SOME PECULIARITIES IN THE ANTIGENIC PROPERTIES OF HUMAN EMBRYONIC TISSUES WHEN CULTURED ON VARIOUS MEDIA

A. M. Eroshkina and V. N. Kolymykova

Division for the Study of Tumor Etiology (Head-Active Member of the AMN SSSR A. D. Timofeevskii) of the Institute of Experimental and Clinical Oncology (Director-Corresponding Member of the AMN SSSR Prof. N. N. Blokhin) of the AMN SSSR, Moscow
(Presented by Active Member of the AMN SSSR N.N. Zhukov-Verezhnikov)
Translated from Byulleten Eksperimental'noi Biologii i Meditsiny Vol. 49, No. 4, pp. 105-109, April, 1960
Original article submitted May 15, 1959

The possibility of changes in the antigenic properties of tissues as a result of heterotransplantation or of growth in tissue cultures on heterogenic media has been studied by many authors. However, the data obtained has been contradictory. Thus, Korngold and Lipari [5], in their investigation of human tumor tissues which had been preliminarily been passed through rat, hamster, and chick embryos for prolonged periods, found that human tumor tissue, successfully growing in an organism of another genus, retains its antigenic properties and does not acquire new ones characteristic of the host organism. On the other hand, V. K. Kozlov [1, 2] noted that with heterotransplantation a tumor loses its original antigenic properties and acquires new ones, characteristic of the proteins in the tissues of the new host.

In studying tissue explants, growing in vitro on heterogenic media, G. V. Suvorova [4] found that with comparatively prolonged cultivation (about 3 months) the explants showed considerable reduction in their original antigenic activity. M. S. Lomakin [3] came to the same conclusions in studying the influence of heterogenic media on the antigenic composition of cultured tissues. According to his data, in the process of cultivation tissues partially lose their initial intrinsic antigenic properties and acquire the antigenic properties of the tissues of the species of animal in whose serum they are cultivated.

The task of the present work was the study of the antigenic properties of normal tissues grown in vitro on heterogenic media. Fundamental interest was directed toward clarifying the possibility that the antigenic properties inherent in human tissues are retained in conditions involving the absence of human proteins in the nutrient medium.

METHOD

The type of material used consisted of 6-12 week old human embryos, from which tissues were taken for explantation from the extremities without the skin covering (i.e., muscular and connective tissue). The culturing was carried out over the course of 3 months in Karrel flasks using chicken plasma, and bovine embryonal extract, and serum; cultures were set up using human serum to serve as controls, while the three experimental groups were calf, rabbit, and horse respectively. The amount of serum in the fluid phase in all groups was equal to 40%. The cultures, both experimental and control, were transplanted at the same time every 14 days. The fluid phase was replaced every 7 days. Twice in each passage, 7 and 14 days after transplantation, the explants were tested for their reaction in complement fixation studies. The fixation reaction was performed with 3 guinea pig sera: 1)Serum immunized against muscle and connective tissue from the human embryo, 2) serum immunized against the muscle and connective tissue of an adult human (tissues taken from individuals who died secondary to accidental trauma), and 3) serum from healthy guinea pigs (as controls for the sera).

Immunization of the guinea pigs was carried out in the following manner. A 10% aqueous-saline extract of the corresponding tissue (embryonal or adult) was injected into the animals, using 0.5, 0.75, 1, 1.5, 2 ml at intervals of 3 to 4 days. Seven days after the last injection the guinea pigs were bled and the obtained sera tested for its reaction in complement fixation studies using the original material. In this fashion, the titer of the sera studied was 1 : 160 to 1 : 320. The active dose of complement was titered before the main experiment in the presence of the serum of healthy guinea pigs and of antigens. The complement fixation reaction was set up with 2 doses of complement. Fixation occurred in the course of 18-20 hours at + 4°.

RESULTS

In the process of cultivation the usual intensive growth of the human embryonic tissue was detected. The growth zone consisted mainly of fibroblasts. In external appearance the control cultures and the experimental one, i.e., the ones growing on the heterogenic sera, did not differ from each other. At the end of the 2nd to the beginning of the 3rd month there was noted a certain lag in the growth of the cultures being cultivated on the horse and calf sera. In the cultures growing on rabbit serum the intensity of the growth was the same as in the controls. The lag in rapidity of growth pointed out in the experi-

Data on the Complement Fixation Reaction of the Extracts from Human Embryonal Tissue Explants Grown on Various Sera

Serum	Dilution	Original material	In human serum, passages 0	1	2	3	4	5	In calf serum, passages 0	1	2	3	4	5
Serum of guinea pigs immunized against muscle and connective tissue from human embryos	10	++++	++++	+	++++	+++	++++	++++	++	++	++++	++++	++++	+++
	20	++++	+++	+	++++	+++	++++	++++	++	+	++++	++++	++++	+++
	40	++++	+++	+	+	++	+++	+++	++	±	++	—	+++	+++
	80	+++	++	+	+	++	++	+++	++	—	++	—	+	—
	160	+	+	+	+	—	+	—	—	—	—	—	—	—
	320	+	+	—	—	—	±	—	—	—	—	—	—	—
Serum of guinea pigs immunized against muscle and connective tissue of adult humans	10	++++	++++	+	++++	+++	++++	++++	+	—	++++	+++	++++	++++
	20	++++	+++	—	++++	+++	++++	++++	—	—	++++	+++	++++	++++
	40	+++	+++	—	++	++	++++	++++	—	—	++++	+++	++++	+++
	80	++	++	—	±	++	++++	++++	—	—	++++	++	+++	++
	160	+	++	—	±	+	+++	+++	—	—	—	++	++	+
	320	—	—	—	—	+	++	++	—	—	—	—	—	—
Serum of healthy pigs	10	—	—	—	—	—	—	—	—	—	—	—	—	—
	20	—	—	—	—	—	—	—	—	—	—	—	—	—
Control antigen	I dose	—	—	—	—	—	—	—	—	—	—	—	—	—
	II »	—	—	—	—	—	—	—	—	—	—	—	—	—

Note. Duration of cultivation per passage: 0) 14 days, 1) 28 days, 2) 42 days, 3) 56 days, 4) 70 days, 5) 84 days.

Data on the Complement Fixation Reaction of the Extracts from Human Embryonal Tissue Explants Grown on Various Sera (continued)

Serum	Dilution	Original material	In horse serum, passages 0	1	2	3	4	5	In rabbit serum, passages 0	1	2	3	4	5
Serum of guinea pigs immunized against muscle and connective tissue from human embryos	10	++++	+++	++++	+++	++	++	++	++	++	+	+++	++++	++++
	20	++++	+++	++++	+++	++	++	+	++	++	—	+++	++++	++++
	40	++++	++	++++	+	++	++	+	++	++	—	++	+++	+++
	80	+++	++	++++	—	++	+	±	++	—	—	—	++	—
	160	+	++	++	—	++	+	—	—	—	—	—	++	—
	320	+	+	—	—	—	—	—	—	—	—	—	—	—
Serum of guinea pigs immunized against muscle and connective tissue of adult humans	10	++++	++	++++	+	++	+	++	++	+	++++	+++	++++	++++
	20	++++	++	+++	—	+	+	++	++	—	++++	+++	+++	++++
	40	+++	++	+++	—	+	+	++	+	—	+	+	+++	++++
	80	++	—	++	—	+	—	+	+	—	+	+	+++	+++
	160	+	—	—	—	—	—	+	+	—	+	±	++	++
	320	—	—	—	—	—	—	—	—	—	—	±	++	—
Serum of healthy pigs	10	—	—	—	—	—	—	—	—	—	—	—	—	—
	20	—	—	—	—	—	—	—	—	—	—	—	—	—
Control antigen	I dose	—	—	—	—	—	—	—	—	—	—	—	—	—
	II »	—	—	—	—	—	—	—	—	—	—	—	—	—

Note. Duration of cultivation per passage: 0) 14 days, 1) 28 days, 2) 42 days, 3) 56 days, 4) 70 days, 5) 84 days.

mental groups was not very great and did not hinder the serological investigations.

In studying the complement fixation reaction of the explant extracts, both experimental and control, it was established that the human embryonal tissues retain the antigenic properties inherent to human tissues throughout all intervals in the course of cultivation, independent of whether or not there is human protein present in the nutrient medium; in addition, the antigenic activity of the explants in relation to sera immunized against human tissues does not decrease in the process of cultivation. The tissues being cultured on human sera gave complement fixation reactions with immunized sera in approximately the same dilutions as the tissues grown on heterogenic media. In prolonged intervals of cultivation there was still not noted any weakening in the antigenic activity of the experimental explants in comparison with the controls (see table).

In studying the data presented in the table on the two immunized sera, the serum against the embryonal tissue and the serum against the tissue of an adult human, differences were noted in the degree of intensity of the complement fixation reactions in those sera with the same antigens at different intervals of cultivation. If the explants of the first passages yielded complement fixation in high dilutions of the antiembryonal serum, then the complement fixation in the presence of extracts from the explants of the 4th to 5th passages (2 $^1/_2$ to 3 months in vitro) occurred even with high dilutions of the serum against the tissues of the adult human. The change in the antigenic activity of the explants in regard to these sera did not evidence a strictly regular character, but, in general, was sufficently clear. In the process of explantation the tissues altered their antigenic composition, which expressed itself by a decrease in the antigenic activity in relation to antiembryonal serum, and, on the other hand, an increase in relation to the serum against the tissues of the adult human.

The indicated changes in the antigenic properties of the cultured tissues were noted in both the control and experimental groups.

Thus, in the process of cultivation human tissues retain the antigenic properties inherent to the human, although they change somewhat, partially losing antigens characteristics of embryonal tissues and acquiring antigens characteristic of the tissues of the adult human.

The possibility of explaining the given phenomenon has still not presented itself; it serves as the subject for our further investigations. It is only possible to state seseveral hypotheses. First, the indicated phenomenon may be related to changes occurring in the embryonal tissues during its comparatively prolonged cultivation. Second, it is not here possible to exclude the influence of the nutrient media, in particular the serum, which was taken from adult animals and naturally had a somewhat different protein composition than the serum of the embryos.

SUMMARY

The human antigenic properties are retained by the human embryonic muscular and connective tissues in culturing for the period of 3 months in homo- or heterogenic medium. The antigenic propertes of these tissues are changed during the process of culturing: There is a gradual weakening of the properties inherent to the embryonic tissues, with the intensification of these characteristic of the muscular and connective tissues of adult.

LITERATURE CITED

[1] V. K. Kozlov, Byull. Éksper. Biol. i Med. 37, 5, 55 (1954).

[2] V. K. Kozlov, Byull. Éksper. Biol. i Med. 37, 4, 56 (1954).

[3] M. S. Lomakin, Byull. Éksper. Biol. i Med. 36, 6, 65 (1953).

[4] G. V. Suvorova, Byull. Éksper. Biol. i Med. 43, 1, 82 (1957).*

[5] L. Korngold and R. Lipari, Cancer. Res. 15, 159 (1955).

*Original Russian pagination. See C.B. translation.