IMMUNOGENICITY OF LEUKEMIC CELLS DURING LOW-TEMPERATURE FREEZING WITH VARIOUS CRYOPROTECTORS

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Aside from their suitability for clinical use, the main requirement of cells used for immunotherapeutic purposes as vaccines is maximal preservation of their immunogenicity, as reflected in the immune response they induce in a recipient.

Leukemia-assocated antigens, like those of the tumor-specific kind, belong to the class of weak antigens [8, 9]. Clinical studies have demonstrated the greater therapeutic effect of viable allogeneic leukemic cells when used for immunotherapy compared with nonviable cells [4]. This led to the study of optimal conditions for long-term preservation of leukemic cells suitable for immunotherapy without any loss of immunogenicity, by programmed freezing to ultra-low temperatures.

During the choice of optimal cryoprotector for use in protecting living cells against injury by cold during freezing and thawing, attention was drawn to dimethylacetamide (DMAC) and polyethylene oxide (PEO), which could most probably be used for freezing cells intended for immunotherapy instead of the glyercol which has hitherto been used. The reason is that both these cryoprotectors need not be washed off after thawing. As the control for these investigations dimethyl sulfoxide (DMSO) was chosen, for it had already been studied previously both by the present writers and by other workers [2, 10].

EXPERIMENTAL METHOD

Human leukemic cells isolated from the peripheral blood of patients with acute lymphoblastic leukemia (ALL) during the period of blastemia, and from patients with chronic lymphatic leukemia (CLL), and also L-1210 murine leukemia cells were used in the investigation. Isolation and preparation of the cells for freezing were done as described previously [2, 5]. The number of viable cells before freezing was 95-100% on staining with supravital dyes. The leukemic suspension was divided into three parts and frozen with one of the three different cryoprotectors, conditions for the use of which were chosen individually.

Cryopreservation with DMAC. The conserving solution containing DMAC was added slowly to the cell supsension in plasma in the proportion of 1:3 (the final concentration of DMAC was 5%). The suspension was carefully mixed and distributed among the containers. Freezing was carried out without preliminary exposure on the UKKM-1M apparatus (USSR) according to the following program: cooling at the rate of 3 deg/min over the range from 20°C to −1°C, the heat pause lasted 2-4 min, after which the temperature was again lowered at the rate of 5-10 deg/min to −100°C, after which the containers were immersed in liquid nitrogen (−196°C) [7].

Cryopreservation with PEO-400. A 15% solution of PEO was added slowly to the cell suspension in plasma in the ratio of one part of solution to three parts of suspension. After mixing the suspension was distributed among the containers. Freezing without preliminary exposure was carried out on the same apparatus by the following program: at 1 deg/min to −40°C, than at 5 deg/min to −80°C, then at 20 deg/min to −120°C, after which the containers were immersed in liquid nitrogen [1].

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TABLE 1. Values of Immunogenicity of Leukemic Cells before and after Cryopreservation

Material for immunization (leukemic cells)	Cryoprotector used	Viability of cell suspension	Levels of immune response	
			titer of cytotoxic antibodies in serum	cytotoxicity of lymphocytes, % (M ± m)
CLL	Native cells	95	1:8	22,6±3,7
	DMSO	93	1:8	25,2±2,4
CLL	Native cells	94	1:2	13,9±1,3
	DMSO	85	1:4	14,5±0,8
	DMAC	50	1:2	12,0±4,2
ALL	Native cells	99	1:16	14,8±0,11
	DMSO	90	1:16	19,2±0,8
	DMAC	49	1:8	19,2±0,34
	PEO	40	1:8	Not tested
L-1210	DMSO	76	1:32	6,5±0,23
	DMAC	51	1:16	6,9±0,13
	DMSO	46	1:16	7,1±0,07
L-1210	PEO	84	1:4	Not tested
	DMAC	95	1:4	The same
	PEO	84	1:2	»
L-1210	DMSO	90	1:4	10,7±0,48
	DMAC	84	1:4	10,3±2,09
	PEO	85	1:2	12,7±0,49
ALL	DMSO	75	1:16	Not tested
	DMAC	60	1:16	The same
	PEO	50	1:16	»

Cryopreservation with DMSO. A conserving solution consisting of 10% DMSO, 10% heated nonimmune healthy human serum from a group AB (IV) blood donor, and 80% of Hanks' solution was added slowly to the cell residue in the ratio of not less than 10 volumes of solution to one volume of residue. After exposure for 15 min freezing was carried out by one of the two programs mentioned above [2].

The cell suspensions were thawed rapidly in a water bath at 40°C with constant shaking. The number of viable cells after thawing varied from 46 to 95% on staining with supravital dyes.

The immunogenicity of the leukemic cells was determined by the immune response induced by these cells in experimental animals. The antibody titer was determined in the complement-dependent cytotoxicity test and the cytotoxicity of splenic lymphocytes was determined against immunized target cells. (CBA × C57BL/6) mice were used for immunization and were given a single intrapertioneal injection of 5×10^7 cells.

Scheme of the Experiment. Leukemic cells from the same patient were injected in equal doses into mice of four groups (5-10 animals in each group): mice of group 1 received native cells, the rest received cells thawed after freezing with the various cryoprotectors (DMSO, DMAC, and PEO). The viability of the cell suspension was taken into consideration. In some experiments the control for the two principal cryoprotectors chosen for study consisted of a group of mice immunized with leukemic cells cryopreserved with DMSO, for their immunogenicity was similar to that of native cells, as the writers showed previously [3]. Mice of group 5 were intact (without immunization, control). Serum and spleen cells for determination of antibodies and cytotoxic lymphocytes were obtained on the 10th day after immunization.

Complement-dependent antibodies in the sera were determined in Terasaki plates by the method described previously [2]. The source of complement was a pool of fresh (from four animals) guinea pig serum. The results of the reaction was estimated by the usual method from the ratio between the numbers of stained and unstained cells. The final antibody titer in the immune sera was given by their last dilution at which a ++ reaction was obtained, i.e., 50% lysis of the target cells. As a control for each test, intact mouse serum (negative control) and standard antilymphocytic serum (from the N. F. Gamaleya Institute of Epidemiology and Microbiology; positive control) were used. To exclude direct toxicity, undiluted immune serum was incubated with target cells without the addition of complement.

The cytotoxicity of mouse splenic lymphocytes was determined by the radiometric method described by the writers previously [6]. The results of the test were expressed as percentages of specific cell lysis, calculated by the equation:

$$\% \text{ specific lysis} = \frac{^{51}\text{Cr in sample} - ^{51}\text{Cr in normal lymphocyte control}}{^{51}\text{Cr in 100\% destroyed cells}}.$$

EXPERIMENTAL RESULTS

The results (Table 1) showed that the titer of cytotoxic antibodies in the sera of mice immunized with freshly isolated native cells and with cells cryopreserved with DMSO was similar. On immunization with the same doses of cells cryopreserved with DMAC and PEO, the titer of cytotoxic antibodies was the same in most experiments.

Analysis of the data shows that the immune response depended on the degree of viability of the injected cell suspension. If only 50% of viable cells were present the antibody titer was usually one dilution lower. If the viability of the cells was equal the results of testing were more often the same.

A comparative study of the cytotoxicity of the lymphocytes of mice when immunized with native cells yielded similar results to those for animals receiving cells cryopreserved with the three cryoprotectors studied.

The results are evidence that the immunogenicity of leukemic cells during preservation with DMAC and PEO is virtually indistinguishable from that of native cells or cells preserved with DMSO. The absence of any lowering of immunogenicity of leukemic cells on cryopreservation with DMAC and PEO, which, unlike DMSO, need not be washed off before use, means that these cryoprotectors can be recommended for freezing leukemic cells intended for immunotherapy.

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