CYTOTOXIC ACTION OF MACROPHAGES ACTIVATED BY BCG ON TUMOR TARGET CELLS *in vitro*

M. M. Vyadro

UDC 615.277.3:/615.385.3.015.2:
615.371:576.852.211

The activating action of liquid BCG vaccine, lyophilized BCG vaccine, and killed BCG cells on macrophages was studied. Activation of mouse peritoneal macrophages was identified by their cytotoxic action *in vitro* on syngeneic tumor target cells labeled with ^{51}Cr. Macrophages obtained from mice two to three weeks after a single injection of liquid BCG vaccine were shown to have a cytotoxic action on tumor cells. A single injection of lyophilized BCG vaccine or killed BCG cells into mice did not cause activation of their macrophages. Two injections of liquid or lyophilized BCG vaccine caused activation of macrophages much earlier — three to five days after the second injection. Intraperitoneal injection of BCG activated macrophages to a greater degree than subcutaneous injection.

KEY WORDS; BCG; macrophages; cytotoxic action; tumor cells.

A role of ever-increasing importance in the mechanism of the effector reactions of antitumor immunity has recently been ascribed to cells of the mononuclear phagocytic system (monocytes, macrophages) [3, 5, 6]. The study of the antitumor properties of activated macrophages, with a cytotoxic action on syngeneic, allogeneic, and even xenogeneic tumor cells, is particularly interesting. One agent with a particularly activating action on macrophages is BCG vaccine. However, the study of different strains of BCG has shown that they differ in their ability to activate macrophages [9, 12, 14]. Moreover, the dynamics of this activation has been inadequately studied. There are no data in the literature on the macrophage-activating properties of the Soviet substrain of BCG.

The object of the investigation described below was accordingly to make a dynamic study of the macrophage-activating action of the Soviet substrain of BCG.

EXPERIMENTAL METHOD

DBA/2 and C57BL/6 mice obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, were used. The mice were given one or two intraperitoneal or subcutaneous injections, each of 6×10^6 cells of an 11-day BCG culture (liquid BCG vaccine) or lyophilized BCG vaccine, or 1 mg of killed BCG cells. All the BCG preparations were obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

At various times after injection of BCG, peritoneal cells (macrophages and lymphocytes) were obtained by irrigating the peritoneal cavity of the mice with 5 ml of medium 199 containing heparin (10 units/ml) and antibiotics (penicillin 100 units/ml, streptomycin 100 μg/ml). Peritoneal macrophages obtained from intact mice served as the control. In the course of the experiments, three to four days before collection of the peritoneal cells, each mouse received an intraperitoneal injection of 2 ml of thioglycol medium. The peritoneal cells obtained were washed three times and suspended in a concentration of 1×10^6 macrophages in 1 ml medium, after which 1 ml of the cell suspension was cultured in Leighton tubes in medium 199 with 10% heated bovine embryonic serum. The monolayer of adherent cells (macrophages) was freed from unattached cells by repeated washing and was used after 24 h as the preparation of effector cells.

Laboratory of Experimental Tumor Therapy, P. A. Gertsen Moscow Oncologic Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Timofeevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 1, pp. 36-39, January, 1979. Original article submitted April 3, 1978.

 0007-4888/79/8701-0042$07.50

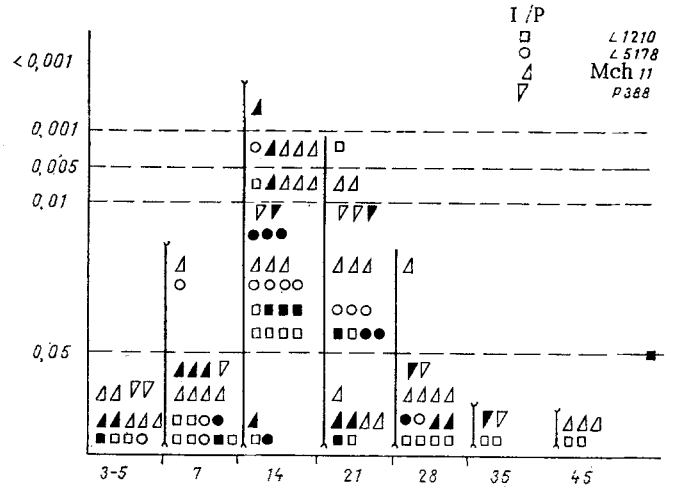

Fig. 1. Results of CTR of macrophages obtained in DBA/2 and C57BL/6 mice immunized with liquid BCG vaccine (one injection) with leukemic and sarcoma target cells. Here and in Figs. 2 and 3: abscissa, days after injection of BCG; ordinate, values of P. Remainder of explanation given on figure.

Activation of macrophages was determined from their cytotoxic action *in vitro* on syngenei tumor target cells labeled with ^{51}Cr. Cells of lymphoblastic leukemias L1210, L5178, and P388, passaged in the ascites form through DBA/2 mice, and cells of ascites sarcoma MCh-11, passaged through C57BL/6 mice, were used as target cells. The tumor cells were incubated in a concentration of 5×10^6–10×10^6 cells to 1 ml medium with 50–100 mCi ^{51}Cr (specific activity $\geq$ 50 mCi/mg) for 30–60 min at 37°C with periodic shaking. The target cells were then washed 3 times with medium 199 cooled to 4°C, counted, and 5×10^4–10×10^4 tumor cells were then incubated with macrophages for 18–20 h at 37°C; the culture medium (1 ml) was then centrifuged and 0.6 ml of supernatant was transferred to tubes for radioactivity counting. The radioactivity of the samples (the number of counts per minute) was determined in gamma-scintillation counters (from Friedicke and Hoepfner, West Germany; or Ames, USA). From three to twelve parallel experimental and control samples were taken. The results were subjected to statistical analysis by the Wilcoxon–Mann-Whitney U criterion. A cytotoxic reaction (CTR) for which the difference between the mean yield of ^{51}Cr in the experiment and control was significant ($P < 0.05$) was considered to be positive.

EXPERIMENTAL RESULTS

The results of the CTR of macrophages from mice immunized once with liquid BCG vaccine with tumor target cells are illustrated in Fig. 1.

As Fig. 1 shows, macrophages obtained two to three weeks after injection of BCG had a cytotoxic action on all tumor target cells tested. Intraperitoneal injection of BCG activated the macrophages more strongly than subcutaneous. For instance, 14 days after intraperitoneal injection of BCG a positive CTR of macrophages with leukemic target cells was observed in 92.3% of experiments, and with sarcoma cells in 100% of experiments, whereas after subcutaneous injection of BCG it was obtained in 87.5 and 75% of experiments respectively; 21 days after intraperitoneal injection of BCG a positive CTR was found in 87.5 and 62.5% of experiments respectively, and after subcutaneous injection of BCG in 80% and 0% of experiments respectively Macrophages obtained 3-5, 7, 28, 35, and 45 days after injection of liquid BCG vaccine had practically no effect on either leukemic or sarcoma target cells ($P > 0.05$).

Lyophilized BCG vaccine had no activating effect on the macrophages (Fig. 2). For instance, a positive CTR of macrophages with leukemic target cells 14 days after injection of lyophilized BCG vaccine was observed in only one of 12 experiments.

Macrophages obtained from DBA/2 mice immunized with killed BCG vaccine likewise had no cytotoxic action on syngeneic leukemic target cells (the data are not shown).

The dynamics of the CTR of the macrophages was different after a second injection of liquid or lyophilized BCG vaccine (Fig. 3). A second intraperitoneal injection of either

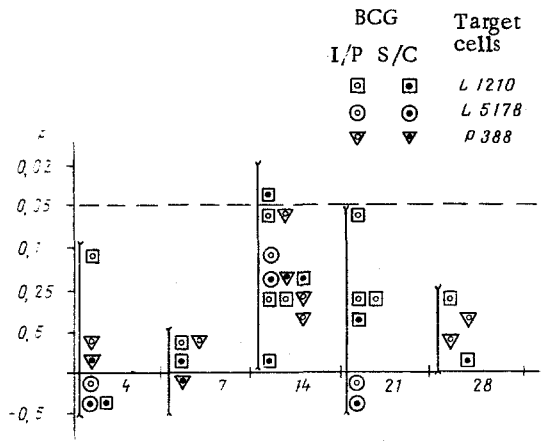

Fig. 2. Results of CTR of macrophages obtained from DBA/2 mice immunized with lyophilized BCG vaccine (a single injection) with leukemic target cells.

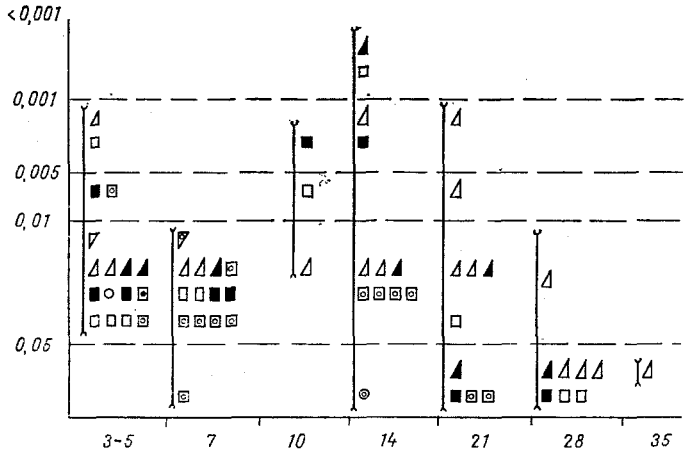

Fig. 3. Results of CTR of macrophages obtained from DBA/2 and C57BL/6 mice immunized with lyophilized or liquid BCG vaccine (two injections) with leukemic and sarcoma target cells. Symbols representing intraperitoneal injection of BCG in Figs. 1 and 2 in this case denote two intraperitoneal injection of BCG; symbols representing subcutaneous injection of BCG in Figs. 1 and 2 in this case represent subcutaneous followed by intraperitoneal injection of BCG.

BCG preparation at an interval of two to three weeks after the first caused the appearance of activated macrophages as early as three to five days after the second injection. However, in this case also, liquid BCG vaccine had a stronger and longer activating effect on macrophages than lyophilized BCG vaccine. For instance, liquid BCG vaccine maintained activation of the macrophages for 3-21 days after the second injection, compared with three to seven days after the second injection of lyophilized BCG vaccine. The macrophage-activating effect of liquid BCG vaccine could no longer be determined 28-35 days after the second injection.

The results support those obtained by other workers who found that BCG induces a population of activated macrophages [2, 4, 10, 11]. The present investigation shows that the liquid vaccine of the Soviet BCG substrain has marked ability to activate macrophages. Activation of macrophages was observed two to three weeks after injection of BCG. After a second injection of BCG activation of macrophages was observed sooner, probably because of the production of macrophage activating factor by sensitized lymphocytes. It was noted that intraperitoneal injection of BCG (i.e., local injection relative to the source of macrophages) activated macrophages more strongly than subcutaneous injection. These findings can be examined in the light of observations by Hibbs [7], who showed that local persistence of antigen is essential

for activation of macrophages. The absence of activating effect after a single injection of lyophilized BCG vaccine, probably associated with a smaller number of living mycobacteria in the lyophilized vaccine than in the BCG culture, can also be explained by the inadequate level of persistence of the antigen. The inability of killed BCG vaccine to activate macrophages also confirms this hypothesis.

The results demonstrate the dynamics of activation of macrophages following administration of BCG, and this must be taken into account when optical schemes of BCG immunotherapy are devised. The antitumor action of activated macrophages can be used in the future for the development of methods of immunotherapy of malignant neoplasms in man with the aid of cells of the mononuclear phagocytic system.

The authors are grateful to L. A. Nesterenko for providing the BCG preparations.

LITERATURE CITED

1. M. M. Vyadro, Usp. Sovrem. Biol., 84, 236 (1977).
2. R. P. Cleveland, M. S. Meltzer, and B. Zbar, J. Nat. Cancer Inst., 52, 1887 (1974).
3. R. Evans and P. Alexander, Nature, 236, 168 (1972).
4. R. N. Germain, R. M. Williams, and B. Benacerraf, J. Nat. Cancer Inst., 53, 709 (1975).
5. G. A. Granger and R. S. Weiser, Science, 151, 97 (1966).
6. J. B. Hibbs, Science, 184, 468 (1974).
7. J. B. Hibbs, Transplantation, 19, 81 (1975).
8. M. H. Levy and E. F. Wheelock, Adv. Cancer Res., 20, 131 (1974).
9. G. B. Mackaness, D. J. Auclair, and P. H. Lagrange, J. Nat. Cancer Inst., 51, 1655 (1973).
10. G. Mathe, O. Halle-Pannenko, J. Florentin, et al., Eur. J. Cancer, 11, 801 (1975).
11. M. S. Meltzer, R. W. Tucker, and A. C. Breuer, Cell Immunol., 17, 30 (1975).
12. I. B. Parr, E. Wheeler, and P. Alexander, J. Nat. Cancer Inst., 59, 1659 (1977).
13. J. S. Remington, J. L. Krahenbuhl, and J. B. Hibbs, in: Mononuclear Phagocytes in Immunity, Infection and Pathology, Oxford (1975), p. 869.
14. S. A. Wolfe, D. E. Tracey, and C. S. Henney, Nature, 262, 584 (1976).