ONCOLOGY

AGGLUTINATION METHOD FOR DETERMINING THE ANTIGENIC SPECIFICITY OF CANCER CELLS

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The reactions of binding of the complement, anaphylaxis, and precipitation are widely used in studies of the antigenic specificity of malignant tumours. The reaction of agglutination has not been applied to such studies. Some authors [3] have attempted to apply it to demonstration of antigenic properties of normal tissues, but this method has not received practical application to studies of this sort.

In our earlier studies [1] of the antiblastic properties of anticancer serum we observed a marked agglutination of Ehrlich ascites tumour cells when these were mixed with the corresponding antiserum. This agglutination was found to be highly specific.

Following from this observation, we commenced work on the elaboration of an agglutination reaction applicable to the determination of antigenic specificity of cancer cells. We describe this method in the present paper, and also give the experimental data concerning the possibilities of this reaction for revealing differences in the antigenic properties of cancerous and normal cells.

Preparation of Cell Suspensions for the Reaction

We used Ehrlich mouse ascites carcinoma cells as our experimental material. We found that freshly prepared suspensions gave the best results, but it is possible, if necessary, to store the material at 4-6° for about 2 days.

In order to avoid clotting and clumping of the ascites fluid we diluted it with 3-5 volumes of physiological saline, centrifuged for a short time, and rejected the supernatant. The cells were resuspended in saline and again centrifuged, and the washing was continued until the supernatant fluid was clear. A homogeneous suspension of tumor cells in saline was then prepared. The optimum concentration of cells was found to correspond with a bacterial standard suspension of 1.5-2 billion.

As controls, we took erythrocytes and cells from the spleen of the same mice from which ascites fluid had been taken; the erythrocytes were washed 3 times before being taken for the agglutination reaction.

Spleen cells were obtained as follows: the spleen was thoroughly comminuted by cutting up with scissors, shaken with 5-7 ml of physiological saline, and the suspension was filtered through 8 thicknesses of muslin. The spleen cell suspension was then further treated to free it of erythrocytes, which is readily achieved by centrifuging for a short time, or by allowing it to settle in a test tube for 30-40 minutes. By either method the spleen cells are in the sediment, while the red cells remain in suspension, and can easily be removed by careful pipetting. This procedure is twice repeated, after which the deposit is practically free of erythrocytes. The spleen cells thus isolated show the morphology of lymphoid spleen tissue, and smears stained with hematoxylin and eosin show isolated round cells of different sizes with deeply stained polymorphic nuclei.

The spleen cell and erythrocyte suspensions were diluted with saline, in the same proportions as the cancer cells, for the agglutination reaction.

Antiserums

Anticancer cell serums were prepared by immunizing rabbits against antigen prepared from Ehrlich ascites tumor cells. These were first washed free of erythrocytes and leucocytes, and were then thoroughly ground up in a mortar, suspended in 15 volumes of saline, and centrifuged for 10 minutes at 2500 r.p.m. The supernatant was used as the antigen for immunizing rabbits.

The antigen was injected intravenously in doses of 1.5-2 ml (5-6 doses) at 3-day intervals, and serum was taken from the rabbits on the 8-10th day after the last injection. Antiserums to spleen cells were prepared analogously, by imminizing rabbits to mouse spleen cells. Only those antiserums were taken for the agglutination reaction which had approximately the same titer of complement-fixing antibodies relative to the corresponding antigen. Both native and adsorbed antiserums were tested in the agglutination reactions.

The antiserums were adsorbed on both formalin treated * and fresh normal mouse tissues. To 1 volume of comminuted and thoroughly washed mouse liver tissue were added 3-4 volumes of antiserum, previously diluted tenfold. The suspension was stirred, and kept for 1-1.5 hours at room temperature, at 37°, or at 4-6°; the incubation time was increased at the lowest temperature. The suspensions were then centrifuged, and the serums were taken for the agglutination reaction.

Agglutination Reactions

The above-described antiserums (native and adsorbed) were taken at dilutions of from 1:10 to 1:320. Three drops (0.1 ml) of diluted antiserum were placed into three rows of test-tubes (diameter 9-10 mm), and the same volume of cancer cell, erythrocyte, or spleen cell suspension was added to each of the rows of tubes; the dilution of the antiserum was therby doubled, and this was taken into account in assessing the results. The systems were then shaken and allowed to stand at constant temperature. Definite agglutionation is usually evident within 1.5-2 hours.

We found that the reaction was most sensitive at 4-6°, with an incubation time of 15-20 hours. Each tube was shaken gently by hand, and the degree of agglutination was assessed in the usual way. We tested the agglutinating power of 15 Ehrlich adenocarcinoma antiserums and of 6 spleen cell antiserums, prepared as described above, in our experiments on antigenic specificity of cancer cells. The agglutination experiments were set up in duplicate or triplicate, and at least one spleen cell antiserum was included in each cancer cell experiment, both native and adsorbed spleen cell antisera being taken.

Since uniform results were obtained in all the agglutination experiments we give here the results of two representative series of experiments only (Table 1 and 2).

TABLE 1

Agglutinationg Power of Mouse Adenocarcinoma Antiserum No. 7.

Dilution of serum	Native serums			Adsorbed serums		
	cells taken for test					
	tumor	erythrocytes	spleen	tumor	erythrocytes	spleen
1:20	++++	++++	++++	++++	—	—
1:40	++++	++++	++++	++++	—	—
1:80	++++	++++	++++	++++	—	—
1:160	++++	±	+	+++	—	—
1:320	++++	—	—	++	—	—
1:640	+++	—	—	±	—	—

*This method was elaborated by P. N. Kosyakov, together with V. S. Korostelova and N. I. Kuznetsova [2].

TABLE 2

Agglutinating Power of Spleen Cell Antiserum No. 34.

Dilution of serum	Native serums			Adsorbed serums		
	cells taken for test					
	tumor	erythrocytes	spleen	tumor	erythrocytes	spleen
1:20	++++	++++	++++	—	++	+++
1:40	++++	++++	++++	—	±	+++
1:80	+++	++++	++++	—	—	++
1:160	++	+++	++++	—	—	±
1:320	±	++	+++	—	—	—
1:640	—	±	+	—	—	—

It is evident from these Tables that the agglutination reactions are sufficiently specific, even for antiserums not treated by adsorption. The untreated anticarcinoma serum agglutinates tumor cells in much higher dilution (1:640) than for spleen cells and erythrocytes (1:80). Conversely, mouse spleen cell antiserums (unadsorbed) agglutinate spleen cells and erythrocytes at much higher dilutions (1:320) than tumor cells (1:80).

The agglutination reactions become still more highly specific when the antiserums are treated by adsorption. Adsorption-treated anticancer serum in high dilution (1:320) agglutinates only tumor cells; spleen cells and erythrocytes not being agglutinated by it even at the lowest dilutions (1:20). Similarly, anti-spleen cell serums do not, after adsorption treatment, have any agglutinating action on tumor cells, although spleen cells and erythrocytes are still agglutinated, although at a lower titer than with untreated serum.

Our experimental results are evidence of the sufficiently high degree of specificity and sensitivity of our technique, which allows of the differentiation of the antigenic specificity of normal and cancer cells. This method is, together with other serological methods, fully applicable to the study of the antigenic specificity of cancer cells, and may serve as an additional method for the study of the immunology of cancer.

LITERATURE CITED

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