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Blood Group Antigens on HeLa Cells shown by Mixed Agglutination

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Summary. The mixed agglutination reaction has been used for investigating the presence of blood group antigens on the surface of human cervical carcinoma cells (HeLa) cultured for eight years *in vitro*.

The H antigen was demonstrated in the absence of A and B. The MN-type antigen has been found as well as Tj^a.

Treatment of HeLa cells with ficin greatly enhanced the reaction of anti-H and anti-Tj^a with the corresponding antigens on HeLa cells. The authors failed to show the following antigens: Rh(D) and Rh(c), S, P, Le^a, Le^b, Lu^a and Lu^b.

INTRODUCTION

THE mixed agglutination reaction (Coombs and Bedford, 1955) has been applied to a preliminary study of the isoantigens on human cervical carcinoma cells (HeLa). This paper records the results of the tests on HeLa cells for the blood group antigens belonging to the systems: ABO, MNS, Rh, P and Jay, Kell, Lewis and Lutheran.

Mixed agglutination of human erythrocytes and HeLa cells can be shown only if both the red cells with known antigens and the HeLa cells possess a common antigen.

The mixed agglutination reaction was previously adapted by Coombs, Bedford and Rouillard (1956) to an investigation of the ABO isoantigens on normal human epidermal cells. It has also been successfully used by Gurner and Coombs (1958) for demonstrating the presence of blood group antigens on human leucocytes.

MATERIALS AND METHODS

GLASSWARE

All glassware was treated with silicone.

DILUENT

Normal rabbit serum absorbed twice with six times washed human O and A red cells, and diluted 1:200 in 0·9 per cent NaCl solution (buffered at *p*H 7·2 with Sørensen's phosphate buffer at a final molarity of 1:1500) was used as the diluent.

HUMAN RED CELLS

Human red cells were obtained from an ear prick into 0·6 per cent tri-sodium citrate in 0·9 per cent saline, centrifuged, washed twice in diluent, and finally resuspended to a 0·5 per cent suspension in diluent.

SERA

All sera were stored at −20° and thawed before use. They were inactivated by heating in a water-bath at 56° for 30 min. In the experiments thirty-two different sera have been used. The species origin and agglutinin titre of the different sera are given in Table 1.

TABLE 1

ANTISERA AND PLANT EXTRACTS USED IN MIXED ERYTHROCYTE—HELA CELL AGGLUTINATION

Antigen	*Agglutinin*		*Result*	
	Source	*Titre*	*Untreated HeLa cells*	*Ficin treated HeLa cells*
A	Natural human anti-A (727)	256	—	—
	Human 'immune' anti-A (1353)	16,000	—	—
B	Natural human anti-B (679)	128	—	—
	Human immune anti-B (2934/25)	16*	—	—
H	*Ulex europeus* seed extract	32	w	+++
	Eel anti-H (P_{42})	4	—	—
	Human anti-H (Marr.)	2	—	—
M	Rabbit anti-M (144)	1000	+++	+++
	Human anti-M (Sm.)	2	—	—
	,, ,, (Sh. 1325)	16	—	—
	,, ,, (Ha.)	2	—	—
	,, ,, (Bl.)	2	—	—
	,, ,, (Ver.)	2	—	—
N	Rabbit anti-N	8	w	w
	Vicia graminea seed extract	8	++	++
	Human anti-N (Mar.)	2	—	—
S	Human anti-S (Gram.)	4	—	—
Rh (D)	Human anti-D (Br.)	32	—	—
	,, ,, (Wo.)	>512	—	—
Rh (c)	Human anti-c (Mar.)	>64	—	—
P	Human anti-P (Flan.)	4	—	—
	,, ,, (Brun.)	8	—	—
Tj^a	Human anti-Tj^a (El.)	8	w	+++
K	Human anti-k (Co.)	4	—	—
Le^a	Human anti-Le^a (Bu.)	4	—	—
	,, ,, (Pu.)	4	—	—
Le^b	Human anti-Le^b (Bo.)	2	—	—
Lu^a	Human anti-Lu^a (Ran.)	4	—	—
Lu^b	Human anti-Lu^b (Rob.)	4	—	—

+++ Strong mixed agglutination
w Weak mixed agglutination
— No mixed agglutination
* Titre by indirect sensitization test 1000.

PLANT AGGLUTININS

Water extracts from the seeds of *Ulex europeus* and *Vicia graminea* (Cazal and Lalaurie, 1952; Ottensooser and Silberschmidt, 1953) were obtained in the following way:

1. The seeds were soaked in distilled water for 18 hours at room temperature.
2. The water was removed and the seeds macerated in a mortar; 1 ml. distilled water per 50 mg. dry seeds was added and the mixture left for 1 hour at room temperature.
3. The mixture was centrifuged at 6000 r.p.m. for 15 min.
4. The clear supernatant fluid was used for experiments after adding 9 mg. NaCl per 1 ml. of extract.

HELA CELLS

The strain of HeLa cells (Gey, Coffman and Kubicek, 1952; see also Scherer, Syverton and Gey, 1953) was originally obtained by Gey *et al.* as an *in vitro* culture on February 8, 1951 from a cervical carcinoma of a patient. The cells have been grown for nearly eight years in many laboratories.

The HeLa cells used in the present experiments were cultured in 200 ml. babies' feeding bottles on the growth medium described by Newton and Stoker (1958). The medium contained 20 per cent human serum.

The HeLa cell suspension was made from a 2 or 3 days old culture. The medium was first discarded and the cell sheet washed three times with 10 ml. of phosphate-buffered saline (PBS) free from Ca^{++} and Mg^{++}. Then 4 ml. of sodium Versenate (Na ethylenediaminetetraacetate) diluted 1:20,000 in PBS were added and the culture kept at 37° C. in an incubator for 20 min. The cell suspension was transferred to a tube centrifuged at 1000 rev./min. for 5 min. and washed once in diluent. Then the cells were resuspended in diluent to give approximately 4×10^6 cells per ml.

FICIN TREATMENT OF HELA CELLS

Ficin solution was prepared according to the following method (see also Haber and Rosenfield, 1957):

50 mg. of Light's ficin were dissolved in 5 ml. of Sørensen's $\frac{M}{15}$ phosphate buffer (*p*H 7·2). The 1 per cent stock solution was stored at −20° C. For use 0·1 per cent ficin in 0·9 per cent buffered saline (NaCl) was prepared.

A volume of HeLa cell suspension (4×10^6 cells per ml.) was mixed with equal volume of 0·1 per cent ficin solution and incubated at 37° C. for 30 min., with gentle shaking every 5 min. to break up the initial clumps of cells.

The cells were then deposited by centrifugation (1000 rev./min. for 5 min.), washed twice in diluent and resuspended in diluent to give 2×10^6 cells per ml.

MIXED AGGLUTINATION TEST

0·1 ml. of a HeLa cell suspension (2×10^6 cells per ml.) was centrifuged and the supernatant fluid removed. To the deposit 0·1 ml. of antiserum or seed extract was added. The tubes were then shaken and put on a slowly rotating machine at 18° C. for 1 hour.

The treated HeLa cells were centrifuged at 1000 rev./min. for 5 min., washed three times with diluent, resuspended in 0·1 ml. diluent, and then an equal volume of a 0·5 per cent suspension of red cells of appropriate group was added. The mixture was centrifuged at 1500 rev./min. for 2 min., the deposited cells taken up with a little supernatant fluid transferred to a glass 3×1 microscope slide, a coverslip laid gently on top and sealed with wax.

All slides were then examined under a phase-contrast microscope using ×100 and ×400 magnifications. Mixed agglutination of human erythrocytes and HeLa cells is illustrated in Fig. 1, and a negative result is shown in Fig. 2.

RESULTS

ABH ANTIGENS

When the naturally occurring iso-agglutinin anti-A and anti-B or human so-called 'immune' anti-A and anti-B were used no positive mixed agglutination was observed with untreated and ficin treated HeLa cells. Also when human anti-H serum and an eel anti-H serum were used no reaction with HeLa cells could be obtained. However, with the Ulex extract a weak agglutination was seen between the HeLa cells and group O red cells. After treatment of the HeLa cells with ficin the Ulex extract brought about a strong mixed agglutination with group O red cells; no reaction being obtained with AB red cells.

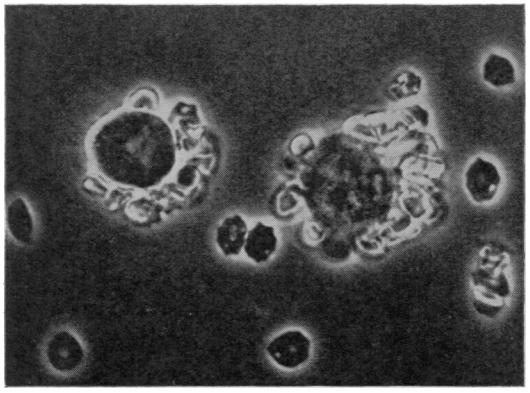

FIG. 1. Mixed agglutination reaction of HeLa cells and human erythrocytes: positive result. HeLa cells exposed to rabbit anti-M serum, washed and human M red cells subsequently added. (Phase-contrast microscopy × 10 eye-piece × 40 dry objective.)

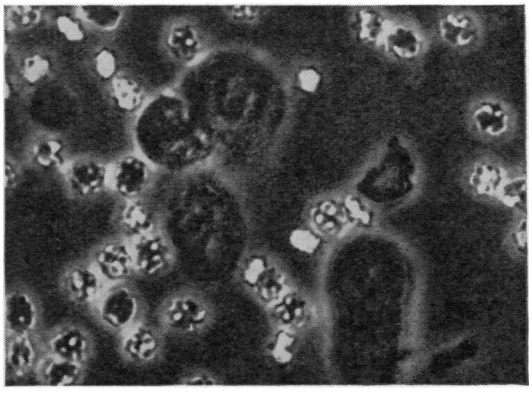

FIG. 2. Mixed agglutination reaction of HeLa cells and human erythrocytes: negative result. HeLa cells exposed to human anti-A serum, washed and human A red cells subsequently added. (Phase-contrast microscopy × 10 eye-piece × 40 dry objective.)

MNS ANTIGENS

A strong rabbit anti-M serum (see Table 1) induced positive mixed agglutination of untreated HeLa cells as well as of treated cells and M erythrocytes. The mixed agglutination could be shown with this particular serum diluted 1:100. No cross-reaction was observed in the mixed agglutination test with N red cells and this anti-M serum diluted 1:2.

With the rabbit anti-N serum, which was not very strong (titre 8), a weak mixed agglutination of HeLa cells and N erythrocytes could be observed.

A positive mixed agglutination was obtained also with N cells using anti-N agglutinin from Vicia seeds; the control with M red cells was negative.

No reaction was observed with five human anti-M sera, one human anti-N and an anti-S serum on untreated or ficin treated HeLa cells. The agglutinin titres of these sera were not very high.

RH ANTIGENS

No mixed agglutination could be shown between HeLa cells and erythrocytes using two samples of anti-D or an anti-c serum. Previous treatment of HeLa cells with ficin did not reveal any masked antigen sites.

P AND JAY ANTIGENS

A very weak erythrocyte-HeLa cell mixed agglutination was observed when anti-Tj^a serum was used. The reaction could be greatly enhanced by previous treatment of the HeLa cells with ficin. Unfortunately, the red cells of Tj^bTj^b genotype were not available to control the specificity of the reaction.

Tests with human anti-P sera, which were approximately as strong in an agglutination test as the anti-Tj^a serum, did not give any positive reaction of untreated or ficin treated HeLa cells.

KELL ANTIGEN

No reaction could be obtained with an anti-K serum and untreated or ficin treated HeLa cells.

LEWIS AND LUTHERAN ANTIGENS

Using heated anti-Le^a, anti-Le^b, anti-Lu^a and anti-Lu^b no unequivocal reaction has been shown with untreated or ficin treated HeLa cells.

With unheated sera a weak mixed clumping resulted, but the reaction could not be referred to the specific iso-antigens on human red cells. With unheated sera the second stage of the mixed agglutination reaction lacks specificity. The tests thus afforded no evidence that antigens of the Lewis and Lutheran systems were present on HeLa cells.

The results of all experiments are summarised in Table 1.

DISCUSSION

The finding of antigens representative of the ABO, MN and Jay blood group systems is in line with what has been found using the *Mixed Agglutination Reaction* on human leucocytes and platelets. The absence of the A and B antigens in the presence of the H antigen is in keeping with the information which we have received from Dr. G. O. Gey (1958) of the Johns Hopkins Hospital that Mrs. 'HeLa' was of blood type O, Rh positive.

Further attempts are being made to demonstrate other individuality antigens on these cells. One is encouraged in this endeavour by the reports of Dausset, Colombani and Evelin (1958), and Moulinier and Servantie (1958), who claim to have shown the Rh(D) antigen to be present on leucocytes using the antiglobulin consumption test. In this connection also it should be mentioned that Chalmers, Coombs, Gurner and Dausset (1959) have shown that leucocyte iso-antibodies, as yet uncharacterized, react strongly with HeLa cells.

The detection of 'marker' antigens on tissue cells of the body and such cells in culture is of importance for many reasons. One may instance the recognition and identification of cells growing in tissue culture and characterizing mutations and transformations. Again, the recent concepts as to the biological changes in cells accompanying malignancy stress the importance of documenting as many 'marker' antigens as possible in order that the deletion of any one may be detected. In this connection Kay (1957) has recently reported that the A antigen is much less easy to detect by the *Mixed Agglutination Reaction* and other methods in carcinoma cells of the human urinary tract than in the normal epithelium of this system.

The *Mixed Agglutination Reaction* would thus be a useful adjunct in a reinvestigation of the work of Hirszfeld, Halber and Laskowski (1929) and Witebsky (1929), who found the A and B antigens in human carcinomata, and Zacho (1932), who found MN antigens in nearly all malignant tumours but not in benign tumours.

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