SPECIES- AND ORGAN-SPECIFIC ANTIGENS IN TISSUE CULTURES OF CONSIDERABLE AGE

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Mammalian and human cells are frequently cultured for a considerable time in nutritive media containing the sera of various animals, i.e., in foreign proteins. It is not clear whether under these conditions the antigens of the cultured cells are changed, or whether the species and organ specificity characteristic of the cells of the organism from which they were obtained are preserved [2, 3, 5]. Some authors [8, 10, 11] found that cells in tissue cultures on heterogenic mixtures preserve their specificity, or change somewhat in type [1, 12]. According to others, the antigenic properties of the cells of explants and of the cell cultures change: the specific antigens are lost, and new antigens, common to many cultures of various origins are produced [4, 6, 7, 9].

We here report an attempt to determine the presence or absence of species- and organ-specific antigens in cells cultured in heterogenic nutritive media for a long time.

EXPERIMENTAL METHOD

To study the antigenic properties of cell cultures we used two strains: 1) a strain consisting of adult rabbit kidney cells; the kidney tissue was treated with trypsin on 8/10/1957 (V. A. Soikin), and cultured in 50 passages on a nutritive mixture consisting of 20% human serum and 80% Hanks solution without antibiotics. Another culture was grown on horse serum and Hanks solution (33 passages); 2) a strain of cells from human-embryo lung was also treated with trypsin on 21/3/1958 (N. A. Kolesnikova); at the start of the experiment 52 passages were made in a medium containing Hanks solution and 20% horse serum.

TABLE 1. Investigation of Species-Specific Antigens from Adult Rabbit Kidney Cells

Animals	Antigen for the test injection	Dose (ml)	Number of animals	Results, as intensity of anaphylactic reaction*
Sensitized by rabbit serum	Antigen from cells from a culture of a strain of adult rabbit kidney cells cultivated on human serum	0.5-0.8	6	From + to ++
"	Rabbit serum	0.3	1	++++
Not sensitized	"	1.5	2	No reaction

TABLE 2. Study of Organ-Specific Antigens of Adult Rabbit Kidney Cells Grown on Heterogenic Nutritive Substrates

Antigen for the test injection	Dose (ml)	Number of animals	Results *
From rabbit kidney tissue	0.5	6	From + to +++
From rabbit kidney tissue cultured on human serum . . .	0.5	6	From + to ++
From rabbit kidney tissue cultured on horse serum	0.5	4	From + to ++

*Intensity of the anaphylactic reaction; + indicates brief scratching of the nose; ++ nose scratched, sneezing, coughing, coat ruffled; +++ same signs more strongly shown, micturition, defurcation, laboured respiration; ++++ effects strongly shown and accompanied by convulsions, sometimes terminating in death of the guinea pigs.

TABLE 3. Extent to which the Organ-Specific Antigens of an Adult Rabbit Kidney Cell Culture Were Preserved

Antigen used for sensitization	First desensitization antigen	Second desensitization antigen	Test antigen	Dose (in ml)	Number of animals	Results (from anaphylactic reaction)
From rabbit kidney tissue	Rabbit serum	From rabbit kidney tissue	Antigen from adult rabbit kidney tissue cultured on human or horse serum	0.5	6	–
"	" "	Antigen from rabbit kidney tissue cultured on human serum	Rabbit kidney tissue	0.5	6	From + to +++
"	" "	Same, on horse serum	"	0.5	4	From + to ++

Both cultures were grown in 1.5-liter vessels containing 180-200 ml of nutritive fluid. By the 8th-12th day after each transplantation, on the wall of the vessel a thin continuous cell layer was formed containing from 9 to 15-20 million cells. The cell layer was scraped off with a metal spatula, and the cell suspension sedimented by centrifugation for 5 minutes at 1000 revs/minute. The material was preserved frozen at −20°. To destroy the cells it was melted, again frozen, and the procedure repeated 4-5 times.

As a control we used rabbit tissue triturated in a mortar with sand.

With the antigens obtained, and also with rabbit and human sera we sensitized guinea pigs by subcutaneous injections, and after 30-45 days we made experiments on anaphylaxis with desensitization, using the method of L. A. Zil'ber. Each antigen was tested to determine whether it was harmless by intravenous injection into guinea pigs of 2-3 times the amount injected in the experiment.

EXPERIMENTAL RESULTS

In the first experiments we attempted to determine whether the cells which had endured prolonged passages on a heterogenic nutritive medium had preserved the antigens specific to rabbits. For this purpose, guinea pigs were sensitized with rabbit serum; after 30 days they received the antigen from the rabbit kidney culture (Table 1).

Similar results were obtained by sensitization of the guinea pigs with human serum and the injection of critical doses of antigen from the human embryo lung cell culture. The cells which had undergone prolonged passage on a heterogenic nutritive medium retained their species-specific antigens.

The object of the following experiments was to find whether cells which had been long cultured in vitro preserved their organ-specificity. For this purpose guinea pigs were sensitized with an antigen of adult rabbit kidney tissue. Because the renal tissue had not been washed free from blood, after 45 days the guinea pigs were desensitized to rabbit serum injected first subcutaneously, and then intravenously, until complete desensitization had been obtained. When the heightened sensitivity of the guinea pigs to the species-specific antigens had been eliminated, antigens from rabbit kidney tissue cultures grown on human serum and on horse serum were injected (Table 2).

From the results given in Table 2 it can be seen that the cells maintained their organ-specificity during culture.

Next, we attempted to find whether the specificity of these tissue-culture cells maintain organ-specificity entirely. For this purpose, the previous experiment was prolonged. The guinea pigs received repeated test antigens, and, because they did not respond to repeated injections, they were given alternately antigens from kidney tissue, and from kidney tissue culture (Table 3).

From the results shown in Table 3 it can be seen that cells which had been kept long in culture had lost some of the organ-specific antigens whereas cells in the normal rabbit kidney retained these antigens completely.

Finally, we need to compare the antigenic composition of cells which have been cultured for long periods on media containing human or horse serum. Guinea pigs were sensitized with antigens of rabbit kidney tissue culture grown with human or with horse serum. To eliminate the heightened sensitivity to species-specific antigens and to

TABLE 4. Antigenic Composition of Cells of Adult Rabbit Kidney Grown on Various Nutritive Substrates

Antigen used for sensitization	Desensitization	First test injection	Dose (in ml)	Number of animals	Results (intensity of reaction)	Second test injection	Dose (in ml)	Results
From rabbit kidney tissue grown on human serum	Rabbit and human serum	Rabbit kidney tissue	0.5	4	+	Antigen of adult rabbit kidney on human serum	0.5	No reaction
"	"	Antigen of adult rabbit kidney on human serum	0.5	4	+	Antigen of adult rabbit kidney on horse serum	0.5	"
"	"	Antigen of adult rabbit kidney on horse serum	0.5	3	+	Antigen of adult rabbit kidney on human serum	0.5	"
Culture of rabbit kidney tissue grown on horse serum	Rabbit and horse serum	Rabbit kidney tissue	0.5	4	From + to ++	Antigen of adult rabbit kidney on horse serum	0.5	"
"	"	Antigen of adult rabbit kidney on horse serum	0.5	6	From + to ++	Antigen of adult rabbit kidney on human serum	0.5	"
"	"	Antigen of adult rabbit kidney on human serum	0.5	4	From + to ++	Antigen of adult rabbit kidney on horse serum	0.5	"

serum from the nutritive medium, the guinea pigs were given rabbit and human or horse serum (as in the previous experiment). After desensitization to these sera, the antigens from the kidney tissue and from kidney tissue-culture on human or horse serum were given (first test injection). Each antigen was injected repeatedly until complete desensitization to it had been obtained, and then the second test injection was given (Table 4).

From Table 4 it can be seen that rabbit kidney cells grown on various nutritive substrates do not differ in their antigenic composition. Further, they contain no new antigens.

SUMMARY

To study the antigenic properties of mammalian cells cultured for a long time on antigenic nutritive media, experiments based on anaphylaxis after desensitization were carried out on guinea pigs. Most of the original species-specificity to adult rabbit kidney cells and human-embryo pulmonary cells was maintained; organ-specificity to rabbit kidney cells was also partially retained. Some organ-specific antigens were lost, but no new ones were acquired.

There was no difference in the antigenic composition of rabbit kidney cells grown by passage through various nutritive (human or equine) serum substrates.

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