Activation of mouse peritoneal macrophages by synthetic glyceroglycolipid liposomes*

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Summary. Liposomes composed of chemically synthesized glyceroglycolipids, such as 1,2-dipalmityl-[β-celllobiosyl-(1'→3)]-glycerol (Cel-DAG), 1,2-dipalmityl-[β-lactosyl-(1'→3)]-glycerol, or 1,2-dipalmityl-[β-maltosyl-(1'→3)]-glycerol, were found to enhance protective immunity against transplantable tumor cells (sarcoma 180) in ICR mice. Peritoneal exudate cells prepared from mice treated in vivo with Cel-DAG showed cytostatic activity in vitro against the mouse leukemia cell line, EL-4. Adherent cells separated from this preparation showed similar activity. Peritoneal cells from polypeptone-injected mice acquired appreciable cytostatic activity when incubated in vitro in the presence of glyceroglycolipid liposomes. The adherent cell fraction alone showed rather weak cytostatic activity when pretreated with the glyceroglycolipids, and full activity was restored by supplementing with the nonadherent cell fraction. The ability of glycolipids to induce tumoricidal effects was affected by cholesterol content: with increasing cholesterol content, the activities decreased. Cholesterol-free glycolipid liposomes were taken more efficiently by macrophages than cholesterol-containing liposomes. Cholesterol modifies the surface property of glyceroglycolipid liposomes. Activation of macrophages is responsible for enhancement of protective immunity against tumor cells by injection of these glycolipids in vivo.

Introduction

Glyceroglycolipids are known to be widely distributed in plant tissues, bacteria, mycoplasma membranes, and to be present in animals in small amounts [7, 13]. We previously investigated the physical properties and barrier functions of synthetic glyceroglycolipids [3] and their surface properties [14]. Like phospholipids, diglycosyl dipalmityglycerol could form liposomes and function as a barrier against small water soluble molecules. Several lines of evidence [4, 7] including electron spin resonance data (to be published), however, suggested that the interaction between head groups on these glyceroglycolipid liposomes is stronger than that on dipalmityl glycerophosphocholine.

It has been shown that lentinan and other polysaccharides inhibited the growth of transplanted tumor cells in mice [1]. These chemically defined β(1→3) glucans did not cause any toxic effects but have been found to enhance protective immunity against tumor cells. For instance, the mechanism of antitumor activity caused by lentinan has been variously explained as follows: activation of alternative complement pathway [18], activation of macrophages [10], tumor destruction by cytotoxic T cells [9], and cell-mediated cytotoxicity via antibody production from activation of helper T cells [2]. Hamuro et al. [8] suggested the significance of the higher structure, which would result from the hydrogen bond, hydrophobic bond, micelle-association, and interchain cohesive force, etc., of the antitumor polysaccharides in relation to their bioactivities. However the molecular mechanism of these antitumor activities of polysaccharides are still unclear.

The sugar residues on the surface of synthetic glyceroglycolipid liposomes may be, in one respect, a good molecular model of antitumor polysaccharides for investigating the importance of their higher structure with respect to the interaction between cellular components and the polysaccharides. On the other hand, very few investigations have been carried out on the biological activities of glyceroglycolipids in spite of the vast variety of structural work. In the present investigation, we present evidence indicating that glyceroglycolipid liposomes but not glyceroglycolipid-cholesterol liposomes induce cytostatic macrophages both in vivo and in vitro.

Materials and methods

Chemicals. RPMI 1640 medium was obtained from Flow Labolatories, Stanmore, Australia. Fetal calf serum (FCS) was purchased from GIBCO, New York, USA. Dipalmitoyl glycerophosphocholine (DPPC), cholesterol (Chol), dicetylphosphate (DCP) and concanavalin A agarose were purchased from Sigma Chemical, St. Louis, Mo., USA. 3H-Thymidine was purchased from the Radiochemical Centre, Amersham, UK. Lentinan was kindly provided by Dr. Chihara, National Cancer Center Research Institute, Tokyo, Japan. The dipalmitoyl glycerophosphocholine (Dialkyl DPPC) was synthesized according to the method previously described [15, 24].

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Assay for cytostatic activity in vitro. The cell-mediated cytostasis assay was employed as follows. Glyceroglycolipid liposomes (volume 0.2 ml) were injected i.p. into the mice, and after 4 days, peritoneal exudate cells were harvested and suspended in RPMI 1640 medium supplemented with 10% FCS. Cells (4 x 10^5) were put into each well of a 96-well culture plate and incubated at 37 °C in a humidified atmosphere of 5% CO₂ for 2 h. EL-4 (2 x 10^4 cells) mouse leukemia cells which were obtained from the National Institute of Health, Japan, and maintained in vitro in RPMI 1640 medium with 10% FCS, were added as target cells. Then 8 h before termination of incubation, cultures were pulsed with 0.1 μCi of 3H-thymidine (20.0 Ci/mmole). The cells were lysed by the addition of one-tenth of the volume of 0.2 M Tris-HCl (pH 7.4) solution containing sodium dodecyl sulfate (1%) and yeast RNA (1.2 mg/ml), and the cell lysate was harvested on a piece of filter paper (Toy0 No. 514). The count of 3H-thymidine incorporated into acid insoluble material was then determined. Cytostatic activity was calculated as follows: Cytostasis = 100 x (dpm in target cells cultures with normal cells - dpm in target cells cultures with test cells)/(dpm in target cells cultures with normal cells).

In experiments of in vitro culture, peritoneal exudate cells which were harvested 4 days after i.p. injection of 2 ml of 10% polypeptide (Dagoeioy Chem. Ltd. Osaka, Japan) solution were used for the cytostasis assay. Peritoneal exudate cells were incubated for adherence at 37 °C for 2 h. Nonadherent cells were removed by washing with RPMI 1640 medium and the remaining cells were used as the macrophage fraction.

Results

Antitumor activity

The results of the antitumor test of glyceroglycolipid liposomes are shown in Fig. 2. Prophylactic effects against death caused by sarcoma 180 tumor cells were appreciably observed when mice were injected i.p. with 3 mg of Lac-DAG liposomes 4 days prior to tumor transplantation. The Dialkyl DPPC liposome which carried the same alkyl chain as glyceroglycolipids did not show any signs of increasing the life-span of sarcoma 180-bearing mice. Specificity of the sugar structure required for the activity was broad, since similar antitumor activities against sarcoma 180 tumor cells were obtained when Cel-DAG or Mal-DAG on days -5, -4, -3, -2, 2, 3, 4, and 5, resulted in 20% and 40% cure of tumor-bearing mice at doses of 0.25 mg and 0.5 mg/day, respectively.

To examine whether glyceroglycolipid liposomes have a direct cytotoxic effect on tumor cells, ascites tumor cells of sarcoma 180 and several kinds of established cell lines including mouse leukemic cell EL-4 were cultured in a medium containing various concentrations of glyceroglycolipid liposomes. The viability of treated cells was then examined after 24 h by measuring the DNA synthesis. The