In vitro Induction of Cell-Mediated Immunity to Murine Leukemia Cells

VI. Adoptive Immunotherapy in Combination with Chemotherapy of Leukemia in Mice, Using Lymphocytes Sensitized in vitro to Leukemia Cells

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Summary. An adoptive chemoimmunotherapeutic model based on the use of chemotherapy and lymphocytes specifically sensitized against tumor cells in vitro was tested in mice transplanted with syngeneic leukemia cells. C57BL/6 and A strain mice were inoculated i.p. or i.v. (day 0) with lethal doses (1 × 10³ - 1 × 10⁹) of EL4 and YAC leukemia cells, respectively. Leukemic mice were subsequently treated (day 1 or day 3) with partially curative doses (80 - 140 mg/kg) of cyclophosphamide (Cy), followed by i.p. or i.v. administration of 1 - 3 × 10⁸ cytotoxic lymphocytes (CL) induced in macro-mixed leukocyte-tumor cell cultures (MLTC). The following results were obtained: untreated mice died with tumor within 20 days; mice receiving sensitized lymphocytes only showed a modest prolongation of survival and only 5 - 15% of the animals were cured; treatment with Cy alone or with Cy and normal lymphocytes prolonged survival considerably and cured 20 - 60% of the mice; mice subjected to Cy in conjunction with in vitro-sensitized lymphoid cells, either syngeneic or allogeneic, had survival rates of 80 - 100% (> 100 days). Under the conditions employed, no severe manifestations of clinical graft-versus-host (GVH) reaction were observed. These findings imply that in vitro-sensitized immunocytes and cytoreductive drugs can operate cumulatively.

Introduction

Adoptive immunotherapy of neoplasms in animals and man with specifically sensitized lymphoid cells has often been shown to be of only limited efficacy, especially when introduced to hosts bearing a large tumor mass (Thompson and Mathé, 1972; Fefer, 1974; Rosenberg and Terry, 1977; Burton and Warner, 1977; Kedar et al., 1978). In contrast, radiation therapy and chemotherapy are highly effective against a large tumor burden but are limited by their nonspecific deleterious action against the host's normal tissues when applied in large doses. Consequently, it has been suggested (Fefer, 1973a) that adoptive immunotherapy, incapable by itself of coping with a large tumor load, might be useful as an adjunct to radiotherapy and chemotherapy, for specific eradication of the residual tumor cells which otherwise may give rise to a recurrence of the disease.

Several studies in experimental models employing such an adoptive chemoimmunotherapeutic modality have already shown encouraging results (Mihich, 1969; Vadlamudi et al., 1971, Gotohda et al., 1974; Fefer et al., 1976). These studies, however, were carried out with lymphoid cells derived from healthy immunized donors, usually syngeneic with the tumor-bearing host. Considering the difficulties of obtaining human lymphocytes that are adequately reactive against human tumor antigens, and the hazards involved in immunizing patients or healthy donors with malignant cells, the clinical application of these experimental models seems doubtful. Another approach with a potential clinical applicability has been considered by several groups, and this involves the use of lymphocytes specifically sensitized in vitro against neoplastic cells. We and several other investigators have demonstrated that lymphoid cells from animals and man can be effectively sensitized in vitro against a variety of tumor cells, including those showing weak immunogenicity in vivo (Wagner and Rollinghoff, 1973; Plata et al., 1975; Kall and Hellstrom, 1975; Burton et al., 1975; Treves et al., 1975; Kedar et al., 1976; McKhann and Jagarlamoodi, 1971; Siegler et al., 1972; Sharma and Terasaki, 1974; Golub and Morton, 1974).

Studies recently conducted in our laboratory have shown that murine lymphocytes endowed with strong cytotoxic capacity for murine leukemic cells can be produced in large quantities using a macro-MLTC tech-
nique (Kedar et al., 1977). In addition, the induction of antitumor response in vitro can be amplified appreciably by introducing immunopotentiating agents to the cultures (Weiss et al., 1976) and by using chemically or enzymatically modified tumor cells (Kedar and Lupu, 1978).

We have also shown that murine (Kedar et al., 1977) as well as human (Kedar et al., in preparation) lymphoid cells and leukemia cells preserved under liquid nitrogen could be used successfully in MLTC to generate effector lymphocytes. Taken together, these findings suggest that this improved MLTC technique can provide a valuable therapeutic tool in clinical trials, particularly where autologous cells are to be employed. Thus, lymphoid cells can be obtained from leukemia patients in the remission period (or from patients with solid tumors), stored, subsequently sensitized in vitro to the cryopreserved autologous tumor cells (or to fresh allogeneic cancer cells of the same histologic type), and reintroduced to the patients before or during relapse.

Previous studies in this series have demonstrated that in vitro-sensitized lymphoid cells are capable of preventing leukemia growth in vivo, when a Winn-type neutralization assay is used (Kedar et al., 1977), but are only of limited therapeutic efficacy when introduced to leukemia-bearing mice (Kedar et al., 1978). The purpose of the present investigation was to evaluate the usefulness of a combined therapeutic regimen consisting of chemotherapy in conjunction with adoptive immunotherapy, using CL generated in vitro. The data presented here show that cyclophosphamide (Cy) and lymphocytes sensitized in vitro to leukemia cells can indeed act cumulatively when given together and are very efficient in curing mice bearing leukemia implants.

Materials and Methods

Most of the materials and methods employed have been detailed by us recently (Kedar et al., 1976, 1977), and will be described here only briefly.

Mice. Male mice of the inbred strains C57BL/6 (H-2b), A (H-2a), BALB/C (H-2d), and F1 hybrids (A × BALB/C) and (A × C57BL/6), 10–16 weeks old, were obtained from the animal colony of the Hebrew University, Hadassah Medical School, Jerusalem. Mice were maintained in metal cages (5–10 per cage) and fed with standard pellets ad libitum. In the adoptive chemoimmunotherapy experiments mice were provided with drinking water containing streptomycin (100 μg/ml), penicillin (100 units/ml), neomycin (5 μg/ml), and kanamycin (5 μg/ml) for up to 14 days after initiation of the experiment. Animals were weighed and carefully observed three or four times weekly, for at least 90 days.

Lymphoid Cells. Single cell suspensions were prepared from spleens of normal donors as described previously (Kedar et al., 1977). Lymphocytes sensitized in vitro were centrifuged over a Ficoll-Hypaque layer (Blyum, 1968), to remove dead cells prior to their i.v. administration.

Tumor Cells. EL4 leukemia induced by dimethylbenzanthracene and YAC leukemia induced by Moloney virus (Klein et al., 1966) were propagated in C57BL/6 and A-strain mice, respectively. Tumor cells were washed three times before use.

Induction of Cytotoxic Lymphocytes (CL) in Macro-Mixed Lymphocyte-Tumor Cell Cultures (MLTC). Cell mixtures consisting of 500 x 10^6 responding spleen cells and 5 x 10^6 normal mitomycin C-inactivated stimulating leukemia cells were prepared in 250 ml of enriched RPMI 1640 medium (Kedar et al., 1977), using 1000 ml tissue culture roller flasks (Corning no. 25130, Corning Glass Works, New York). The culture medium was supplemented with 15% heat-inactivated fetal calf serum, 1 x 10^-4 M 2-mercaptoethanol, 10 mM Hepes buffer, and antibiotics. Cultures were incubated upright, for 6–7 days, in a humidified 7% CO2 incubator at 37 °C. For controls, splenocytes were incubated under identical conditions but without leukemic cells. After incubation, cells were washed twice, counted for viability in 0.1% Trypan blue, and adjusted to the required cell concentration.

51Cr Release Assay. The 51Cr cytotoxicity assay was performed as described previously (Kedar et al., 1977). Briefly, cell mixtures containing 2 x 10^5 51Cr-labeled leukemic target cells and 6 x 10^5 normal or sensitized lymphocytes (in a total volume of 0.5 ml) were placed (in triplicate) in 10 x 70 mm plastic tubes. Tubes were centrifuged for 3 min at 1200 rpm and then incubated for 3 h at 37 °C in a 7% CO2 incubator. After incubation, 1 ml cold medium was added, the cells were spun down, and the supernatants collected for counting. Percentage specific lysis was calculated as described previously (Kedar et al., 1976).

Neutralization Assay (Winn Assay). Cell mixtures consisting of 2 x 10^6 leukemia cells and 4 x 10^6 in vitro-sensitized lymphocytes were inoculated s.c. into recipients syngeneic with the tumor cells. Under these conditions, 90–100% of the mice survived tumor free for 90 or more days. Survivors were refractory to challenge with a lethal tumor dose (1 x 10^3) and their splenocytes exhibited a greater capacity to generate syngeneic cytotoxic reactivity in vitro than did splenocytes from normal donors (Kedar et al., 1978).

Chemotherapy. Cyclophosphamide (Cy) (Taro Pharmaceutical Industry Ltd., Haifa, Israel) was dissolved in sterile saline and administered in a single i.p. injection. The doses employed ranged from 2–4 mg per mouse (approximately 80–140 mg/kg).

Adaptive Immunotherapy in Combination with Chemotherapy. Mice (age 3–4 months, average weight 24 g) were divided into groups of 15–30 animals each and treated as follows: 1) tumor only; 2) tumor + Cy; 3) tumor + Cy + lymphocytes cultured alone; 4) tumor + Cy + lymphocytes sensitized in vitro to leukemia cells; 5) tumor + sensitized lymphocytes; and 6) Cy + sensitized lymphocytes. Tumor cells were injected s.c., i.p., or i.v. (in 0.2 ml) on day 0 in doses ranging from 1 x 10^3 to 1 x 10^5 cells. Cy (80–140 mg/kg) was administered i.p. (in 0.2 ml) on day 1 or 3. Lymphocytes from syngeneic or allogeneic donors were administered (1–3 x 10^5) i.v. or i.p. (in 0.2 and 1.0 ml, respectively) 16–24 h after Cy treatment. Mice were weighed and inspected carefully four times weekly for 90 days or longer for tumor growth and for GVH pathology. Each experiment was repeated two or three times. No attempt was made to examine the surviving mice for chimerism.