THE STUDY OF THE ANTIGENIC PROPERTIES OF CELLS OF THE HeLa STRAIN USING THE AGGLUTINATION REACTION

(UDC 578.085.23)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 57, No. 6, pp. 73-75, June, 1964
Original article submitted March 15, 1963

The method of monolayer cultures has been applied widely in various fields of experimental biology and medicine. However, the nature of the cells of these cultures has not yet been explained [5]. In particular, insufficient attention has been paid to the study of the antigenic composition of the cells of human cultures, and the results which have been obtained are conflicting [8,9,11-13].

The agglutination reaction has recently been used successfully to determine the antigenic structures of the species and carcinoma specificity of the cells of mouse tumors [4,6,7,10]. When the same method is used to investigate the cells of monolayer cultures of human tissues, in addition it is possible to study the group (A,B,O), type (M,N) and other antigens found in human tissues.

We used the agglutination reaction to analyze the antigenic composition of the surface of the cells of a culture of the HeLa strain. This culture was obtained by Hayem originally in 1951 from the tissue of a squamous-cell carcinoma of the cervix uteri of a woman (with blood group 0), and is very widely used at the present time for investigations of various types [1-3,5].

METHOD

In setting up the experiments on agglutination of the cells of human monolayer cultures we used a modification of the method proposed by M. M. Kapichnikov [4].

For each reaction suspensions of cells of strain cultures of HeLa or human amnion or fibroblasts were prepared in Hanks's solution. The cultures of these strains were grown in Roux Flasks for 7-10 days (in medium 199 with 10% calf serum), after which they were removed from the glass with a 0.02% versene solution; the resulting suspension was centrifuged for 5-10 min at 500-1000 rpm, and the cell residue was then diluted with Hanks's solution to a concentration of 0.5×10^7 cells/ml. In the control series a 2-3% suspension of human erythrocytes of group A, B, O and type M and N was used.

The experiments were carried out in agglutination tubes with different antisera in dilutions of between 1:4 and 1:512. Forensic medical sera against antigens A, B, O, M, N, and Rh were used (immune rabbit sera and also human sera α and β were taken. Additionally, by means of immunization of rabbits, antisera were obtained against cells of the strain cultures of HeLa, fibroblasts, and amnion cells. Immunization of 9 rabbits was carried out by the usual scheme [4,6]. Altogether the animals received 8 intravenous injections of 10^7 cells of the corresponding cultures each, and on the 6th day after the last injection the animals were given antisera.

Antisera agglutinating HeLa cells to dilutions of 1:128-1:256 and amnion cells and fibroblasts to dilutions of 1:64-1:128 were chosen for analysis.

For control purposes a serum was used which was taken from the rabbits before immunization, in ordinary doses (approximately 0.15 ml), and to it were added suspensions of HeLa cells, amnion cells, fibroblasts, and erythrocytes in the standard dose (approximately 0.05 ml).

Results of the Study of Cells of Human Monolayer Cultures in the Agglutination Reaction

Sera used	Cell suspensions			Human erythrocytes
	Strains of cultures			
	HeLa	Amnion	Fibroblasts	
Sera against cells of monolayer cultures of:				
HeLa	1:128-1:256	1:64-1:128	1:32-1:64	1:128-1:256
Amnion	1:32-1:64	1:64-1:128	1:32-1:64	–
Fibroblasts	1:32-1:64	1:32-1:64	1:64-1:128	–
Hemagglutinating sera against antigens				
A	1:4-1:16	–	–	–
B	1:4-1:16	–	–	–
O (H)	1:8-1:32	–	–	–
M	1:4-1:16	–	–	–
N	1:4-1:16	–	–	–
Normal rabbit serum (control)	1:8-1:16	1:8-1:16	1:8-1:16	1:8

Note. The numbers denote titers of agglutination of cells by corresponding antisera, the lines – no reaction performed.

The results of the experiments were most often read after exposure of the samples for 24-48 h at 4°. The main index used was the titers, i.e., the maximal dilutions of the sera in which they still reacted with the corresponding cells.

RESULTS

A preliminary series of experiments was carried out in order to develop a method of optimal agglutination of the cells of the monolayer cultures. It was found that incubation at 37° for 20 min-2 h leads to a marked increase in the nonspecific agglutination of these cells. The use of physiological saline, and also centrifugation at over 1000 rpm, are highly undesirable because of the lack of resistance of the culture cells. The most definite results were obtained by the use of cultures grown for 5-7 days in Roux flasks seeded with cells in a dose of 0.5×10^7. By the discovery of these conditions it was possible to adopt the method described and to obtain the reproducible results given in the table.

The strains HeLa, amnion, and fibroblasts, in cross experiments with three antisera, revealed a fairly similar ability to agglutinate at titers of sera down to 1:64-1:256. In the control series normal rabbit serum gave nonspecific reactions in a titer of 1:8-1:16.

Hemagglutinating sera against antigens A, B, O, M, N, and Rh caused agglutination in titers close to the control values (1:4-1:16), disregarding a certain tendency for the reaction to be intensified with antisera against antigens O(H) (to a titer of 1:32). A most interesting fact from our point of view was that human erythrocytes, irrespective of whether they contained antigens A, B, O, M, or N, were agglutinated by serum against HeLa cells in a fairly high titer (1:128-1:256), whereas in a control test with untreated rabbit serum agglutination was observed only in a titer of 1:8-1:16.

It may be concluded from these results that cells of HeLa cultures, despite their very long period of cultivation, preserved certain antigens in common with human erythrocytes and with the cells of other human cultures. There is every reason to suppose that it is these antigenic structures which determine the species specificity of the human cells and tissues. Meanwhile, in the cells of the HeLa strain, it was not possible to detect antigens A, B, O (H), M, N, or Rh, although there are reports in the literature of the presence of O and Rh antigens in this culture [12]. To obtain a final explanation of the last problem, further investigations are required, using more sensitive methods. These experiments shed light on the nature of the variability of human cells in monolayer cultures.

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