Changes in Nonspecific Lymphoid (NK, K, T Cell) Cytotoxicity Following BCG Immunisation of Healthy Subjects

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Summary. Nonspecific cytotoxicity was investigated in five healthy subjects following a single BCG immunisation (day 0). A decline in white cell, lymphocyte, and monocyte counts occurred at day 2, followed by increases back to baseline levels. Lymphoid NK, K, and to a lesser extent T cell cytotoxicity exhibited a similar pattern in the five subjects; a decrease at day 2, followed by recovery by day 7. An overshoot on days 10 and 14 for NK cytotoxicity was then observed, with ADCC activity still significantly increased at day 21. A decline towards baseline values was seen at day 28. The implications for immunotherapy scheduling are discussed.

Introduction

Interest is increasing in the potential of BCG as an agent for use in cancer immunotherapy. Little information is available concerning the effects of BCG on nonspecific lymphoid cytotoxicity in humans, and few reports based on animal models have been published. A nonmacrophage, 'natural cytotoxic cell' population has been demonstrated in the peritoneal exudates of mice 2–15 days after intraperitoneal infection with BCG organisms, but not in unimmunised animals (Wolfe et al., 1976). Augmented natural killer cytotoxicity in athymic and conventional mice also followed inoculation of mouse tumour cells, murine viruses and intraperitoneal BCG (Herberman et al., 1977). The cytotoxicity reached a peak 3 days after inoculation and then declined rapidly. Preliminary results are now published describing the effects of BCG in five healthy volunteers on NK, K, and T cell cytotoxicity in a sequential investigation.

Materials and Methods

Five healthy volunteers in the hospital service (median age 31 years, range 28–40) were immunised with BCG after informed consent had been given. The vaccine (percutaneous, Glaxo) was reconstituted with 0.3 ml sterile water (average number of organisms 1.5 × 10^8) and administered by multiple puncture gun: five applications of vaccine (100 needle punctures, 2 mm depth) were given on each limb. All subjects demonstrated a positive delayed hypersensitivity skin reaction at the vaccination sites.

Peripheral venous blood was taken immediately before immunisation and at 2, 4, 7, 10, 14, 21, and 28 days after immunisation. Assays were set up with freshly separated lymphocytes and no samples were missed. The immunisation date was staggered to avoid errors arising from an assay involving all subjects on the same day.

Lymphocytes were prepared from heparinised blood, incubated with finely divided iron and submitted to Ficoll-Triosil gradient centrifugation. The methods used for immunological assessment have been described previously (Thatcher et al., 1977). Briefly, the cytotoxic assay used ^51^Cr-labelled Chang cells (2 × 10^5^ ml) as a nonspecific target. These cells were reacted with lymphocytes alone (DCC, direct cellular cytotoxicity), with lymphocytes and rabbit anti-Chang serum (10^-5^ dilution) (ADCC, antibody-dependent cellular cytotoxicity), and with lymphocytes and PHA (3 μg/ml) (PCC, PHA cellular cytotoxicity). Lymphocytes were used in concentrations of 3 × 10^5^/ml and 5 × 10^5^/ml concentrations. Spontaneous and maximal (with water) release of isotope was determined for each experiment. The mean corrected percentage ^51^Cr release after a 20-h incubation was obtained for triplicate tubes from the equation:

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\text{Experimental } ^{51}\text{Cr} \% \text{ release} - \text{Spontaneous } ^{51}\text{Cr} \% \text{ release} \]

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\text{Maximal } ^{51}\text{Cr} \% \text{ release} - \text{Spontaneous } ^{51}\text{Cr} \% \text{ release} \]

The numbers of lymphocytes required to give 33.3% target cell lysis for DCC and 50% lysis for ADCC and PCC were determined and the number of 'lytic units' in the subjects' blood was then determined for DCC, ADCC, and PCC by use of the peripheral blood lymphocyte count (Cerottini et al., 1975). Friedman's two-way non-parametric analysis of variance was performed for each assay to detect statistically significant differences between the pre- and post-immunisation values for the five subjects.

Results

The total white blood cell count (P < 0.01), lymphocyte count (P < 0.01), and monocyte count (P < 0.05) all
showed statistically significant changes following immunisation. There was a decrease in all three cell counts at day 2 and also at day 4 for the lymphocyte count, and recovery occurred by day 7–10 (Fig. 1a–c).