REPOPULATION OF DENUDED TRACHEAL GRAFTS WITH ALVEOLAR TYPE II CELLS

Abstract -- Repopulation of denuded heterotopic tracheal grafts with populations of specific epithelial cell types is one approach to study the differentiation potential of various cell types. This technique has been adopted to delineate the differentiation pathways of alveolar type II cells isolated from rat lungs. Under the conditions of this experiment, the reestablished epithelial lining was alveolar-like, however, ultrastructural analysis of the cells showed them to be like Clara cells. These preliminary results suggest that the secretory cells of the lung parenchyma and terminal airways may share a common ancestry.

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The lining of the alveoli is composed of type I and type II cells. The type I cell is squamoid and is thought to be terminally differentiated from type II cells. Type II cells are secretory cells that produce and secrete a surfactant material covering the lining of the alveolus. Type II cells are capable of undergoing transformation, forming peripheral adenocarcinomas. Adenocarcinomas in the lung periphery can also arise from Clara cells of the terminal bronchiolar lining. These tumors account for 5 to 10% of human lung tumors and are thought to be largely independent of cigarette smoking. Peripheral lung tumors are the type found spontaneously in many animal species and also following exposure to respirable carcinogens. The differentiation pathways of alveolar type II cells have been largely determined from histological studies following injury to the epithelial lining of the alveolus. It is frequently difficult to follow the lineage of particular cell types using histological approaches on tissues derived from organs undergoing repair. The ability to isolate type II cells in high purity, and their subsequent use as an inoculum to repopulate denuded heterotopic tracheal grafts, has been exploited as a method to delineate the differentiation pathways of rat alveolar type II cells.

METHODS

Male Fischer 344/N rats from the Institute's breeding colony were killed by CO_2 anesthesia. The lung vasculature was perfused and lungs lavaged with saline. The trachea and lungs were removed and inflated with a mixture of hyaluronidase and cytochalasin B and incubated for 60 min at 37°C, after which the mixture was removed and saved, and replaced with a pronase solution, which was incubated for a further 30 min. The pronase solution was also removed and saved. The lungs were cut up into small pieces, which were washed in medium (RPMI) containing 10% fetal calf serum. This medium was saved and the remaining lung tissue incubated with a mixture of elastase and collagenase for 30 min. The resultant mixture was passed through a 70 μm nylon mesh and the medium saved. All of the saved samples were pooled and placed in a Celsep (Brinkman Instruments, NY) chamber containing a continuous Ficol gradient (1-2%). The fraction containing the highest purity type II cells (as previously confirmed by electron microscopy) was analyzed for cell number and viability by using a hematocytometer. Cytospin preparations from this fraction were prepared and stained with a modified Papanicolaou stain to identify alveolar type II cells. Viability was determined by trypan blue dye exclusion. The cell concentration was adjusted to 2×10^5 per mL; 0.5 mL of this suspension was used as the inoculum to repopulate denuded tracheal grafts. The

grafts were prepared according to the procedure described by Terazghi et al.[1] The inoculated grafts were implanted subcutaneously in the scapular region of host male Fischer 344/N rats. Additional grafts were inoculated with medium alone to verify that the grafts were completely denuded of epithelial cells. The animals were left for 5 wk before they were killed by CO_2 anesthesia and the tracheal grafts removed for histological and electron microscopical examination. Paraffin embedded tissue was stained with hematoxylin and alcian blue or with PAS (periodic acid schiff reagent). Resin embedded sections were stained with uranyl acetate and examined by electron microscopy.

RESULTS

Isolation of type II alveolar cells using density-gradient sedimentation produced a cell fraction containing 85-88% alveolar type II cells. The purity of the sample was determined by electron microscopy. The non-alveolar type II cells were predominantly macrophages (7-11%) and inflammatory cells (lymphocytes and polymorphonuclear leukocytes); ciliated, Clara and brush cells formed 2-3% of this cell fraction. The isolated type II cells had an ultrastructural appearance (Fig. 1) similar to that found in cells lining the alveolus of the normal rat lung.

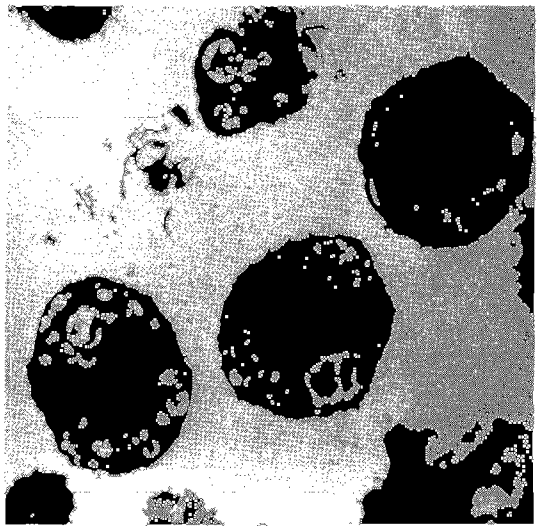


Figure 1. Electron micrograph of isolated type II alveolar cells. The cells contain the characteristic lamellar bodies and have an ultrastructural appearance of type II cells found in the intact lung.

Light microscopy examination of the type II cell-inoculated tracheal grafts showed that an epithelial lining had been reestablished. The epithelial lining was composed of flat squamoid cells and spherical cells that had a superficial appearance similar to the alveolar lining seen in the intact lung (Fig. 2). Inoculation of the grafts with medium alone resulted in the lumen of the graft being filled with loose connective tissue comprised of fibroblasts, macrophages and small capillaries. Electron microscopy revealed that the cells of the repopulated trachea contained secretory granules similar to those seen in mature Clara cells; the cells also contained prominent rough endoplasmic reticulum and mitochondria (Fig. 3). The epithelial nature of these cells was confirmed by the presence of cell junctions. Macrophages were frequently seen in association with the epithelial lining. The macrophages had well developed microvilli and numerous lysosomes; their ultrastructural appearance was markedly different from that of the adjacent epithelial cells.

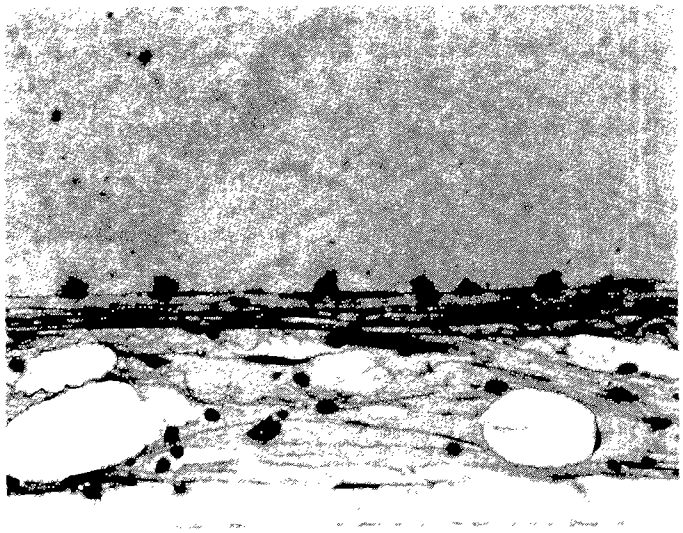


Figure 2. Light micrograph showing the alveolar-like epithelial lining re-established after inoculating type II cells five weeks previously.

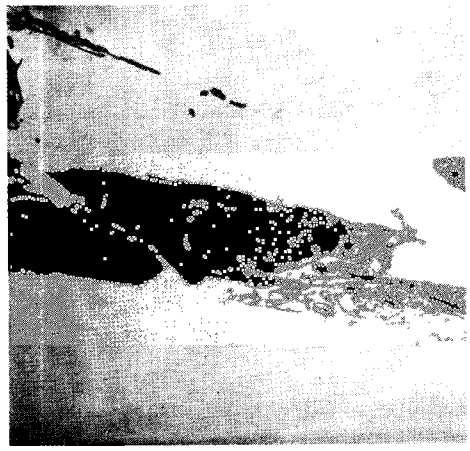
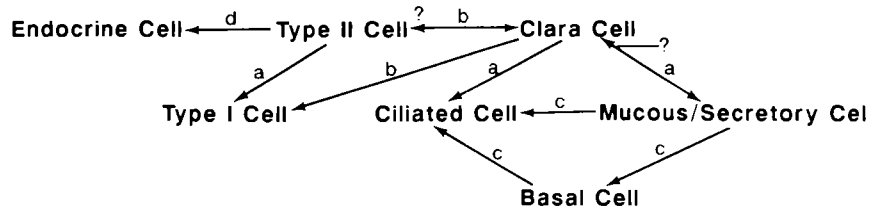


Figure 3. Electron micrograph of the reestablished epithelium showing the electron dense granules similar to those seen in mature Clara cells of the intact epithelium.

CONCLUSION

This study confirms the utility of tracheal repopulation studies in determining the differentiation pathways of pulmonary epithelial cells. Inoculation of denuded tracheal grafts with highly enriched populations of type II cells resulted in an epithelial lining that superficially resembled the alveolar lining in normal lungs. However, instead of being composed of type II cells, the epithelium was made up of Clara-like cells. These results suggest that the type II alveolar cells and Clara cells share a common ancestry. It is well documented that type II cells are the progenitor cell in the peripheral lung and give rise to the alveolar type I cells. Schuller[2] has demonstrated that, under certain circumstances, type II cells can differentiate towards endocrine-like cells containing characteristic dense core granules. The cell fractions used in this latter study contained 12-15% contaminating cells that may have influenced the outcome of the experiment. The majority of contaminating cells in the present study were macrophages and inflammatory cells that would not be expected to contribute to the repopulation of the denuded tracheas. The contaminating mixture of epithelial cells accounts for only 2-3% of the total cell fraction and would be insufficient in number to repopulate the trachea grafts in their own right. If the contaminating Clara cells possessed great replicative capacity, the reestablished epithelium would be expected to contain ciliated cells. Clara cell progeny have

been shown to include ciliated cells following injury to bronchiolar epithelial lining. Isolated Clara cells have also been shown to be capable of establishing an epithelial lining similar to bronchiolar epithelium following their inoculation into denuded tracheal grafts, and seem capable of mucous differentiation, as muco/Clara cells have been identified following toxicant injury to the lower airways. The muco/Clara cells possess mucous granules similar to those found in the secretory cells of the upper airways and electron-dense secretory granules seen in Clara cells. A companion study to this report (1986-87 Annual Report, LMF-120, pp. 508-512) shows that the secretory cell of the rat trachea is the major proliferative and progenitorial cell and is capable of giving rise to all the major cell types encountered in the upper airways. These latter results confirm those of McDowell and Trump[3] who showed that following a denuding injury to hamster trachea, secretory cells are the major cell types involved in its repair. This complex of differentiation pathways may have common or interrelated steps (Fig. 4), with an undifferentiated secretory cell playing the pivotal role, its differentiation determined by microenvironmental influences. This plasticity of development may also be evident in lung tumor cells that can co-express a combination of or all the features associated with epidermoid carcinoma (cytokeratin bundles), adenocarcinomas (secretory granules) and small cell lung cancer (dense core granules).[3]



a) Confirmed by several reports in established literature
b) Present experiment
c) Companion study (1987 - 1988 Annual Report)
d Single study of Schuller, 1988
?) No Evidence

Figure 4. Proposed scheme for the interrelationship between the differentiation pathways of the major epithelial cell types found in the lung.

REFERENCES

1. Terazghi M., P. Nettesheim, and M. L. Williams. Repopulation of Denuded Tracheal Grafts with Normal, Preneoplastic, and Neoplastic Epithelial Cell Populations, Cancer Res. 38: 3563-3565, 1978.

2. Schuller, H. M. Mechanisms of Neuroendocrine Lung Carcinogenesis, Proceedings of Biology, Toxicology, and Carcinogenesis of Respiratory Epithelium Symposium, Albuquerque, NM, November 14-16, 1988.

3. McDowell, E. M. and B. F. Trump. Histogenesis of Preneoplastic Lesions in Tracheobronchial Epithelium, Surv. Synth. Pathol. Res. 2: 235-279, 1983.