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Immunotherapy of Guinea Pigs with a Transplanted Hepatoma: Comparison of Intralesionally Administered Killed BCG Cells and BCG Cell Walls

ELIYAHU YARKONI* AND HERBERT J. RAPP

Laboratory of Immunobiology, National Cancer Institute, Bethesda, Maryland 20205

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Heat-killed whole BCG cells (KC) and BCG cell walls (CW) were each tested in emulsified form for their potency to cause regression of a transplanted guinea pig hepatoma. On a weight basis, KC were at least as effective as CW in causing tumor regression and elimination of microscopic lymph node metastasis, and they, as well as purified protein derivative of mycobacteria, provoked delayed cutaneous hypersensitivity reactions in animals immunized with CW or with KC. On a weight basis, KC were as active as CW in eliciting delayed cutaneous hypersensitivity in sensitized guinea pigs whether the animals were immunized with CW or with KC. In unimmunized animals the inflammatory response to intradermally administered KC was similar to that induced by CW. Because KC are easier to prepare than CW, it is suggested that whole killed BCG might be used instead of CW in clinical trials of cancer treatment requiring administration of nonliving mycobacteria.

Nonviable but immunotherapeutically active mycobacterial preparations might be useful clinically, because one possible undesirable effect of administering living BCG intralesionally to cancer patients is systemic BCG infection. Nonliving whole BCG cells (KC) and BCG cell walls (CW) have been found active in experimental immunotherapy of cancer (2-4, 7-9). CW were derived from BCG as described in reference 1. The study reported here was undertaken to compare (i) the efficacy of emulsified KC and CW in producing regression of the line 10 guinea pig hepatoma and (ii) their immunogenicity as judged by delayed cutaneous hypersensitivity (DCH).

KC and CW were derived from the same lot of BCG, Tice substrain, and were purchased from ITR Biomedical Research, University of Illinois at the Medical Center, Chicago. The ultrasonic method for preparing injectable emulsions of mycobacterial components has been described previously (8). Each emulsion containing a given concentration of KC or CW was prepared separately and identically so that all vaccine preparations contained the same amount of mineral oil (3.3%) and Tween 80 (0.2%) and differed only in KC or CW content. Emulsions were infiltrated into the growing tumor in 0.4-ml volumes. All experiments were done with tumor line 10, an ascitic variant derived from a hepatocarcinoma induced by diethylnitrosamine in a strain 2 guinea pig. Inoculation of 10^6 tumor cells intradermally resulted in progressive intradermal tumor growth, and by 1 week tumor cells were present in the axillary draining lymph nodes; guinea pigs usually died 2 to 3 months later (5). Animals were treated by a single intralesional injection of emulsified KC or CW 7 or 14 days (Table 1, experiments 1 and 2, respectively) after tumor implantation, when the average diameter of the tumor was 9 and 14 mm, respectively. Control animals received injections of emulsion lacking mycobacterial components. In each of the two separate experiments, comparable cure rates were produced by KC and CW.

In addition to determining the relative therapeutic efficacy of emulsified KC and CW, we tested their capacity to induce DCH. DCH reactions were measured 24 h after injection of saline suspensions of purified protein derivative (PPD), KC, or CW. The diameters of the skin reactions were measured and used to calculate the average diameter of the DCH reaction to each challenge. Tumor-free animals from the first experiment treated with 0.1 or 0.9 mg of emulsified KC or CW were divided into groups of four or five, and graded doses of saline suspensions of PPD, KC, and CW were tested by intradermal administration in 0.1-ml volumes (Fig. 1 and 2). Animals in a control group consisting of normal animals were tested in the

same way. No skin reactions were found in the normal animals challenged with saline suspensions of PPD (up to 0.002 mg), KC, or CW (up to 0.01 mg). KC or CW injected intradermally in saline suspension induced mild inflammatory reactions in control animals at a dose of 0.05 mg (4.0 ± 0.7 mm and 3 ± 0.7 mm in diameter,

TABLE 1. *Tumor regression induced by KC and CW*

Expt[a]	Material injected	Dose (mg)	No. of tumor-free animals/no. of animals treated (90 days)
1	Emulsion		0/9
	KC	0.9	10/10
		0.3	10/10
		0.1	8/10
		0.03	6/9
	CW	0.9	10/10
		0.3	10/10
		0.1	4/9
		0.03	5/9
2	Emulsion		0/10
	KC	0.9	9/10
		0.3	7/9
		0.1	2/9
	CW	0.9	7/10
		0.3	4/9
		0.1	2/9

[a] In experiment 1, emulsified preparations were injected intralesionally into 7-day-old line 10 tumors, about 9 mm in diameter. In experiment 2, emulsified preparations were injected into 14-day-old tumors, about 14 mm in diameter. In each experiment there were no statistically significant differences in the cure rates of animals treated with equivalent doses of KC or CW, as evaluated by the Wilcoxon nonparametric rank test (6).

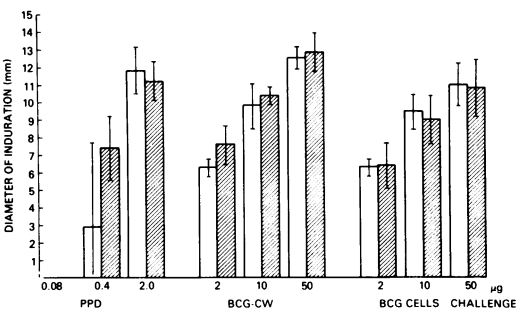

FIG. 1. *Delayed cutaneous hypersensitivity reactions to saline suspensions of PPD, CW, and KC in guinea pigs presensitized with 0.1 mg of intralesionally administered emulsions of CW (open bars) or KC (hatched bars).*

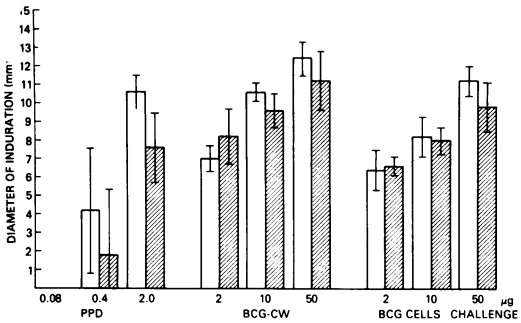

FIG. 2. *DCH reactions to saline suspensions of CW and KC in guinea pigs presensitized with 0.9 mg of intralesionally administered emulsions of CW (open bars) or KC (hatched bars).*

respectively). Emulsified KC and CW at the doses used for immunization were comparably immunogenic. The degree of skin reactivity elicited in these animals by each dose of KC suspended in saline was not significantly different from that found with an equivalent dose of CW in saline (Fig. 1 and 2).

The studies reported here showed that the mycobacterial components KC and CW administered intralesionally in emulsified form were each capable of curing a significant number of animals (up to 100%) with a transplanted hepatoma at a time when there was local spread of disease to draining lymph nodes. Guinea pigs cured by intratumoral injection of either KC or CW exhibited qualitatively and quantitatively similar DCH reactions to PPD, KC, and CW.

The lack of apparent therapeutic advantage of CW over KC in the guinea pig tumor model, as well as the relative complexity of preparation of CW, suggested that KC might be used instead of CW in clinical trials of cancer immunotherapy in which initial treatment consists of infiltration of a mycobacterial component into a primary solid tumor.

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