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Spontaneous and lectin-dependent cellular cytotoxicity by lymphocyte subpopulations against cell lines susceptible or resistant to spontaneous cytotoxicity

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SUMMARY

In the present study we have investigated whether cell lines, which are resistant to spontaneous cytotoxicity, can be killed by lectin–dependent cellular cytotoxicity (LDCC), the expression of EAC (7S) receptors on LDCC effector cells, and the relationship between spontaneous cytotoxicity and LDCC when EAC (7S) receptor–positive or –negative subpopulations are tested against different target cell lines.

We found that target cells which are resistant to spontaneous cytotoxicity can, indeed, be killed by LDCC, and that there is an inverse relationship between spontaneous cytotoxicity and LDCC, both when unfractionated lymphocytes were tested against different target cells and when fractionated cells were tested against cell lines highly susceptible to spontaneous cytotoxicity.

The implications of these findings, in relation to the relative specificity of the spontaneous cytotoxicity system, are discussed.

INTRODUCTION

There exists a number of ways whereby human peripheral lymphocytes can become cytotoxic for antigen-bearing target cells. Specific T-cell cytotoxicity can arise during the course of an acute virus infection, as demonstrated in the infectious mononucleosis syndrome (Svedmyr & Jondal, 1975); or cytotoxicity may be triggered by certain lectins or by target cell–specific IgG antibodies (Holm, 1967; Perlmann, Perlmann & Wigzell, 1972). Apart from T-cell cytotoxicity, lectin-dependent cellular cytotoxicity (LDCC) and antibody-dependent cellular cytotoxicity (ADCC), it has recently been shown that normal lymphocytes may be spontaneously cytotoxic against certain target cells (Jondal, 1975; Peter *et al.*, 1975; Pross & Baines, 1977). The effector cells involved in the last kind of cytotoxicity have been called natural killer cells in the mouse, or spontaneous killer cells in humans (Jondal, 1975; Pross & Baines, 1977).

In all of these cytotoxicity systems, a considerable amount of work has been done in order to characterize the involved effector cells by cell surface markers (Bonnard & West, 1977). It is thus clear that the killer cells responsible for ADCC and spontaneous cytotoxicity express Fc receptors, and partly complement receptors, whereas reports are more conflicting regarding the Fc receptor expression on LDCC effector cells. Some results thus indicate that most LDCC effector cells primarily belong to the Fc receptor-positive lymphocyte population (Bonavida, Robins & Saxon, 1977; Bonnard & West, 1977; Hallberg, 1974), whereas Waller, Campbell & MacLennan, 1976 have shown that Fc receptor-negative cells also can be cytotoxic in the presence of PHA.

In the present paper we have been interested in LDCC in relation to spontaneous cytotoxicity for the

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following reasons: as we and others have been able to demonstrate that SK cells display a certain degree of specificity, in the sense that they kill certain target cells better than others, we wanted to investigate whether this resistance, on part of the SK-resistant target cells, was caused by an unspecific resistance to cell-mediated lysis or if these target cells were killed in the presence of cell surface agglutinins, such as concanavalin A (Con A) or phytohaemagglutinin (PHA). We thus argued that if we could demonstrate that SK-resistant target cells were killed by LDCC, it would support the idea that lack of SK killing may be related to lack of recognition which, in turn, would support the concept that there is a certain degree of specificity in the recognition process in the SK system. Besides this main question of the specificity in the SK system, we also studied the expression of surface receptors on LDCC effector cells when tested against target cells with a variable susceptibility to spontaneous cytotoxicity.

We did indeed find that SK-resistant target cells are susceptible to Con A- and PHA-dependent LDCC and, furthermore, that characterization of LDCC effector cells by cell surface markers requires that one takes into consideration the particular target cell employed.

MATERIALS AND METHODS

Purification of lymphocytes. Lymphocytes were purified by centrifugation of heparinized whole blood on Ficoll–Hypaque gradients (Jondal, 1974). Phagocytic cells were removed by the iron carbonyl powder technique (Jondal, 1974). All lymphocyte donors used in the present investigation were adult, healthy laboratory workers.

Fractionation of effector cells. The effector cells were fractionated according to the expression of EAC(7S) receptors, as described elsewhere (Jondal, 1976). Briefly, sheep RBC (4%) were treated with rabbit IgG anti-SRBC, using a maximum subagglutinating titre with shaking for 1 hr at room temperature. The IgG-treated cells were then washed twice and, as a 4% solution, mixed with 1 part of a 1 : 2 diluted fresh A-strain mouse serum and incubated for 30 min at 37°C. After two washes the erythrocyte–antibody–complement (EAC) mixture was mixed with the lymphocytes, in 3·0 ml of medium, producing a concentration of 6×10^6 cells per ml, in siliconized 15 ml tubes. The ratio of EAC cells to lymphocytes was 5 : 1, and the cell mixture pelleted and incubated for 30 min at 37°C. The cells were gently resuspended and the percentage of rosette-forming cells calculated. The cell suspension was subsequently layered over 3·0 ml of cold standard Ficoll–Hypaque and centrifuged at 850 *g* for 15 min. The cells at the interface, which were depleted of receptor-bearing cells, were then collected and the percentage of rosette-forming cells determined in the pelleted fraction. In all of the experiments reported in the present paper there was less than 1% of contaminating rosette-forming cells in the depleted fractions, and the purity of the rosette-forming cell fraction, in the pellet, was higher than 90%.

Maintenance and origin of target cells (Table 1). Target cells were maintained as suspension cultures in RPMI 1640 medium

TABLE 1. Cell lines differ in their susceptibility to spontaneous and lectin-induced cytotoxicity

Classification of target cells	Susceptibility to spontaneous cytotoxicity	Susceptibility to LDCC	Representative cell line used in the present investigation
(1)	High	Low	MOLT-4, a T-cell line derived from acute lymphatic leukaemia (Minowada, Ohnuma & Moore, 1972)
	Intermediate	Intermediate	P3HR-I, a B-cell line derived from Burkitt's lymphoma (Hinuma & Grace, 1967)
(2)	Low	High	Raji, a B-cell line derived from Burkitt's lymphoma (Epstein *et al.*, 1966)
	Low	Intermediate	109, a B-cell line derived from normal lymphocytes (see legend)
(3)	Low	Low	253, a B-cell line derived from normal lymphocytes (see legend)

Cell lines which differed markedly in their susceptibility to spontaneous cytotoxicity were tested for lectin-dependent cytotoxicity (mostly Con A) and divided into there representative subgroups. The two cell lines derived from normal lymphocytes, 109 and 253, were established by Dr Celsa Spina at the Department of Microbiology and Immunology, UCLA, USA.

with 10% foetal calf serum and antibiotics, and subcultured twice weekly. MOLT-4 is a T-cell line derived from acute lymphocytic leukaemia. P3HR-1 and Raji are two B-cell lines derived from African Burkitt's lymphoma. 109 and 253 are two standard EBV-carrying B-cell lines derived from normal lymphocytes.

Spontaneous cytotoxicity tests. Cytotoxicity tests were done in standard V-shaped ninety-six hole microplates in a total volume of 150 μl of RPMI 1640 medium supplemented with 10% heat-inactivated foetal calf serum (Svedmyr & Jondal, 1975). In each well 10^4 ^{51}Cr-labelled cells were used as targets. At the end of a 3–5 hr incubation period, 50 μl of the supernatant was collected from each well and the cytotoxicity estimated from amount released radioactivity. The cytotoxicity was calculated according to the following formula:

(test release)− (spontaneous release)/(80% of total label)− (spontaneous release) × 100.

LDCC test. LDCC tests were performed as described for the spontaneous cytotoxicity tests. PHA (Wellcome Research Laboratories, Beckenham, England) was used at a final concentration of 1 : 500 and Con A (Pharmacia, Uppsala, Sweden) at a final concentration of 2·0 μg/ml. These concentrations had earlier been found to be optimal in inducing LDCC as well as in triggering DNA synthesis.

RESULTS

Classification of cell lines according to their susceptibility to spontaneous and lectin-induced cytotoxicity

As will be demonstrated below, the different target cells could be classified according to their susceptibility to spontaneous killing and to lectin-induced cytotoxicity. Type 1 target cells, such as MOLT-4 and P3HR-I, could be killed both by spontaneous cytotoxicity and by lectin-induced cytotoxicity. Type 2 target cells, such as Raji and 109, were relatively resistant to spontaneous cytotoxicity but susceptible to lectin-induced cytotoxicity. Type 3 target cells, such as 253, could not be killed either by spontaneous killer cells or by lectin-induced killer cells.

Spontaneous and Con A-induced cytotoxicity against types 1 *and* 2 *target cells with unseparated lymphocytes*

As seen in Fig. 1, very little additional cytotoxicity could be triggered by Con A using unseparated lymphocytes against MOLT-4. Almost the mirror image can be seen with the SK-resistant target cell Raji, against which almost all cytotoxicity is Con A-dependent.

With target line P3HR-1, an intermediate result is obtained, in the sense that this particular cell line seems to be equally susceptible to spontaneous and to lectin-induced cytotoxicity.

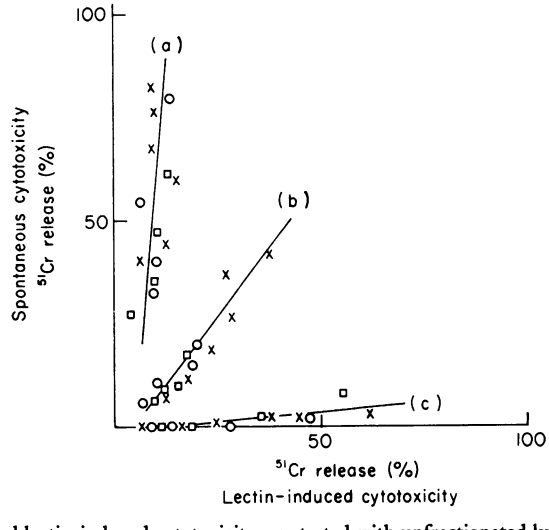

FIG. 1. Spontaneous and lectin-induced cytotoxicity was tested with unfractionated lymphocytes against three different cell lines in three separate experiments. Six (×) or four (□, ○) different lymphocyte : target ratios were tested starting from 40 : 1 with 1 : 2 dilution steps. Con A LDCC was done with a final concentration of 2·0 μg/ml. The cytotoxicity assay was run for 4 hr. Cell lines: (a) MOLT-4; (b) P3HR-I; (c) Raji.

Spontaneous and lectin-induced cytotoxicity against type 1 *target cells with EAC* (7*S*) *receptor-positive and -negative lymphocyte subpopulations*

From Figs 2 and 3 it is clear that there is a relationship between the different lymphocyte subpopulations' capacities to mediate spontaneous cytotoxicity and lectin-induced cytotoxicity against type 1 target cells. Thus lymphocytes which are most active in causing spontaneous cytotoxicity respond by an almost negligible increase of killing in the presence of Con A or PHA. In contrast, lymphocytes which display little spontaneous cytotoxicity give a rather strong cytotoxic response in the presence of PHA or Con A. Unfractionated lymphocytes give, as in Fig. 1, an intermediate result.

Spontaneous and lectin-induced cytotoxicity against types 2 *and* 3 *target cells with EAC*(7*S*) *receptor-positive and -negative lymphocyte subpopulations*

From Table 2, it is clear that type 2 target cells, such as Raji and 109, are killed to an equal degree in the presence of Con A or PHA. In this particular experiment, it appears as if separated lymphocytes are less active against Raji cells in the presence of PHA. The reason for this is unclear. With type 3 target cells, which are mostly resistant to cell-mediated lysis, no cytotoxicity is seen.

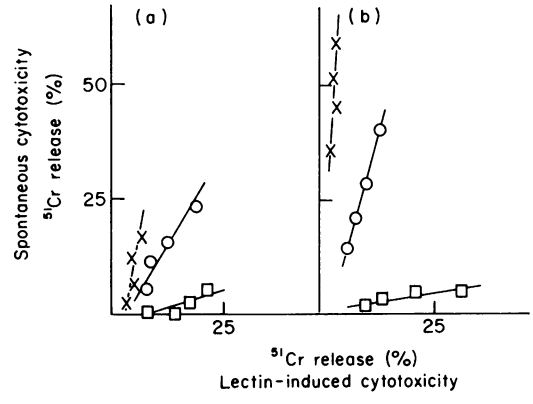

FIG. 2. Spontaneous and lectin induced cytotoxicity was tested with unfractionated (○), EAC(7S), receptor-positive (×) and EAC(7S) receptor-negative (□) lymphocytes against two different target cells at four different lymphocyte : target cell ratios, starting from 20 : 1 dilution steps. The final concentration of Con A was 2·0 μg/ml and the cytotoxicity assay was run for 3·5 hr. (a) P3HR-I (b); MOLT-4.

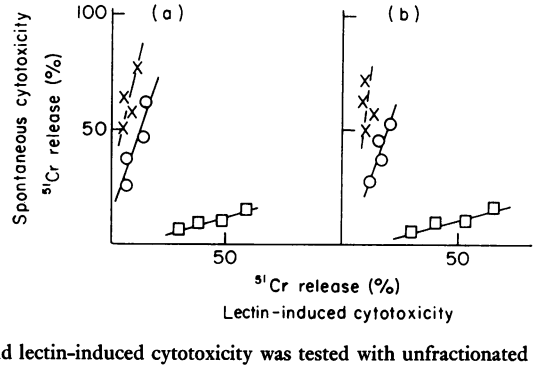

FIG. 3. Spontaneous and lectin-induced cytotoxicity was tested with unfractionated (○), EAC(7S) receptor-positive (×) and EAC(7S) receptor-negative (□) lymphocytes against MOLT-4 cells, using PHA at a final concentration of 1 : 500 and Con A at a final concentration of 2·0 μg/ml. Four different lymphocyte : target cell ratios were used starting from 40 : 1 with 1 : 2 dilution steps. As in Fig. 2 the cytoxicity assay was run for 3·5 hr. (a) MOLT-4 (Con A); (b) MOLT-4 (PHA).

TABLE 2. Spontaneous and lectin-induced cytotoxicity against type 2 and 3 target cells with EAC(7S) receptor-positive and -negative lymphocyte subpopulations

Target cell	Effector : target ratio	Unseparated			EAC(7S)-positive			EAC(7S)-negative		
		0	Con A	PHA	0	Con A	PHA	0	Con A	PHA
Raji (same experiment as in Fig. 3)	30 : 1	1	57	53	0	48	21	0	41	20
	15 : 1	3	47	32	0	44	18	0	28	17
	7·5 : 1	2	31	21	1	30	12	−1	21	13
	3·75 : 1	−1	17	15	1	19	9	−1	13	8
109 (same experiment as in Fig. 2)	20 : 1	5	36		4	30		5	26	
	10 : 1	1	25		1	21		3	22	
	5 : 1	0	20		−1	14		1	18	
	2·5 : 1	−1	16		−1	10		−1	12	
253 (same experiment as in Fig. 2)	20 : 1	3	5		4	6		3	1	
	10 : 1	−1	2		2	2		2	1	
	5 : 1	−2	1		3	3		2	−1	
	2·5 : 1	−1	0		1	1		3	0	

Spontaneous and lectin-induced cytotoxicity was tested against Raji, 109 and 253 target cells in two separate experiments. Four different lymphocyte : target cell ratios were used as indicated in the table.

DISCUSSION

In the interpretation of the present results one can think in terms of cellular recognition. It is thus possible that SK cells have surface receptors which enable them to recognize surface structures predominantly expressed on SK-susceptible target cells and that they, by virtue of these receptors, establish contact with and kill these target cells. SK cells may thus have all necessary requirements to express their full cytotoxic potential against susceptible target cells, and may have very little to gain by the addition of cell surface agglutinants such as PHA and Con A. On the other hand, if target cells which are relatively SK resistant lack these surface structures, these may become susceptible when an artificial bridge, between lymphocyte and target, is created by PHA or Con A. Target cells such as cell line 253 may, for obscure reasons, have a cell membrane configuration that does not allow rapid cell-mediated lysis as detected in the present system.

Furthermore, it is clear that EAC(7S) receptor-negative non-SK lymphocytes have the capacity to kill in LDCC. With target cells that are resistant to spontaneous cytotoxicity, non-SK lymphocytes and SK lymphocytes are about equal as LDCC effector cells.

A main point which can be made in the present paper is, in our minds, the fact that SK-resistant target cells, such as Raji and 109, can be killed by LDCC. If interpreted in terms of recognition, as above, it would thus give support to the idea that SK-susceptible target cells express surface structures which are recognized by the SK system. We find this all the more interesting as lately we have been able to show that target cells derived from leukaemia tumours are much more susceptible to SK-killing than target cells derived from normal lymphocytes (Jondal, Spina & Targan, 1978; Jondal & Targan, 1978). (In the present work the tumour-derived Raji target cell is an exception from this rule; see Jondal & Targan, 1978.) Although, at the present time, we have no idea about how the specificity of the SK system is determined (whether it is related to clones of different killer cells or to quantitative or qualitative differences in the expression of surface receptors and/or surface antigens), our findings indicate that the SK system somehow may be able to discriminate malignant from normal target cells.

Furthermore, one would like to learn how SK cells mediate their cytotoxicity, how they are generated and to what major subpopulation they belong. Whereas cytophilically absorbed antibodies have been proposed in initiating an ADCC-like killing by some workers (Akira & Takasugi, 1977), others have had

more difficulty in finding a similar mechanism with other targets. With regard to the nature of the SK cells, it seems most reasonable, at the present time, to think in terms of a T-cell origin (Bonnard & West, 1977; West *et al.*, 1977). In relation to the generation process, leading to the formation of SK cells, we have recently been able to show that antigens expressed on autologous or allogeneic B-cell lines may trigger the induction of killer cells that display a similar target cell selectivity as that of the SK system (Jondal & Targan, 1978). One possibility is thus that B cell-associated antigens, which also are expressed on other cell types, may be involved in the continuous generation of a lymphocyte subpopulation which is important, as one of many other factors, in tumour surveillance. Hopefully, in the continuation of this type of research, it will be possible to define how the SK system may be selectively activated *in vivo*.

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