DISTRIBUTION OF ABO BLOOD GROUP ANTIGENS IN SUBCELLULAR FRACTIONS OF HUMAN TISSUES AND CELLS IN CULTURE

Yu. V. Zykov, V. T. Timofeev,
G. P. Tribulev, and Yu. T. Aleksanyan

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Antigens of the ABO system have been found in cytoplasmic fractions of human tissues and cells cultivated for long periods in vitro. They have been found in fractions of mitochondria, microsomes, and cell juice, but in the latter the O-antigen is found inconstantly. Antigens of the ABO system were not found in ribosomes from human tissues. B antigen similar to human B-isoantigen was found in the subcellular fractions of rat tissues.

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Several workers have demonstrated the presence of A-, B-, and O-isoantigens not only in erythrocytes, but also in normal and tumor tissues, and also in transplantable lines of human cells [3, 4, 5, 12, 14]. Only isolated studies have been made of the presence of isoantigens in tissue fractions [6, 13, 15], and we know of no investigation of these antigens in subcellular fractions of human cells cultivated for long periods in vitro. At the same time, we have previously demonstrated that isoantigens can be detected in cells cultivated in vitro after their destruction by repeated freezing and thawing [8].

The object of the present investigation was to study antigens of the ABO system in cytoplasmic fractions isolated from tissues and from transplantable lines of human cells.

EXPERIMENTAL METHOD

The test objects consisted of normal human tissues and tumor tissues (adenocarcinoma of the stomach) taken from persons of known blood group, and human cell lines HeLa (carcinoma of the cervix uteri), CaVe (carcinoma of the stomach), A-1 (amnion), No. 580 (embryonic skin), and Tg-33 (fallopian tube). Simultaneous studies were made of certain normal and neoplastic rat tissues and also of cells of a transplantable line of carcinoma of the rat kidney (RPK) obtained from a tumor of strain RA [1], in which B-antigen similar to human B-isoantigen had previously been detected [10].

To obtain the fractions the tissue material was carefully minced and repeatedly washed with cold water to remove erythrocytes. The tissues and cells were then homogenized in a Potter's glass homogenizer with Teflon pestle. To one part of tissue by weight 10 parts of the following medium were used: 0.25 M sucrose, 0.05 M $MgCl_2$, 0.1 M tris-HCl buffer, pH 7.4. The resulting homogenates were filtered through two or three layers of gauze and then centrifuged twice at 1500 g to remove tissue debris, undestroyed cells, and nuclei. The method of differential centrifugation [17] was used to separate fractions of mitochondria (12,000 g, 15 min) and microsomes and cell juice (105,000 g, 60 min) from the supernatant. Ribosomes were separated by treating the microsomal fractions with sodium desoxycholate solution in a final concentration of of 0.5% (105 000 g, 120 min). All granular fractions were twice reprecipitated in the same medium under the conditions described above and resuspended. After determination of their protein content [16], the suspensions were diluted to a concentration of 1 mg protein/ml.

Immunologic analysis of the isoantigens in mitochondrial, microsomal, and ribosomal fractions was carried out by the method of specific absorption of antibodies [5], and in the soluble fraction (cell juice) by fractional exhaustion of standard sera [2]. At the same time, an express test was used, i.e., titration was carried out without 15-min intervals, and in both cases identical results were obtained.

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TABLE 1. Antigens of ABO System in Subcellular Fractions of Human Cell Lines in Culture

Material studied		Hemagglutinin		
cell line	Fractions	Anti-O	α	β
HeLa	Mc	+	—	—
	Ms	+	—	—
	CJ	—	—	—
CaVe	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+
A-1	Mc	+	—	—
	Ms	+	—	—
	CJ	—	—	—
580	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+
Tg-33	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+

Legend (here and in Tables 2 and 3): Mc—mitochondria, Ms—microsomes, CJ—cell juice; (+) specific absorption of hemagglutinin (antigen present), (-) absence of antigen.

TABLE 2. Antigens of the ABO System in Subcellular Fractions of Normal and Neoplastic Human Tissues

Material tested		Hemagglutinin		
Tissue group	Fractions	Anti-O	α	β
0	Mc	+	—	—
	Ms	+	—	—
	Rs	—	—	—
	CJ	±	—	—
A	Mc	—	+	—
	Ms	—	+	—
	Rs	—	—	—
	CJ	—	+	—
B	Mc	—	—	+
	Ms	—	—	+
	Rs	—	—	—
	CJ	—	—	+
AB	Mc	—	+	+
	Ms	—	+	+
	Rs	—	—	—
	CJ	—	+	+

Legend: Rs denotes ribosomes; remainder of legend as in Table 1.

The reagents used were isosera of groups A (β) and B (α) with a titer of 1:32, and also anti-0 (H) phytohemagglutinin in a titer of 1:24, extracted from seeds of Cytisus sessilifolius.

In the absorption experiments, the residue obtained by centrifugation of 2 ml of suspension of the granular fraction was treated with 10 drops of isoserum (α, β) or phytohemagglutinin (anti-0). The contents of the tubes were thoroughly mixed and allowed to stand for 18-20 h at 4°. Next day, after centrifugation (under the appropriate conditions) the supernatant was aspirated and titrated in dilutions of 2, 3, 4, 6, 8, 12, 16, 24, 32, and 48 times. Next, one drop of a 2% suspension of standard 0, A, or B erythrocytes was added to the tubes. The results were read after centrifugation for 1 min at 1500 rpm (~400 g). Antigen was considered to have been found if the titer of the corresponding agglutinin was 3 dilutions higher than in the control. All experiments were repeated several times and their reproducibility was good.

EXPERIMENTAL RESULTS

The results of investigation of group antigens of the ABO system in cytoplasmic fractions isolated from human cells of lines HeLa, CaVe, A-1, No. 580, and Tg-33 are given in Table 1.

As Table 1 shows, the presence of B-isoantigen was established in all the studied fractions of human cells (CaVe, No. 580, Tg-33). Meanwhile, 0 (H)-isoantigen was discovered only in the mitochondria and microsomes of cell lines HeLa and A-1, and failure of attempts to detect it in the fractions of the cell juice was evidently due to the special nature of this antigen [11].

The study of tissues obtained from 13 patients with adenocarcinoma at operation (4 patients of blood group 0, 4 group A, 3 group B, 2 group AB) showed no differences in the content of isoantigens in fractions isolated either from macroscopically unchanged (normal) tissue or from tumor tissue from the resected stomach of the same patient. The distribution of isoantigens among the fractions in accordance with blood group of the tissue is illustrated in Table 2.

The results given in Table 2 are very similar to those described above: A- and B-isoantigens were found in mitochondria, microsomes, and cell juice, whereas 0-antigen was clearly detected in the first two fractions but not always in the third. In addition, the fact was noted that group antigens of the ABO system

TABLE 3. B Antigen Similar to Human B Isoantigen in Subcellular Fractions of Rat Tissues and Cells

Material tested		Hemagglutinin		
Tissues and cells	Fractions	Anti-O	α	β
Liver	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+
Kidney	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+
Tumor RA	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+
Cell RPK	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+
Tumor RPK	Mc	—	—	+
	Ms	—	—	+
	CJ	—	—	+

could not be detected in the ribosomal fraction. It was considered that this was due to the absence of elements of membranes in the ribosomal fraction.

As well as human cells and tissues, subcellular fractions of normal rat tissues (liver, kidney), tissues of transplantable kidney carcinoma strain RA, cells of line RPK obtained from this tumor, and also tissues of a tumor developing after injection of cells of line RPK into rats were investigated (Table 3).

A B antigen similar to human B isoantigen was distinctly found in the cytoplasmic fractions of all these objects. The results obtained confirm previously published data and indicate maintenance of a steady level of isoantigens during malignant change and explantation [4, 8].

When optimal conditions for the absorption experiments were selected, suspensions of fractions containing from 1 to 10 mg protein were tested; the best results were obtained with doses of 2-3 mg. Large doses of the fractions frequently caused a nonspecific decrease in the antibody titer.

A similar phenomenon was observed when fractions preserved for long periods (3-4 weeks at 4°) were used, but their preliminary treatment with human group AB serum and heating at 100° for 10 min [9] largely abolished the nonspecific antibody fixation. The same treatment of freshly isolated fractions had no effect, it will be noted, on the results of the reactions, thus confirming once more the thermostability of antigens of the AB0 system.

Hence, the results decribed show that A-, B-, and 0-isoantigens are present in subcellular fractions isolated both from human tissues and from human cells cultivated in vitro for long periods, and also that they persist during malignant change and explantation. This suggests that isoantigens are stable immunogenetic markers and that they can be used in experiments to study transformation and hybridization of somatic cells.

The study of the distribution of group antigens among the cytoplasmic fractions showed that A- and B-antigens are found in mitochondrial microsomal and soluble fractions, in contrast to 0 (H)-antigen, which was not always discovered in the cell juice. No isoantigens could be found in the ribosomal fractions.

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