Effect of Bacillus Calmette-Guérin on Mammary Tumor Formation and Cellular Immunity in Dimethylbenz(a)anthracene-treated Rats

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SUMMARY

The effects of nonspecific stimulation of the reticuloendothelial system by Bacillus Calmette-Guérin (BCG) on rat mammary carcinogenesis were studied. A single feeding of dimethylbenz(a)anthracene (DMBA) to female Sprague-Dawley rats at the age of 50 days induced an immediate depression of spleen weight and reduction of the number of mononuclear spleen cells and the formation of multiple mammary carcinomas after a latency period of 6 weeks. The spleen weight returned to the control values within 3 weeks of the DMBA feeding, but the number of mononuclear spleen cells remained below the control values throughout the latency period of 6 weeks.

BCG given on the same day as DMBA counteracted the effect of the latter on the spleen. It decreased both amplitude and duration of the carcinogen-induced depression of spleen weight; moreover, it actually increased the absolute number of mononuclear spleen cells above the control values, namely, those recorded in rats receiving neither DMBA nor BCG. When BCG was given before or within 6 weeks (latency period) after the DMBA feeding, spleen weight and spleen cell counts, although usually higher than in animals not receiving BCG, did not exceed the control values. When given 6 weeks after DMBA, BCG slightly increased both parameters above the control values.

Tumor appearance was delayed only in animals treated with BCG on the same day as the DMBA feeding. However, the ultimate tumor incidence in the BCG-treated and untreated groups was similar. When BCG was administered after the appearance of the first tumor, it accelerated the formation of additional tumors. BCG had no effect, in any of these circumstances, on the growth rate of established tumors.

The effect of BCG on the induction of mammary tumors by DMBA did not seem to be mediated through endocrine mechanisms.

INTRODUCTION

Much evidence accumulating in recent years has shown that experimental tumors induced by a virus or chemical carcinogen may contain tumor-specific antigens (12, 24) and that the immune responsiveness of the host is depressed during the latency period of carcinogenesis (6, 14, 21). On the other hand, stimulation of the RES during the latency period can delay tumor appearance and sometimes even completely prevent it (15, 16, 23).

The purpose of this communication is to report the effects of single doses of BCG given at various times before and after a carcinogenic administration of DMBA on the cellularity of the RES and on mammary tumor formation in the rat.

MATERIALS AND METHODS

Virgin female rats from a random-bred Sprague-Dawley strain received a single intragastric instillation of 20 mg DMBA in sesame oil at the age of 50 days for the induction of mammary tumors.

Experiment 1. The 1st experiment was devised to study the effect of DMBA and BCG on the RES by measuring the weight of the spleen and the total number of mononuclear cells contained in it. At various times before and after the DMBA feeding (−21, 0, 7, 21, and 42 days), groups of animals were treated with a single BCG injection (1 mg of a living strain given s.c. in 0.5 ml of the culture medium in the interscapular area). An additional group was composed of rats receiving BCG immediately after appearance of the first palpable tumor. The BCG-treated groups were compared to untreated groups (DMBA without BCG) and to control groups (no DMBA and no BCG). Randomization into the several groups was always carried out among rats born on the same day. At various times after the BCG treatment, splenectomy was performed on 4
RESULTS

Experiment 1. The effects of DMBA and BCG on spleen weight and number of mononuclear spleen cells are shown on Charts 1 to 4. The results are expressed, for ease of reading, as percentage of the values obtained in the control animals. Statistical analysis was carried out by the Student t test.

Feeding DMBA produced a significant decrease in the spleen weight, which reached its minimum after 5 days (Chart 1). This decrease remained significant until Day 14 (p < 0.01). Thereafter, the spleen weight progressively increased, reaching the control value on Day 20 and significantly surpassing this value on Day 24 (p < 0.05).

animals in each group. The spleens were weighed and minced, and single-cell suspensions were prepared by gentle stirring in a standard amount of 0.9% NaCl solution. The cells were counted in a hemocytometer, and differential counts were obtained from smears of the cell suspension after staining by the method of May-Grünwald-Giemsa.

Experiment 2. In this experiment, the effect of BCG on the appearance of the DMBA-induced mammary tumors was studied. The time of administration of DMBA and BCG in the several groups, as well as the method of randomization, was the same as in the groups of Experiment 1. The animals were examined weekly for the presence of palpable breast tumors.
Effect of a single injection of BCG on the appearance of
DMBA-induced mammary carcinomas in the rat

When BCG was given 21 days before DMBA, the general
course was similar, except that the decrease in spleen weight
was smaller both in amplitude and duration: the spleen weight
values were already higher than in the control group.

When BCG was given on the same day as DMBA, the
depression in spleen weight was even smaller, a significant
decrease ($p<0.05$) being found only on Day 5; on Day 14 the
values were already higher than in the control group.

Small and temporary increases in spleen weight above the
values obtained in untreated animals were observed when BCG
was given 7, 21, or 42 days after DMBA feeding (Chart 2).
Similar effects were observed when BCG was given to rats
already bearing 1 tumor.

The number of mononuclear cells per spleen followed the
same general pattern as the spleen weight (Charts 3 and 4).
However, the decrease in cell number induced by DMBA in
untreated animals was greater and lasted longer when
compared to the corresponding decrease in spleen weight.
Minimal counts amounting to only 15% of the control values
were recorded on Day 14; the depression remained significant
($p<0.02$) until Day 35, which is less than 1 week before the
first tumors became palpable in the corresponding group of
Experiment 2.

BCG given 21 days before DMBA again significantly
reduced both amplitude and duration of the carcinogen-induced depression in number of spleen cells.
However, the most striking effect occurred when BCG was
given on the same day as DMBA. Cell number per spleen
increased immediately and significantly above the control
values for at least 10 days ($p<0.01$); it did not differ from
the controls thereafter. In this group, BCG more efficiently
counteracted the effect of DMBA on the spleen, when
estimated by measuring total mononuclear cell number rather
than weight. BCG treatment on Days 7 or 42 or BCG given to
animals already bearing 1 tumor was uniformly followed by an
increase in spleen number as compared to the untreated
animals. BCG was without detectable effect when given on
Day 21, at a time when spontaneous recovery was occurring.

Experiment 2. In the untreated group of rats (DMBA
without BCG), the first tumors appeared 6 weeks after
administration of the carcinogen. The number of tumor-bearing rats in the different groups after 14 or 18 weeks
is shown in Table 1.

BCG treatment markedly reduced the proportion of
tumor-bearing rats, but only in the group where it was given
on the same day as DMBA. This effect was highly significant at
the 14th week of carcinogenesis, but was no longer detectable
after 4 additional weeks of observation. BCG, in this group
only, increased the number of mononuclear spleen cells over
the control values in the early phase of carcinogenesis.

Since the rat mammary tumors induced by DMBA are
hormone-dependent, a possible effect of BCG on endocrine
functions in these rats was envisaged. Therefore, they were
submitted to autopsy and much attention was paid to the
weight and histological appearance of the various endocrine
glands as compared to the untreated group. No significant
difference was observed. Furthermore, in experiments not
detailed here, it was found that BCG administered together
with DMBA did not significantly alter the frequency of
adrenal necrosis observed 6 days later (9). In another
experiment, it was found that BCG administered at the age of
50 days did not influence the estrus cycle, as estimated by
vaginal exfoliative cytology. Finally, it was found that BCG
did not influence the growth rate of the rats, as measured by
the body weight curves.

In contrast to the inhibitory effect of BCG on tumor
formation when given at the same time as the carcinogen, BCG
given to rats immediately after appearance of the first tumor
increased significantly the proportion of rats developing
additional tumors.

BCG given either 21 days before or 7, 21, or 42 days after
DMBA feeding did not influence the number of tumor-bearing
rats.

In none of the groups did BCG treatment either increase or
decrease the rate of growth of the tumors once they became
established.

Table 1

<table>
<thead>
<tr>
<th>Time of BCG administration (days)</th>
<th>No. of rats with tumors</th>
<th>Weeks after DMBA administration</th>
<th>Statistical significance$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMBA, -21</td>
<td>BCG treated: 13/20</td>
<td>BCG untreated: 17/19</td>
<td>14</td>
</tr>
<tr>
<td>DMBA</td>
<td>6/20</td>
<td>17/19</td>
<td>14</td>
</tr>
<tr>
<td>DMBA, +7</td>
<td>13/20</td>
<td>17/19</td>
<td>18</td>
</tr>
<tr>
<td>DMBA, +21</td>
<td>15/20</td>
<td>13/17</td>
<td>14</td>
</tr>
<tr>
<td>DMBA, +42</td>
<td>12/19</td>
<td>13/17</td>
<td>14</td>
</tr>
<tr>
<td>I tumor already present$^c$</td>
<td>11/20</td>
<td>16/20</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>13/14</td>
<td>7/15</td>
<td>14</td>
</tr>
</tbody>
</table>

$^a$ Based on chi squares.
$^b$ n.s., not significant.
$^c$ in this group, the numbers refer to the rats bearing new tumors in addition to the one already present.
$^d$ Eight weeks after BCG administration.
DISCUSSION

Animals infected with an oncogenic virus or treated with a chemical carcinogen show a marked immunological deficiency throughout the latent period before their tumor becomes detectable (7, 21). Both cellular and humoral immune responses are impaired; rejection of incompatible skin homografts is delayed (7, 14, 26) and antibody formation against such a variety of antigens as Escherichia coli (5), human albumin (7), and sheep erythrocytes (5, 7, 27) is less than in control animals.

These studies also indicate an inverse relationship between the susceptibility of mice to Friend virus-induced leukemogenesis and immune competence (6, 22).

Furthermore, Stjernswärd (27) was able to show that, at least in CBA mice, only the carcinogenic members of hydrocarbon families decreased the number of antibody-forming cells, suggesting a possible relationship between the carcinogenic and immunosuppressive properties of a given chemical.

Thus, it seems that the immunodepressive effect of carcinogens may favor the development of antigenic tumor cells, induced either by the carcinogen itself, by an associated virus (10), or as a result of spontaneous cell mutation.

On the other hand, BCG has been shown to enhance the activity of the RES as judged by the clearance of carbon particles (3), to stimulate the formation of antibodies against unrelated viral or bacterial antigens (17), to accelerate the rejection of incompatible grafts (29) or tumor transplants (18), and to delay the appearance of primary induced tumors when given before or shortly after tumor induction (16, 23).

BCG can also correct the depressive effect of carcinogens on the number of in vitro plaque-forming cells (28).

Changes in spleen weight similar to those described herein have been reported by Cawein and Sydnon (4) using the same carcinogen in the same rat strain. He also documented the changes in the peripheral blood lymphocytosis following DMBA administration.

In our rats a profound depletion of splenic mononuclear cells promptly developed during the first days after feeding DMBA and persisted throughout the latent period of carcinogenesis. Recovery of normal cellularity was coincident with the appearance of the first palpable tumors.

With one exception, BCG treatment did not prevent the carcinogen-induced spleen cell depletion, but often limited its magnitude and duration. This, however, did not affect the incidence or the clinical evolution of the mammary tumors. The exception is that in the group of rats with a delayed tumor incidence BCG treatment not only prevented the carcinogen-induced mononuclear cell depletion but actually increased the number of mononuclear spleen cells during the first 10 days of the carcinogenesis. In a limited number of assays it was found that the proportion of hemolytic plaque-forming cells after immunization with sheep erythrocytes, estimated by the technique of Jerne et al. (11), was increased in this group of rats; however, the number of determinations was too small to allow any definite conclusion (W. F. Piessens, unpublished results).

Our results thus suggest that the delayed appearance of mammary tumors is mediated by nonspecific stimulation of the RES during the early phase of carcinogenesis, and this occurs only when BCG is given on the same day as the carcinogen. Even when BCG is given as early as 1 week after DMBA, there is no delay in the tumor appearance, probably because the effect on spleen cellularity lasts hardly longer than 2 weeks, which is quite a short period as compared to the shortest latent period of about 6 weeks in this system of carcinogenesis.

One puzzling effect of BCG treatment is its apparent tumor-enhancing effect when it is given to rats already bearing 1 tumor. No apparent immune deficiency was demonstrated in this group of rats, and their increase in splenic cellularity after BCG treatment was almost identical, both in amplitude and duration, to that observed in nontumor-bearing rats of the same age (Chart 4). The possible mechanisms of tumor enhancement by nonspecific stimulation of the RES have been fully discussed elsewhere (19, 20).

Since the majority of rat mammary tumors induced by DMBA are hormone dependent (8), the possibility must be considered that the observed differences are due to a modification of the hormonal environment. We observed no differences in body weight, rectal temperature, or post mortem microscopic appearance of endocrine glands (hypophysis, adrenals, ovaries, and pancreas) between the BCG-treated and untreated groups.

In addition, the findings reported here that BCG had no effect on the frequency of the DMBA-induced adenocortical necroses described by Huggins and Morii (9) and did not influence the estrus cycle in 50-day-old female rats were not in favor of an indirect effect of BCG through endocrine mechanisms.

Finally, to the best of our knowledge, no hormonal modifications induced by BCG vaccination have ever been recorded. It seems unlikely, therefore, that hormonal modifications played a major role, if any, in these experiments.

CONCLUSIONS

The present study adds further evidence in favor of the hypothesis that immunodepression probably plays an important role in experimental and possibly also in human carcinogenesis. The finding of cellular immune deficiencies both in animals during the latency period and in human cancer patients with manifest disease (1, 25) and the increased incidence of experimental (2, 30) and human (13) cancers after prolonged immunosuppressive therapy could be invoked as additional evidence to support this hypothesis.

The possible beneficial effects of active immunotherapy, even nonspecific as with BCG or other vaccines, should be thoroughly investigated in animals before such treatment can be safely used in human beings. As long as the exact mechanisms of the cytotoxic versus enhancing effects are not elucidated, immunotherapy in human cancer should be used only in strictly controlled clinical trials.
ACKNOWLEDGMENTS

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REFERENCES

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