THE EFFECT OF THE TITER OF IMMUNE ANTIBODIES ON THE GROWTH OF TUMOR AND NORMAL TISSUES IN VITRO

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There is a relatively large number of experimental investigations devoted to the question of the action of immune sera on tissue growth in vitro. However, the data furnished by these studies are very conflicting. Thus, R. Ludford [11], H. Phelps [14] and others deny specificity of action of antitumor sera in vitro. M. S. Lomakin [2], A. D. Timofeevskii [6], P. P. Filatov [7], K. Fellinger [8], D. Imagava [9], D. T. Imagava, J. T. Syverton, J. J. Bittner [10], T. Lumsden [12], T. Lumsden, T. F. Macrae, E. Skipper [13], K. Takeda [15] and others on the contrary consider that immune sera can suppress the growth of tumor cells in tissue cultures. Unfortunately the majority of the authors cited do not give precise information concerning the titer of the sera used by them.

With the data of our previous work [4], [5] on the inverse relationship of intensity of tissue growth in vitro and the level of the titer of normal nutrient medium antibodies in mind, we undertook to establish in the present work, begun as far back as 1954, the importance of the titer value and specificity of immune antibodies for the growth of tumor and normal tissues in vitro.

EXPERIMENTAL METHOD

White mouse spleen served as a specimen of normal tissue. Tumor tissue was that of the subcutaneous form of Ehrlich's adenocarcinoma. The tumor and normal tissues were explanted in Carrel dishes. Into each dish were placed 4-5 explants measuring approximately 1 mm^3.

The nutrient media used included cock plasma, chick embryo extract, Tyrode solution and rabbit serum, normal or immune, of different titer and specificity: antitumor sera with antibody titer of 1:20 +++, 1:40++(+), 1:640+++, 1:800+++; antispleen (white mouse) sera with antibody content of 1:40+++, 1:400++, 1:800 +++ and 1:800+++(+); antiliver (white mouse) sera with titer of 1:20++, 1:40++, 1:360+++, 1:400+++. The antibody titer values cited here are given only with respect to specific antigens, since these sera reacted with other non-specific antigens giving a lower titer. The Ehrlich adenocarcinoma explant and that of white mouse spleen were cultured simultaneously in normal and all the immune sera mentioned above.

A total of 8 series of experiments was carried out; the number of explants in separate series varied from 235 to 347. The total number of explants reached 2475. All the dishes containing explants were subjected to microscopic examination on the 2nd, 4th, 6th, 8th, 10th and 14th day from the start of culturing. On the 6th, 10th and 14th day drawings were made (with the help of the drawing apparatus "RA") of the fragments and their zones of growth, and the ratio of the area of growth zone to the area of the tissue fragment was calculated according to the method described in our previous communication [4].

TABLE 1

Intensity of Growth of Ehrlich Adenocarcinoma Tissue on the 10th Day of Culturing

No. of series	Total no. of explants	No. of explants in each series	Ratio of area of growth zone and area of Ehrlich adenocarcinoma explant						
			sera						
			anti-Ehrlich's adenocarcinoma		antispleen (white mouse)		antihepatic (white mouse)		Normal non-immune
			serum titer						
			1:20—1:40	1:640—1:800	1:40	1:800	1:20—1:40	1:360—1:400	
			experimental group						
			I	II	III	IV	V	VI	VII
1	1284	136	6.76	1.57	6.65	2.49	5.92	6.12	5.93
2		173	5.99	1.21	5.73	1.78	6.41	5.82	6.06
3		170	6.83	1.65	6.17	2.01	6.12	6.45	6.25
4		158	6.44	1.40	6.36	2.51	5.78	6.19	5.73
5		172	6.0	1.42	5.77	1.89	6.01	6.07	6.73
6		170	6.96	1.17	6.20	2.37	5.80	5.95	6.21
7		164	6.69	1.74	6.63	2.12	6.22	5.68	5.87
8		141	6.89	1.20	6.69	2.63	5.69	5.98	6.01
		Mean	6.57	1.42	6.27	2.22	5.99	6.03	6.09

Groups I and VII P = 0.014, II and VII P = ∞, IV and VII P = ∞, III and VII, V and VII, VI and VII P not significant.

EXPERIMENTAL RESULTS

Observations on the growth of explants during the time intervals mentioned revealed the following consistent features: signs of growth appeared more rapidly (on the 2nd-4th day) in the case of tumor and normal tissue explants cultured in media with normal, antisera and with sera with a low titer of antitumor (1:20-1:40) and antispleen (1:40) antibodies; when Ehrlich's adenocarcinoma and white mouse spleen tissue were cultured in nutrient media with a high content of antitumor and antispleen antibodies the sign of growth appeared later (4th-6th-8th day).

Since the numerical material collected is vast, only the mean data for each series of experiments, obtained on examination of the preparations on the 10th day of culturing, are given. The data obtained on the 6th-14th day of tissue growth in vitro did not differ substantially from the data obtained on the 10th day.

Table 1 shows the mean ratios of the area of the growth zone to the area of Ehrlich's adenocarcinoma explants in separate series of experiments determined on the 10th day of culturing the tissue in nutrient media containing normal and immune antitumor, antispleen and antihepatic sera of various titers.

As can be seen from Table 1 the same intensity of growth of adenocarcinoma explants was observed when they were cultured in nutrient media containing normal sera, antihepatic sera of low and high titer and antispleen sera of low antibody titer (1:40).

When tumor tissue was explanted on specific antitumor antiserum of low titer (1:20-1:40) some increase of growth was noted in the cultured tissue as compared with the control. When Ehrlich's adenocarcinoma was cultured in antisera with a high titer of antitumor (1:640-1:800) or antispleen (1:800) antibodies the intensity of growth of the explants was markedly lower than in the case of controls (a 4.2-2.7 fold decline).

Data shown in Table 1 were treated statistically by the Fischer-Student method. It was found that the difference between the action of normal serum (control) and anti-Ehrlich's adenocarcinoma sera (titer 1:20-1:40

TABLE 2

Intensity of Growth of White Mouse Spleen Tissue on the 10th Day of Culturing

No. of series	Total no. of explants	No. of explants in each series	Ratio of area of growth zone and area of spleen explant						
			sera						
			antispleen (white mouse)		anti-Ehrlich's adenocarcinoma		antihepatic (white mouse)		Normal nonimmunized
			1 : 40	1 : 800	1:20—1 : 40	1:640—1:800	1: 20—1 : 40	1:360—1:400	
			groups of experiments						
			I	II	III	IV	V	VI	VII
1	1191	99	9.99	1.1	10.7	2.06	—	—	9.7
2		174	8.05	0.92	8.61	3.18	9.47	8.21	7.38
3		153	10.08	1.38	7.99	2.90	8.75	8.14	10.74
4		142	9.47	0.6	8.85	3.80	9.20	8.07	8.73
5		141	10.56	1.07	10.11	3.36	10.61	11.08	9.05
6		154	9.75	1.2	9.45	3.96	8.99	9.40	10.30
7		173	9.95	1.49	9.97	3.98	8.29	8.58	8.99
8		153	8.67	1.13	7.51	1.38	8.83	10.01	10.56
		Mean	9.56	1.11	9.07	3.37	9.16	9.21	9.43

Groups II and VII P = ∞, Groups IV and VII P = ∞, Groups I and VII, III and VII, V and VII, VI and VII P not significant.

and 1:640-1:800) and antispleen (white mouse) serum (titer 1:800) was undoubtedly statistically significant (P = 0.014 and P = ∞). Data obtained on comparison of the same control with the result of the action of antihepatic sera and antispleen (white mouse) serum (titer 1:40) were shown to be statistically nonsignificant (P > 0.05).

Table 2 presents data obtained in separate series of experiments on culturing white mouse spleen tissue for 10 days on nutrient media containing antispleen, antitumor and antihepatic sera of different titer, and also sera of nonimmune rabbits.

It follows from Table 2 that the rate of growth of spleen tissue (white mouse)in vitro in the presence of antihepatic sera of low and high titers, as well as of antispleen and antitumor sera with a low content of specific antibodies (1:20-1:40) is almost the same as the rate of growth of the same tissue in normal sera, used as controls. The differences in mean coefficients in these groups compared with control is found to be statistically nonsignificant when the material is treated by the Fischer-Student method (P > 0.05). When spleen tissue is cultured on nutrient media containing antispleen antisera with a high antibody titer (1:800) a marked growth-inhibiting effect was observed (8.4-fold lowering of the ratios as compared with the control). Explantation of spleen tissue in the presence of antitumor antibodies with titers of 1:640 and 1:800 also caused a reduction in the rate of growth (2.8-fold). The differences between the data of these groups and the control are statistically significant (P = ∞).

Since in all the series of experiments all the ingredients, with the exception of the sera, in the nutrient media were identical, the results obtained suggest a definite relationship between the titer of specific immune antibodies in the nutrient medium and the rate of growth of explants of both tumor and normal tissues. Thus, sera with a high titer of specific antibodies delay the appearance of the manifestations of growth and lower the intensity of growth in explants of the corresponding tissues. Sera with a low titer of antibodies exert no inhibiting effect on the growth of tissues in vitro. Antitumor sera with a low content of specific antibodies even exerted a growth-stimulating effect on Ehrlich's adenocracinoma under our experimental conditions. It must also be taken into account that when there is similarity of antigen structure of the cultured tumor and normal tissues (e. g. tissue of Ehrlich's adenocarcinoma and white mouse spleen [1, 3]) antisera immune with respect to them and possessing a sufficiently high antibody titer may exert a growth inhibiting cross effect on the tissues being cultured.

SUMMARY

The tissue of subcutaneous form of Ehrlich's adenocarcinoma (explant 1284) and the spleen of a white mouse (explant 1191) were cultured in vitro on nutrient media containing normal or immune antitumor, antisplenic and antihepatic sera of low and high titers. The results of this work demonstrate that sera with high titer of specific antibodies retard the appearance of manifestations of growth and affect its intensity in the explants of corresponding tissues. Low titer sera do not possess these properties. On the contrary, antitumor sera with a low content of specific antibodies have a growth stimulating effect on the tissues of Ehrlich's adenocarcinoma.

LITERATURE CITED

[1] V. S. Korosteleva, Problems of Immunology of Normal and Malignant Tissues,* Moscow, pp. 94-104 (1956).

[2] M. S. Lomakin, Biull. Ekptl. Biol. i Med., No. 9, pp. 40-44 (1956).**

[3] I. N. Maiskii, The Biologic Basis of Anticancer Immunity,* Moscow, pp. 27 (1955).

[4] G. V. Suvorova, Biull. Eksptl. Biol. i Med., No. 11, pp. 58-61 (1953).

[5] G. V. Suvorova, Ibid., No. 12, 62-64 (1953).

[6] A. D. Timofeevskii, Explantation of Human Tumors,* Moscow, p. 136 (1947).

[7] P. P. Filatov, "Antimetastatic Action of Immune Antitumor Sera Under Experimental Conditions",* Thesis, Moscow (1956); Thesis, Moscow (1957).

[8] K. Fellinger, Arch. exper. Zellforsch., Bd. 13, S. 310-319 (1933).

[9] D. Imagava, Proc. Am. Ass. Canc. Res. v. 1, p. 26 (1953).

[10] D. T. Imagava, J. T. Syverton and J. Bittner, J, Cancer Res. v. 14, pp. 8-11 (1954).

[11] R. Ludford, J. Arch. exper. Zellforsch. Bd. 14, S. 42-55 (1933).

[12] T. Lumsden, Am. J. Canc. v. 15, pp. 563-640 (1931).

[13] T. Lumsden, T. F. Macrae and E. Skipper, J. Path. a. Bact. v. 39, pp. 595-607 (1934).

[14] H. J. Phelps, Am. J. Canc. v. 31, pp. 441-445 (1937).

[15] K. Takeda, Gann. Japan J. Res. v. 46, N. 4, pp. 567-584 (1955).

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