EFFECT OF EXTRACTS FROM CULTIVATED CELLS ON GROWTH OF TRANSPLANTABLE HUMAN CELLS OF LINE Tg_{33}

I. I. Kochergina

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The action of different doses of protein in extracts obtained from cultures of Tg_{33}, HeLa-K-41, and L cells on cells of line Tg_{33} was studied. The effect of extract of Tg_{33} cell culture was shown to depend on the dose: with a small dose (25 μg/ml) growth was stimulated, with an average dose (50 μg/ml) there was no effect, and with large doses (75 and 100 μg/ml) growth was inhibited. Extracts obtained from HeLa-K-41 and L-cell cultures, when added to a culture of Tg_{33} cells, had an equal inhibitory action on growth despite differences in the protein content of the extracts (25, 50, 75, and 100 μg/ml).

Investigations [7, 8, 10] have shown that the phenomena of allogenic inhibition and syngeneic preference play an important role in carcinogenesis and tissue transplantation. These phenomena have been reproduced both in vivo and in vitro, with the use of inbred strains of mice and cell cultures of animal origin [2, 3, 7, 10]. The investigation of these phenomena on human cell models is particularly interesting [1, 9].

The object of the investigation described below was to study the action of extracts obtained from cell cultures of different origin on growth of human cells of line Tg_{33}.*

EXPERIMENTAL

A culture of Tg_{33} cells was seeded in Wassermann tubes at the rate of 50,000 cells to 2 ml of Eagle's medium with the addition of 10% bovine serum. The cells were grown in some tubes on coverslips in order to obtain stained specimens.

Cell extracts were obtained from cultures of Tg_{33}, HeLa-K-41,† and L cells. The cell suspension obtained after 1-2 months was freed from versene and washed in Hanks' solution. The cells were broken up by repeated freezing and thawing in acetone with dry ice and centrifuged at 1000 rpm for 30 min. The supernatant was used in the experiments and its protein content was determined by Lowry's method. On the second day of growth, extracts from Tg_{33}, HeLa-K-41, and L cells were added to the Tg_{33} cells in doses of 25, 50, 75, and 100 μg/ml medium. The medium was changed in the control tubes. On the fifth day of growth (the third day after treatment) the living and dead cells were counted in a Goryaev's chamber after staining with 1% trypan blue, and the proliferation index was calculated. Cells grown on coverslips were washed with warm (37°C) Hanks' solution and fixed in Bouin's mixture for 1 h, after which they were stained with Mayer's hematoxylin.

The mitotic index (number of mitoses per 3000 cells) was calculated in the fixed and stained specimens. To determine the relative size of the nuclei, Khesin's method [4] was used. The outlines of the nuclei (100 nuclei per specimen) were traced on standard paper by means of a drawing apparatus (ocular

*Cell line Tg_{33} was obtained by Barski [6] in 1961 from the histologically normal fallopian tube of a woman.
† Clone HeLa-K-41 was obtained by Sharyi [5] in 1966.

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TABLE 1. Proliferation Index of Culture of Tg_{33} Cells after Treatment with Extracts of Tg_{33}, HeLa-K-41, and L Cells with Different Protein Content

Protein dose in extracts (μg)	Name of extract	n	M	±m	P
25	Control Tg_{33} + Tg_{33}	8	14,5	0,5	
	HeLa-K-41	8	11,2	0,7	=0,001
	L	8	10,5	0,7	<0,001
	Technical control	8	12,8	0,5	
	Tg_{33}	8	14,5	0,5	<0,05
	HeLa-K-41	8	11,2	0,6	<0,05
	L	8	10,5	0,7	<0,02
50	Control Tg_{33} + Tg_{33}	4	11,0	0,8	
	HeLa-K-41	4	9,5	0,3	<0,2
	L	4	9,4	0,7	<0,2
	Technical control	4	12,1	0,2	
	Tg_{33}	4	11,0	0,8	<0,5
	HeLa-K-41	4	9,5	0,3	<0,001
	L	4	9,4	0,7	<0,01
75	Control Tg_{33} + Tg_{33}	5	10,6	0,2	
	HeLa-K-41	5	9,7	0,1	>0,5
	L	5	9,3	0,2	>0,5
	Technical control	5	13,5	0,5	
	Tg_{33}	5	10,6	0,2	<0,001
	HeLa-K-41	5	9,7	0,1	<0,001
	L	5	9,3	0,2	<0,001
100	Control Tg_{33} + Tg_{33}	3	9,0	0,2	
	HeLa-K-41	3	8,5	0,1	<0,1
	L	3	7,1	1,1	<0,2
	Technical control	3	11,7	0,8	
	Tg_{33}	3	9,0	0,2	<0,05
	HeLa-K-41	3	8,5	0,1	<0,02
	L	3	7,1	1,1	<0,05

TABLE 2. Relative Size of Nuclei of Tg_{33} Cell Culture after Treatment with Extracts of Cell Cultures with Different Protein Content

Name of extract	n	M	±m	P
Dose 25 μg				
Tg_{33}	3	4,1	0,02	<0,01
HeLa-K-41	3	3,0	0,2	=0,01
L	3	3,3	0,2	<0,02
Control	3	4,8	0,02	
Dose 50 μg				
Tg_{33}	3	5,5	0,1	<0,2
HeLa-K-41	3	3,5	0,2	<0,5
L	3	4,0	0,02	>0,5
Control	3	4,2	0,5	
Dose 75 μg				
Tg_{33}	3	2,7	0,2	<0,02
HeLa-K-41	3	2,9	0,4	<0,01
L	3	3,1	0,03	<0,02
Control	3	4,8	0,2	
Dose 100 μg				
Tg_{33}	3	3,3	0,2	<0,05
HeLa-K-41	3	3,4	0,03	<0,05
L	3	3,1	0,03	<0,05
Control	3	4,2	0,5	

15, objective 90), and the shapes of the nuclei were cut out and weighed on analytical scales. Statistical analysis was carried out by Student's method.

EXPERIMENTAL RESULTS

Extracts Containing 25 μg Protein. The experiments showed that autogeneic Tg_{33} extract stimulates growth of a Tg_{33} cell culture. For instance, the proliferation index after addition of this extract was significantly higher than the control and also than the proliferation indices obtained after the addition of allogeneic (HeLa-K-41) and xenogeneic (L) extracts (Table 1).

The mitotic activity of the Tg cells was significantly greater after the addition of autogeneic extract than in the control (P = 0.05, n = 3) (Fig. 1). The stimulant action of the autogeneic extract was also confirmed by an increase in the relative size of the nuclei (Table 2). With reference to all indices the autogeneic extract with a small dose of protein thus led to significant stimulation of growth of the Tg_{33} cell culture. The phenomenon of autogeneic stimulation is evidently attributable to the fact that extracts obtained from a culture of Tg_{33} cells are genetically homogeneous with respect to cells of line Tg_{33}.

Extracts from cells of HeLa-K-41 and L cultures significantly inhibited growth of the Tg_{33} cells, as shown by statistical analysis of the results obtained for the proliferation indices

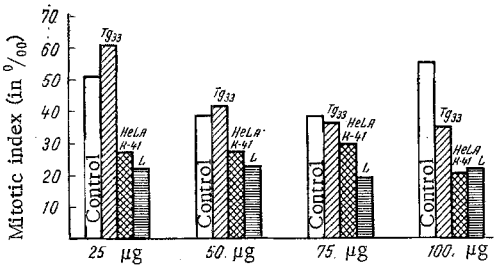

Fig. 1. Effect of dose of protein in extract on mitotic activity of culture of Tg_{33} cells.

(Table 1), the mitotic activity ($P < 0.02$, $P < 0.01$; $n = 3$), and the relative size of the nuclei ($P < 0.01$ and $P = 0.01$) (Table 2).

Extracts Containing 50 μg Protein. With this dose a tendency was observed for the stimulant effect on growth to be abolished after the addition of the autogeneic extract (Fig. 1). The differences between the indices of proliferation and relative size of the nuclei after addition of the Tg_{33} extract and the control were not statistically significant ($P < 0.5$, $P < 0.2$). By contrast with the above indices, the mitotic activity of the Tg_{33} cell culture was significantly higher after the addition of autogeneic extract than in the control ($P < 0.05$). Allogeneic (HeLa-K-41) and xenogeneic (L) extracts inhibited growth of the test culture, as is confirmed by statistical analysis of the results for the proliferation and mitotic indices.

Extract Containing 75 μg Protein. After the addition of autogeneic extract in a dose of 75 μg protein, the stimulant action of the extract on growth was converted into inhibitory (Fig. 1). Tg_{33} extract, when added to the culture of Tg_{33} cells, significantly inhibited the growth of this culture, as confirmed by statistical analysis of the results for the proliferation index ($P < 0.001$) and the relative size of the nuclei ($P < 0.02$). The allogeneic and xenogeneic extracts significantly inhibited growth of the Tg_{33} cell culture as reflected by all the growth indices.

Extracts Containing 100 μg Protein. Extracts containing the largest dose of protein significantly inhibited growth of the Tg_{33} cell culture on the addition of all the extracts (Tg_{33}, HeLa-K-41, and L).

Experiments on a culture of human cells (Tg_{33}) thus showed that the effect of an extract obtained from cells of a Tg_{33} culture depended, so far as all the growth indices are concerned, on its dose: with a small dose (25 μg/ml) growth was stimulated, with an average dose (50 μg/ml) there was no effect, and with large doses (75 and 100 μg/ml) growth was inhibited.

Extracts obtained from cultures of HeLa-K-41 and L cells, when added to Tg_{33} cells, exhibited the same inhibitory action on growth even if the extracts contained different quantities of protein (25, 50, 75, and 100 μg/ml).

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