherapy of urinary tract tumors with BCG has only recently been explored. Soloway (11) showed no decreased induction of an experimental FANFT bladder tumor after chronic administration of BCG. Most recently, Morales *et al.* (9) has shown that intraperitoneal administration of BCG failed to protect against FANFT tumor development. In this study we have shown that immunization with BCG produced no effect on the indicence of tumor takes, whereas there was an enhancement of tumor growth in the BCG-treated mice. Many of the factors that detemine the success or failure of BCG immunotherapy in animal models have been defined. Chee *et al.* (3) documented that the beneficial effect of BCG immunization was dependent upon the dose of BCG inoculated. They found, upon using the B-16 mouse melanoma tumor, that mice preimmunized with a high dose (0.5mg) of BCG had an accelerated growth rate, whereas, with a lower dose (0.005mg) of the BCG, tumors grew at a reduced rate. The variable effect of BCG immunization may be related to the tumor burden present. A study by Bsnsal *et al.* (1) showed that administration of BCG 2 weeks prior to or at the time of isografting of a polyoma tumor resulted in inhibition of tumor growth, but when BCG was inoculated when the tumor was palpable, there was an enhancement of the tumor growth. The various routes of BCG administration have variable effects on tumor growth. It was shown by Pimm *et al.* (10) that growth of intrapleurally injected cells of immunogenic methylcholanthrene-induced rat sarcomas was suppressed by intrapleural injection of BCG, in contrast, injection of BCG intravenously or subcutaneously enhanced rather than supressed, intrapleural tumor growth. Thus, several factors such as the substrain, the dose, the time and route of administration, etc., appear to be responsible for these opposite tumor activities. BCG was injected intralesionally in our experimental model in the hope that proximity is best achieved by intratumor injection. However, protection was not seen as compared to those receiving BCG injection at other sites.

The reasons for these opposite inhibiting and stimulating effects are presently not known. Several investigators (1, 2, 3, 8, 15) provide some proposed explanations for the mechanism (s) of tumor enhancement following

immunostimulation. (a) Antigenic competition can exist between BCG and the tumor. Some tumor lines have antigenic determinants that crossreact with antiserum to BCG, and some animals immunized with BCG may form antibodies that cross-react with antigenic determinants present on the tumor cells. (b) The induction of serum-blocking factors may occur in BCG-immunized animals. Although inoculation of BCG into experimental animals before they receive a tumor isograft heightens their resistance to the isograft, a similar administration of BCG into animals which already carry a tumor, has a weaker effect or none at all or sometimes even causes enhanced tumor growth. This latter effect of the BCG treatment might be due to an increased production of serum-blocking factors in animals already producing such factors at the time of treatment. (c) Immunostimulation by BCG and other related agents causes a marked alteration in the morphological structure and functional activity of lymphoid organs. Such an alteration, especially in the local relative balance of B and T-cells, could result in an inappropriate immunological response to a tumor cell inoculum, with the end result being an enhancement of tumor growth. (d) If stimulation of tumor growth by the immune response depends on the relative degree of the host's immunity, any agent that can vary the immune response of a tumor-bearing host over an appreciable range should be capable of both stimulating and inhibiting tumor growth. BCG is such an agent.

Wepsic *et al.* (15) give us warnings that if immunotherapy with BCG and other agents is performed following tumor resection and curative chemo and irradiation therapy, one must be cognizant of the fact that, on occasion such immunostimulation may be a form of functional immunosuppression, with the end result being an increase in the growth rate of any residual tumor and the subsequent clinical deterioration of the patient. Moreover, immunoresponses should be monitored in such patients to detect any immunosuppressive effect of BCG therapy (8).

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REFERENCES

1) Bsnsal SC and Sjogren HO: Effects of BCG on various facets of the immune response against polyoma tumors in rats. Int J Cancer 11:162, 1973.
2) Borsos T and Rapp HJ: Brief communication: Antigenic relationship Cancer. Inst 51: 1085, 1973.
3) Chee DO and Bodurtha AJ: Facilitation and inhibition of B-16 melanoma by BCG *in vivo* and by lymphoid cells from BCG-treated mice *in vitro*. Int J Cancer 14: 137, 1974.
4) deKernion JB, Ramming K and Fraser K: Immunotherapy and chemoimmunotherapy of the FANFT bladder tumor. Proc Am Assoc Cancer Res 18: 116, 1977.
5) Javadpour N, Hyatt CL and Soares T: Immunological features of a carcinogen-induced murine bladder cancer: *In vivo* and *in vitro* studies. J Surg Oncol 11: 153, 1979.
6) Lamoureux G, Poisson R and Desrosiers M: An antagonistic side effect of BCG immunotherapy: Induction of immunological anergy. In BCG in Cancer Immunotherapy, G Lamoureux, R Turcotte and V Portelance (Editors), pp, 167—178. Grune and Stratton Inc., New York, 1976.
7) Lamoureux G and Poisson R: BCG and immunological anergy. Lancet i: 989, 1974.

8) Levy NL Mahaley MS and Day ED: Serum-mediated blocking of cell-mediated anti-tumour immunity in a melanoma patient: Association with BCG immunotherapy and clinical deterioration. Int J Cancer 10: 244, 1972.
9) Morales A Djeu J and Herberman RB: Immunization by irradiated whole cells or cell extracts against an experimental bladder tumor. Invest Uro 17: 310, 1980.
10) Pimm MV and Baldwin RW: BCG therapy of pleural and peritoneal growth of transplanted rat tumors. Int J Cancer 15: 260, 1975.
11) Soloway MS: Intravesical and systemic chemotherapy of murine bladder cancer. Cancer Res 37: 2918, 1977.
12) Soloway MS, Missenkom I, McCallum LW and Murphy WM: Single and sequential combination intravesical chemotherapy of murine bladder cancer. Urology 2: 169, 1982.
13) Stjernsward J: Immune status of the primary host toward its own methyl-cholanthrene-induced sarcomas. J Natl Cancer Inst 40: 13, 1968.
14) Turcotte R and Quevillon: Opposite activities of BCG phenotypes in experimental tumors. In BCG in cancer immunotherapy, G Lamoureux, R Turcotte and V Portelance (Editors), pp, 15—21. Grune and Stratton Inc., New York, 1976.
15) Wepsic HT, Harris S, Sander J, Alaimo J and Morris H: Enhancement of tumor growth following immunization with Bacillus Calmette-Guerin cell walls. Cancer Res 36: 1950, 1976.