LONG-TERM ORGAN CULTURES OF THE PRIMORDIAL MOUSE RESPIRATORY TRACT AND EFFECT OF ISOLATION FROM MESENCHYME

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Epithelial-mesenchymal interactions play an important role in morphogenesis and histogenesis of organs, and in particular, of the lungs. Organ cultures are a convenient model with which to study the role of epithelial-mesenchymal interactions in these processes [4, 5]. Experiments on short-term cultures have shown that the mesenchyme of the lungs induces and maintains morphogenesis of the epithelial anlage of the respiratory tract (RT): removal of the mesenchyme from the lungs of 11-12-day mouse embryos led to cessation of branching, spreading, and fragmentation of the epithelial tree [6, 9].

To study the late effects of disturbance of epithelial-mesenchymal interactions on morphogenesis and histogenesis of RT, long-term organ culture of different parts of the primordial respiratory epithelium of mice, both intact and after isolation from the mesenchyme, was carried out.

EXPERIMENTAL METHOD

Experiments were carried out on 13-day mouse embryos of the A strain, from which the trachea, extrapulmonary bronchi, and lungs were removed en bloc and transferred into a Petri dish containing medium L-15, where the respiratory tract was divided into its various parts and (or) the mesenchyme was removed (Fig. 1). The mesenchyme was removed mechanically (with a sharp instrument) under a binocular loupe, without the use of enzymes. Intact extrapulmonary tracheobronchial regions of RT (35 explants), intact anlagen of the lobes of the lungs (35), the complex of trachea – extra pulmonary bronchi – lungs, after removal of the mesenchyme (31), the extrapulmonary RT after removal of the mesenchyme (34), and the intrapulmonary RT, after removal of the mesenchyme (34), were explanted into organ culture. The explants were cultured on filters of the AUFS type by the method in [1]. After 35-40 days in culture the explants, both intact and isolated from mesenchyme (experimental), were fixed in Bouin's fluid without removal from the filter, taken through butanol, and embedded in paraffin wax. Serial sections through the cultures (5-6 μ) were stained with hematoxylin and eosin. The chi-square test was used for statistical analysis of the results.

EXPERIMENTAL RESULTS

After culture for 35-40 days the explants of the intact lobes on the whole preserved their characteristic structure plan. Ability of the bronchial epithelium to survive was greater than that of the epithelium of the alveolar structures. The latter was atrophied to a considerable degree in virtually all explants. Meanwhile, in 38.4% of the explants, hyperplasia of the bronchial epithelium of the lobes of the lungs was found. Explants of the extrapulmonary tracheobronchial portion of RT at this stage of culture had acquired the shape of large cysts, often filled with homogeneous eosinophilic contents (Fig. 2). The lining epithelium as a rule was cubical (less frequently cylindrical), and in the largest cysts it was atrophied and desquamated in some places. All cyst-like explants showed hyperplasia of the epithelium adjacent to the filter. Only a small proportion (2.8%) of explants had small cavities lined with cubical epithelium, with only slight hyperplasia in areas adjacent to the

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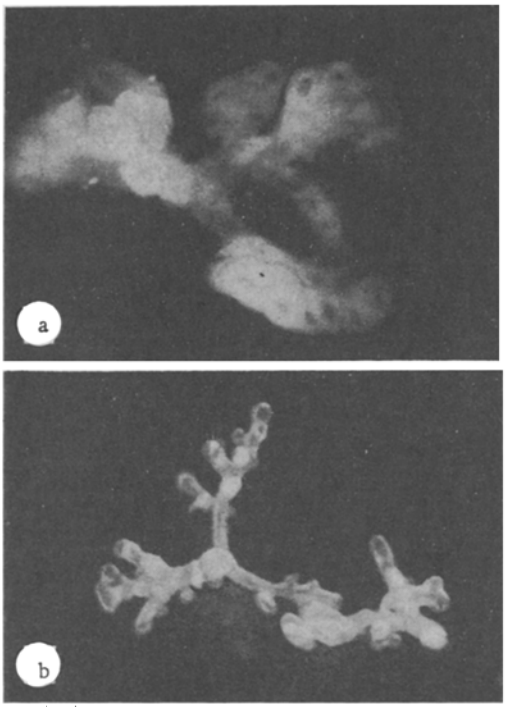

Fig. 1

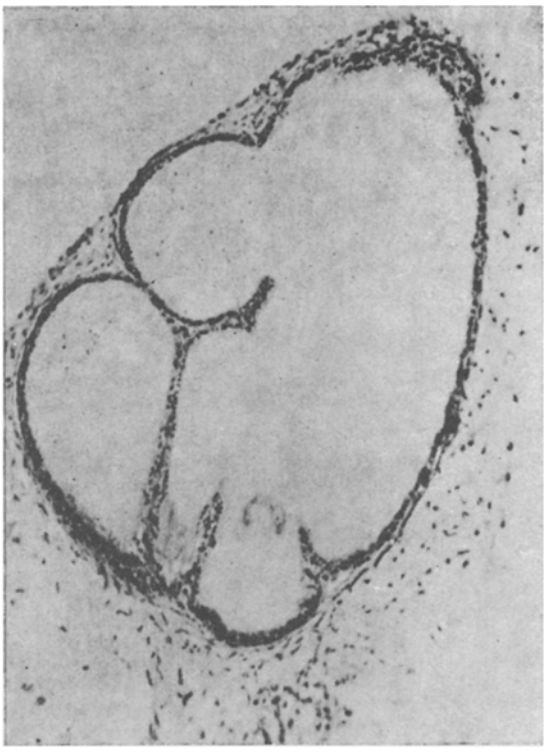

Fig. 2

Fig. 1. Trachea — extrapulmonary bronchi — lungs complex: a) Intact complex, b) after removal of mesenchyme. Unstained preparation. 36 ×.

Fig. 2. Explant of intact extrapulmonary RT (35 days in culture). Hematoxylin—eosin, 78 ×.

filter. The mesenchymal component in intact explants of different parts of RT consisted of one to three layers of elongated cells surrounding epithelial structures. A high proportion of mesenchymal cells had migrated onto the surface of the filter, to form a backing layer between the epithelium and the filter. In these sites hyperplasia of the epithelium was observed in the cyst-like explants. Many explants of the trachea—extrapulmonary bronchi—lungs complex, isolated from mesenchyme, died toward the end of the 3rd week in culture. In all 11 experimental explants which preserved their viability for 35-40 days in culture, besides an epithelial component, a mesenchymal component also was found in a small number of them, in the form of a backing layer between the epithelium and the filter. It is evident that individual mesenchymal cells, remaining after removal, migrated onto the filter and formed loose fibrous connective tissue beneath the epithelium during long-term culture. Most of the surviving explants (72.7%) consisted of a single layer of cubical, normal, or somewhat atrophied epithelium. Only in one explant (9.1%) did the epithelial component consist of several layers of cubical cells, spread above a mesenchymal backing. The remaining two explants (18.2%) consisted of a mass of atypical basophilic cells with a high nucleoplasmic ratio and with many mitoses. Microinvasion of proliferating cells into the partially restored mesenchymal backing and extensive growth over the filter were observed in such cases (Fig. 3). Among the basophilic cells there were microfoci of squamous epithelial cells, showing signs of keratinization in some places. Marked squamous-cell metaplasia was observed in the surface layers of the atypical proliferating masses, and also in the case of extensive growth of cells over the filter. In long-term organ cultures isolated from mesenchyme, the ability of the intrapulmonary epithelium of RT to survive was much less than that of the extrapulmonary epithelium: total necrosis developed in 67.6% and 22.6% of these explants, respectively ($P < 0.001$). Most explants which survived consisted of a single layer or two to four layers of cubical epithelium, supported on a mesenchymal backing. Explants of extrapulmonary RT more frequently consisted of several layers than explants from the intrapulmonary portion (41.9 and 5.9%, respectively, $P < 0.001$), which correlates with the overall rate of survival of explants of these regions of RT. Atypical proliferation of basophilic cells, with areas of squamous-cell metaplasia were found more often in explants of the extrapulmonary RT (12.9%) than in those of the intrapulmonary part (2.9%; $P < 0.1$).

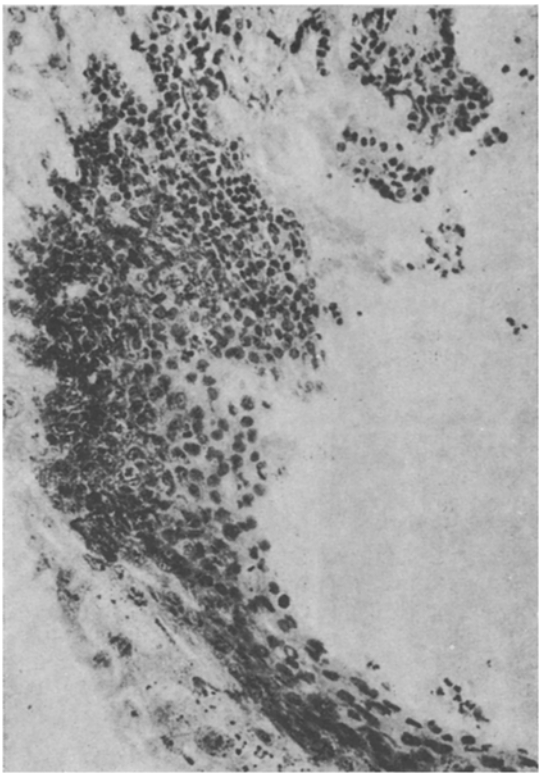

Fig. 3. Explant of extrapulmonary RT after removal of mesenchyme. Focus of proliferation of basophilic cells with regions of squamous-cell metaplasia. Mitoses and microinvasion of proliferating cells into supporting connective tissue can be seen. Hematoxylin–eosin, 140 ×.

It will be clear from these results that different parts of the intact RT preserve their viability and their characteristic organotypical structure in organ culture for a long time. Cystic structures appearing in explants of the tracheobronchial extrapulmonary RT are evidently the result of *in vitro* realization of morphogenetic powers, aimed at maintaining the hollow structure of these parts of RT. Hyperplasia of the epithelium, developing at points of contact of the cyst-like explants with the filter, is evidently the result of the inducing effect of the underlying layer of mesenchyme, formed by migrating cells, whose proliferative activity, as has been shown previously [2], is significantly higher than that of the mesenchymal cells of the explant itself. The writers' previous experiments showed that increased proliferative activity of the mesenchyme during organogenesis of the lungs causes increased proliferation of the epithelium [3]. Clearly in the present experiments also, a similar phenomenon took place. Long-term culture of the intact anlage revealed a higher rate of survival of the bronchial epithelium than of the epithelium of the alveolar structures. Complete removal of the mesenchyme led to death of the majority of explants of the different parts of RT, in agreement with results obtained by other workers in short-term cultures [6, 9]. We found that the presence of a few undetached mesenchymal cells prevents death of the epithelium for a long time. However, the growth powers of the epithelium, especially of the intrapulmonary part of RT were sharply reduced compared with those of the intact explants. In all the experimental explants morphogenesis and the spatial organization of the epithelial and mesenchymal cells were disturbed. In the overwhelming majority of cases, histiotypical growth of the layers of epithelium over a mesenchymal backing essentially was observed. Foci of proliferation of atypical basophilic cells with regions of squamous-cell metaplasia, appearing in isolated cases, were similar in their morphology, in the abundance of their mitoses, and in their capacity for microinvasion and extensive growth, to basal-cell proliferation of the epithelium of RT in regenerative and neoplastic processes observed *in vivo* [7, 8]. Separate culture of the different parts of RT showed that cells of the extrapulmonary tracheobronchial epithelium, whose growth potential, like that of intact RT, is higher than that of the epithelium of the distal portion, was the source of these atypical foci of proliferation.

Long-term organ cultures of the primordial mouse RT, both intact and isolated from mesenchyme, were thus obtained. As a result, differences were discovered in the growth powers of the proximal and distal portions of RT and it was shown that long-term disturbance of epithelial-mesenchymal interactions leads to cessation of morphogenesis and to a reduction in the growth powers of the epithelium, and also that important deviations in differentiation and bkological properties of the cells arise in epithelium deprived of the normal inducing influence of the mesenchyme, as is shown by the appearance of foci of proliferation of atypical cells in the experimental cultures.

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CORRELATION BETWEEN CIRCADIAN RHYTHMS OF LYMPHOCYTE RECIRCULATION AND cAMP SYNTHESIS

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One of the principal processes supporting function of the immune system is continuous redistribution of cells, in accordance with a regular spatiotemporal organization that is reflected in the circadian rhythms of the number of cells in the lymphoid organs and the number of lymphocytes in the peripheral blood [3]. Recirculation and migration of individual subpopulations of lymphoid cells are synchronized with each other in a definite manner, and also with cyclic fluctuations of the functional state of the lymphocytes and the associated level of intracellular metabolism [3, 8-10]. In our opinion, the study of correlation between circadian rhythms of recirculation of immunocompetent cells, and the turnover of cyclic nucleotides — universal regulators of differentiation and function of cells [1], in them is a matter of great interest. In particular, it has been shown that differentiation of lymphocytes, mainly T cells, is accompanied by elevation of the intracellular cAMP level, with immediate participation of adenylate cyclase (AC), the enzyme catalyzing conversion of ATP into cAMP [4, 11].

Analysis of correlations between circadian rhythms of the parameters of distribution of lymphocytes and rhythms of AC activity in them could help to elucidate the principles and mechanisms of regulation of temporal coordination between such fundamental processes in the immune system as cellular migration, recirculation, and differentiation. The investigation described below was devoted to a study of these problems.

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