EFFECT OF IMMUNE SERA ON PROLIFERATIVE ACTIVITY OF L CELL CULTURES

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The effect of humoral immunity on tumor growth has been the subject of many investigations. They have shown that antisera against tumor cells modify their proliferative activity. Under certain conditions tumor growth is inhibited, under others it is stimulated as a result of treatment with antisera [1-3, 5]. The mechanism of action of antibodies on proliferative activity of tumor cells has not yet been fully explained. Antibodies can either change physiological processes in tumor cells or they can act indirectly through the immune system of the tumor carrier [5]. It has been suggested that the character of the change produced in tumor growth by antibodies (inhibition or stimulation) may depend on their quantity, methods of their isolation, or their class [2-4].

The objective of this investigation was to study the effect of different concentrations of antibodies, and also of whole anti-L-serum on proliferative activity of L-cells.

EXPERIMENTAL METHODS

L cells were cultured in 100-ml flasks in medium No. 199 with 10% bovine serum (BS). Subcultures were made once a week.

Rabbits weighing 3-3.5 kg were used for immunization; $2 \cdot 10^7$ L cells, washed 3 times with Hanks' solution, in 1 ml medium No. 199 were mixed with 1 ml of Freund's complete adjuvant (Difco) and injected into the footpads of the rabbits' hind limbs. A second injection of $2 \cdot 10^7$ cells was given 2 weeks later into the auricular vein. After 10 days serum was obtained, inactivated at 56°C for 30 min, and kept at −20°C. The cytotoxic titer of the anti-L-sera was 1:64. Sera from unimmunized rabbits served as the control.

The globulin fraction of the antisera was obtained by salting out with ammonium sulfate at 40% saturation. Immunoglobulins were then passed through a column with DEAE-cellulose to isolate the IgG fractions, which were concentrated on an Amicon PM-30b filter. The resulting IgG were dialyzed overnight against physiological saline.

The proliferative activity of L cells treated with antisera (AS) or IgG was determined in two series of experiments. In series I the L cells were incubated with different dilutions of AS (1:2, 1:4, 1:64, 1:128) or IgG (3200, 1600, 400, 50 ml) for 30 min at 37°C, after which the cells were washed to remove reagents and transferred in a concentration of $4 \cdot 10^4$-$6 \cdot 10^4$/ml into penicillin flasks or on to plastic plates with wells with a capacity of 0.2 ml (with 5 times less cell concentrations respectively — $1 \cdot 10^4$ per well). In the experiments of series II the cells were incubated in the presence of different concentrations of AS and IgG. The culture medium contained 10% BS or 10% embryonic calf serum (ECS). The cultures were incubated for 24 to 72 h. Four hours before harvesting of the cells ^{3}H-thymidine (^{3}HT) was added to the culture medium in a concentration of 1 μCi/ml. The radioactivity of the cells was counted in cpm.

EXPERIMENTAL RESULTS

Data on incorporation of ^{3}HT into L cell cultures treated with AS are summarized in Table 1. A difference in principle was found between the action of low (1:2, 1:4) and high

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TABLE 1. Incorporation of ^{3}HT into L Cell Cultures Treated with Antisera

Experimental conditions	Duration of observation (h)	Dilution of serum 1/2	1/4	1/64	1.128
Reagents not washed off	24	4226±321 / 8748±698	10 550±680 / 9017±236	18 542±1643 / 9526±383	13 323±2905 / 8838±650
		$P<0,05$	$>0,05$	$<0,01$	$>0,05$
	48	5714±241 / 11 100±1163	16 125±1173 / 12 625±3499	32 619±5446 / 9447±815	25 726±3437 / 7858±471
		$P<0,05$	$>0,05$	$<0,02$	$<0,01$
	72	4949±518 / 10 970±1503	10 315±1586 / 13 257±702	67 497±8686 / 11 762±1503	55 688±1847 / 12 785±1021
		$P<0,05$	$<0,05$	$<0,01$	$<0,001$
Reagents washed off	48	15 236±3762 / 7921±1694	15 386±3873 / 9557±3232	—	—
		$P<0,05$	$>0,05$		
	72	15 910±4348 / 6900±1324	19 292±6954 / 10 815±2748	—	—
		$P<0,05$	$>0,05$		

Legend. Mean number of cpm per L cell culture (data of three experiments) treated with anti-L-sera (numerator) and with intact sera (denominator); P) significance of differences between them.

TABLE 2. Incorporation of ^{3}HT into L Cell Cultures Treated with IgG (M ± m)

Experimental conditions	Concentration, μg/ml 3200	1600	400	50
Without washing off	9375±108 / 5164±417	15 044±2289 / 7117±2601	8233±966 / 3321±317	4266±115 / 3688±95
P	$<0,01$	$<0,05$	$<0,02$	$>0,05$
With washing off	20 130±3229 / 5164±417	20 836±2438 / 6333±592	—	—
P	$<0,05$	$<0,05$	—	—

Legend. Mean number of cpm in 24-h culture of L cells treated with IgG isolated from anti-L-sera (numerator) and from intact sera (denominator) from one well (data of three experiments) are given.

(1:64, 1:128) dilutions of AS on DNA synthesis in the L cells during culture. Low dilutions of AS inhibited, whereas high dilutions stimulated proliferative activity of the L cell cultures.

Examination of the action of low dilutions of AS on proliferative activity of L cells when the reagents were constantly present in a dilution of 1:2 in the culture medium revealed a decrease in incorporation of ^{3}HT into the cell culture by half. This decrease was found at all times of culture. With a dilution of AS in the incubation medium of 1:4, a statistically significant decrease in ^{3}HT incorporation was found only on the 3rd day of culture. Incubation of the cells with low dilutions of AS followed by washing to remove it led to abolition of the effect of inhibition of proliferative activity. In that case a tendency was found for ^{3}HT incorporation into the cultures to increase. This was probably due to the reduction in the AS concentration in the culture medium and on the surface of the tumor cells, which evidently led to an increase in their proliferative activity.

When the L cells were treated with AS in high dilutions ^{3}HT incorporation into the cell cultures was increased to two to five times. The increase itself increased toward the later periods of observation.

Replacement of BS in the incubation medium by ECS led to stimulation instead of inhibition in the presence of low dilutions of AS, whether or not it was washed out. The increase in incorporation of ^{3}HT into L cell cultures treated with AS in high dilutions (1:64) in in-

cubation medium with 10% ECS was even more marked (from three to seven times) depending on the time of incubation.

During culture of L cells treated with normal rabbit serum or with serum obtained after immunization of rabbits with sheep's red blood cells, no statistically significant differences in ^{3}HT incorporation into the cells were observed either in medium with 10% BS or in medium with 10% ECS. Accordingly, serum of intact rabbits was used in the experiments as the control reagent.

When the number of cells in L cultures treated with AS was determined, no statistically significant differences were found between the experimental and control groups. However, under the influence of low dilutions of AS a tendency was observed for ^{3}HT incorporation to be reduced. Under the influence of high dilutions of AS there was a tendency for ^{3}HT incorporation to increase.

Data on incorporation of ^{3}HT into cultures treated with IgG are given in Table 2. High (3200 μg/ml) and low (50 μg/ml) concentrations of IgG isolated from AS stimulated ^{3}HT incorporation into the L cell cultures. Washing out the IgG did not affect their stimulating action on proliferation.

The main purpose of this investigation was to establish in general that AS against surface antigens of L cells have an action on their proliferative activity. To solve this problem it was unnecessary to use antisera with narrow specificity, and for that reason the AS were not exhausted.

The results indicate that high concentrations (low dilutions) of AS inhibit, whereas low concentrations (high dilutions) activate proliferative activity of L cells. ECS abolishes the inhibitory action of high AS concentrations; hence, it follows that the direction of the effect can be varied by using reagents causing stimulation of proliferation, or certain components of serum. The change in proliferative activity of the cells under the influence of AS is undoubtedly connected with the action of a specific IgG fraction. However, the suppressive action of high concentrations is also determined by other components of the antiserum.

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