MOUSE-STRAIN DIFFERENCE IN IMMUNOPROPHYLACTIC AND IMMUNOTHERAPEUTIC EFFECTS OF BCG ON CARCINOGEN-INDUCED AUTOCHTHONOUS TUMORS

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SUMMARY: The prophylactic and therapeutic effects of BCG on the tumors induced by 3-methylcholanthrene (MCA) were studied comparatively between two inbred mouse strains, SWM/Ms and C3H/He, the first tumor appeared 5 weeks after MCA and the cumulative tumor incidence reached almost 100% within 20 weeks. On the other hand, the first tumor appeared 8 weeks after MCA in SWM/Ms, the number of tumor-bearers increased more slowly than in C3H/He, and the final tumor incidence (at 30 weeks) was about 90-80%. Single subcutaneous injection with BCG 2 weeks prior to MCA significantly protected SWM/Ms from tumor development, but not in C3H/He. These tumors, once appeared grew progressively and killed the hosts equally in both the strains. Intratumor (i.t.) injection with BCG showed more or less therapeutic effects in SWM/Ms; most tumors regressed or retarded after BCG. The time period after tumor-appearance to tumor-death was prolonged in most of SWM/Ms mice given i.t. injection with BCG, except a few mice that died earlier than non-treated controls after BCG. Contrary, no therapeutic effect of i.t. injection with BCG was observed in C3H/He. Different host responses to BCG between SWM/Ms and C3H/He were found by the peritoneal macrophage disappearance test and the footpad reaction test; SWM/Ms was a high-responder to BCG and C3H/He was a low-responder. The marked differences between SWM/Ms and C3H/He in prophylactic and therapeutic effects of BCG on the autochthonous tumors were discussed in terms of difference of the host immune response to BCG that is defined genetically.

INTRODUCTION

A huge number of reports have been published on the immunotherapeutic and immunoprophylactic effects of BCG against human cancers (Bast et al., 1974; Mastrangelo, Berd and Bellet, 1976). Although early studies (Davignon et al., 1970; Rosenthal et al., 1972) indicated that mortality from leukemia could be reduced by BCG vaccination, most studies failed to confirm these observations. General conclusion is difficult to be reached regarding the prophylactic efficacy of BCG against human cancer. The most convincing therapeutic effects of BCG seem to be demonstrated in melanoma given intratumor (i.t.) injection.
with BCG (Morton et al., 1970; Pinsky et al., 1973, 1976; Guttetman et al., 1976). However, efficacy of i.t. injection with BCG on miscellaneous tumors is too incomplete to allow any valid conclusion (Mastrangelo et al., 1976). To evaluate properly the prophylactic and therapeutic efficacy of BCG, information from animal experiments using autochthonous tumor systems must be important at this stage of study.

Previously we reported such effects of BCG on 3-methylcholanthrene (MCA)-induced autochthonous tumors in SWM/Ms mice (Tokunaga et al., 1974). During the observation period of 35 weeks after single subcutaneous injections with MCA, more than 80% of control mice developed tumors. Intradermal (i.d.) injection with BCG 2 weeks before MCA administration significantly decreased tumor incidence. BCG injected directly into the tumors within 3 days of tumor detection showed therapeutic effects on about 50% of the tumors, while control tumors without BCG treatment grew progressively and killed the hosts. A similar experiment was repeated using C3H/He mice instead of SWM/Ms mice in expectation of obtaining results similar to the previous ones. However, neither prophylactic nor therapeutic effect was observed. Therefore, similar experiments were repeated using both SWM/Ms and C3H/He mice simultaneously. The present paper describes the results of these experiments and explains the different antitumor effects of BCG on these two mouse strains in terms of different host response to BCG.

**Materials and Methods**

**Mice:** Inbred SWM/Ms mice were obtained from the National Institute of Genetics, Mishima, Shizuoka, Japan in 1971, and have been bred in this laboratory. Inbred C3H/He mice were purchased from Funabashi Animal Farm, Chiba, Japan. All the mice were female and about 8 weeks of age at the beginning of experiments. They were fed with commercial pellets and tap water ad libitum.

**MCA administration:** Mice were given subcutaneous (s.c.) injections on the right rump with 0.5-mg MCA dissolved in 0.1-ml olive-oil.

**Tumor incidence:** In the previous work, we noticed that tumors with diameters of over 5 mm grew progressively, resulting in consistent death of the hosts (Kataoka et al., 1972; Tokunaga et al., 1974). Therefore, tumor incidence was expressed as the ratio of the number of mice bearing tumors bigger than 5 mm in diameter to the total number of mice. Some mice died without visible tumors during the observation period. The probability that an animal would remain tumor free was estimated every week, with deaths of tumor-free animals counted by the statistical analysis of the life-table method (Pike, 1966; Tokunaga et al., 1974).

**BCG:** Lyophilized BCG manufactured by the Japan BCG Laboratory, Tokyo, Japan, was suspended in physiologic saline, washed once and adjusted to an appropriate concentration of viable units.
Intratumor injection of BCG: When a tumor nodule grew over 5 mm in diameter, 0.1 ml of the BCG suspension was injected through a Mantoux’s needle into the tumor within 3 days of the detection; a half volume was injected into the center of tumor and the other half into the base of tumor.

Evaluation of the effect of i.t. injection with BCG: Tumors responded to the i.t. injections with BCG in either of the following four ways: (a) tumors regressed and disappeared completely and no tumor recurrence was observed throughout the observation period (regression and cure); (b) tumors regressed and disappeared, completely or almost completely, but afterwards they began to develop progressively at the same or distant sites (regression and recurrence); (c) tumors grew very slowly and the hosts survived for 10 or more weeks after BCG administration (retardation), and (d) tumors grew progressively at almost an equal rate to untreated tumors (progression).

Peritoneal macrophage disappearance test: The method of Nelson and Boyden (1963) was used principally. Mice given s.c. injection with $2 \times 10^7$ viable units of BCG 2 weeks before were injected intraperitoneally with 10 µg PPD or with saline as a control. Four hours after the injections, the peritoneal cells were harvested by washing the peritoneal cavities with 10 ml of Hanks' balanced salt solution (HBSS). The harvested cells were smeared on slide glasses, stained with Giemsa’s solution, and counted differentially. About 500 cells were counted in each sample. The results were expressed as percentage of the macrophage counts to the total cell counts.

Footpad reaction with PPD: Mice receiving s.c. injection with $2 \times 10^7$ viable units of BCG 2 weeks before were injected intradermally with 10 µg PPD in 0.05 ml saline into a hind footpad. Saline was injected into the other hind footpad as a control. The footpad swelling was measured by a dial-thickness gauge (Peacock, Ozaki Co. Ltd., Tokyo, Japan) 24 hr after injection. The difference in the thickness expressed in 0.1 mm between the two hind footpads was designated as the footpad reaction (FPR).

**Results**

*Effects of BCG Sensitization of Mice on Incidence of MCA-induced Tumors*

Exp. I. One hundred and ninety-four C3H/He mice were devided into four groups (A, B, C and D) containing 50, 47, 50 and 47 mice, respectively. All the mice were given s.c. injection with MCA. Two weeks before MCA, groups A and C received intradermal (i.d.) inoculation on the left rump with about $5 \times 10^7$ viable units of BCG. Groups B and C received i.d. injections of the same units of BCG 5 weeks after MCA. Group D received no BCG. Tumors were palpated and measured twice a week, and the dates of tumor appearance and the diameters of all tumors were recorded. Observation was continued until the 30th week after MCA injection. Eight mice died without visible tumors...
Fig. 1. Estimated percentage of cumulative incidence of MCA-induced tumors in C3H/He mice with or without BCG-sensitization. Group A of C3H (○—○) was given BCG 2 weeks before MCA injection. Group B of C3H (△—△) was given BCG 5 weeks after MCA. Group C of C3H (▲—▲) was given BCG 2 weeks before and 5 weeks after MCA. Group D of C3H (●—●) received no BCG.

during the observation period; in group A, two died in 9 weeks after MCA; in group B one each in 6, 8 and 13 weeks; in group C, one in 14 weeks; in group D, one each in 6, 9 and 10 weeks after MCA treatment. The probability that an animal would remain tumor free was estimated every week by the statistical analysis of the life-table method, and the cumulative tumor incidence (%) calculated statistically for each group are plotted in Fig. 1.

The first tumors appeared 5 weeks after MCA injection, and the number of tumor-bearing animals increased rapidly from 8 to 11 weeks. In 13 weeks, almost all animals produced tumors despite of BCG administration. The calculated tumor incidence at the end of the experiment was 98.0% in group A, 95.6% in group B, 98.0% in group C and 97.1% in group D. There existed no significant difference in the tumor incidence among the four groups throughout the observation period. A similar experiment performed previously using SWM/Ms mice (Tokunaga et al., 1974) indicated that the estimated final tumor
incidence in SWM/Ms given BCG 2 weeks prior to MCA was only 43.5%, while that of the control group without BCG was 83.7%; the difference between these incidences was statistically significant (P=0.01).

Exp. II. In order to confirm the differences in tumor incidence and of prophylactic effect of BCG between these two strains, SWM/Ms and C3H/He, a similar experiment was repeated. Sixty SWM/Ms and 57 C3H/He mice were given s.c. injection with MCA and divided into two groups; one group (30 mice each) of both the strains received i.d. injection with about $5 \times 10^7$ viable units of BCG 2 weeks prior to MCA (groups SWM-BCG and C3H-BCG), and the other (30 of SWM/Ms and 27 of C3H/He) remained as untreated controls (groups SWM-control and C3H-control).

Eight mice died without visible tumors within the observation period of 30 weeks after MCA; in group SWM-BCG, one each died in 7 and 13 weeks after MCA treatment; in group SWM-control, one each in 4, 7, 13 and 15 weeks; in group C3H-BCG, none died; in group C3H-control, one each in 4 and 7
The estimated percentages of cumulative tumor incidence for each group were calculated with adjustment for non-tumor deaths (Fig. 2).

In C3H-control, the first tumors appeared 5 weeks after MCA injection; the number of tumor-bearing mice increased rapidly. Within 20 weeks, 100% of animals bore tumors. In SWM-control group, on the other hand, the first tumors appeared 8 weeks after MCA and the number of tumor-bearers increased relatively slowly till the 23rd week after MCA. The tumor incidence at the end of the experiment was 92.2%. All of C3H-control group died within 21 weeks after MCA, except one that died in 25 weeks. On the other hand, eight mice of SWM-control group survived for more than 21 weeks; one of the eight mice died in 24 weeks after MCA, four died in 25 weeks, one died in 28 weeks and two survived for more than 30 weeks. Differences in the cumulative incidence 10, 11 and 20 weeks after MCA between C3H-control and SWM-control groups were significant by both the Fisher's exact test and the $\chi^2$ test at a probability of 0.05, and those in 8 and 13 weeks were significant at a probability of 0.01.

In C3H-BCG group, the first tumor appeared also in 5 weeks, and all mice died within 21 weeks; exceptionally two died in 22 weeks. Difference in the tumor incidence between C3H-control and C3H-BCG was insignificant throughout the experimental period ($P=0.05$). In SWM-BCG group, on the contrary, the first tumor appeared 7 weeks after MCA, and the number of tumor-bearers increased more slowly than SWM-control. Differences in the tumor incidence between SWM-control and SWM-BCG were significant in 9, 10 and 11 weeks after MCA ($P=0.05$) and in 12, 14 and 18 weeks ($P=0.10$). Thus, prophylactic effect of BCG against MCA-induced tumors was clear in SWM/Ms but not in C3H/He.

Effects of BCG Injection into MCA-induced Tumors

In the course of Exp. I described above, BCG was injected directly into all of the tumors grown to 5 mm or more in diameter except for group D. In group D, three out of four tumors were given i.t. injection with BCG as they developed and the rest were left untreated as control; 34 of 44 tumors were given BCG and 10 were the controls. The results are summarized in Table I. As references, our previous data obtained in similar experiments with SWM/Ms (Tokunaga et al., 1974) are included in the table. No tumor regression occurred after i.t. injection with BCG in any of the groups of C3H/He or the control group of SWM/Ms, while four of 12 tumors (33.3%) regressed, at least once, in SWM/Ms presensitized with BCG (group A in Table I) and five of 30 (16.7%) in SWM/Ms without presensitization (a part of group D). More than 80% of tumors in C3H/He grew progressively and killed the hosts within 10 weeks after tumor appearance, regardless of presensitization or i.t. injection with BCG.

In the course of Exp. II described above, BCG was injected also directly
### Table I

*Effects of intratumor injection with BCG on MCA-induced tumors (I)*

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Group*</th>
<th>Number of mice given MCA</th>
<th>Death of tumor-free mice</th>
<th>Number of tumor-bearing mice</th>
<th>Regression and Retardation Progression</th>
<th>Death after i.t. with BCG**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cure (%) Recurrence (%) (%) (%)</td>
<td></td>
</tr>
<tr>
<td>C3H/He</td>
<td>A</td>
<td>50</td>
<td>2</td>
<td>48</td>
<td>0 0 5 (10.4) 42 (87.5) 1 (2.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>47</td>
<td>2</td>
<td>44</td>
<td>0 0 3 (6.8) 39 (88.6) 2 (4.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>50</td>
<td>1</td>
<td>49</td>
<td>0 0 9 (18.4) 40 (81.6) 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>47</td>
<td>3</td>
<td>34</td>
<td>0 0 6 (17.6) 28 (87.4) 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200 2 (20.0) 8 (80.0) 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>** Mice died within 2 weeks after i.t. injection with BCG.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*** See above (*).</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>**** These data were reported previously (Tokunaga et al., 1974).</td>
<td></td>
</tr>
<tr>
<td>SWM/Ms*****</td>
<td>A</td>
<td>30</td>
<td>4</td>
<td>12</td>
<td>1 (8.3) 3 (25.0) 3 (25.0) 3 (25.0) 2 (16.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>60</td>
<td>2</td>
<td>30</td>
<td>0 0 5 (16.7) 8 (26.7) 17 (56.7) 0</td>
<td></td>
</tr>
</tbody>
</table>

* Group A and C received BCG i.d. 2 weeks before MCA. Group B and C received BCG i.d. 5 weeks after MCA. Group D received only MCA as control. All tumor-bearing mice received i.t. injection with BCG within 3 days when a tumor grew to more than 5 mm in diameter, except in the mice marked with *** in group D, which were given no i.t. injection with BCG.

** Mice died within 2 weeks after i.t. injection with BCG.

*** See above (*).

**** These data were reported previously (Tokunaga et al., 1974).

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![Fig. 3](image_url)

**Fig. 3.** Growth rates of MCA-induced autochthonous tumors in normal SWM/Ms (17 mice) and C3H/He (23 mice) during first 4 weeks after tumor appearance. Mice having died within 4 weeks after BCG injection were removed from calculation. Vertical bars represent S.D. from the means of the tumor sizes (mm in diameter).
TABLE II

Effects of intratumor injection with BCG on MCA-induced tumors (II)

<table>
<thead>
<tr>
<th>Group*</th>
<th>Number of mice given MCA</th>
<th>Death of tumor-free mice</th>
<th>Number of tumor-bearing mice</th>
<th>Regression and Recurrence (%)</th>
<th>Retardation (%)</th>
<th>Progression (%)</th>
<th>Death after i.t. injection with BCG**</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H-control</td>
<td>27</td>
<td>2</td>
<td>25</td>
<td>0</td>
<td>4 (16.0)</td>
<td>21 (84.0)</td>
<td>0</td>
</tr>
<tr>
<td>C3H-BCG</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>4 (13.3)</td>
<td>25 (83.3)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>SWM-control</td>
<td>30</td>
<td>4</td>
<td>24</td>
<td>0</td>
<td>4 (16.7)</td>
<td>20 (83.3)</td>
<td>0</td>
</tr>
<tr>
<td>SWM-BCG</td>
<td>30</td>
<td>2</td>
<td>23</td>
<td>2 (8.7)</td>
<td>10 (43.5)</td>
<td>4 (17.4)</td>
<td>5 (21.7)</td>
</tr>
</tbody>
</table>

* C3H-BCG and SWM-BCG received i.d. injection with BCG 2 weeks before MCA, and were given i.t. injection with BCG within 3 days after tumor appearance. C3H-control and SWM-control received MCA only.

** Mice died within 2 weeks after i.t. injection with BCG.

Fig. 4. Survival rates of tumor-bearing C3H/He and SWM/Ms after tumor appearance. Solid lines represent control groups without BCG. Dotted lines represent groups, presentized with BCG 2 weeks before MCA and given i.t. injection with BCG within 3 days after tumor appearance. Data were obtained from 24 C3H-control, 29 C3H-BCG, 24 SWM-control and 23 SWM-BCG mice.

into the tumors produced in mice of C3H-BCG and SWM-BCG groups, when tumors grew to over 5 mm in diameter. Average growth rates of tumors of SWM-control (24 mice) and C3H-control (25 mice) were compared. The mean values of tumor sizes ± S.D. are shown in Fig. 3. Variations among the sizes of individual tumors were large in both the strains, and no significant difference in the growth rate seemed to exist between the two strains. The results of i.t. injections with BCG are shown in Table II. No tumor regression was observed in C3H-BCG, while four mice out of 23 in SWM-BCG suppressed tumors after i.t. injection with BCG. In addition, 43.5% of SWM-BCG inhibited tumor growth and survived for more than 10 weeks after BCG.
TABLE III

**Delayed type hypersensitivity of SWM/Ms and C3H/He to BCG**

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Peritoneal macrophage disappearance*</th>
<th>Footpad reaction**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (saline)</td>
<td>PPD</td>
</tr>
<tr>
<td>SWM-Ms</td>
<td>79.0±3.5</td>
<td>25.3±5.6</td>
</tr>
<tr>
<td>C3H/He</td>
<td>76.0±3.2</td>
<td>56.2±4.6</td>
</tr>
</tbody>
</table>

* Mice given i.d. injection with BCG 2 weeks earlier were injected i.p. with 10 μg PPD or saline. Four hours later, the peritoneal cells were harvested, smeared and stained with Giemsa. Percentages (±S.D.) of the macrophage to the total cells counted (at least 500 cells per mouse).

** Mice given s.c. injection with BCG 2 weeks earlier were injected with 10 μg PPD or saline into a hind footpad. The footpad swelling was measured 24 hr after PPD.

The dates of tumor appearance and of tumor death of each tumor-bearing mouse were also recorded. The survival rates of both the control and the BCG groups are indicated in Fig. 4. Mean time periods from tumor appearance to tumor death were 6.4±3.1 weeks in SWM-control (24 mice) and 7.5±2.1 weeks in C3H-control (25 mice); there seems to be no significant difference between them. On the other hand, BCG obviously prolonged the survival time in SWM-BCG but not in C3H-BCG. It was also noticed that six mice, one C3H-BCG and five SWM-BCG, died within 2 weeks after BCG.

Host Responses to BCG

In order to know possible differences in host immune response to BCG between SWM/Ms and C3H/Ms, the peritoneal macrophage disappearance test and the footpad reaction test were performed using those mice sensitized with BCG 2 weeks earlier. The results are shown in Table III.

In the peritoneal macrophage disappearance test, the loss of the macrophages with PPD injection was remarkable in SWM/Ms, while it was weak in C3H/He.

In the footpad reaction test with PPD, it was also shown that SWM/Ms was a high responder strain to BCG, while C3H/He was a low responder.

Discussion

A large number of reports on the antitumor effects of BCG against experimental tumors have been published (Bast et al., 1974, 1976). They suggested that a variety of factors influence largely the immunoprophylactic and immunotherapeutic effects of BCG. For instance, the administration timing of BCG in response to oncogenic agents is an important factor for successful prophylactic effects of BCG against tumors (Old et al., 1961; Lemonde et al., 1966; Sjogren
For successful immunotherapy, several factors, such as small tumor burden, direct contact of BCG with tumor cells, adequate number of BCG injected, and the immunologic ability of the host to respond to BCG and tumor-associated antigens, are required (Bast et al., 1974, 1976; Zbar et al., 1976). Importance of the ability of the host to develop immune response to BCG antigens for eradication of tumor cells was evidenced by the facts that BCG did not produce regression of tumor transplants in guinea pigs treated with antilymphocyte serum, and in these animals, PPD sensitivity failed to develop (Hanna et al., 1973), and that immunosuppressed mice with antithymocyte serum, with cortisone acetate or by thymectomy and sublethal irradiation did not develop PPD hypersensitivity and permitted the tumor growth after inoculation with the mixtures of BCG and tumor cells (Chung et al., 1973). Patients with melanoma who responded to intralesional injection with BCG were reported to be PPD-positive, whereas non-responders usually remained PPD-negative (Morton et al., 1974; Bast et al., 1974; Mastrangelo et al., 1976). The present data also indicate that the host immune response to BCG is a critical factor for successful prophylaxis and therapy with BCG against tumors. As shown in Table III, SWM/Ms was found to be a high-responder strain to BCG and C3H/He was a low-responder, as judged from the results of either the peritoneal macrophage disappearance test or the footpad reaction test. Presensitization with BCG 2 weeks prior to MCA injection protected the high-responder mice from tumor development, but did not protect the low-responders. Intratumor injection with BCG resulted in regression or retardation of tumor and in prolongation of survival time in most of the high-responders but not in the low-responders.

Mode of anti-tumor action of BCG has been elucidated fairly well in a form of "local immunotherapy", where BCG is introduced into the area of tumor, such as by i.t. injection (Zbar et al., 1976; Bast et al., 1974, 1976; Baldwin, Hopper and Pimm, 1976; Tokunaga et al., 1977). It involves at least the following three different mechanisms, which arise sequentially after BCG: (1) non-specific inflammation caused by BCG, in which the effector cells are mainly macrophages activated directly with BCG (Yamamoto and Tokunaga, 1978), (2) delayed type hypersensitivity reaction specific to BCG, in which the effector cells are macrophages activated by lymphokines released from BCG-sensitized T lymphocytes that were stimulated by BCG, and (3) induction of tumor-specific immunity mediated by killer lymphocytes sensitized with tumor-associated antigen. Tumor cells are destroyed at the site of an intense granulomatous reaction by the 1st and the 2nd mechanisms as innocent bystanders, and by the 3rd mechanisms as specific target cells. BCG-activated macrophages ingesting tumor cell debris that were produced by the former two mechanisms may play an important role in induction of the 3rd mechanism; obviously, all of these anti-tumor mechanisms are by-products of the host responses to BCG infection.

The SWM/Ms is an inbred strain derived from Swiss mice and established
in the National Institute of Genetics, Mishima, Japan. Although the histocompatibility of SWM/Ms is unknown, it is obvious that this strain is histoincompatible to C3H/He (H-2b), because antiserum prepared in C3H/He against SWM/Ms spleen cells was cytotoxic to the SWM/Ms lymphocytes, and *vice versa*, and a transplantable fibrosarcoma, K5 (Tokunaga et al., 1972), that is syngeneic to SWM/Ms, was never taken by C3H/He (Nakamura, R. M. and Tokunaga, T., unpublished data). As described in the present paper, these two strains were shown to respond differently to BCG. Genetic analysis of the immune response to BCG in F1, F2 and backcross of these two strains showed that the high responsiveness to BCG observed in SWM/Ms was a feature of dominant over low responsiveness in C3H/He, and transmitted according to the Mendel’s Laws (Nakamura and Tokunaga, 1978). Obviously, tumor incidence with MCA was also higher in C3H/He than SWM/Ms, as indicated in this paper. Whether or not the high incidence of tumor in C3H/He links with the low responsiveness to BCG or the histocompatibility or both is unknown and under study.

The spleen cells from SWM/Ms mice receiving single subcutaneous injections with BCG 2 weeks before responded well to PPD in vitro, while those from C3H/He treated by the same way did not (Nakamura et al., 1978). This means that production of BCG-sensitized T lymphocytes after BCG may be inhibited in C3H/He. Our previous data (Tokunaga and Yamamoto, 1977) obtained with athymic nude mice and their syngeneic tumor, KKN-1, indicated that growing solid tumors did not regress after i.t. injection with BCG in nu/nu mice, while did so in nu/+ in the same genetic background, suggesting that the 1st mechanism described above alone can not inhibit the growth of once established, growing tumors; the 2nd or the 3rd or both the mechanisms connected with T cell functions must be required. These may explain the fact that autochthonous tumors in C3H/He were not regressed by i.t. injection with BCG, in terms of lack of the 2nd mechanism, and therefore the 3rd, too, in these mice after BCG.

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