Increased incidence of lung metastases of sarcoma 276 bearing XVII/Berlin mice after total body irradiation and BCG

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Combined prophylactic treatment by total body irradiation and BCG produce an increase in lung metastasis incidence and pleural effusions in a syngeneic murine tumor model. The prophylactic effect of BCG on metastasis formation is abolished independently of the order of pretreatments. Several possible causative mechanisms underlying this phenomenon and the clinical implications are discussed.

Introduction

Recently, immune stimulants, in particular BCG, have been used to provide nonspecific stimulation of tumor host defenses against various human tumors [6, 8]. With certain tumors, this nonspecific immune therapy has sometimes been combined with radiation therapy [5, 10, 12, 14]. Clinical studies have been done in bronchogenic carcinoma [12, 14], mammary carcinoma [5] and carcinoma of the bladder [10]. Experiments of Pouillart et al. [13] concerning survival times of tumor-free mice showed a significant increase in lifespan when BCG was administered 24 hours before lethal total body irradiation. The present experiments were performed to examine the influence of total body irradiation and BCG treatment on metastasis development. For this purpose we used the i.v. injection of tumor cells as experimental metastatic model [3].

Material and methods

Animals

Male mice of the inbred strain XVII/Berlin, weighing 22 ± 1 g and aged 10–12 weeks.

Tumor

Syngeneic sarcoma 276 passaged in ascitic form and originally induced by benzo[a]pyrene [1].

Induction of metastases

Metastases were produced by i.v. injection of 0.5 × 10⁶ sarcoma ascitic cells/0.25 ml Hanks’ saline. The metastatic spread was quantified by the percentage of metastatic animals and the organ distribution of metastases. On day 50 after tumor cell injection the surviving animals (about 30 per cent of animals pretreated with physiological saline) were killed and evaluated together with the animals which died from tumor metastases.
The animals received total body irradiation with 250 kV X-rays (total dose 4 Gy). (The irradiation was performed by Dr. K. Welker, Academy of Sciences of the GDR, Central Institute of Cancer Research.)

**BCG**

The lyophilized BCG vaccine (*Mycobacterium bovis*, BCG strain, S4 substrain) was obtained from the BCG-Institute, Jena; it contained about $15 \times 10^6$ germs/mg half-dry weight [16]. The animals received a single i.v. treatment of 3 mg of the lyophilized vaccine/0.5 ml physiological saline per animal.

**Experimental regimes**

Two regimes were used which varied with regard to the order of pretreatment:

- **Experiment 1.** Irradiation 14 days before tumor cell injection and then BCG 10 days before tumor cell injection;
- **Experiment 2.** BCG, 10 (B) or 14 (A) days before tumor cell injection and then irradiation 4 days before tumor cell injection.

Doses used were similar with each component and physiological saline, respectively, served as controls.

**Results**

The results of the two experimental arrangements are listed in tables 1 and 2. The animals pretreated solely with BCG show the expected metastasis-prophylactic effect, which is recognizable from the distinctly lower values for the percentage of animals bearing metastases. However, pretreatment with both total body irradiation and BCG caused a 100 per cent metastasis incidence. The prophylactic BCG effect is completely abolished irrespective of the order of pretreatment.

Regarding the metastasis pattern, all three control groups, i.e. animals pretreated only with physiological saline, BCG, or total body irradiation, gave a very low

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Number of animals bearing metastases/total</th>
<th>Metastases incidence (per cent)</th>
<th>Lungs</th>
<th>Pleural effusions</th>
<th>Organs of the greater circulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body irradiation and BCG</td>
<td>20/20</td>
<td>$100^a$</td>
<td>95$^b$</td>
<td>70$^c$</td>
<td>100</td>
</tr>
<tr>
<td>Irradiation</td>
<td>21/22</td>
<td>95</td>
<td>5</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>BCG</td>
<td>15/23</td>
<td>$65^a$</td>
<td>13</td>
<td>13</td>
<td>93</td>
</tr>
<tr>
<td>Physiological saline</td>
<td>21/25</td>
<td>84</td>
<td>5</td>
<td>10</td>
<td>95</td>
</tr>
</tbody>
</table>

Checking the differences between two frequency values [15].

$^a$ Statistically significant for $P<0.01$.

$^b$ Statistically significant as compared to the three control groups for $P<0.001$.

$^c$ Statistically significant as compared to the three control groups for $P<0.01$.

Table 1. Experiment 1: Total body irradiation and subsequent BCG.