STUDY OF THE SPECIES-SPECIFIC SERUM ANTIGEN OF STABLE HUMAN CELL LINES

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The presence of a species-specific antigen is a stable sign and one which is taken into account in investigations concerned with heterotransplantation of tissues and prolonged cultivation of animal and human cells [1, 2, 3, 9]. In these conditions loss or attenuation of the species-specific serum antigens has been reported in the heterotransplanted tissue [3, 5, 6] and in cells cultivated for long periods in artificial nutrient media containing heterogenic sera [8, 11, 12, 14]. Information in the literature on the preservation of the species-specific antigen of human serum in cells transplanted over long periods is contradictory [13, 14].

In the present investigation the existence of serum and species-specific antigens was investigated in monolayer cultures of human cells grown for long periods in media with heterogenic sera.

EXPERIMENTAL METHOD

For the study of the first problem, the gel-diffusion reaction [8] with crossed adsorption as described by Bjerklund [2] was used. Saline extracts from cultures of human (CaVe, 580) and mouse (line L) cells grown on medium No. 199 with the addition of 10% bovine (b), human (h), or rabbit (r) serum were used as antigen. Saline extracts of fresh tissue from a carcinoma of the human stomach and normal sera (h, b, and r) were also used. The antigens were prepared by the method described previously [12]. The protein content in the antigens used in the investigation was determined by Lowry's method.

To study the second problem, the rapid cytotoxic test was used, as described by Green and coworkers [10].

Rabbit sera precipitating human and bovine protein (titer 1 :10,000) and sera against cultures of cells of lines CaVe and 580 (r), obtained by the method of immunological purification [7, 12] with a titer of 1 :16 in the gel-diffusion reaction were used in both tests.

EXPERIMENTAL RESULTS

In the gel-precipitation reaction the serum precipitating human protein reacted clearly with antigens from the tissue of the carcinoma of the human stomach and the human serum, forming 5-10 and 15-18 precipitation lines respectively. In addition, this antiserum reacted with antigens of cultures of CaVe (h; 5-7 bands), CaVe (b), and 580 (b; 4-5) cells and with cultures of mouse L cells (b; 1-2), and also with antigens of bovine serum (3-5), to some extent common with the tissue antigens of carcinoma of the human stomach and with antigens of human serum (see the table). During growth on a medium with rabbit serum no precipitation lines identical with antigens of bovine and human serum were formed, but lines appeared with antigens of CaVe (h) cells.

After absorption with normal bovine serum, the serum precipitating human protein did not form precipitation lines with antigens of CaVe (b), 580 (b), and L (b) cells, although it did form lines with antigens of CaVe (h), normal serum, and tissue of carcinoma of the human stomach. Identical results were obtained after absorption of this immune serum with antigens of cultures of L (b), CaVe (b), and 580 (b).

The serum precipitating bovine protein formed precipitation lines with antigens of stomach tissue, serum, and cultures of human CaVe (b), 580 (b), and CaVe (h) cells and mouse L (b) cells, but reacted very weakly or hardly at all with CaVe (r), 580 (r) and L (r) antigens. After absorption of this antiserum with antigens of human serum and with CaVe (b) and L (b) cultures, precipitation bands appeared only with bovine serum antigens.

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Study of Species-Specific Serum Antigens of Monolayer Cultures of Human Cells in the Gel-Diffusion Reaction

Antiserum	Antigens										
	Carcinoma of human stomach	Of sera			Of culture of human cells				Of mouse		
		h	b	r	CaVe (h)	CaVe (b)	580 (b)	CaVe (r)	580 (r)	L (b)	L (r)
Precipitating human protein											
Before absorption	5-10	15-18	3-5	–	5-7	4-5	4-5	–	–	1-2	–
After absorption with antigens of											
Bovine serum	5	5-8	–	–	5	–	–	–	–	–	–
Cells of mouse L (b)	5-6	5-8	–	–	5-6	–	–	–	–	–	–
CaVe (b) and 580 (b) cells	5-6	5-8	–	–	5-6	–	–	–	–	–	–
Precipitating bovine protein											
Before absorption	2-3	3	15-16	–	2-3	5-6	5-6	(1)	(1)	5-6	(1)
After absorption with antigens of											
Human serum	–	–	2-5	–	–	–	–	–	–	–	–
CaVe (b) and L (b) cells	–	–	5-7	–	–	–	–	–	–	–	–
Against culture of CaVe (r) cells											
Before absorption	1-2	(1)	(1-2)	–	4	4	3-4	3	3	(1)	–
After absorption with antigens of bovine serum	1	–	–	–	3	3	2-3	3	2	–	–
Normal rabbit serum	–	–	–	–	–	–	–	–	–	–	–

Legend: The figures show the relative number of precipitation lines; (1) precipitation lines appear inconstantly; – absence of reaction.

The rabbit serum against cells of line CaVe (r) reacted clearly with antigens of CaVe (r) 580 (r), CaVe (b), and 580 (b) cells, and of stomach tissue and reacted very weakly with L (b) antigens and with antigens of human and bovine sera. After absorption of this antiserum with antigens of bovine serum, the precipitation lines with the antigens of bovine and human sera disappeared, but in the reactions with tissue from carcinoma of the stomach and with cells of lines CaVe and 580, common features were observed.

Hence, in the gel-diffusion reaction no antigens of human serum could be found in cells cultivated for long periods in a medium with borine or rabbit serum. However, this does not signify the loss of human speciies-specific antigens in the monolayer cultures, as is clear from the results of the rapid cytotoxic test. This showed that sera against CaVe and 580 cells are toxic for those cells when grown in a medium with bovine, rabbit, or human serum, but do not act on mouse L cells. At the same time, the serum precipitating human protein had no toxic action on the cell cultures studied.

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