EFFECT OF ACTINOMYCIN D ON CYTOTOXIC ACTIVITY OF IMMUNE LYMPHOCYTES IN TISSUE CULTURE

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Treatment of immune mouse lymphocytes with actinomycin D does not reduce the cytotoxic activity of the lymphocytes on allogenic target cells regardless of the specificity and dose of the lymphocytes, the concentration of actinomycin D, and the incubation time with the target cells. The cytotoxic effect of immune lymphocytes treated with actinomycin D retains immunologic specificity and is independent of the nonspecific toxicity associated with such treatment.

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Lymphocytes of animals immunized with allogenic cells are specifically adsorbed on these cells and destroy them when incubated together [1, 16, 19]. The processes taking place under these conditions in the lymphocytes and target cells have not been studied. Differences in the immunologic specificity of the lymphocytes and antibodies [3] and the absence of a connection between the cytotoxic effect of the lymphocytes and the secreted γ-globulin [4] indicate that the lymphocyte receptors and antibodies differ in nature. It is not clear whether these receptors are present in immune lymphocytes in a ready-made form or whether they are synthesized after repeated contact between immune lymphocytes and antigens of the target cells. Suppression of activity of immune lymphocytes in vivo [10] and in vitro [11, 20] by inhibitors of DNA and RNA synthesis may in some cases be the result not of depression of synthesis in the cells, but of their delayed death under the action of inhibitors [5].

The object of the present investigation was to study the effect of actinomycin D, an inhibitor of the synthesis of DNA-dependent RNA [12], on the intensity and specificity of the cytotoxic effect of immune lymphocytes.

EXPERIMENTAL METHOD

The lines of mice and the actinomycin D (AD) used in the experiments were described in a previous paper [5]. The tumors—sarcomas MKh11, MKh19, and DMBA2—were induced by chemical carcinogens in mice of lines C57BL/10, C3H, and B10.D2 respectively.

Immune lymphocytes obtained from the regional lymph glands 8 days after a single immunization with the cells of the allogenic tumor and normal lymphocytes of mice of the same line were washed, counted, and suspended in a concentration of $5 \cdot 10^7$/ml in medium No. 199 containing or not containing AD. After incubation in a water bath for 60 min at 37° the cells were washed four times with Hanks' solution, suspended in medium No. 199, recounted, and injected into cultures of peritoneal macrophages of mice belonging to various lines [1]. After incubation at 37° for 6 or 18 h, the culture medium with the lymphocytes was removed and the number of living macrophages in the cultures determined by staining them with a mixture of 0.1% eosin and trypan blue solution [2]. The cytotoxic effect (CE) was calculated by the formula:

$$[(a-b)/a] \times 100,$$

where a and b represent the number of living macrophages after incubation with normal (a) and immune (b) lymphocytes subjected to the same treatment (mean of 4 determinations).

EXPERIMENTAL RESULTS

Normal lymphocytes treated with AD (3 μg/ml) destroyed not more than 3% of the target cells when incubated together for 6-9 h, and 40-55% when incubated for 18 h. The results of 10 experiments to study

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TABLE 1. Intensity and Specificity of Cytotoxic Effect of Immune Lymphocytes Treated with Actinomycin D

Source of target cells	Number of lymphocytes ($\times 10^6$)	Cytotoxic effect (in %) with AD in concentration of		
		0 μg/ml	1,5 μg/ml	2,5—3,5 μg/ml
Corresponding line*	15—20	41,2	47,1	82
		86,6		91
		97,0		94
		87,7		100
		90,5	100	
		100		100
		49,4		53,6 ‡
		52,9 ‡		74,8 ‡
		72,6 ‡		77,7
	10	7,9 **	—14,1**	>96
	5	51,2		>95
Mean	15—20	75,3		82,0
Noncorresponding line†	15—20	20,9 **	24,6	6,4 **
		5,2 **		1,8 **
		—5,9 **		10,6 **
		1,2 **		—4,1 **
		—1,9**‡		—4,9**‡
		2,0**‡		11,7**‡
Mean		3,6		3,9

* Line used as donor in immunization or analogous to donor in its H-2 locus (for example, anti-C57BL/10 lymphocytes, CC57BR target cells; lines C57BL/10 and CC57BR have the same H-2^b allele).
† Line used as recipient in immunization or unrelated line (for example, B10.D2 anti-C57BL/10 lymphocytes, target cells of line B10.D2 or A).
‡ Incubation of lymphocytes with target cells for 6-9 h, in remainder of experiments for 18-20 h.
**P > 0.05; in other cases P < 0.01 (t-test)

TABLE 2. Effect of AD on Cytotoxic Activity of Immune Lymphocytes with Identical Nonspecific Cytotoxic Background

Type of lymphocytes	Dose (millions)	Number of living macrophages ($\times 10^6$)	Cytotoxic effect (in %)
Immune	8	9,5±1,2	91,6
	4	54±3,5	50.5
	2	73±4,5	33 †
Normal	4	109±15,5	control
Immune + normal AD*	8+8	<3	<93
	4+4	12,7±2,2	80,8
	2+2	49,3±4,9	27 †
Immune AD*+ normal	8+8	<5	91
	4+4	15,6±2,8	76,4
	2++	45,5±5,8	32,6
Normal AD*+ normal	8+8	53,7±3,7	control
	4+4	66,0±1,1	
	2+2	67,5±3,2	

* Lymphocytes treated with AD (3 μg/ml) and washed.
† P < 0.02, in the other cases P < 0.01

the effect of AD on the activity of immune lymphocytes are given in Table 1. B10.D2 anti-C57BL/10, C3H anti-C57BL/10, and CC57BR anti-C3H lymphocytes lowered after treatment with AD in concentrations of between 0.25 and 3.5 μg/ml, irrespective of the dose of lymphocytes (from 5 to 20 million) and the period of incubation with the target cells (6 or 18 h). Conversely, treatment of the immune lymphocytes with AD led to a slight increase in their CE.

As Table 1 shows, immune lymphocytes treated with AD destroyed only the corresponding targets and had no specific cytotoxic action on macrophages of the recipient lines or of unrelated lines. The CE of immune lymphocytes treated with AD thus retains its immunologic specificity.

Although the CE of immune lymphocytes treated with AD was much greater than the nonspecific toxicity of normal lymphocytes treated with AD, the possibility cannot be ruled out that because of the nonspecific cytotoxic "background" (in the 18-h experiments) the decrease in activity of the immune lymphocytes could not be detected. To test this hypothesis, mixtures of normal and immune lymphocytes, untreated and treated with AD, were added to the cultures so that the nonspecific ("background") toxicity was equal in all cases (Table 2). Under these conditions the CE of the immune lymphocytes was equal regardless of whether they were treated with AD or not. Table 2 shows that the nonspecific toxicity produced by treatment of the lymphocytes with AD did not affect the specific activity of the immune lymphocytes even when they were used in the minimally active dose (2 million).

Treatment of lymphocytes with AD irreversibly depresses the synthesis of cell RNA. The intensity of this depression depends on the AD concentration: results obtained by various workers show that 1 μg/ml depresses the synthesis of total RNA by 75% [15], 80-83% [17], or more than 90% [7]; 3-5 μg/ml depresses its synthesis by 92-95% [7, 17]. Synthesis of mRNA and rRNA is most sensitive to the action of these concentrations of AD [7, 15, 18].

The absence of effect of AD (1.3-3.5 μg/ml) on the CE of immune lymphocytes demonstrated in the present investigation indicates that the CE is exhibited without synthesis of mRNA essential for the formation in vitro of antibodies, especially those of type 19S [8, 14, 18]. The protein synthesis essential for CE [6, 11] evidently takes place in the lymphocytes on a preformed RNA template. These results are in agreement with data indicating the inability of AD to depress the CE of normal lymphocytes in the presence of phytohemagglutinin [13], but they do not agree with data indicating depression of the activity of immune lymphocytes in vivo by mitomycin C (100 μg/ml) and AD (12.5 μg/ml) [10] and in vitro by imuran (azothioprine) (5-10 μg/ml) [20] and AD (10 μg/ml) [11]. The high concentrations of inhibitors in these cases not only were able to depress mRNA synthesis, but also to produce other effects leading to death of the lymphocytes.

Preservation of activity of the immune lymphocytes despite depression of RNA synthesis in them indicates that the possible transfer of newly synthesized RNA from lymphocyte to target cells [9] is not essential for CE.

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