SENSITIVITY OF PHASES OF THE MITOTIC CYCLE OF TUMOR CELLS TO THE ACTION OF IMMUNE LYMPHOCYTES

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The cytotoxic action of immune lymphocytes (IL) on synchronized L tumor cells was studied in all phases of the mitotic cycle. A cytotoxic action was observed only after the addition of IL to L-cells at the very beginning of the G_1 phase. Destruction of the L-cells was observed after 30 min. The addition of IL to target cells 180 min or more after the beginning of G_1, and also their addition to cells in the S, G_2, and M phases, had no cytotoxic action, not only after 30 min, but throughout the duration of each of these phases. The addition of IL to L-cells in all phases of the cycle except early G_1 led to the same cytotoxic effect as in unsynchronized L-cells. The importance of the high sensitivity of early G_1 to the cytotoxic action of IL for cell physiology and the need for allowing for it during immunotherapy of tumors are discussed.

Tumor cells in different phases of the mitotic cycle are known to differ in their sensitivity to chemical and physical agents and also to cytotoxic antibodies. However, nothing is yet known about the action of immune lymphocytes on the various phases of the cell cycles.

The sensitivity of target cells (malignant fibroblasts of strain L) in various stages of the mitotic cycle to the action of immune and normal lymphocytes was investigated by the cytotoxic test in vitro and the results are described below.

EXPERIMENTAL METHOD

A system of the Rosenau and Moon type [5] used for the experiments was described earlier [1, 2].

Transplantable L-cells and lymphocytes of immune C57BL/6j mice were used.

The L-cells were seeded in tubes at the rate of 100,000 cells to 1 ml medium No. 199 with 20% bovine serum, inactivated at 56°C for 30 min and incubated at 37°C for 24 h. An unsynchronized culture was used in the control. Some of the cells were synchronized by the addition of an excess of thymidine (2 mg/ml), and 20 h later they were washed three times with Hank's solution at pH 7.2. The degree of synchronization was determined in preliminary experiments by the method of incorporation of thymidine-H^3 (0.5 μCi/ml) for 20 min at 37°C, followed by coating the preparations with type M emulsion and exposing them for 7 days. The resulting preparations were stained with methyl green-pyronine (by Brachet's method), 500 cells were counted, and the percentage of labeled cells determined. The degree of synchronization averaged 80-85%.

The duration of the phases of the mitotic cycle for the investigated strain of L-cells was as follows: G_1) 10 h; S) 6 h; G_2) 3 h; M) 30 min. These figures agree well with data in the literature [6].

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TABLE 1. Interaction between Immune and Normal Lymphocytes and Unsynchronized Culture of Tumor Cells and with a Synchronized Culture in the G_1 Phase

Experimental conditions	Duration of interaction between lymphocytes and L-cells						
	30 min			3 h	6 h	10 h	48 h
	experiment 1	experiment 2	experiment 4*	experiment 2	experiment 2	experiment 2	experiment 2
Unsynchronized culture							
1. Target L-cells	60±3,3	370±42,7	180±7,0	417±16,0	400±16,0	470±24,0	370±10,0
2. Target L-cells + normal lymphocytes	64±3,0	373±5,0	189±9,0	415±19,2 353±19,0	377±12,4 214±30,0	510±44,0 120±13,1	370±12,0 27±2,6
3. Target L-cells + immune lymphocytes	56±2,9	402±40,0	167±11,5	0,1	<0,01	<0,01	<0,01
P_{2-3}	>0,05	>0,05	>0,05				
Synchronized culture							
4. Target L-cells	65±3,0	371±10,0	168±11,0	401±21,9	411±24,0	400±26,0	305±12,1
5. Target L-cells + normal lymphocytes	70±4,9	378±25,4	163±10,5	339±30,4 117±27,2	340±23,1 183±17,7	405±31,7 150±26,7	341±15,0 68±8,0
6. Target L-cells + immune lymphocytes	40±2,5	30±5,1	127±15,3	<0,01	<0,01	<0,01	<0,01
P_{5-6}	<0,01	<0,001	>0,05				

*Lymphocytes added 3 h after beginning of G_1 phase.

Note to Tables 1 and 2: Results of corresponding experiments not included in the tables were similar to those presented.

TABLE 2. Interaction between Immune and Normal Lymphocytes and an Unsynchronized Culture of Tumor Cells and with a Synchronized Culture in Various Phase of the Mitotic Cycle

Experimental conditions	Duration of interaction between lymphocytes and L-cells								
	30 min	3 h	6 h	48 h	30 min	3 h	48 h	30 min	48h
Unsynchronized culture									
1. Target L-cells	135±20,1	65±9,6	121±11,0	270±2,9	96±18,5	96±6,0	95±6,0	501±11,3	653±18,0
2. Target L-cells + normal lymphocytes	149±13,8	81±20,5	117±14,0	328±4,1	120±8,4 112±9,9	96±5,7 91±7,6	42±3,0 6±1,1	508±10,9 494±9,4	681±21,3 406±28,1
3. Target L-cells + immune lymphocytes	151±18,2	84±10,0	113±9,1	173±1,9	>0,05	>0,05	<0,01	>0,05	<0,01
P_{2-3}	>0,05	>0,05	>0,05	<0,01					
Synchronized culture									
	Phase S				Phase G_2			Phase M	
4. Target L-cells	109±10,4	76±8,1	114±12,7	151±2,3	70±11,0	85±13,7	99±8,3	400±11,8	803±22,4
5. Target L-cells + normal lymphocytes	113±18,1	79±4,4	117±8,8	168±4,0	71±11,0 68±7,0	93±8,0 88±8,1	33±2,7 4±0,9	408±9,7 435±7,6	834±20,9 584±13,8
6. Target L-cells + immune lymphocytes	111±12,3	86±4,9	115±12,4	95±1,6	>0,05	>0,05	<0,01	>0,01	<0,01
P_{5-6}	>0,05	>0,05	>0,05	<0,01					

Mice of strain C56BL/6j were immunized in a single session by intraperitoneal and subcutaneous injection of 55 million cells per mouse at each of 11 different points, in a total volume of 2 ml. On the 8th-9th day after immunization lymphocytes were obtained from the lymph glands and transferred to a Potter's glass homogenizer. The concentration of the lymphocytes was made up with medium No. 199 to 4 million per ml.

An experiment was performed for each phase of the mitotic cycle. In each phase of the cycle old medium was poured from the tubes containing L-cells, and immune and normal lymphocytes were added at the rate of 1 ml per tube. The time of exposure of the lymphocytes to the L-cells varied from 30 min to 48 h; the number of living L-cells in each tube was then counted by staining with trypan blue and eosin.

EXPERIMENTAL RESULTS

These investigations showed that the sensitivity of the synchronized tumor cells to the cytotoxic action of the immune lymphocytes depends on the phase of the mitotic cycle.

The cytotoxic effect was well defined only in the G_1 stage of the cell cycle after interaction for 30 min between the immune lymphocytes and the target L-cells. Moreover, if the lymphocytes were added to the synchronized culture at the very beginning of the G_1 phase, the cytotoxic action was strongest, but if they were added 3 h after the beginning of the G_1 phase either the action was not significant or it could not be observed (Table 1).

In the other phases of the mitotic cycle (S, G_2, M) the cytotoxic effect was ill-defined not only after interaction for 30 min between the immune lymphocytes and the L-cells but also after their interaction throughout the phase.

On incubation of immune lymphocytes with a synchronized culture of tumor cells for 48 h a cytotoxic effect was observed independently of the phase of the cell cycle (Table 2).

The investigation of the sensitivity of synchronized target cells of strain L in various phases of the mitotic cycle to the action of immune lymphocytes thus showed that a cytotoxic action is possible only at the very beginning of the cell cycle – in the G_1 phase.

It is not yet known whether this maximal sensitivity of tumor cells in the G_1 stage is connected with development of the antigens of the H_2 locus in this phase or whether it reflects the state of the cells at the very beginning of the G_1 phase, and the problem is at present being studied [3, 4, 7].

Treatment with interferon is known to increase the sensitivity of the cells to the cytotoxic action of both double-helical RNA and lymphocytes. The writers suggest that the cytotoxic action of the lymphocytes is connected with the formation of double-helical forms of RNA and with RNA-dependent RNA synthesis in the target cells. This can be tested in the synchronized system described above. It has also been found that interferon itself can synchronize cell cultures.

The results point to the importance, in principle, of allowing for the differential sensitivity of tumor cells in different phases of the mitotic cycle during the immunotherapy of cancer in order to increase its effectiveness.

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