EFFECT OF CELL AND TISSUE EXTRACTS ON GROWTH OF CELL CULTURES

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UDC 612.6-085.23-06:612.015.21

The action of extracts of different origin on growth of human (Tg_{33}) and mouse (L) cell structures was studied. Autogeneic extract from Tg_{33} had a stimulant action and allogeneic and xenogeneic extracts from HeLa K-41 and L an inhibitory action on growth of cells of a Tg_{33} culture. Extracts from C3H mouse spleen cells and L cells stimulated growth of L cells (syngeneic and autogeneic action). Extracts from C57BL mouse spleens and from Tg_{33} cells inhibited growth of the culture (allogeneic and xenogeneic effects).

During the study of the mechanisms of transplantation immunity in recent years increasing attention has been paid to the phenomena of allogeneic inhibition and syngeneic preference [2, 8]. Investigations [3, 4, 7-10] have shown that mouse tissue homogenates and extracts of syngeneic and allogeneic origin respectively can stimulate or inhibit proliferation of cells in vivo and in vitro. The nature of these phenomena is not yet clear and further study is required, in particular, in human cell cultures.

The object of the present investigation was to study the action of cell and tissue extracts on growth of human (Tg_{33}) and mouse (L) cell cultures. An extract from Tg_{33} and L cells, i.e., from the same tissues as were being investigated, was used for the first time.

EXPERIMENTAL METHOD

The experiments were carried out by Hellström's method [7] modified slightly in the authors' laboratory. A culture of Tg_{33} and L cells was subcultured at the rate of 50,000 cells into test tubes containing 2 ml Eagle's medium and medium No. 199 with the addition of 10% bovine serum. Cell extracts were obtained from cells of strains Tg_{33},* HeLa K-41,† and L, and tissue extracts from spleens of C3H and C57BL mice.

The spleen tissues were disintegrated in a manual homogenizer. The cell suspension and the suspension of spleens in Hanks's solution were then frozen and thawed 15 times in acetone with dry ice, and centrifuged at 1000 rpm for 30 min. Protein in the resulting extracts 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 and extracts from C3H and C57BL mouse spleens and extracts from L and Tg_{33} cells were added to the L cells. The dose of protein in the added extracts was 50 μg/2 ml medium for both lines. The medium was changed in the control tubes. On the 5th day of growth (the 3rd day after treatment) the living and dead cells were counted, using 1% trypan blue solution, and the coefficient of proliferation was calculated [4]. The mitotic index was studied in stained films. The results were analyzed statistically by the Fisher – Student method [1].

*Tg_{33} is a strain obtained by Barski in 1961 from the histologically normal fallopian tube of a woman [6] and generously made available to the authors in 1965.
†HeLa clone K-41 was obtained by N. I. Sharyi in 1966 [5].

Group of Immunogenetics and Biology of Antigens, Research Laboratory of Experimental Immubiology, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 3, pp. 86-88, March, 1971. Original article submitted July 17, 1970.

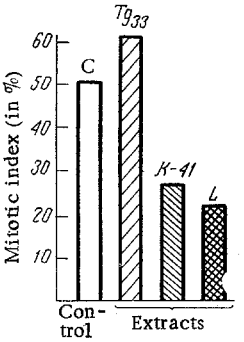

Fig. 1. Mitotic activity of culture of Tg_{33} cells after treatment with cell extracts of different origins: C, control; Tg_{33}, HeLA K-41, L, extracts from cells of corresponding cultures.

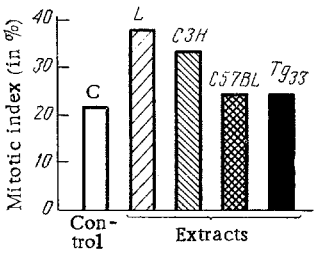

Fig. 2. Mitotic activity of cultures of L cells after treatment with extracts of different origins: C, control; L, Tg_{33}, C3H, C57BL, extracts from cells of corresponding lines and mouse spleens.

EXPERIMENTAL RESULTS

Action of Cell Extracts on Tg_{33} Culture. These experiments showed that extracts from Tg_{33} cells stimulated growth of this same culture (mean coefficient of proliferation 14.5), while extracts from HeLa K-41 and L cells inhibited growth of the Tg_{33} culture (coefficients of proliferation 11.2 and 10.2 respectively, control 12.8).

Statistical analysis of the results* revealed significant differences between the numbers of living cells after the action of extracts from Tg_{33}, HeLa K-41, and L cells on the culture of Tg_{33} cells. Comparison of the effects of extracts of allogeneic (HeLa K-41) and xenogeneic (L) origins with the effects of extract from cells of the same line (Tg_{33}) showed that in 7 of the 8 experiments the difference was significant. Comparison of the results after the action of HeLa K-41 extract with the control (Tg_{33} without addition of extracts) revealed a significant difference in 5 experiments, and after the action of L extract in 6 experiments (Table 1). These findings correspond to the results of a study of mitotic activity of Tg_{33} cells. The mitotic index after the action of Tg_{33} extract was $61^0/_{00}$, and after the action of HeLa K-41 extract $27.6^0/_{00}$, after the action of L extract it was $22^0/_{00}$, and in the control $51^0/_{00}$ (Fig. 1).

Action of Cell and Tissue Extracts on Culture of L Cells. After addition of extracts of C3H mouse spleens and of L cells, stimulation of growth of the L cells was observed (mean coefficients of proliferation 5.1 and 6.2 respectively). Comparison of the action of extracts from L and Tg_{33} cells showed a significant difference in the number of living cells in all 7 experiments. When the results of addition of the tissue

*The result is considered significant if $1-P=0.950$ or more [1].

TABLE 1. Statistical Significance of Differences (1-P) in Numbers of Living Cells after Action of Extracts of Different Origins on Line Tg_{33}

Expt. No.	Extr. from Tg_{33} / Extr. from HeLa K-41	Extr. from Tg_{33} / Extr. from L	Extr. from HeLa K-41 / Control	Extr. from L / Control
1	0,647	0,952	0,637	0,992
2	0,969	0,953	0,687	0,789
3	0,969	1	0,227	0,901
4	1	1	0,997	0,991
5	1	1	0,991	0,976
6	0,995	0,969	0,976	0,950
7	0,965	0,908	1	0,994
8	1	1	0,986	0,962

TABLE 2. Statistical Significance of Differences (1-P) in Numbers of Living Cells after Action of Extracts of Different Origins on Line L

Expt. No.	Extr. from C3H / Extr. from C57BL	Extract from L / Extr. from Tg_{33}	Extr. from C 57BL / Control	Extr. from Tg_{33} / Control	Extr. from C3H / Control
1	1	1	0,996	0,562	0,988
2	0,940	1	1	1	1
3	0,998	1	0,657	0,856	0,993
4	1	1	1	1	0,965
5	1	1	1	1	0,994
6	1	1	1	1	0,618
7	1	1	1	1	1

extracts were compared the difference was significant in 6 of the 7 experiments. Comparison of the action of extracts of allogeneic, syngeneic, and xenogeneic origin with the control also revealed significant differences (Table 2). The results of calculations of the mitotic index confirmed the growth-stimulating action of extracts from cells of the same line (Fig. 2).

Hence, the experiments on cultures of Tg_{33} cells showed that extracts of cells of the same line stimulate the growth of these cells, while extracts from cultures of HeLa K-41 cells and L cells inhibit growth of Tg 33 cultures.

Experiments on cultures of L cells showed that tissue extracts from C3H mouse spleens and from cells of the same line stimulate growth of strain L, while extracts of C53BL mouse spleen tissues and human cells inhibit growth of cells of line L.

LITERATURE CITED

1. A. D. Timofeevskii (Editor), Models and Methods in Experimental Oncology [in Russian], Moscow (1960), p. 240.
2. R. V. Petrov, Introduction to Nonspecific Immunology [in Russian], Novosibirsk (1968).
3. I. I. Podoplelov, I. P. Kosova, et al., in: Transplantation of Organs and Tissues [in Russian], Minsk (1969), p. 27.
4. I. I. Podoplelov, I. P. Kosova, et al., in: Proceedings of an All-Union Conference on General Immunology and Immunity against Viruses, to Commemorate the 125th Anniversary of the Birth of I. I. Mechnikov [in Russian], Part 1, Moscow (1970), p. 87.
5. N. I. Sharyi, in: Proceedings of a Conference of Junior Scientific Staff of the Institute of Experimental Biology [in Russian], Moscow (1966), p. 141.
6. G. Barski, et al., Ann. Inst. Pasteur, 109, 173 (1965).
7. J. Hellström and K. E. Hellström, Ann. New York Acad. Sci., 129, 724 (1966).
8. K. E. Hellström, J. Hellström, et al., Nature, 204, 661 (1964).
9. N. N. Holmgrem and D. Merchant, J. Nat. Cancer Inst., 40, 561 (1968).
10. G. Möller and E. Möller, Ann. New York Acad. Sci., 129, 735 (1966).