COMPARATIVE STUDY OF THE ACTION OF C57BL AND CBA MOUSE LIVER EXTRACTS ON HEPATOCYTE ADHESION

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Disturbance of adhesive interactions between cells is observed in the course of various pathological processes, including those connected with invasive growth [1]. For example, reduction of the force of adhesion has been found between cells in tumors [5] and in target cells in animals genetically predisposed to spontaneous carcinogenesis [2]. However, changes in the molecular mechanisms of cellular adhesion taking place in pathological processes have so far received little study.

The aim of this investigation was to compare the action of preparations obtained from the liver of CBA mice, predisposed to spontaneous carcinogenesis, and of C57BL mice, genetically resistant with respect, on adhesion between hapatocytes.

EXPERIMENTAL METHOD

Male CBA and C57BL mice weighing 18-20 g were used.

Four preparations were obtained by the method suggested previously for isolating macromolecular adhesive factors from rat liver and lung [3], by extracting the mouse liver with the solution not containing Ca^{2+}, at 4 and 20°C.

To study dose dependence, each preparation was successively diluted 10, 10^2, 10^3, and so on, times.

The action of the preparations on cellular adhesion was investigated by the following methods: 1) intact liver (after decapitation), 2) liver with functions weakened beforehand after perfusion with a Ca^{2+}-free solution, and 3) a suspension of hepatocytes.

Methods 1 and 2 were carried out in a similar way to that described previously [3, 4]. The hepatocyte suspension was obtained by Seglen's method [6]. The viability of the cells in suspension was assessed by the trypan blue test. 0.1 ml of suspension with a density of 10^6-10^7 cells/ml was added to 1 ml of medium 199 containing 20% bovine serum and 0.1 ml of the test preparation in the corresponding concentration, and the sample was inhibited at 37°C for 1.5 h and dispersed. In all methods the dispersion procedure was carried out under standard conditions — in 0.1 ml of 0.1% trypan blue in Hanks' solution, using a special glass disintegrator with 50-μ gap. During investigation by methods 2 and 3, after dispersion the number of isolated single cells (N_c) and the number of cell nuclei (N_n) were counted in order to determine values of the coefficient of disconnection (K_d), equal to the ratio of N_c to the sum of N_c and N_n. At each experimental point at least 10 tissue fragments (methods 1 and 2) or six aliquots of hepatocyte suspension were counted. Each experiment was repeated at least 3 times. The results were compared by Student's test.

EXPERIMENTAL RESULTS

Comparative study of the intact liver of C57BL and CBA mice, based on quantitative evaluation of adhesive interactions between cells when the tissue is exposed to deformation procedures, revealed no differences in adhesion of hepatocytes of these two lines of mice. As the data in Table 1 show, the value of K_d for the intact liver is largely determined by the state of intercellular junctions in the tissue: when they are disturbed (methods 2 and 3) K_d has closely similar values, which differ significantly

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TABLE 1. Dependence of K_d on State of Intercellular Junctions

Test model	K_d ($x \pm s$)		K_d after incubation in medium 199 (37°C, 1.5 h)		K_d after incubation in bovine serum (37°C, 1.5 h)	
	C57Bl	CBA	C57Bl	CBA	C57Bl	CBA
1 Liver after decapitation of animal	0	0	0,02±0,01	0,02±0,01	0,02±0,01	0,02±0,01
2. Liver perfused with Ca^{++}-containing Ringer's soln.	0,02±0,01	0,02±0,01	Not det.	Not det.	Not det.	Not det.
3 Liver perfused with Ca^{++}-containing Ringer's solution	0,65±0,01	0,65±0,01	0,46±0,01	0,43±0,01	0,42±0,02	0,46±0,01
4. Hepatocyte suspension	0,52±0,04	0,50±0,02	0,50±0,02	0,51±0,02	0,52±0,04	0,50±0,02

TABLE 2. Action of C57BL and CBA Mouse Liver Extracts on Adhesive Properties of Hepatocytes

Line of mice from which extract was obtained	Temperature of obtaining extract	Degree of dilution of extract	Number of isolated cell nuclei (2×10^5) per milligram tissue (method 1)		Values of K_d ($x \pm \bar{S}$)			
					liver with weakened junctions (method 2)		hepatocyte suspension (method 3)	
			C57Bl	CBA	C57Bl	CBA	C58Bl	CBA
C57Bl	4 °C	Control	34±11	48±3	0,47±0,02	0,41±0,04	0,68±0,03	
		10^1—10^3	35±2					
		10^4	27±1					
		10^5	19±2	43±1	0,45±0,01	0,45±0,01	0,72±0,02	Not determined
		10^6	24±2					
		10^7	27±3					
		10^8—10^{13}	33±1					
CBA	4 °C	Control	33±2	45±3	0,33±0,01	0,58±0,02	Not det.	0,76±0,02
		10^2—10^{13}	31±1	46±2	0,24±0,02	0,57±0,01		0,80±0,04
C57Bl	20 °C	Control	27±2	78±4	0,47±0,01	0,52±0,01	0,58±0,02	
		10^1—10^2	36±3	79±5	0,42±0,01	0,53±0,02		Not det.
		10^3		78±3	0,42±0,01			
		10^4		55±3	0,32±0,02	0,48±0,02	0,55±0,02	
		10^5	36±3		0,30±0,02	0,41±0,04		
		10^6		64±4	0,36±0,02	0,51±0,02		
		10^7—10^8			0,43±0,02			
		10^9	34±3	53±1	0,43±0,02			
		10^{10}	16±2		0,43±0,02			
		10^{11}	33±1					
		10^{12}	18±1					
		10^{13}	26±1	58±7				
CBA	20 °C	Control	82±2	68±2	0,51±0,02	0,41±0,02		0,66±0,02
		10^1—10^2	70±3	57±3				
		10^3	63±4					
		10^4	54±2	57±3		0,42±0,01	Not det.	
		10^5—10^{10}	79±1		0,47±0,01			0,62±0,01
		10^{11}		46±3				
		10^{12}		43±6				
		10^{13}		47±2				
		10^{14}—10^{15}		63±10				

Legend. Asterisk indicates protein concentration in extracts obtained at 4°C varied from 1 to 2 mg/ml, and in preparations obtained at 20°C, from 2 to 4 mg/ml.

from values of K_d for tissue with undisturbed intercellular junctions (method 1 and after perfusion with Ca^{2+}-containing solution).

Considerable differences in hepatocyte adhesion were found in C57BL and CBA mice when the action of preparations obtained from the liver of mice of these two lines on adhesion was studied (Table 2).

Extracts obtained from the liver of C57BL mice at 4°C were found to be adhesively active, but only on cells of the intact liver of this same line of mice (method 1). Liver preparations obtained in a similar way from CBA mice did not affect adhesion of hepatocytes from the two lines of mice. Extracts obtained at 20°C from mouse liver of the two lines had an effect on the adhesive properties of hepatocytes from the intact liver of both lines of mice (method 1), but only the liver preparation from C57BL mice was active against adhesion of liver cells with previously weakened junctions (method 2). None of the preparations tested caused any change in the values of K_d for hepatocytes in suspension (method 3).

The results suggest that preparations obtained from the liver of C57BL mice at different temperatures include macromolecular adhesive factors which differ from one another. The discovery of adhesive activity in preparations obtained at 4°C on a model of the intact liver and the absence of any biological effect on models of the perfused liver or in a hepatocyte suspension indicate that their action is effected only when the cell surface is intact and, consequently, adhesive factors contained in them are located at the periphery of the cell surface. Since preparations obtained at 20°C exhibited adhesive activity on models of the intact and perfused liver, but had no action on adhesion of hepatocytes in suspension, it can be postulated that the adhesive factors present in them are semiintegral components of the plasma membrane.

The results of this comparative study of adhesively active preparations of C57BL mouse liver and preparations obtained in the same way from the CBA mouse liver indicate a disturbance of adhesive interactions between liver cells in CBA mice, which are evidently connected with changes in the properties of components of the cell surface, involved in adhesive processes.

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CHANGES IN LYSOSOME COMPOSITION IN HEPATOCYTES CULTURED IN VITRO AS A MODEL OF PATHOLOGICAL STATES

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Destructive processes in cells largely involve the functioning of an extremely heterogeneous class of cell organelles, namely lysosomes, but much is unknown about the regulation of their activity. Much research has been published to show that lysosomal hydrolases are released into the cell cytosol in various pathological states [3]. There is morphological evidence of the development of autophagy and an increase in the number of lysosomes in cells during pathology [7, 8]. However, it is not clear what morphological changes in lysosomes ought to reflect this process of acid hydrolase release. A model for studying processes of anoxia, ischemia, substrate starvation, and rehabilitation, using a primary monolayer culture of hepatocytes from 3-day-old rats was developed previously [1]. It was shown on this model that incubation of hepatocytes in Hanks' buffered salt solution for 20 min leads to an increase in nonsedimented activity of acid phosphatase, the marker enzyme of lysosomes. Reproduction of anoxia in Hanks' solution for 1 h potentiates this effect, and subsequent rehabilitation for 1 h restores normal values.

The above model was used in the present investigation to study changes in the composition of the lysosomal population in hepatocytes by light microscopy.

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