DISTRIBUTION OF TRANSPLANTATION ANTIGENS IN SUBCELLULAR FRACTIONS OF SOME MOUSE ORGANS

A. A. Yarilin

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Fractions of cell membranes and microsomes isolated from the liver, kidneys and spleen of C57BL/6 mice possess the highest alloantigenic activity. The hyaloplasm and nuclei have no alloantigens. The specific alloantigenic activity of splenic cell fractions is much higher than that of fractions of the liver and kidneys.

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The importance of alloantigens in development of immunologic response to tissue transplantation is accepted unequivocally by most investigators. The number and localization of the transplantation antigens are evidently reflected in the manifestation of transplantation immunity [12]. The results of experimental studies of distribution of alloantigens in subcellular fractions of various organs are highly contradictory [1, 2, 10].

Distribution of alloantigens in subcellular structures was investigated in the present study by the absorption of cytotoxic antibodies test.

EXPERIMENTAL METHOD

The concentration of transplanation antigens was determined in the liver, kidneys, and spleen of adult male C57BL/6 mice. The organs were perfused with buffered physiological saline, pH 7.4, chopped finely with scissors, and homogenized. The homogenate was treated by differential centrifugation in accordance with the scheme adopted in this laboratory [1]. The supernatant obtained after sedimentation of the nuclei in 2.2 M sucrose was dialyzed against physiological saline, pH 7.4, and the cell membranes were isolated from it by the method of Emmelot and Bos [5] by centrifugation in a sucrose density gradient (1.16, 1.2, 1.22, 2.2 M). The membranes were thereby concentrated between the 1.2 and 1.22 M sucrose layers [5-7]. In some experiments, lipoproteins were isolated from the organs by Davies's method [4].

The alloantigenic activity of the samples was determined by absorption of cytotoxic isoantibodies. The isoantibodies were obtained by immunizing male BALB/c mice with spleen cells from male C57BL/6 mice (10^8 cells per injection, 5-10 intraperitoneal injections at intervals of 10 days). The cytotoxic activity of the sera was determined by the method of Gorer and O'Gorman [6], and the recommendations of B. D. Brondz were followed during the performance of the experiments. The unit of antibody activity was taken to be the dose of serum causing death of 50% of cells in a suspension containing 2×10^6 cells/ml. Absorption of cytotoxins was carried out at 37° for 1 h in a mixture of equal quantities of antibodies (in a dilution giving death of 75% of cells in the suspension) and the antigen sample. An absorption curve was plotted from the results of determination of the decrease in cytotoxicity of the serum after incubation with different doses of the sample, and this was subsequently used to estimate the quantity of antigen absorbing 1 unit activity of the antibodies. This dose was taken as the unit of alloantigenic activity.

EXPERIMENTAL RESULTS

The results of investigation of the distribution of alloantigens in subcellular fractions of homogenates from various mouse organs are given in Table 1.

The alloantigenic activity of spleen homogenate in these experiments was between twice and three times higher than that of liver and kidney homogenate. Undestroyed spleen and kidney cells absorbed only

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TABLE 1. Distribution of Transplantation Antigens in Subcellular Fractions of Spleen, Liver, and Kidneys of C57BL/6 Mice

Organ	Fractions	Protein yield (in mg/g fresh weight of organ)	Protein cont. in various frac. of homog. (%)	Isoantigenic activity		
				No. of units activity/mg protein of sample (specific activity)	No. of units in whole frac. calc. per gram fresh wt. of organ	Activity of fraction (in %)
Spleen	Whole cells ($1.5 \cdot 10^6$)	77,0	100,0	1,2±0,5	92,4	36,4
	Homogenate	77,0±12,5	100,0	3,3±0,8	254,1	100,0
	Residue after centrifugation at 1500 g	49,1±10,8	63,6	2,5±0,5	127,7	50,3
	nuclei			<0,5		
	membranes			17,0±0,8	51,0	20,1
	Mitochondria	4,2±0,9	5,4	10,0±0,6	42,0	16,5
	Microsomes	3,3±0,8	4,3	17,0±0,7	56,1	22,1
	Davies's lipoprotein	3,5±0,8	4,5	20,0±0,6	70,0	27,5
	Hyaloplasm	32,9±10,1	22,6	0	0	0
Liver	Whole cells ($1.8 \cdot 10^8$)	118,5	100,0	1,0±0,6	118,5	77,4
	Homogenate	118,5±15,1	100,0	1,3±0,3	153,05	100,0
	Residue after centrifugation at 1500 g	30,5±9,2	25,7	2,4±0,8	73,2	41,3
	nuclei			<0,5		
	membranes			2,3±0,5		
	Mitochondria	9,0±1,8	7,7	3,0±0,5	27,0	17,6
	Microsomes	7,6±0,9	6,4	2,2±0,3	16,7	10,9
	Hyaloplasm	71,4±16,6	60,2	0	0	0
Kidneys	Whole cells ($6.5 \cdot 10^8$)	107,5	100,0	0,7±0,6	75,2	50,0
	Homogenate	107,5±13,2	100,0	1,4±0,4	150,5	100,0
	Residue after centrifugation at 1500 g	40,1±10,4	37,3	2,1±0,4	84,2	56,6
	nuclei			<0,5		
	membranes			2,3±0,5		
	Mitochondria	11,2±1,6	10,4	1,4±0,3	15,7	10,4
	Microsomes	5,8±0,9	5,0	2,5±0,5	14,5	9,6
	Hyaloplasm	42,9±15,0	47,3	0	0	0

half the quantity of antibodies absorbed by homogenate with the equivalent protein content; a suspension of liver cells had almost the same specific activity as the homogenate. A considerable proportion of alloantigens of the investigated organs was sedimented at 1500 g. Further separation of this fraction showed that its alloantigenic activity is mainly contained in the cell membranes; the alloantigenicity of the nuclei was extremely low. In the tests of the spleen and kidneys, the specific alloantigenic activity of the microsomes was higher than that of the mitochondria. In the liver, on the other hand, the mitochondria were almost twice as active as the microsomes. The highest specific alloantigenic activity was shown by samples of lipoprotein isolated by Davies's method. The cell hyaloplasm of all the investigated organs contained no transplantation antigens.

A high content of transplantation antigens in the spleen has been repeatedly stressed by various workers [2, 3, 10]. Alloantigenic activity of the liver is the subject of highly conflicting opinions: some workers [3] found almost the same content of alloantigens in the liver as in the spleen, while others [5, 13] emphasized the extremely low alloantigenicity of this organ. According to data in the literature [3], the content of transplantation antigens in the kidneys is even lower than in the liver.

The results of these investigations to determine alloantigens in subcellular fractions are in substantial agreement with those described in the literature [2, 3, 9, 10]. In some cases comparison is rendered difficult by the fact that the fraction of light mitochondria (lysosomes) was not isolated in the present investigation, and according to findings obtained in one laboratory [3-6], this fraction possesses particularly high alloantigenicity. When the subcellular components were fractionated by the method used in the present investigation, lysosomes appeared in the microsomal fraction. This may have been responsible

for the greater (1.7-1.8 times) activity of the spleen and kidney microsomes than of their mitochondria. However, there is evidence to show that pure microsomes contain large quantities of alloantigens [7, 10]. The low activity of the liver microsomes can easily be explained by the presence of a factor blocking alloantigens [11] in the cell lysosomes of this organ. A possible explanation of the contradictory nature of results for the total content of alloantigens in the liver may be that this factor varies in its activity depending on conditions of treatment of the liver. The relatively high activity of the liver mitochondria in the present experiments is not in agreement with estimates made by other workers [2, 3]. A high content of alloantigens in cell membranes has already been described in many investigations using immunochemical and immunomorphological methods [7, 8, 12]. However, the existing view that activity of granule fractions is the result of their contamination by fragments of membranes cannot be reconciled with observations [7] of high activity of the intracellular components completely freed from membranes, or with the higher activity of fractions of certain cytoplasmic granules discovered in the presence experiments compared with the activity of fractions containing mainly cell membranes. Most probably the membranes of the cytoplasmic granules, which are immunologically related to cell membranes [15], like the cell membranes themselves, contain large quantities of alloantigens.

The investigation was carried out by means of serum containing antibodies against several H-2 specificities (2, 5, 22, 23) and not against H-2 antigens. Since the distribution of individual specificities within the cell may differ, the conclusions drawn must be limited to the distribution of the strongest antigens distinguishing line C57BL/6 from line BALB/c.

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