status, most probably a higher level of estrogen receptors in the uterus of CBA mice than of CBA57BL/6 and C3HA mice It has also been shown that injection of DUH into the sensitive CBA strain raises the level of estrogen receptors [1], which also increases the sensitivity of the uterus to tumor development in the present of additional injection of ED.

Our results thus suggest that the uterine tissue of CBA mice is significantly more sensitive to estrogen than the uterus of C57BL/6 and C3HA mice, and this may be one cause of induction of hormone-dependent sarcoma of the uterus in CBA mice, and also of the significant potentiation of this induction by injection of exogenous hormone.

LITERATURE CITED

1. O. V. Morozova and N. E. Kushlinskii, Éksp. Onkol., No. 4, 54 (1987).
2. L. S. Trukhanova, Éksp. Onkol., No. 6, 21 (1986).
3. V. S. Turusov, A. B. Linnik, and O. V. Morozova, Byull. Éksp. Biol. Med., No. 11, 596 (1980).
4. V. S. Turusov, N. S. Lanko, V. A. Krutovskikh, and Y. D. Parfenov, Carcinogenesis, **3**, 603 (1982).

DYNAMICS OF PROLIFERATIVE ACTIVITY OF ORGANOTYPICAL EPITHELIAL-MESENCHYMAL RECOMBINANT CULTURES FROM EMBRYONIC LUNGS OF INTACT AND URETHANE-TREATED MICE

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Transplacental exposure of the developing organism to chemical carcinogens takes place against the background of active intercellular and intertissue interaction, regulating the processes of morphogenesis, proliferation, and differentiation. In normal ontogeny the mesenchyme is an inducer of development of the epithelial anlage and, in particular, of the lungs [6, 10]. The role of epithelial-mesenchymal interaction and of each of these tissue components in induced carcinogenesis of the lungs and other organs is unknown. To study this problem we developed an experimental model, consisting of a culture of organotypical epithelial-mesenchymal recombinance, obtained from dissociated lungs of intact and experimental mouse embryos, receiving a pulmonotropic carcinogen transplacentally [4, 7, 9]. One of the early stages of induced adenoma of the lungs is an increase in proliferative activity of the epithelial cells [2, 5, 8]. We used this criterion to study epithelial-mesenchymal interactions and the role of each of these tissue components in the realization of transplacental carcinogenic effects.

This paper describes a study of the dynamics of proliferative activity of cells in cultures of organotypical epithelial-mesenchymal recombinants, formed from dissociated embryonic lungs of line A mice, either intact or receiving urethane transplacentally.

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TABLE 1. LI of Cells in Cultures of Organotypical Aggregates Obtained by Recombination of Epithelium and Mesenchyme of Embryonic Lungs from Intact and Experimental Line A Mice

Duration of experiment, days	Type of aggregate	Epithelium				Mesenchyme			
		number of cells		LI, %	p	number of cells		LI, %	p
		total	labeled			total	labeled		
4	EC MC	2251	122	5,4		1327	343	25,8	
	EC ME	2313	345	14,9	<0,001	1072	565	52,7	<0,001
	EE MC	2880	418	16,1	<0,001	2214	533	24,1	>0,1
	EE ME	2392	810	33,8	<0,001	791	350	44,2	<0,001
7	EC MC	3706	237	6,4		575	113	19,7	
	EC ME	2615	177	6,8	>0,1	986	271	27,5	<0,001
	EE MC	2157	211	9,8	<0,001	894	199	22,3	>0,1
	EE ME	3218	307	9,5	<0,001	973	304	31,2	<0,001
14	EC MC	2314	107	4,6		1124	127	11,3	
	EC ME	1972	168	8,5	<0,001	1167	212	18,2	<0,001
	EE MC	1179	92	7,8	<0,05	820	176	21,2	<0,001
	EE ME	2311	239	10,3	<0,001	1096	208	19,0	<0,001

Legend. Significance of differences determined by comparison with intact control (EC MC).

EXPERIMENTAL METHOD

The lungs of 17-18-day embryos of intact and experimental line A mice were used. Urethane was injected subcutaneously into the mothers in physiological saline in dose of 1 g/kg body weight (total 2 g/kg on the 15th and 16th days of pregnancy). To separate the embryonic lungs into fractions of epithelial and mesenchymal cells, we used a modified method [7], consisting of a combination of enzymic and mechanical treatment of finely shredded lobes of the lungs, followed by fractional sedimentation of epithelial complexes from a suspension of single mesenchymal cells, under stipulated conditions of centrifugation. As a result four cell fractions were obtained: epithelium – from intact control (EC) and experimental (EE) embryos, mesenchyme from intact control (MC) and experimental embryos (ME). To obtain organotypical aggregates the following recombination was used: 1) both tissue components from lungs of intact embryos (EC MC); 2) epithelium from intact and mesenchyme from experimental embryos (EC MC); 3) epithelium from experimental, mesenchyme from control embryos (EE MC); 4) both tissue components from experimental embryos (EE ME) The cells were aggregated by our modification of the "hanging drop" method [9]. After 4 days the aggregates which had formed were transferred from the hanging drop to the surface of Millipore filters and their culture continued by our modification of the organ cultures method [1]. The cultures were studied after 4, 7, and 14 days of the experiment. ^{3}H-Thymidine was added to the nutrient medium in a concentration of 1 μCi/ml 24 h before the end of the experiment. The cultures were fixed with Bouin's fluid and treated by histological methods; serial sections were covered with type M photographic emulsion. After appropriate treatment the sections were stained with hematoxylin. The labeling index (LI) of the epithelial and mesenchymal cells was determined in different types of aggregates, by counting virtually all cells in serial sections. The results were subjected to statistical analysis by the chi-square method.

EXPERIMENTAL RESULTS

In all types of aggregates obtained from the epithelium and mesenchyme of lungs of both intact and experimental mouse embryos, and also from recombinants of these tissue components, DNA-synthesizing epithelial and mesenchymal cells were found at all times of cultivation.

LI was comparatively low in intact control aggregates obtained from epithelium and mesenchyme of the lungs of intact embryos (EC MC) and remained virtually unchanged throughout the period in culture (Table 1). LI of the mesenchyme in these aggregates on the 4th day was almost 5 times higher (25.8%) than LI of the epithelium (5.4%). During subsequent cultivation LI of the mesenchyme gradually decreased, although it still remained 2-3 times higher than LI of the epithelium.

In experimental aggregates obtained from tissue components of the lungs of the experimental embryos (EE ME) LI of the epithelium on the 4th day of culture reached 33.8%, i.e., more than 6 times higher than the control level (5.4%). LI of the epithelium in aggregates of this kind decreased significantly with the duration of the experiment, remaining only 1.5-2.2 times higher than the corresponding control value. LI of the experimental mesenchyme on the 4th day exceeded the control level by only 1.7 times. At the latter stages LI of the experimental mesenchyme gradually decreased, and under these circumstances the difference between the experiment and control was virtually unchanged.

In aggregates obtained by recombination of the tissue components of the lungs of intact and experimental mouse embryos, the role of epithelial-mesenchymal interactions and of each of these tissue components in the realization of the transplacental growth-stimulating effect of urethane was manifested. For instance, on aggregation of intact epithelium with experimental mesenchyme (EC ME) LI of this epithelium after 4 days of culture was 3 times higher (14.9%) than in the case of intact epithelium (5.4%), aggregated with intact mesenchyme (EC MC). With increasing duration of the experiment the stimulating action of the experimental mesenchyme on intact epithelium diminished (7th day) and on the 14th day it again increased almost twofold (Table 1). During aggregation of the experimental epithelium with intact mesenchyme (EE MC) its LI fell significantly compared with the experimental epithelium, aggregated with the experimental mesenchyme (EE ME), although under these circumstances it was higher than the control level (EC MC).

It is thus clear from the results described above that in different types of aggregates (EC MC, EE MC, EC ME, EE ME) intact and experimental mesenchyme had a significant effect on the proliferative activity of both intact and experimental epithelium aggregated with it: intact mesenchyme depressed the proliferative activity of the experimental epithelium, bringing it close to the control level, whereas the experimental mesenchyme increased the proliferative activity of intact epithelium, bringing it close to the level of experimental epithelium.

Mice of the A line are sensitive to spontaneous and induced tumor (adenoma) formation in the lungs. Pulmonotropic carcinogens induce adenomas of the lungs in them with high frequency, however administered, including transplacentally [3]. During organ culture of the embryonic lungs of line A mice exposed transplacentally to the action of pulmonotroptic carcinogens, especially urethane, all stages of adenoma formation known in experiments on mice develop [8]. Under these circumstances, in the early stages of culture, LI of the epithelial cells rose significantly in experimental explants of embryonic lungs of line A mice exposed transplacentally to the action of urethane. In this investigation the transplacental action of urethane had a growth-stimulating effect not only on epithelial target cells, but also on mesenchymal cells. Similar results were obtained in our previous experiments with benz(a)pyrene [2]. All these findings point to an important role of epithelial-mesenchymal interactions and, in particular, of the mesenchyme in the realization of transplacental pulmonotropic effects of carcinogens. The mesenchyme of the lungs, which normally induces the development of the epithelial analage, when stimulated by a carcinogen, can evidently enhance the stimulating action of the carcinogen on proliferative activity of the epithelium in the early stages of transplacental carcinogenesis.

LITERATURE CITED

1. E. Antoshina and T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 4, 119 (1982).
2. T. G. Gor'kova and T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 7, 69 (1986).
3. L. A. Gritsyute, Experimental Tumors of the Lungs [in Russian], Moscow (1975).
4. T. S. Kolesnichenko and E. E. Antoshina, Byull. Éksp. Biol. Med., No. 1, 97 (1978).
5. T. S. Kolesnickenko and N. B. Gusina, Byull. Éksp. Biol. Med., No. 10, 426 (1978).
6. T. S. Kolesnichenko and A. L. Medvinskii, Ontogenez, **20**, No. 4, 381 (1989).
7. T. S Kolesnichenko, E. E. Antoshina, and A. L. Medvinskii, Ontogenez, **15**, No, 5, 522 (1984).
8. T. S. Koleshichenko and L. M. Shabad, Neoplasma, **26**, No. 4, 369 (1979).
9. A. L. Uedvinskii and T. S. Kolesnichenko, Byull Éksp. Biol. Med., No. 3, 379 (1981).
10. J. Masters, Devel. Biol., **51**, 98 (1976).