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CELL STRUCTURE AND PROLIFERATIVE ACTIVITY OF ORGAN CULTURES OF NORMAL EMBRYONIC LUNG TISSUE OF MICE RESISTANT (C57BL) AND PREDISPOSED (A) TO LUNG TUMORS

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KEY WORDS: embryonic lung; epithelium; mesenchyme; organ culture.

Mice of lines A and C57BL differ in being predisposed and resistant respectively to spontaneous and induced carcinogenesis of the lung [1, 10]. Interlinear differences in sensitivity of mice to pulmonotropic carcinogens are known to be manifested as early as during prenatal ontogeny and they persist in isolated lung tissue when explanted in organ culture [3-5, 9, 10]. It has been found that the ability of organ cultures of normal embryonic lungs of A mice to survive is significantly less than that of C57BL mice [3-5]. Among the many factors influencing realization of carcinogenic effects the most important, as we know, are the degree of differentiation and the proliferative activity of cells in target organs [15]. This, in particular, explains the high sensitivity of embryonic tissues to transplacental carcinogenic action [10, 11, 14, 15]. The present writers have shown that an essential role in the realization of transplacental carcinogenic influences on embryonic mouse lungs of the sensitive line A is played not only by epithelial target cells, but also by mesenchymal cells and interaction between these tissue components [6-8]. The same factors are important for survival, growth, and differentiation of tissue explanted into culture [12]. An essential factor for normal organogenesis of the lungs is direct contact of the epithelial anlage with the mesenchyme, with a definite ratio between the numbers of cells of these tissue components; the limiting factor, moreover, is the presence of a "critical mass" of mesenchyme [3].

On the basis of the facts given above we postulate that local factors such as proliferative activity and the numerical ratio between epithelial and mesenchymal cells, and also the character of interaction between the tissue components in ontogeny may also play an important role in the realization of sensitivity of mice of a particular line to the development of lung tumors. It was accordingly decided to investigate these characteristics of lung tissue in mice of lines A and C57BL under normal conditions and during induced carcinogenesis. This paper gives the results of a comparative study of the relative numbers of epithelial and mesenchymal cells in organ cultures of embryonic lungs from mice of these lines.

EXPERIMENTAL METHOD

Minced lungs of 17-day A and C57BL mouse embryos, transplanted on to the surface of membrane filters (of the AUFS type, pore diameter 0.6 μ), resting on tantalum gauze platforms, placed in deep watch glasses, were used for culture. Into each watch glass was poured 1.5-2.0 ml of nutrient medium of the following composition: 71.5 ml of medium 199, 25 ml of bovine serum, 2.5 ml of concentrated extract of 11-day chick embryos, 1 ml of 40% glucose solution, per 100 ml of mixture. The material was cultured at 37°C in a constant gas mixture of atmospheric air and 5% CO_2; the nutrient medium was changed every 3-4 days. The explants were studied after 3 and 15 days of culture. ^{3}H-thymidine was added to the cultures on the

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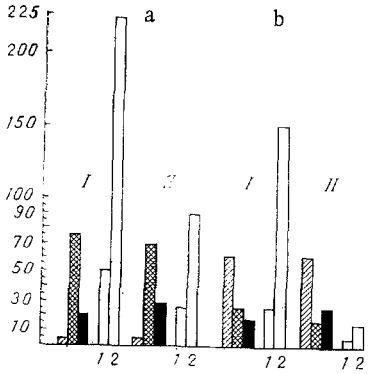

Fig. 1. Cell composition of embryonic lung extracts from A (I) and C57BL (II) mice after culture for 3 days (a) and 15 days (b). Ordinate, number of cells (in %). Oblique shading, EA; cross-hatching, EB; black columns, ME; unshaded columns, MF. 1) Number of MF cells relative to total number of cells in explants; 2) number of MF cells relative to number of ME cells.

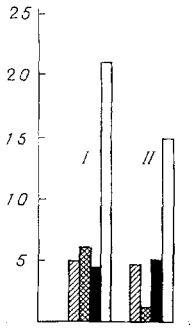

Fig. 2. LI (in %) of cells of embryonic lung explants from A and C57BL mice. Legend as to Fig. 1.

14th day of the experiment in a concentration of 1 μCi/ml medium, and 24 h later they were fixed with 70% ethyl alcohol and treated histologically. Dewaxed serial sections 4 μ thick were coated with type M photographic emulsion, exposed for 2 weeks at 4°C, then developed and stained with hematoxylin and eosin. To characterize the cell structure of the explants quantitatively and determine the labeling index (LI) virtually all the epithelial and mesenchymal cells of the explant itself and mesenchymal cells which had migrated on to the surface of the filter were counted in serial sections. Altogether eight series of experiments were carried out on embryonic lung explants from A mice and more than 70,000 cells were counted; seven series of experiments were carried out on embryonic lung explants from C57BL mice and about 60,000 cells were counted in the early and late stages of culture. The number of different types of cells in the cultures was expressed as percentages of the total number of cells of the explant proper, taken as 100. The chi-square test was used for statistical analysis of the results.

EXPERIMENTAL RESULTS

The cell mass of explants from mice of both lines consisted mainly of epithelial cells of alveolar (EA) and bronchiolar structures (EB), and to a lesser degree, of fibroblast-like mesenchymal cells surrounding them and in direct contact with the epithelium (ME). Many of the fibroblast-like mesenchymal cells had migrated from the explants on to the filter surface and they formed an extensive zone of growth (MF).

After culture for 3 days the number of EA cells in embryonic lung extracts from mice of both lines was small compared with that of EB cells (Fig. 1). The number of ME cells in explants from A mice was about 10% less ($P < 0.01$), but the number of MF cells in them was significantly greater ($P < 0.001$) than in explants from C57BL mice. After 15 days the proportion of EA cells in embryonic lung explants from both lines of mice was considerably increased whereas the proportion of EB cells was reduced. On average the number of cells of the epithelial components in explants of both lines was increased, but not significantly, and the number of ME cells of the mesenchymal components was reduced correspondingly. However, under these circumstances the C57BL explants continued to have a higher proportion of ME cells than explants from line A mice ($P < 0.001$), whereas they contained significantly fewer MF cells than the line A explants ($P < 0.001$). An autoradiographic study of the cultures showed that on the 15th day of the experiment LI of EA cells and of ME cells was low and almost equal (about 5%) in lung explants from both lines of mice (Fig. 2). However, LI of EB cells in line A embryonic lungs was 6 times higher than in line C57BL ($P < 0.001$). LI of MF cells was highest of all, especially in line A explants ($P < 0.001$).

Analysis of the results showed that interlinear differences in the cell structure of mouse embryonic lung explants are manifested in the early stages of culture and they continue during the course of organotypical differentiation, as is shown by the increase in the number of alveolus-like structures and in the number of EA cells in the late stages of the experiment. These differences were reflected quantitatively in the ratio between epithelial and mesenchymal components of lung tissue and between the proliferative activities of their cells. Incidentally, proliferative activity of EB cells was significantly higher in embryonic lungs of line A mice, sensitive to carcinogenesis, and the number of these cells also was greater, than in embryos of the resistant C57BL line (see Figs. 1 and 2). These data agree with views on the important role of proliferative activity of cells for realization of carcinogenic effects in target organs [15]. Similar principles also were found with respect to the mesenchymal component of the lungs. As was mentioned above, in cultures it was represented by two fractions of fibroblast-like cells, similar in their morphology but differing in certain properties and in their number in embryonic lungs of mice of these two lines. These were ME cells in direct contact with the epithelium of organotypical structures of the explants. Their proliferative activity was comparatively low and virtually identical in explants of the two lines. The other fraction consisted of mesenchymal cells (MF) capable of migration and of extensive growth on the filter. Their proliferative activity was several times higher than that of ME cells (Fig. 2), and this was evidently connected with their extensive type of growth and disturbance of epithelial-mesenchymal interactions. However, in lung cultures from mice of line A the proliferative activity of MF cells and their number were substantially higher than in line C57BL. In the period of organogenesis of mammalian lungs significant changes are known to take place in the relative numbers and proliferative activity of cells of the epithelial anlage and mesenchyme [2]. In lungs of 17-day mouse embryos (which were used in this investigation) *in situ*, the most highly differentiated fibroblast-like mesenchymal cells are in direct contact with epithelium of presumptive respiratory passages. The less differentiated mesenchymal cells, free from contact with epithelium, form small islands of loose interstitial tissue. Under conditions of culture it is evidently these cells which migrate from the explant on to the filter and form the zone of growth. Another possibility is that those mesenchymal cells whose contact with epithelium is weaker and (or) is disturbed under extremal conditions such as explantation and culture *in vitro*, may also migrate. In this connection the interlinear differences in proliferative activity under relative numbers of ME and MF cells found in this investigation may be taken as evidence of differences in epithelial-mesenchymal interaction in the target organ of mice of the strains used. The results of this investigation confirm our hypothesis on the role of these factors in realization of the genetically determined sensitivity of mouse lung tissue to carcinogenesis, which is formed actually during prenatal ontogeny. These data also confirm previous results indicating an important role of the mesenchyme of the mouse lungs in the realization of transplacental carcinogenic influences [6-8].

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TRANSPLANTATION OF HUMAN EMBRYONIC TISSUES INTO NUDE MICE

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Tumors have now been successfully transplanted directly from man into nude mice [1, 3]. However, normal human tissues have been successfully transplanted into them extremely rarely. As regards normal adult human tissues, only skin has been successfully transplanted into nude mice [6]. Human tumor cells from tissue culture proliferate well in nude mice [4, 5], but no such proliferation could be obtained when normal human embryonic fibroblasts were transplanted into them from culture [2].

In the investigation described below tissue from human embryonic and fetal skin and muscle, stomach, large intestine, and liver was transplanted into nude mice.

EXPERIMENTAL METHOD

Nude mice based on line BALB/c, aged 8-10 weeks and reared by ourselves, were used. The different tissues for transplantation were obtained from 6-8-week human embryos and 5-8-month human fetuses. The material was injected subcutaneously into the mice in the form of a suspension in a dose of 0.5 ml, which contained 150 mg of human tissue. Sections were cut from the transplants 20-40 days after injection, and stained with hematoxylin and eosin, and picrofuchsine.

EXPERIMENTAL RESULTS

Transplants of liver, stomach, large intestine, and skin and muscle tissue taken from 8-10-week human embryos grew rapidly. If the tissue was taken from 5-8-month human fetuses the transplants grew much more slowly.

The transplants of embryonic liver consisted of a system of cavities (honeycomb) lined with cylindrical, cubical, and thickened epithelium. The cavities were separated by connective-tissue septa of varied thickness. Accumulations of cells resembling hematopoietic tissue were present in the lumen of some cavities. The transplant thus preserved the structure of the embryonic liver (Fig. 1a). The transplant of the fetal liver had a honeycombed struc-

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