Differences in Biological Activity among Batches of Lyophilized Tice Bacillus Calmette-Guérin and Their Association with Clinical Course in Stage I Lung Cancer

James A. Bennett, Howard Gruft, Martin F. McKneally, Daniel Zelterman, and Ray G. Crispen


ABSTRACT

Various measures of biological activity were studied in batches of lyophilized Tice Bacillus Calmette-Guérin (BCG) that have been used intrapleurally as adjuvant therapy in surgically resected Stage I lung cancer patients by our own group, the North American Lung Cancer Study Group, and the Ludwig Lung Cancer Study Group. The biological activities of BCG that were studied were: (a) inhibition of solid tumor growth in mice following s.c. inoculation of BCG admixed with methylcholanthrene-induced fibrosarcoma cells (MC 43); (b) protection against tumor colonization of lungs and prolongation of survival in mice pretreated intrapleurally with BCG and later inoculated i.v. with a lethal dose of MC 43 tumor cells; and (c) growth properties in vitro, including a determination of the relative amounts of growing and nongrowing material and the sensitivity of growing material to inhibition by isoniazid. Significant differences in biological activity among batches of BCG were found. Some batches of BCG protected mice against circulating tumor cells, while others did not. Batches of BCG which protected mice against circulating tumor cells were found to have greater sensitivity to isoniazid and higher viability than batches which did not elicit this antitumor activity. There were also trends of some batches of BCG outperforming others in the clinic. Cumulative disease-free interval of patients was longer with batches which protected mice against circulating tumor cells than with batches which did not protect mice against circulating tumor cells. The results of this study suggest that preclinical testing of BCG for antitumor activity may improve the efficacy of this agent in future clinical trials.

INTRODUCTION

BCG, an attenuated strain of Mycobacterium tuberculosis subsp. bovis, was originally developed by Calmette and Guérin as a vaccine against tuberculosis in humans (7). Since its development, it has also been found to be a nonspecific stimulant of the reticuloendothelial system (3, 11), an immunological adjuvant (17, 21), and an antitumor agent (10, 28). The relative activity in all of these settings is dependent in part on the preparation of BCG that is used. Hart (9) discussed the importance of prepa-

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3 The abbreviations used are: BCG, Bacillus Calmette-Guérin; LCSG, North American Lung Cancer Study Group; CFU, colony-forming units; HBSS, Hanks' balanced salt solution; INH, isoniazid.

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MATERIALS AND METHODS

**Mice**

Male C3H/HeJ mice were purchased from The Jackson Laboratory when they were 5 weeks old. Mice were not used in experiments until they were 8 to 10 weeks old.

**Experimental Tumor**

A syngeneic, methylcholanthrene-induced fibrosarcoma, MC 43, was kindly supplied by Dr. Charles McKhann (Yale University) and was maintained in serial passage by s.c. inoculation of 10^5 tumor cells into the flank of C3H/HeJ mice. Single-cell suspension of tumor was prepared by enzymatic digestion with 0.14% collagenase and 0.03% DNase as described by Peters et al. (19).

**Bacillus Calmette-Guérin**

**Seed Lot System.** Lyophilized Tice BCG was generously supplied by ITR Biomedical Research. Genetic drift of the BCG organism was minimized by utilizing the seed lot system as outlined by the WHO (25). The master seed lot of BCG at ITR is Lot 12 obtained from the Pasteur Institute in 1934. All seed lots are produced from this master seed lot. Each reactivation of the master seed lot is designated by a number, and this number makes up the first part of the number identifying a lot or batch of BCG. For example, the 74th reactivation of the master seed lot is designated Seed Lot 74. Each lot or batch of BCG is produced from the same seed lot until that seed lot is used up. Each reactivation of a batch of BCG is designated by a number, and this number makes up the second part of the number identifying a lot or batch of BCG. One of the BCG batches used in this study was 74 62. This batch was the 62nd reactivation of Seed Lot 74.

**Preparation of BCG Vaccine.** Ampules of Seed Lots IL74 and IL105 were reconstituted with distilled water and planted on potato plugs in Sauton's medium in Roux tubes. After incubating at 36°C for 14 days, the veal growth was transferred to the surface of 130 ml of Sauton's medium in 250-ml flasks. After 6 days of further incubation, the pellicle growth from the surface of the flasks was transferred to 500-ml of Sauton's medium in 2500-ml low form flasks and incubated for 9 days at 36°C. The medium was then removed by aspiration, and 4 kg of 6-mm stainless steel balls were added to the flask. The flask was rotated around a horizontal axis at 30 rpm for 6 min. A lactose:salts freeze-drying medium was added at the level of 250 to 300 ml/ampul depending on the growth. After allowing the solution to wash the balls, the suspended growth was collected from all the flasks into a 5-liter bulk container. From the bulk container, the vaccine was dispensed aseptically into ampuls and freeze dried overnight. Ampuls were stoppered under vacuum and glass sealed in an oxygen:gas flame. Each ampul was tested for vacuum by a high-voltage spark tester before storing at -20°C.

Each lot or batch was then tested for the following characteristics required to meet the standards for licensed BCG vaccine: identity; potency (in vitro); potency (in vivo); pathogenicity; immunogenicity; safety; stability; and residual moisture (5). One lot (IL 10572 L) was prepared to contain 1 x 10^7 CFU/ampul rather than the 5 x 10^6 CFU/ampul contained in the other lots. All lots were shipped by air express and reached their destination in less than 48 hr. Lots were stored at 4° until they were used, and all lots were used well before their expiration date. Vials of lyophilized BCG were reconstituted with 1 ml of sterile water and diluted to the proper concentration with sterile HBSS immediately prior to their use.

**Assays of Biological Activity in Batches of BCG**

**Inhibition of Solid Tumor Growth.** MC 43 tumor cells were injected s.c. either alone or in direct admixture with BCG in the right flank of C3H mice. For each batch of BCG, 4 groups of 10 mice each were given, respectively, 10^6 tumor cells, 10^5 tumor cells plus 10^6 BCG, 10^5 tumor cells plus 10^5 BCG, or 10^5 tumor cells plus 10^6 BCG. These numbers of tumor cells and BCG organisms were determined from previous experiments with this model in which the 100:1 BCG:tumor cell ratio routinely resulted in inhibition of tumor growth, the 10:1 ratio occasionally resulted in inhibition of tumor growth, and the 1:1 ratio routinely did not result in inhibition of tumor growth (15). Tumor growth was determined by caliper measurement of 2 perpendicular diameters 10, 15, 20, 25, and 30 days postinjection. Mice were checked daily for survival.

**Inhibition of Circulating Tumor Cell Growth.** For each batch of BCG, 80 C3H mice were given injections intraperitoneally in groups of 20 with 10^6, 10^5, or 10^4 BCG organisms in a total volume of 0.2 ml. Controls were given injections of 0.2 ml of HBSS. Fourteen days later, mice were challenged i.v. with 5 x 10^6 MC 43 tumor cells. Fourteen days after challenge, 10 mice from each group were sacrificed, and tumor growth in their lungs was determined by insufflating the lungs with 15% India ink, sizing, and counting tumor foci with an automatic bacterial counter (Model 870: Artex Systems Corp., Farmingdale, N. Y.). The remaining 10 mice in each group were checked each day for survival. We have described previously the requirements of dose, route, and schedule of BCG for inhibition of tumor colonization of lungs in this model (6). Active BCG preparations had been found previously to routinely reduce lung tumor growth at a 2:1 BCG:tumor cell ratio, occasionally reduce lung tumor growth at a 1:5 BCG:tumor cell ratio, and usually did not reduce lung tumor growth at a 1:50 BCG:tumor cell ratio.

**Growth of BCG In Vitro.** BCG batches were reconstituted with 1 ml of sterile water, and a 0.1-ml aliquot of this suspension was diluted 10-fold in sterile HBSS for growth determinations (a 2-fold dilution was made for the low-dose Batch 105 72 L). One-half of this suspension was dried on a 0.45-μm Gelman filter for 30 min, and the tared weight was determined on a Mettler balance. The remaining half of the suspension was diluted 100-fold (10-fold in the case of 105 72 L) for growth determinations. Serial 10-fold dilutions were made from this suspension. One hundred-μl aliquots of each dilution were placed onto Dubos-Davis agar for quantitation of CFU and onto Middlebrook 7H10 agar that contained, respectively, no INH, INH (0.02 μg/ml), INH (0.05 μg/ml), and INH (0.1 μg/ml) for determination of sensitivity to INH. Quantitation of growth was made 3 weeks later. A count of between 10 and 90 colonies at a particular dilution was used in reporting the data. Growth differences in the presence and absence of INH were clearest at the 0.05-μg/ml concentration of INH, and these values are reported in "Results." Batches that were most sensitive to the growth-inhibitory effects of INH (0.05 μg/ml) required a 1-log reduction in dilution for determination of CFU.

**Experimental Design**

We selected what we suspected to be active and inactive batches of BCG based on the clinical progress of patients who had received these particular batches of BCG. Dr. Ray Crispen, Director of BCG Production at ITR Biomedical Research, supplied us with the appropriate batches of BCG. He coded them so that we were unaware of the clinical activity associated with each batch during testing, and we did not inform him of the clinical activity associated with each batch when we requested shipment of these batches.

As results were obtained and analyzed, the number of assays was decreased in order to focus on those which appeared to be most promising in terms of their correlation with clinical course. In the first part of the study, different batches of BCG were compared for their effect on solid tumor growth, colonization of lungs by circulating tumor cells, survival of mice bearing circulating tumor cells, and their growth characteristics in vitro. In the second part of the study, batches of BCG were compared for their effect on survival of mice bearing circulating tumor cells and for their growth characteristics in vitro. In the third part of the study, all of the batches of BCG were compared in one large experiment for their effect on survival of mice bearing circulating tumor cells. These parts are numbered 1, 2, and 3 in the tables. In all parts of the study, the batches of BCG were coded so that the investigators carrying out

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the analysis of biological activity in each batch of BCG were unaware of
the clinical activity associated with these batches of BCG.

RESULTS

Twelve batches of BCG have been used in the Albany study of
intrapleural BCG for surgically resected Stage I lung cancer
patients. The recurrence curves for BCG-treated and control
patients are shown in Chart 1A. The batches of BCG and the
clinical course of patients who received a particular batch of
BCG are shown in Chart 1B. There is a striking difference in the
clinical course associated with Batches 74 15 and 74 62. Four
patients have received Batch 7415. One patient developed re-
currence 5 years after treatment with this batch of BCG and
remains alive 8 years after treatment. Another patient de-
veloped a recurrence 6.5 years after treatment and died 6 months
later. The remaining 2 patients are alive with no evidence of
cancer 8 years after treatment. In contrast, 5 patients received
Batch 7462, and all 5 of these patients have suffered recurrences
less than 3 years after treatment.

Four batches of BCG have been used in the LCSG study of
intrapleural BCG for surgically resected Stage I lung cancer
patients. Batches 105 48 and 105 50 were used in the first 46
patients who received BCG (24 patients received Batch 105 48,
and 22 patients received Batch 105 50). Batch 105 53 was used
in the next 132 patients, and Batch 105 89 was used in the last
38 patients who received BCG. Patients who received Batches
105 48 and 105 50 experienced a slightly more favorable clinical
course than did control patients. Patients who received Batches
105 53 and 105 89 experienced a slightly less favorable clinical
course than did control patients (Chart 2). There are no statisti-
cally significant differences between the treatment groups and
the control group in any of these comparisons. The p values
derived by the Mantel-Haenzel test (14) are 0.105, 0.596, 0.242,
and 0.421, respectively, for Batches 105 48, 105 50, 105 53,
and 105 89. However, a trend of certain batches of BCG out-
performing other batches of BCG does exist. For instance, in a
nonrandomized comparison of survival, patients who received
Batches 105 48 and 105 50 survived longer than did those who
received Batches 105 53 and 105 89 (p = 0.057).

One batch of BCG, 105 72 L, has been used in the Ludwig
study of intrapleural BCG for surgically resected lung cancer
patients. Toxicity was associated with this batch of BCG in
pneumonectomized patients, and there appears to be no differ-
ence in survival between patients who received BCG intrapleu-
really and control patients at this time (12).

There are several possible explanations that could contribute
to differences in clinical benefit among Stage I lung cancer
patients who received different batches of lyophilized Tice BCG.
Random variation, undetected maldistribution of risk factors in
patients receiving therapy, the methods used in delivery of
therapy and supportive care after therapy, and the biological
activity in batches of BCG that were given to patients could all
play a role. We have investigated various measures of biological
activity in the batches of lyophilized Tice BCG used in the clinical
studies described above in order to determine whether there are
differences in biological activity and whether these differences
could explain differences in clinical course of patients who re-
cived these batches of BCG.

Effect of Various Batches of BCG on Solid Tumor Growth.
The results of inoculating tumor admixed with various amounts

of a particular batch of BCG are shown in Chart 3. Data were
analyzed using the multivariate regression package (26). Multi-
ivariate regression was used to take into account the serial
dependencies of multiple observations taken over time on the
same mice. Preliminary inspection of Chart 3 revealed exponen-
tial growth patterns, so data analysis reported here is for loga-
arithms of tumor sizes.

A comparison of mean log tumor size of controls in all experi-
ments revealed that tumor growth was not uniform across all
experiments [F = 6.40; d.f. = (20, 170); p < 0.0005]. Tumor
growth in control mice was particularly slow in the experiment
testing Batch 74 62. Elimination of this experiment from further
Four BCG Batches and All Controls

Chart 2. LCSG trial of intrapleural BCG as adjuvant immunotherapy for surgically resected State I non-small-cell lung cancer patients. Time to recurrence for each batch of BCG and all controls. This chart was compiled by Dr. Mitchell Gail of the LCSG and is included in this paper with permission from that group.

Chart 3. Growth of MC 43 tumor cells following s.c. inoculation of tumor alone or admixed with BCG. ■, 10^6 tumor cells; ●, 10^6 tumor cells + 10^6 BCG; ▲, 10^6 tumor cells + 10^6 BCG; ×, 10^6 tumor cells + 10^6 BCG. Number at top of each panel, serial number for each batch of BCG. Numbers in parentheses, the number of mice monitored for survival in this experiment did not have the shortest survival when compared to control mice in other experiments. There did not appear to be a level of tumor colonies in the lung that was uniformly lethal for all mice in these experiments, suggesting that other factors such as the location of the tumor colony in the lung or tumor colonization of other organs may be more directly related to survival than number of tumor colonies in the lung. There was no evidence of tumor colonies in visceral organs. However, there were symptoms of central nervous system involvement, such as loss of balance, immediately prior to death in some mice, suggesting that the brain rather than the lung may have been the lethal site of metastasis in these mice.

When batches were retested for their effect on survival of mice bearing circulating tumor cells (Part 2), Batches 74 62, 105 89, and 105 72 L again did not significantly prolong survival, whereas Batches 105 48 and 105 50 did prolong survival. Interestingly, Batch 105 53 prolonged survival in its first test but did not prolong survival upon retesting. This indicates that not only was there batch-to-batch variation within Tice BCG, but it was also possible to have vial-to-vial variation within a given batch of Tice BCG.

These batches were all tested again in one large experiment where all mice were inoculated with the same preparation of MC 43 tumor cells (Part 3). This experiment was carried out in an attempt to minimize the variation that might have been caused by different preparations of MC 43 tumor cells. Batches 74 62, 105 53, 105 89, and 105 2 L did not significantly prolong survival, whereas Batches 105 48 and 105 50 did significantly prolong survival. This was consistent with the results obtained in our previous experiments. The survival of control mice in this experiment still varied in spite of carrying out this experiment with one preparation of tumor cells. However, this variation could be explained. The order of testing these batches was 105 50, 105 48, 105 53, 105 89, 74 62, and 105 72 L. The first 3 were tested in the late morning, and the last 3 were set up in the afternoon. The morning control survival times were all 18 days, and the afternoon control survival times were 20.5 and 21.5 days. Viability of the tumor was tested by trypan blue dye exclusion before inoculation into each group of 40 animals used to test a batch of BCG. All animals received 5 x 10^6 viable MC 43 tumor cells i.v. Nevertheless, the virulence of the MC 43 analysis greatly improved comparability of controls [F = 2.39; d.f. = (16, 128); p = 0.005] but by no means established a pattern of uniform tumor growth in control mice across all remaining experiments.

There was a striking inhibition in the rate of tumor growth with all batches of BCG [F = 3.43; d.f. = (16, 541); p < 0.005], but batches whose controls exhibited larger tumor burdens tended to exhibit larger tumor sizes, and vice versa. Smaller mean log tumor sizes resulted from increases in BCG concentration at every time point measured [F = 19.02; d.f. = (12, 488); p < 0.005]. This reduction in mean log tumor size resulted from a combination of inhibition in rate of tumor growth and reduction in tumor takes by BCG. All mice that grew tumors eventually died from that tumor. Those animals that did not grow tumors lived a normal life span. There were no cases of tumors growing and then regressing. There appeared to be no obvious difference in the antitumor activity of one batch of BCG over another in terms of prolongation of survival or reduction in tumor growth as measured by this assay.

Effect of Various Batches of BCG on Circulating Tumor Cells. There was variation from experiment to experiment in the extent of tumor colonization of lungs and in the survival of mice given injections i.v. of 5 x 10^6 MC 43 tumor cells (Table 1). The most extensive colonization of lungs occurred in the control group of the experiment testing Batch 105 50. Interestingly, mice monitored for survival in this experiment did not have the shortest survival when compared to control mice in other experiments. Pretreatment of mice with BCG intrapleurally significantly reduced tumor growth in lungs and prolonged survival when Batches 74 15, 105 48, 105 50, and 105 53 were used. This was most apparent with the highest dose of BCG. There was no reduction in tumor growth or prolongation of survival by pretreatment with Batches 74 62 and 105 89. In fact, these 2 batches of BCG enhanced tumor growth at the low dose of BCG. The BCG-induced reduction in tumor colonization of lungs correlated with prolongation of survival within a given experiment but not between experiments. There did not appear to be a level of tumor colonies in the lung that was uniformly lethal for all mice in these experiments, suggesting that other factors such as the location of the tumor colony in the lung or tumor colonization of other organs may be more directly related to survival than number of tumor colonies in the lung. There was no evidence of tumor colonies in visceral organs. However, there were symptoms of central nervous system involvement, such as loss of balance, immediately prior to death in some mice, suggesting that the brain rather than the lung may have been the lethal site of metastasis in these mice.
tumor appeared to diminish slightly with the passage of time, and the loss in virulence could not be explained by a loss in viability. In summary, there appeared to be differences among batches of BCG in their ability to protect mice against circulating tumor cells.

In order to verify this apparent difference in antitumor activity among batches of BCG, an analysis of variance was carried out on the data that were used to generate Table 1. The mean survival time for all mice was 20 days and was slightly skewed to the right but judged to be adequately normally distributed. All mice were challenged i.v. with \(5 \times 10^6\) MC 43 tumor cells, and their survival was monitored. In Part 1 of the study, 10 mice from each group were sacrificed 14 days after tumor challenge, and their lungs were examined for evidence of tumor colonies. Quantitation of tumor colonies in the lung was carried out using the technique of Wexler (24). Statistical significance was determined using the \(\chi^2\) median test.

### Table 1

<table>
<thead>
<tr>
<th>BCG batch</th>
<th>Part of study</th>
<th>0.9% NaCl solution</th>
<th>(10^6) BCG</th>
<th>(10^5) BCG</th>
<th>(10^4) BCG</th>
<th>(10^3) BCG</th>
<th>(10^2) BCG</th>
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<td>74 15</td>
<td>1</td>
<td>18</td>
<td>18</td>
<td>20</td>
<td>(22)</td>
<td>340</td>
<td>261</td>
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<td>23</td>
<td>19.5</td>
<td>21</td>
<td>21.5</td>
<td>275</td>
<td>480</td>
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<td>21.5</td>
<td>19</td>
<td>19</td>
<td>24.5</td>
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</tr>
<tr>
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<td>19.5</td>
<td>21</td>
<td>(28)</td>
<td>338</td>
<td>403</td>
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<td>16</td>
<td>16</td>
<td>(19)</td>
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</tr>
<tr>
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<td>3</td>
<td>18</td>
<td>18</td>
<td>21</td>
<td>(25)</td>
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<td>23</td>
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<td>(28)</td>
<td>708</td>
<td>(463)</td>
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<td>21</td>
<td>16.5</td>
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<td>105 89</td>
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<td>17</td>
<td></td>
<td>18</td>
<td>570</td>
<td>685</td>
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<td>105 72 L</td>
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<td>16</td>
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<td>19</td>
<td>19</td>
<td>24</td>
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*Numbers in parentheses, median survival times that were significantly greater \(p < 0.05\) than their controls or median number of tumor colonies that were significantly less than their controls.*

### Table 2

<table>
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<tr>
<th>Source(s) of variation</th>
<th>d.f.</th>
<th>Mean square</th>
<th>(F)</th>
<th>(p)</th>
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<td>74.22</td>
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<td>Batches</td>
<td>6</td>
<td>235.2</td>
<td>15.66</td>
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<td>Treatment</td>
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<td>639.8</td>
<td>42.59</td>
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<td>6</td>
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<td>Batch (\times) treatment*</td>
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<td>Test (\times) batch</td>
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<td>Test (\times) batch (\times) treatment</td>
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<td>36.91</td>
<td>2.46</td>
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<td>Error</td>
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<td>Total</td>
<td>636</td>
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*Significant interaction between batches and treatment (see "Results").*
J. A. Bennett et al.

Table 3

<table>
<thead>
<tr>
<th>BCG batch</th>
<th>Part of study (mg/vial)</th>
<th>CFU x 10^7/vial</th>
<th>CFU/mg wet wt</th>
<th>CFU/μg with isoniazid, 0.05 mg/ml</th>
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<td>74 15</td>
<td>1</td>
<td>13.0 ± 0.7</td>
<td>0.65</td>
<td>8.2 ± 2.2</td>
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<td>74 62</td>
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<td>19.8 ± 0.4</td>
<td>0.47</td>
<td>36.0 ± 6</td>
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<td>4.5 ± 0.3</td>
<td>0.50</td>
<td>29.2 ± 1.6</td>
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<td>105 48</td>
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<td>21.0 ± 6</td>
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<td>18.8 ± 0.6</td>
<td>0.82</td>
<td>4.9 ± 4</td>
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<td>105 50</td>
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<td>26 ± 6</td>
<td>1.18</td>
<td>9.5 ± 0.9</td>
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<td>12 ± 0.8</td>
<td>0.88</td>
<td>8.6 ± 1.3</td>
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<td>26 ± 1</td>
<td>1.85</td>
<td>10.5 ± 0.7</td>
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<td>14.7 ± 0.9</td>
<td>0.98</td>
<td>15.8 ± 1.2</td>
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<td>0.45</td>
<td>22 ± 1</td>
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<td></td>
<td>2</td>
<td>9.7 ± 0.4</td>
<td>0.81</td>
<td>12 ± 1.5</td>
</tr>
<tr>
<td>105 72 L</td>
<td>1</td>
<td>0.30 ± 0.01</td>
<td>0.09</td>
<td>26 ± 4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.48 ± 0.05</td>
<td>0.34</td>
<td>12 ± 0.8</td>
</tr>
</tbody>
</table>

- The suspension of BCG (0.5 ml) was dried on a 0.45-μm Gelman filter for 30 min and weighed on a Mettler balance.
- Serial dilutions of BCG were grown in Dubos-Davis agar, and colonies were enumerated after 21 days of growth.
- Serial dilutions of BCG were grown in Middlebrook 7H10 agar for 21 min and weighed on a Mettler balance.
- There were also differences in sensitivity of batches to the growth-inhibitory effects of INH (0.05 μg/ml).
- Batch 74 62 was least sensitive, and Batch 105 48 was most sensitive to the growth-inhibitory effects of INH. All batches were inhibited to a similar extent by INH (0.1 /μg/ml). None of these batches would be considered resistant to INH by present criteria used to determine clinical resistance, that is, more than 1% of control growth in the presence of INH (0.1 μg/ml) (4). When second vials of these batches were shipped at a later time to us and retested, there was vial-to-vial variation in at least one of the above parameters in all of the batches except Batch 105 48.

When a batch of BCG prolonged the survival of mice bearing circulating tumor cells, it did so at the highest dose of BCG that was tested in this assay (10^6 CFU; Table 1). Sensitivity of BCG to INH correlated with protection of mice against circulating tumor cells (Chart 4; correlation coefficient, -0.69; p < 0.05). Those batches of BCG that were inhibited in their growth by INH (0.05 μg/ml) to less than 12% of control growth provided a significant increase in the life span of tumor-bearing mice. An increase in the life span of tumor-bearing mice to approximately 120% of control or greater was a significant increase (p < 0.05).

Relationship of Clinical Course to BCG Biological Activity. The relationship of clinical course of surgically resected lung cancer patients who received a particular batch of BCG intrapleurally and the biological activity of that batch of BCG is shown in Table 4. Cumulative disease-free interval of patients was longer with batches which protected mice against circulating tumor cells than with batches which did not protect mice against circulating tumor cells. A similar association with clinical course was found when the sensitivity of a batch of BCG to the growth-inhibitory effects of INH was measured. There was less of an association between clinical course and CFU/mg, wet weight.

DISCUSSION

The results of this study demonstrated that there were differences in the biological activity among the batches of lyophilized Tice BCG which were given intrapleurally to surgically resected Stage I lung cancer patients in 3 clinical trials. There were statistically significant differences among batches of BCG in their...
The variability in antitumor activity in preparations of BCG is not surprising when the following points are taken into consideration. (a) BCG is a living organism subject to the genetic and epigenetic changes inherent in rapidly dividing organisms. (b) BCG is not standardized for its antitumor activity, but rather for vaccine activity in guinea pigs, and its induction of tuberculin sensitivity in humans (5). There is no requirement to test BCG for antitumor activity, even though it may be used for this purpose. (c) There are steps in the production and handling of BCG where variation between batches might arise. Inconsistency in incubation and harvest conditions during the reactivation and growth of a secondary seed lot into a batch could be one source of variation. Studies have shown that preparations of *Corynebacterium parvum* that were harvested during early phases of growth in culture elicited less splenomegaly and antitumor activity in mice than did preparations that were harvested during later phases of growth in culture (Ref. 23; Footnote 4). BCG may undergo similar changes during its growth in culture. Another source of variation between batches might be that slight changes in the cooling and drying elements during freeze-drying could introduce changes in viability and perhaps biological activity. Additional sources of variability could be mishandling of temperature conditions during shipping and storage and technical differences in the clinic during reconstitution of lyophilized BCG.

Two factors will determine the antitumor response elicited by BCG, the genetic and epigenetic potential of the host to develop immunity to line 1 hepatoma by BCG: comparison of different BCG preparations. Cancer (Phila.), 46: 488–496, 1980.


**ACKNOWLEDGMENTS**

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**REFERENCES**


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**Table 4**

<table>
<thead>
<tr>
<th>Study group</th>
<th>BCG batch</th>
<th>Clinical performance of BCG batch</th>
<th>Protection of mice against circulating tumor cells</th>
<th>CFU/mg wet wt</th>
<th>Sensitivity to isoniazid (12%) of control growth</th>
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</thead>
<tbody>
<tr>
<td>Albany</td>
<td>74 15</td>
<td>Good</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Albany</td>
<td>74 62</td>
<td>Bad</td>
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<td>LCSG</td>
<td>105 48</td>
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<td>Yes, yes, yes</td>
<td>Yes, yes</td>
<td>Yes, yes</td>
</tr>
<tr>
<td>LCSG</td>
<td>105 50</td>
<td>Good</td>
<td>Yes, yes, yes</td>
<td>Yes, yes</td>
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</tr>
<tr>
<td>LCSG</td>
<td>105 53</td>
<td>Bad</td>
<td>No, yes, yes</td>
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<td>No, no</td>
</tr>
<tr>
<td>LCSG</td>
<td>105 89</td>
<td>Bad</td>
<td>No, no, no</td>
<td>No, no</td>
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</tr>
<tr>
<td>Ludwig</td>
<td>105 72 L</td>
<td>Bad</td>
<td>No, no, no</td>
<td>No, no</td>
<td>No, no</td>
</tr>
</tbody>
</table>

* "Good" and "bad" in reference to the clinical performance of BCG batches are relative terms. They were chosen to describe the following clinical results: Batch 74 15 outperformed Batch 74 62 in the Albany study; Batches 105 48 and 105 50 outperformed Batches 105 53 and 105 89 in the LCSG study; and Batch 105 72 L was associated with cases of atypical toxicity and unimproved clinical course in the Ludwig study.
J. A. Bennett et al.


Differences in Biological Activity among Batches of Lyophilized Tice *Bacillus Calmette-Guérin* and Their Association with Clinical Course in Stage I Lung Cancer

James A. Bennett, Howard Gruft, Martin F. McKneally, et al.


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