ANTIGENS OF THE A, B, O SYSTEM IN PRIMARY CULTURES AND TRANSPLANTED LINES OF HUMAN CELLS

V. T. Timofeev, G. P. Tribulev, I. I. Podoplelov, and Yu. T. Aleksanyan

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The presence of the isoantigens A, B, O, M, N, etc. in human tissues has been demonstrated previously [3, 4]. However, the problem of whether these antigens are preserved in human cells during growth in vitro has not been finally settled [2, 9, 11-19]. The solution of this complex problem is also interesting because recently antigens have been regarded as natural genetic markers of somatic cells [19, etc.].

The object of the present investigation was to study A, B, and O antigens in monolayer cultures of human cells by means of a group of immunologic methods.

EXPERIMENTAL METHODS

The experimental material consisted of primary monolayer cultures obtained from human embryonic tissues and also of transplantable lines of human cells (HeLa, Liver, CaVe, 580, A-1). A line of transplantable rat kidney cancer (REC) cells obtained from the carcinoma PA strain, in which B antigen was found before cultivation [10], was used as the control.

Primary cultures were obtained from embryos aged 10-12 weeks, in the tissues and erythrocytes of which group antigens had been detected beforehand. The transplantable cell lines were not tagged by isoantigen before the investigation, except for the HeLa line, obtained from uterine carcinoma tissue from a woman of blood group O.

The primary cultures were studied from 6 to 60 days, for later the cells began to degenerate. A nutrient medium of the following composition was used for growing the cultures: medium No. 199 (50%), lactalbumin hydrolyzate solution (50%), bovine serum (20%). The transplantable cell lines were cultivated in medium No. 199 with 10% bovine serum. The cells were detached from the glass with a 0.02% versene solution.

The investigation was carried out by using a group of immunologic reactions: absorption of antibodies, mixed agglutination, and the method of fractional exhaustion of standard sera.

The following reagents were used in the experiments: 1) isoagglutinating α and β sera (titer 1 : 16-1 : 32), anti-A, anti-B, and anti-O immune sera (titer 1 : 16), and also anti-O (H) phytohemagglutinin obtained from broom seeds; 2) a 2% suspension of standard erythrocytes of groups A, B, and O. The experiments were repeated not less than 4-6 times.

The antigens were prepared by freezing (the tubes with the material were placed in acetone with dry ice) and thawing the cells 15 times, followed by heating them at 100° on a water bath for 10 min. This procedure enabled the detection of antigens which, as a rule, would not otherwise be found [5, 8]. For the absorption reaction [6], the cell residue obtained after repeated freezing and thawing was used, and for the factional exhaustion of standard sera [1], the supernatant (cell extract) was used. Before the absorption experiments the antigenic material was treated with human serum from a person with blood group IV to prevent nonspecific antibody fixation [7].

The mixed agglutination reaction was performed by the method described elsewhere [15], except that Hanks' solution was used instead of phosphate buffer. Monolayer cultures of cells grown in Wasserman

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TABLE 1. Antigens of the A, B, O System in Monolayer Cultures of Human Cells

Test object	absorption of antibodies					mixed agglutination					fractional exhaustion				
Transplantable lines	α	β	anti-A	anti-B	anti-O	α	β	anti-A	anti-B	anti-O	α	β	anti-A	anti-B	anti-O
HeLa	—	—	—	—	+	—	—	—	—	+	—	—	—	—	—
Liver	+	+	+	+	—	+	+	+	+	—	+	+	+	+	—
CaVe	—	+	—	+	—	—	+	—	+	—	—	+	—	+	—
580	—	+	—	+	—	—	+	—	+	—	—	+	—	+	—
A-1	—	—	—	—	+	+	—	+	—	—	—	—	—	—	—
Primary cultures туры [2]:															
118(A)	+	—	+	—	—	+	—	+	—	—	+	—	+	—	—
87(B)	—	+	—	+	—	—	+	—	+	—	—	+	—	+	—
119(0)	—	—	—	—	+	—	—	—	—	+	—	—	—	—	—
126(AB)	+	+	+	+	—	+	+	+	+	—	+	+	+	+	—
RKC	—	+	—	+	—	·	·	·	·	·	—	+	—	+	—

Legend: + Presence of antigen, — absence, · no experiment carried out.
* Similar results were obtained with anti-) (H) phytochemagglutinin from broom seeds.
† The blood group of the embryos from which these primary cultures were obtained is indicated in parentheses.
‡ The transplantable line of rat carcinoma cells was used as control.

tubes for 3-4 days were used for the experiments. The indicator system was a 0.5% suspension of human erythrocytes of groups A, B, and O, treated beforehand with a 0.25% trypsin solution at 37° for 30 min.

EXPERIMENTAL RESULTS

Altogether 121 primary cultures of human embryonic cells (of which 43 belonged to group O, 37 to group A, 28 — B, and 13 — AB) and six transplantable cell lines (HeLa, Liver, CaVe, 580, A-1, and RKC) were studied. The results obtained are indicated in Table 1.

The method of fractional absorption of standard sera in saline cell extracts revealed A and B antigens, but no O antigen could be found. The B antigen was more clearly defined than the A antigen.

Somewhat conflicting results were obtained when A-1 cells were investigated, for the absorption method revealed only O antigen in these cells and the mixed agglutination method revealed only A antigen. The reason may be that when the latter method is used, nonspecific reactions are sometimes observed [16].

The results of these investigations thus showed that antigens of the ABO system persist in primary cultures obtained from tissues preliminarily marked by these antigens, and are found in cells cultivated for a long time in vitro, as follows: HeLa and A-1 cells — O antigen; 380, CaVe, and RKC cells — B, and Liver cells — AB. However, the detection of isoantigens by the absorption reaction requires destruction of the cells by repeated freezing and thawing, and heating followed by treatment with group IV serum enable clearer results to be obtained. These results suggest that the localization of these antigens in the cells both of primary cultures (after growth for 3-4 weeks) and of transplantable lines is topographically deeper.

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