CHARACTERISTICS OF ANTIGENIC COMPOSITION ON INTRA- AND INTERSPECIFIC HYBRID CELLS OF MOUSE HEPATOMA XXIIa

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The study of the antigenic composition of hybrids of somatic cells in culture is interesting because they are widely used for the investigation of some important problems in modern biology — genetics of somatic cells, cell differentiation, the biology of malignant growths, and so on [4, 7, 9, 12-14]. The presence of antigens of species specificity, Forssman antigen, and an antigen similar to human B isoantigen has been established in mouse hepatoma XXIIa cells in long-term culture [1].

The object of this investigation was to study the above-mentioned antigens in hybrids of mouse hepatoma XXIIa cells in culture.

EXPERIMENTAL METHOD

Cell line MHXXIIa [2], obtained from transplantable mouse hepatoma XXIIa, was used. An intraspecific hybrid was obtained by fusion of microcells (mc) of clone 625 of line L, resistant to ethidium bromide [3], with hepatoma cells (H). To obtain microcells, the method described in [8, 10] was used. Interspecific hybrids of cultured cells were obtained by fusion of hepatoma cells with complete hamster (RJK) cells or microcells. A clonal culture of hamster cells not containing the enzyme thymidine kinase (TK) was resistant to the action of ethidium bromide (EB) and ouabain (OUA). Complete hepatoma cells were used to obtain both intra- and interspecific hybrids. Cell fusion was carried out in suspension with the aid of polyethylene-glycol solution (mol. wt. 1450). Clonal cultures of complete and microcellular hybrids of mouse hepatoma XXIIa were isolated by the method described in [10] with the aid of selective media (EB; EB + HAT, EB + HAT + OUA), corresponding to the genetic markers of the parental cells. The hybrid origin of the isolated clones was tested karyologically.

To study antigen composition the biomass of both hybrid and parental cells, grown on Eagle's nutrient medium with 10% bovine serum, was collected. The presence of antigens determining mouse species-specificity in the cells in culture was studied by the cytotoxic express test [11] and the cell agglutination test [5] using rabbit antisera against saline extract of mouse liver tissues. Male chinchilla rabbits weighing 3 kg were used for immunization. Antigen was injected subcutaneously into the rabbits in a dose of 40 mg protein, mixed with Freund's adjuvant. After 21 days, the antigen was injected intramuscularly in a dose of 60 mg protein. The third injection of antigen was given 1 week later, intramuscularly, in a dose of 80 mg protein. Antigens for the 2nd and 3rd injections were given without Freund's adjuvant. Antisera were obtained on the 10th day after the last injection. During the experiments, immune sera exhausted with sheep's red blood cells (SRBC) were used to remove antibodies against Forssman antigen, which are usually present in rabbit sera. Immune rabbit sera treated in this way had a cytotoxic action on mouse cells in culture and did not react with a culture of hamster cells (according to the details of this method, a cytotoxic action was considered to be positive if no fewer than 50% of dead cells were present). To study the presence of Forssman antigen in parental and hybrid cells, a combination of immunologic methods was used: the antibody absorption test, the cytotoxic express test [11], and the cell agglutination test [5]. Rabbit antisera against SRBC and normal rabbit sera were used in the tests. To obtain immune sera, rabbits were immunized

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TABLE 1. Antigenic Composition of Parental and Hybrid Cells

Name of cultures and their origin	Antigen		
	of mouse species-specificity	Forssman	similar to human B isoantigen
Parental cells, mouse —			
MHXXIIa (H)	+	+	+
Mouse — L 625	+	+	+
Hamster — RJK	—	+	—
Cells of intraspecific hybrid HL 625 mc	+	+	+
Cells of interspecific hybrids:			
HRJK mc — I—II—3	+	+	+
HRJK mc — 2	+	+	+
HRJK — 3	+	+	+
HRJK — 5	+	+	+

Legend. +) Antigen present, —) antigen absent.

subcutaneously with SRBC in a dose of 10^{10} 3 times at intervals of 10 days. Antisera were obtained on the 10th day after the last injection. Both immune and normal rabbit sera had marked ability to hemolyze SRBC. In the antibody absorption test, rabbit antisera against SRBC were incubated overnight at 4°C with the test objects (residues of cultured cells). The supernatant obtained by centrifugation at 3000 rpm for about 10 min was used for the SRBC hemolysis test. Absence of hemolysis indicated the presence of Forssman antigen in the test object. Liver tissue from a Forssman-negative animal (rat) was used as the control in the antibody absorption test. By means of the method of specific absorption of α- and β-antibodies described in [6] parental and hybrid cells were tested for the presence of antigen similar to human B isoantigen. The test for detecting antigen similar to human A isoantigen in the test objects was used as the control. A residue of cultured cells disintegrated by frequent (15 times) freezing and thawing was used in the experiments. All test objects were saturated beforehand with human group IV serum to prevent nonspecific binding of α and β antibodies. To identify specific absorption of hemagglutinins, the test of hemagglutination of absorbed α and β sera with human test erythrocytes of groups II and III was carried out.

EXPERIMENTAL RESULTS

The results of a study of antigens determining mouse species-specificity, Forssman antigen, and antigen similar to human B isoantigen in the parental cells and cells of intra- and interspecific hybrids of mouse hepatoma XXIIa are summarized in Table 1.

As the results of the investigations showed, rabbit antiserum against saline extract of mouse liver tissues had a marked cytotoxic action on mouse hepatoma cells and a clonal culture of mouse fibroblasts (L 625) in culture. However, the same antiserum had no visible cytotoxic action on hamster cells (RJK) in culture. This antiserum agglutinated mouse hepatoma cells and L 625 cells in titers of up to 1:256, but did not agglutinate hamster cells in culture. These results are evidence that mast cells in culture contain species-specific antigens and that there is no antigenic similarity between mouse and hamster cells.

Both antisera against SRBC and normal rabbit sera had a marked cytotoxic action on cells of strains MHXXIIa, L 625, and RJK. However, after exhaustion with SRBC neither immune nor normal rabbit sera had any cytotoxic action on these cells. Rabbit antisera against SRBC agglutinated cells of strain MHXXIIa, mouse fibroblasts, and hamster cells in titers up to 1:128-1:256. After absorption with SRBC, the antisera lost their ability to agglutinate the test cells. Immune rabbit sera absorbed with cells of strains MHXXIIa, L 625, and RJK did not cause hemolysis of SRBC. The results obtained by this combination of immunologic methods points to the presence of Forssman antigen in the test objects.

Specific absorption of β-antibodies by mouse hepatoma cells and mouse fibroblasts in culture was demonstrated by the method of specific absorption of α- and β-antibodies. Hamster cells in culture did not bind β-antigen. α-Hemagglutinins were not found by any of the objects

mentioned. These findings indicate the presence of antigens similar to human B isoantigen in cells of strain MHXXIIa and in mouse fibroblasts and the absence of this antigen in RJK hamster cells in culture.

The use of the cytotoxic express test showed the presence of antigens of mouse species-specificity in cells both of the intraspecific microcellular hybrid (HG 625 mc) and of the microcellular (HRJK mc – 1-11-3 and HRJK mc – 2) and whole-cell (HRJK – 3 and HRJK – 5) interspecific hybrids.

The antibody absorption test and cytotoxic express tests revealed the presence of Forssman antigen in cells of both intraspecific and interspecific microcellular and whole-cell hybrids.

In all the hybrid cells mentioned above, an antigen similar to human B isoantigen was found by the method of specific absorption of β-antibodies.

The presence of antigens determining mouse species-specificity and of antigens similar to human B isoantigen in the hybrid cells is evidence of preservation of the corresponding chromosomes, responsible for synthesis of these antigens, in all four interspecific hybrid clones. The discovery of these antigens in hybrid clones could also indicate that the chromosomes of this partner (hamster), while taking part in the formation of interspecific hybrids, evidently have no repressive action on the corresponding genes of the hepatoma cells. It is impossible to interpret these data on inheritance of Forssman antigen by interspecific hybrid cells because these antigens were found in the cells of both partners.

The results of these experiments thus indicate that antigens of species-specificity, Forssman antigen, and antigens similar to human isoantigen are expressed in cells of intra- and interspecific hybrids of mouse hepatoma XXIIa. These antigens can be used as phenotypic markers of hybrid cells in culture.

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