Antitumor Activity of Two BCG Vaccine Preparations Against the Lewis Lung Carcinoma in Mice

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Summary. Two BCG vaccine preparations were prepared following different production methods. Immuno-BCG Pasteur F was produced by surface culture on Sauton medium; BCG-RIV was a homogeneous stirred deep culture.

The antitumor effects of the two BCG vaccines were investigated on the Lewis lung carcinoma (3LL) in C57Bl/6 mice. A direct relationship exists in this tumor model between the log_10 dose of single-cell suspension inoculated subcutaneously in the hind footpad of mice and the onset and the degree of local tumor growth and the time of death, which is directly related to the lung metastases. No significant difference from control mice was observed in the two groups of BCG-immunized mice when 3LL tumor cells were injected 2 weeks after BCG immunization. When varying numbers of viable units of the two BCG vaccines were injected together with 10^5 tumor cells in separate groups of normal mice, a dose-dependent local reaction was observed with Immuno-BCG Pasteur F, which was associated with a delay in the onset and development of tumor growth and an increase in the mean survival time. The local inflammatory reaction produced with BCG-RIV was of lower magnitude, and only the highest concentration (1.8 x 10^6 viable units) led to some delay in tumor occurrence and mortality. The antitumor effect of a specific local delayed-type hypersensitivity (DTH) elicited by varying amounts of the two BCG preparations injected together with 10^6 tumor cells in separate groups of normal or BCG-immunized mice showed that the challenge injection of Immuno-BCG Pasteur F was in all cases more effective than the BCG-RIV, but these two vaccines were more effective in BCG-RIV-immunized mice than in Immuno-BCG F Pasteur-immunized mice.

When the same number of viable units within each BCG vaccine was used as a criterion of comparison, Immuno-BCG Pasteur F produced a higher specific and nonspecific local inflammatory reaction (which was associated with a local antitumor effect) than BCG-RIV. But within 2 weeks, the latter was much better able to sensitize the mice to mycobacterial antigens. This was confirmed by the evaluation of local granuloma formation and tuberculin hypersensitivity. BCG vaccines prepared as surface-grown pellets and mechanically dispersed always sensitized mice to a lesser degree and after a much longer period of time than did the well-dispersed deep-cultured vaccine.

Introduction

The use of bacterial vaccines for cancer immunotherapy requires methods to check their suitability for this purpose. Several authors have described methods for evaluating the stimulating potency of different strains of mycobacteria with in vivo or in vitro experimental systems [1]. Previously, it was shown that normal and BCG-preimmunized C57Bl/6 mice behave similarly when challenged with SC injections of a single-cell suspension of 1 x 10^5 tumor cells of the lung Lewis carcinoma (3LL) [7]. Also, when a mixture of BCG vaccine and 10^6 tumor cells was injected together in the same footpad, only a minor delay was observed in these mice compared with those receiving tumor cells only. However, when this mixture (BCG + tumor cells) was injected in previously BCG-sensitized mice, the local specific inflammatory reaction, which fulfills all criteria of a delayed-type hypersensitivity (DTH) reaction, enabled the mice to completely destroy the tumor cell inoculum and all mice survived. All mice from control groups died within 3–4 weeks. Parameters of the
antitumor effect of a DTH reaction were then tested and it was found that its highest efficacy was achieved when the eliciting antigen was injected concurrently with the tumor cells in mice presenting a tuberculin-type DTH but not a Jones Mote-like DTH reaction, even when modified by the use of cyclophosphamide. Moreover, only particulate antigens – such as living or heat-killed BCG – and not soluble antigens – such as tuberculin or culture filtrates – were able to elicit the antitumor effect in a DTH reaction. An analogous finding was also observed when Listeria monocytogenes was inoculated in the site of a DTH reaction: a protective effect was detected only in the tuberculin-type DTH reaction, and this seems to be related to the recruitment and activation of mononuclear phagocytes [6]. Thus, this antitumor effect of a tuberculin-type DTH reaction can be used for testing the ability of different strains of BCG for antitumor therapy. Two distinct parameters can be studied: one is the ability to induce the state of sensitization, and the second is the efficacy to elicit the specific immune local inflammatory reaction. This report concerns the results of such a test in which two BCG substrains were compared: Immuno-BCG Pasteur F from the Pasteur Institute (France) and BCG-RIV from the Rijks Instituut voor de Volksgezondheid (Holland). It is shown that these two BCG substrains differ and there is an inverse relationship between the ability to induce sensitization and the capacity to elicit a DTH reaction. The same BCG preparations were examined for immunostimulating properties, safety, and antitumor activity against a murine fibrosarcoma (Kreeftenberg JG et al. 1981, submitted for publication).

Material and Methods

1. Animals. Specific pathogen-free inbred C57Bl/6 female mice from the Pasteur Institute Breeding Unit were used for all tumor experiments; NCS female mice were also used for the study of the specific immune response against BCG.

2. BCG Vaccines. Two BCG vaccines were tested: Immuno-BCG Pasteur F and BCG-RIV. Immuno-BCG Pasteur F (lot 02-1978) was produced from strain BCG Pasteur Paris 1173 P2 secondary lot A by surface culture on Sauton medium. The bacillary mass was collected by filtration and homogenization was performed with stainless steel balls. The vaccine was resuspended in Dubos medium with 5% glycerol and 5% human albumin as recently described [5]. The vaccine was stored in a frozen state at -70 °C [10]. This batch contains 766 \times 10^6 culturable particles BCG or 75 mg semidry weight/ml, which corresponds to 15 mg dry weight/ml.

BCG-RIV (lot 057) vaccine was prepared by and obtained from Dr. P. A. Van Hemert (Rijks Instituut voor de Volksgezondheid, Bilthoven, Neederlands). This vaccine was grown in homogenously stirred deep culture from seedlot P3, prepared from strain BCG Pasteur Paris 1173 P2. Bacteria were collected by centrifugation and resuspended in freeze-drying medium without the use of a ball-mill (Kreeftenberg JG et al. 1981, submitted for publication), and then freeze-dried. This batch contains 75 \times 10^6 viable BCG per vial, which corresponds to 0.25 mg dry weight per vial. In some experiments, another lot of BCG-RIV was used (lot P24): each freeze-dried ampoule contained 0.54 mg BCG (dry weight), corresponding to 16.5 \times 10^6 viable units.

3. Tumor Cells. Single-cell suspensions of 3LL, a malignant metastasizing tumor, which arose spontaneously in a C57Bl/6 mouse, were kindly donated by B. Hevin (Institut Pasteur). Suspensions of a known concentration of tumor cells (viability always 95%) in culture medium and living BCG were mixed at room temperature for 10 min and were then injected in a volume of 0.04 ml into the left hind footpads (LHFP) of normal or BCG-immunized mice.

4. BCG Immunization. Mice were immunized SC with a single injection of a known number of live BCG organisms in the nape of the neck, and 14 days later the animals were challenged with a mixture of \(10^5\) tumor cells and varying concentrations of the two BCG substrains.

5. Delayed Local Reaction (DLR). The development and decay of the DLR in mice is characteristic of the formation of a thymus-dependent local granuloma [9]. Mice received injections of varying concentrations of the two BCG preparations into the LHFP, in a volume of 0.04 ml. After this the footpad swellings were regularly measured individually with a dial caliper reading to 0.05 mm. Reactions were expressed as the difference in thickness between feet that had received the BCG injection and those that had not. The results are expressed as previously described [9].

6. Delayed-type Hypersensitivity. The DTH reaction was measured as described elsewhere [12]. In brief, variations of footpad thickness were measured after the injection of 0.04 ml saline containing 200 tuberculin units of reconstituted lyophilized purified protein derivative (PPD) into the RHFP. Reactions were expressed as the difference in thickness of feet before and 24 h after the injection of the eliciting antigen.

7. Viable BCG in the Draining Nodes. Counts of viable BCG were performed in the popliteal draining nodes 28 days after the injections of varying concentrations of BCG preparations in the LHFP, by plating dilutions of homogenized nodes on Middlebrook 7H10 medium [12]. These counts were expressed to the log10 and the geometric mean per group was calculated.

Results

1. Tumor Growth in Normal and in BCG-immunized Mice

Separate groups of mice were inoculated in the nape of the neck with 0.1 ml saline alone or containing 7.6 \times 10^6 viable units of Immuno-BCG Pasteur F or 7.5 \times 10^6 viable units of BCG-RIV, and 14 days later all mice were challenged with an injection of \(10^5\) 3LL tumor cells in the LHFP. Tumor growth in normal