Prevention by the MER Tubercle Bacillus Fraction of Immunosuppression Induced by Cancer Chemotherapeutic Agents

II. Contact Hypersensitivity in Guinea Pigs and Mice Treated with Cyclophosphamide*

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Summary. Contact hypersensitivity (CH) to 2,4-dinitrofluorobenzene (DNFB) was induced in guinea pigs and mice by DNFB skin application. Development of CH was suppressed in both species either by cyclophosphamide (CY) treatment after sensitization or by single intravenous injection of dinitrobenzene-sulfonate (DNBS) before sensitization (hapten-induced tolerance). Additional treatment schedules were employed in guinea pigs, with the following results: Suppression of CH by injection of DNBS concomitant with sensitization; abrogation of hapten-induced tolerance by administration of CY before sensitization; and potentiation of CH skin reactivity by administration of CY before sensitization.

Pretreatment by two injections of the methanol extraction residue (MER) tubercle bacillus fraction restored significantly the ability of CY treated animals to respond to DNFB sensitization. In contrast, administration of MER either by one injection before sensitization, concomitant with DNFB, or after sensitization did not prevent immunosuppression by CY.

MER treatment was not effective in reversing hapten-induced tolerance in mice, and had only an occasional effect on this process in guinea pigs. Abrogation of hapten-induced tolerance and potentiation of DNFB sensitization by CY in guinea pigs were also not influenced by MER treatment.

Introduction

The phenomenon of contact hypersensitivity (CH) is well recognized as one of the manifestations of cell-mediated immunological (CMI) responses. A large number of simple chemical compounds can induce contact sensitization following epicutaneous application. Among them, dinitrofluorobenzene (DNFB) is widely used in experimental animals (Turk et al., 1972; Phanuphak et al., 1974a and b, 1975; Claman, 1976); a compound of similar structure, dinitrochlorobenzene (DNCB), is also known as a potent sensitizer in human beings (Miller and Levis, 1973a and b).

T lymphocytes play a major role in the development and manifestation of CMI, including CH (Parrot et al., 1970; Pritchard and Micklem, 1972; Asherson et al., 1973; Zembala and Asherson, 1973a). Experiments performed in mice showed that at least two specific T-cell subpopulations, “active T cells” and “suppressor T cells”, are involved in CMI response (Zembala and Asherson, 1973a; Asherson and Zembala, 1974) and the outcome of contact sensitization is determined by the interactions between different categories of lymphocytes. Unresponsiveness to CH can be induced by injection of isolated haptenic determinants (“hapten induced tolerance”) before, or concomitant with, specific sensitization (Polak, 1976; Asherson and Zembala, 1974), or, by cyclophosphamide (CY) given after sensitization (Turk, 1964; Turk et al., 1976). Hapten-induced tolerance has been interpreted as arising from a preferential promotion of suppressor cell subpopulations, and CY suppression from damage to proliferating reactive lymphoid cells. Rather special situations are represented by the prevention of hapten-induced tolerance by CY (Polak and Turk, 1974) and by the potentiation of DNFB and DNCB sensitization which results from the administration of CY prior to sensitization (Turk et al., 1972; Polak, 1975). In these cases as well, the effects of the agents can be explained by the differential susceptibilities of variously functioning lymphocyte subpopulations.

The occurrence and potentiation of CMI are of special interest in neoplasia in view of the prominence of cell-mediated immunological responses in the attack...
against many types of tumor cells. The immunotherapeutic action of various "adjuvant" substances may indeed result from the potentiation of CMI reactivity towards neoplastic clones.

One of the nonspecific immunotherapeutic agents now employed widely in both human and experimental neoplasia is the methanol extraction residue (MER) fraction of BCG tubercle bacilli. A large body of experimental data has shown the capabilities of MER as an immunomodulator and as a stimulator of resistance to a variety of solid and leukemia tumors (Weiss and Yashphe, 1973; Weiss, 1976; Ben-Efraim, 1977).

Evidence has already developed that the beneficial effect of MER in at least some instances of human malignant diseases is associated with a potentiation of CMI in the treated patients (Izak et al., 1977). Accordingly, the development of experimental systems for the evaluation of the influence of MER on defined CMI responsiveness is of considerable importance for the elaboration of the agent's mode of action in immunotherapy.

The system of CH is especially suitable for such investigations because it represents, under certain conditions, a "pure" state of CMI, probably not affected by detectable amounts of antibody. Another ready possibility provided by the system of CH is analysis of the effects of the immunomodulator in animals submitted to a combined treatment, i.e., immunosuppressive anticancer drugs and immunomodulator, a treatment combination analogous to the joint chemoinmunotherapeutic schedules that are gaining increasing popularity clinically.

The present investigation was undertaken to evaluate the influence of MER on experimental CH to DNFB in guinea pigs and mice. The influence of MER was assayed both when given alone and when in combination with either CY or dinitrobenzenesulphonate (DNBS) immunosuppressive treatment; in some experiments, the action of MER was also studied in sensitized animals submitted to both DNBS and CY treatments.

Materials and Methods

Animals. Female albino randomly-bred Hartley strain guinea pigs, weighing 300–500 g, and young adult female mice (8–10 weeks old) of the BALB/c inbred strain were employed in these experiments. The animals were obtained from the Loewenstein Breeding Colony, Yoknam, Israel; mice originated from the breeding stock of the Cancer Research Genetics Laboratory of the University of California at Berkeley.

Test Compounds. DNFB (1-fluoro-2,4-dinitrobenzene) and DNBS (Na salt of dinitrobenzenesulphonate) were obtained from Eastman Kodak, Rochester, N.Y., and CY (cyclophosphamide, "cytophosphan") from Taro, Haifa, Israel. The MER preparation was from a standard batch formulated for clinical use and made available by the National Cancer Institute of the N.I.H.-U.S.P.H.S., Bethesda, Md.

Sensitization and Testing

a) Guinea Pigs. Sensitization was performed by epicutaneous application of 0.05 ml of a 10% w/v solution of DNFB in acetone; olive oil (1:1) on the right ear (Turk et al., 1972). Skin tests were by epicutaneous painting on the flanks performed on either the 8th or 14th day (as indicated in Results) after sensitization. Three concentrations of DNFB in acetone; olive oil (4:1) were employed for testing: 0.25, 0.5, and 1%, in 0.05 ml per skin site. The intensity of the skin reactions was assessed after 24 h and was expressed according to a scale described previously (Polak et al., 1975), as follows:

0 = no detectable local reaction;
0.5 = slight redness or some red spots;
1.0 = confluent redness;
2.0 = confluent redness and swelling.

The degree of sensitivity of the individual subject was expressed as the sum of the intensity of reactions at all 3 concentrations used. The degree of sensitivity of each group was taken as the average of the individual reactions.

b) Mice. The method employed was as previously described (Phanuphak et al., 1974a). A solution of 0.5% DNFB w/v in acetone; olive oil (4:1) was applied to the shaved abdomen on two consecutive days (days 0 and 1), 0.02 ml at each application. Ear skin tests were performed on day 5 by applying 0.04 ml of a 0.2% DNFB solution in acetone; olive oil (4:1) equally divided between both sides of the ear. The degree of sensitization was evaluated by comparing the thickness of the ear before testing with that measured 24, 48, 72, and 96 h after testing. The maximum reactions were found to occur on 48 and 72 h. The results here presented are those seen during this time interval and are expressed as the increment of ear thickness (as measured by the "Oditest" micrometer, H. C. Kroepelin GmbH, Schlichtern, Germany); Mean ear thickness of the animals after testing minus mean ear thickness of the same animals before testing. The degree of thickness is given in units of × 10^-2 cm.

Treatments. Treatment of guinea pigs with CY was either by a single intraperitoneal (ip) injection (250 mg/kg body weight) of a freshly prepared solution or by serial daily injections, each of 20 mg/kg body weight, for a total quantity of 140 mg/kg to 300 mg/kg. The times of treatment are specified in Results for each experiment. Mice received a freshly prepared solution of CY in a volume of 0.5 ml ip twice, on days 1 and 2 after sensitization, for a total of 2–5 mg CY per mouse.

DNBS treatment of guinea pigs was by introducing into the ear vein a neutral solution of DNBS in PBS + 1 N NaHCO₃. The quantity injected was 500 mg DNBS/kg body weight, unless specified otherwise. The injection was given either 14 days before sensitization or on the day of sensitization. DNBS treatment of mice was by injection of 0.5 ml of a neutral solution of 2–20 mg/ml into the tail vein, on day 7 before sensitization.

Experiments were also performed in guinea pigs with combined DNBS-CY application. In these instances, the DNBS injection was given iv (500 mg/kg either 14 days before or on the day of sensitization) and CY was injected ip (250 mg/kg) on day 3 before sensitization.

MER was administered to guinea pigs either alone or in combination with one of the other manipulations. The MER suspension was injected ip, 1 mg/ml per animal at each treatment. The time of injection was either before sensitization (prior treatment), on the day