Epithelial Hyperplasia in Organotypical Aggregate Cultures from Mouse Embryonal Lung Cells

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The incidence of epithelial hyperplasia is studied in cultures of aggregates from epithelial and mesenchymal cells of embryonal lungs of intact and urethrane-treated Asn and C57Bl mice (transplacental urethrane, 3 g/kg). In the control, epithelial hyperplasia was detected in 5.9% Asn and in 9.9% C57Bl mice. In experimental aggregates epithelial hyperplasia was 54.3% in Asn and 39.8% in C57Bl mice ($p<0.001$). In aggregates with only one tissue component from experimental and another from control embryos, the incidence of epithelial hyperplasia was lower than in experimental animals: 27.1-28.3% ($p<0.001$) in Asn and 16.7-35.1% ($p<0.05$) in C57Bl mice.

Key Words: *blastomogenesis; lungs, epithelial mesenchymal reactions*

Dissociated embryonal lung cells of intact and urethrane-treated (transplacentally) mice form under certain conditions organotypical aggregates capable of growing and differentiating in organ cultures [1,3, 5,6,8]. Urethrane is a pulmonotropic carcinogen for mice, causing adenomogenesis upon any route of administration, including the transplacental [2,9,10]. Autoradiographic study of transplacental effect of urethrane on the aggregates showed a drastic increase in proliferative activity of epithelial target cells and mesenchyma. The latter affected the realization of early blastomogenic effect in experimental aggregates [4,5]. In this paper we present the results of morphological study of transplacental effect of urethrane on aggregates of epithelial and mesenchymal embryonal lung cells of Asn and C57Bl mice sensitive and resistant to lung blastomogenesis, respectively.

MATERIALS AND METHODS

Epithelial-mesenchymal aggregates were prepared from the lungs of 18-day embryos of Asn and C57Bl mice. Urethrane was injected to females three times on days 15, 16, and 17 of pregnancy subcutaneously in 10% normal saline in a dose of 1 g/kg. Embryonal lung epithelium (E) and mesenchyma (M) were separated by enzymatic and mechanical treatment of a grind organ, followed by fractionated sedimentation of epithelial complexes from suspension of individual mesenchymal cells [5]. The following combinations of isolated tissue components of embryonal lungs of control (intact) and experimental mouse embryos were used to prepare aggregates: epithelium and mesenchyma of control embryos (EcMc); epithelium from control and mesenchyma from experimental embryos (EcMe); epithelium from experimental and mesenchyma from control embryos (EeMc); and epithelium and mesenchyma from experimental animals (EeMe). Mixed cell suspensions were cultured for 4 days in special vessels by the modified suspended droplet method [8], after which formed aggregates were transferred onto Millipore filters and cultured by the organ culture method for 21 days. After histological processing, serial sections of aggregates (4 μ) were stained with hematoxylin and eosin and examined under a light microscope. Some aggregates were cultured in medium with ^{3}H-thymidine (1 mCi/ml) for 24 h. After treatment, serial sections

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were stained with hematoxylin and examined under a light microscope. Results were statistically processed using the ξ^2 test.

RESULTS

After explantation of formed aggregates in organ culture, epithelial growth and differentiation were observed with formation of alveoli- and bronchiole-like structures [3] (Fig. 1). These processes were paralleled by hyperplastic proliferation of epithelium with intense incorporation of ^{3}H-thymidine (Fig. 2). The incidence of epithelial hyperplasia depended on the type of aggregation and strain of donor mice. Epithelial hyperplasia in EcMc aggregates from Asn and C57Bl mice was observed in 5.87 and 9.97% cases, respectively. Epithelial hyperplasia was most often observed in EeMe aggregates: 54.3% in Asn mice, which is almost 10 times higher than in the control, and 39.8% in C57Bl mice — 4 times higher than in the control (Table 1). The incidence of hyperplasia in EeMc and EcMe aggregates from sensitive Asn mice was two times lower than in EeMe aggregates but 4-5 times higher than in the control. A similar, although weaker tendency was observed in aggregates from resistant C57Bl mice (Table 1).

Squamous-cell epithelial metaplasia (SEM) with and without keratinization was detected in few experimental aggregates from embryonal lungs of both mouse strains. For C57Bl aggregates its incidence was no higher than 0.37-0.50%, which is statistically negligible. In Asn strain the incidence of SEM was statistically significant and its fluctuations in different types of aggregates followed the same tendency as epithelial hyperplasia: 0.76% in EcMe ($p<0.1$), 0.99% in EeMc ($p<0.05$), and 3.83% in EeMe ($p<0.001$). No SEM were found in control aggregates.

Thus, hyperplasia developed more often and SEM rarely after long culturing of experimental aggregates prepared by recombination of dissociated epithelial and mesenchymal embryonal lung cells of intact and/or urethrane-treated mouse embryos. Epithelial hyperplasia is the initial morphological stage of pulmonary adenomogenesis induced by different carcinogens, including urethrane, in mice [2]. Hyperplastic changes in the epithelium are observed in lung adenomogenesis induced by transplacental urethrane and other carcinogens in organ cultures of experimental mouse embryonal lungs [9,10,12]. It is therefore obvious that high incidence of epithelial hyperplasia in experimental aggregates (EeMe, EeMc, and EcMe) and its low level in the control (EcMc) is a result of the initial stage of adenomogenesis of the lungs induced by transplacental urethrane. Like *in vivo,* the effect depends on the strain sensitivity of donor mice to pulmonary blastomogenesis. The in-

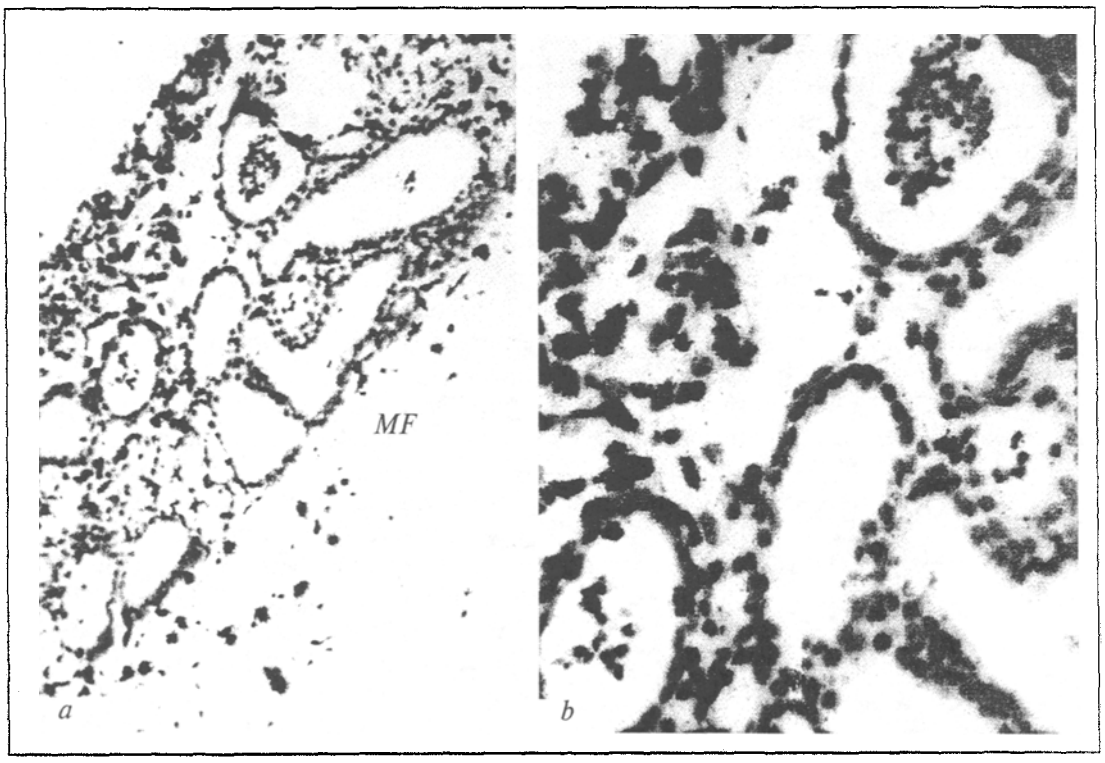

Fig. 1. Organotypical structures in aggregates of dissociated embryonal lung cells of intact Asn mice. Hematoxylin and eosin staining. *a*) aggregate on a Millipore filter (MF). Aggregation tissue consists of alveoli- and bronchiole-like structures with desquamated cells in cavities. ×200; *b*) the same fragment, ×500.

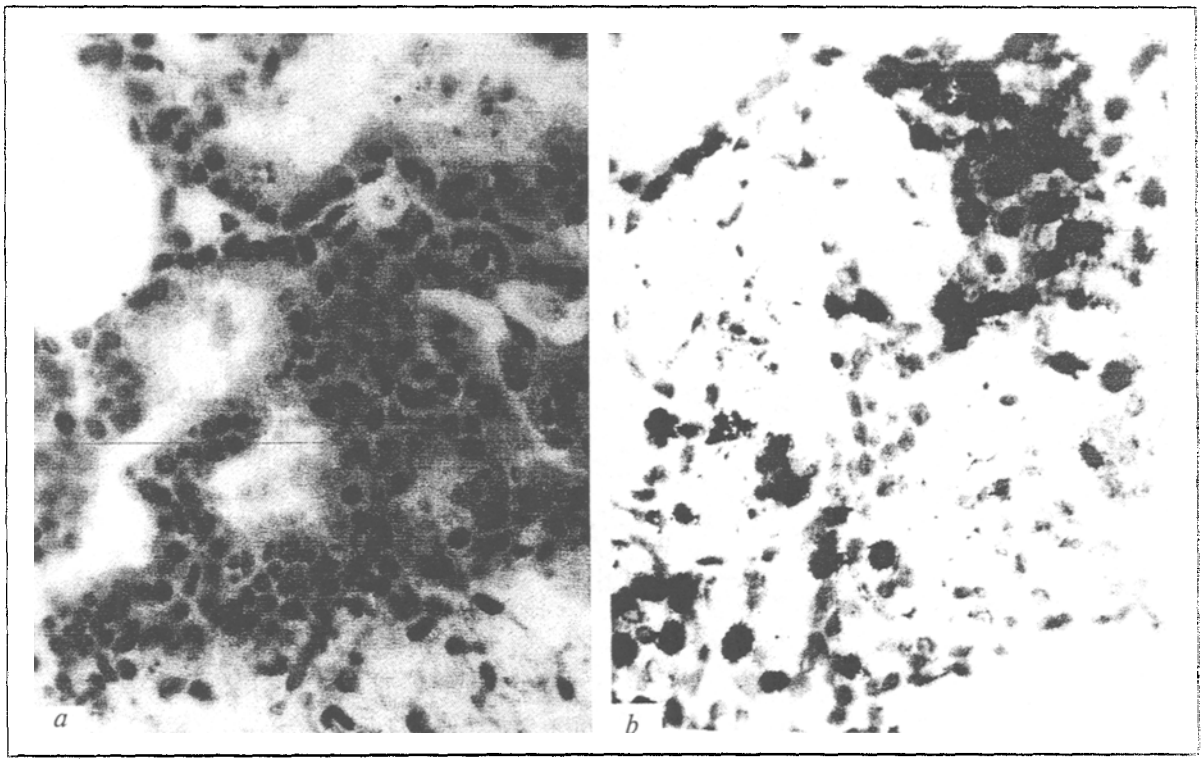

Fig. 2. Aggregate from experimental (transplacental urethrane, 3 g/kg) dissociated embryonal lungs cells of Asn mice. Hematoxylin and eosin staining, ×500. *a*) epithelial hyperplasia; *b*) accumulation of ^{3}H-thymidine-labeled cells in a focus of epithelial hyperplasia.

cidence of epithelial hyperplasia in aggregates from dissociated lungs of experimental embryos of resistant C57Bl mice was lower (39.8%) in comparison with the sensitive Asn strain (54.3%, $p<0.001$). The highest incidence of epithelial hyperplasia was observed in EeMe aggregates from both strains, in which both tissue components were exposed to transplacental urethrane (Table 1). The incidence of hyperplasia in EeMc and EcMe aggregates from sensitive Asn mice was two times lower than in EeMe but still several times higher than in EcMc. In resistant C57Bl mice this regularity was observed in EeMc aggregates, while in EcMe aggregates the incidence of epithelial hyperplasia was several times higher than in the control and close to the level in EeMe aggregates. Thus, the use of epithelial-mesenchymal recombinations from intact and experimental embryonal lungs showed that urethrane-treated mesenchyma of embryonal lungs of both mouse strains can induce hyperplastic proliferation of intact epithelium. Experimental epithelium aggregated with intact mesenchyma is less capable of hyperplastic proliferation. Our results agree with the data obtained by autoradiography at early stages of adenomogenesis induced in this model. This indicates that epithelial-mesenchymal relations play an important role in the realization of pulmonotropic blastomogenic effect of urethrane. The results demonstrate an important role of the stroma in the development of epithelial tumors in the lungs. In contrast to epithelial hyperplasia,

Table 1. Incidence of Epithelial Hyperplasia in Aggregates of Dissociated Cells of Embryonal Lungs of Intact and/or Experimental Asn and C57Bl Mice

Aggregate	Asn		C57Bl	
	total number of aggregates	epithelial hyperplasia, %	total number of aggregates	epithelial hyperplasia, %
EcMe	426	5.9	271	9.9
EcMe	456	28.1**	251	35.1**
EeMc	403	27.3**	270	16.7*
EeMe	392	54.3**	264	39.8**

Note. *$p<0.05$, **$p<0.001$ *vs.* EcMc.

SEM found in some experimental aggregates was never before observed in experiments with urethrane on animals or organ cultures of lung tissue explants. The development of SEM in aggregates can be caused by altered quantitative ratio of the epithelial and mesenchymal cells in comparison with that in the organ *in situ*. Such alterations can completely arrest the respiratory tract morphogenesis or impair the differentiation of SEM [7,11].

REFERENCES

1. E. E. Antoshina and T. S. Kolesnichenko, *Byull. Eksp. Biol. Med.*, **93**, No. 4, 119-121 (1982).
2. L. A. Gritsyute, *Experimental Lung Tumors* [in Russian], Moscow (1975).
3. T. S. Kolesnichenko and E. E. Antoshina, *Byull. Eksp. Biol. Med.*, **99**, No. 1, 97-99 (1985).
4. T. S. Kolesnichenko and E. E. Antoshina, *Ibid.*, **112**, No. 12, 683-685 (1991).
5. T. S. Kolesnichenko, E. E. Antoshina, and A. L. Medvinskii, *Ontogenez*, **15**, No. 5, 522-528 (1984).
6. T. S. Kolesnichenko and A. L. Medvinskii, *Ibid.*, **13**, No. 1, 83-87 (1982).
7. T. S. Kolesnichenko and A. L. Medvinskii, *Ibid.*, **20**, No. 4, 381-389 (1989).
8. A. L. Medvinskii and T. S. Kolesnichenko, *Byull. Eksp. Biol. Med.*, **91**, No. 3, 379-381 (1981).
9. T. S. Kolesnichenko, in: *Modulation of Experimental Carcinogenesis*, Lyons (1983), No. 51, pp. 81-98.
10. T. S. Kolesnichenko and L. M. Shabad, *Neoplasma*, **26**, No. 4, 369-379 (1979).
11. J. R. W. Masters, *Dev. Biol.*, **51**, 98-101 (1976).
12. V. S. Turusov, T. S. Kolesnichenko, and T. G. Gor'kova, *Exp. Toxic Pathol.*, **47**, 244-246 (1995).