SEARCH FOR STEM CELLS FOR PULMONARY ALVEOLAR EPITHELIUM

MAREK CEGIELSKI¹, IRENEUSZ CAŁKOSIŃSKI², PIOTR DZIEGIEL¹ AND MACIEJ ZABEL¹

¹Department of Histology and Embryology, ²Department of Physiology, Medical University of Wroclaw, 50-368 Wroclaw, Poland

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Abstract

The present study was aimed at detecting actively dividing cells which might correspond to stem cells of the epithelium in pulmonary alveoli. The studies were performed on rats in which pneumonia was induced by intra-pleural injection of carrageenin and which, subsequently, were treated with bromodeoxyuridine (BrdU) to identify actively dividing cells. Lung samples served to prepare paraffin sections and the material was fixed for studies in electron microscope (EM). In the paraffin sections, immunocytochemical reactions were performed using anti-BrdU antibodies and colloidal gold, if the reaction was monitored by EM. The highest number of BrdU-labelled cells was noted on the 5th d of the experiment. As for localisation, few of them corresponded to the surface cells of pulmonary alveoli. The remaining labelled cells were present in the interstitium, close to blood vessels, in vessel lumen and in inter-alveolar septa. Positive results of the immunocytochemical reaction noted by light and electron microscopy as well as distribution of the labelled cells pointed to the involvement of several heterogeneous populations of stem cells, which participate in the regeneration of the respiratory epithelium.

Key words: rats, stem cells, lung, alveolar epithelium.

Recent years of studies on stem cells represented a period of an enormous progress and new approach to the role, significance, and functioning of the cells. On one hand, the new perspective has opened up using the cells in therapy, on the other hand, their role have been observed to be increasingly important in renewal and regeneration of mature tissues and organs. The processes of regeneration and renewal are linked to persistent presence of stem cells in tissues and organs, with their heterogeneous populations circulating in blood and when needed being released from bone marrow (5, 9).

Mature respiratory epithelium represents a morphological structure consisting of flat type I cells (pneumocytes type I), cuboid type II cells (pneumocytes type II). Pneumocytes of type I form a thin lining of pulmonary alveoli and pneumocytes of type II synthesise a surfactant, the substance which controls surface tension in pulmonary alveoli. Pneumocytes of type II are extremely rare and their free surface forms numerous low, thick microvilli, providing the cells with a brush outlook (hence their name of brush cells). Till now, pneumocytes of type II are thought to be responsible for the regeneration of pneumocytes type I (4). However, it seems improbable that fully differentiated, mature surfactant-producing cells in certain conditions start to proliferate and provide source for renewal of pneumocytes type I. Occasionally, the cells of type II are termed stem cells of alveolar surface. Nevertheless, it remains to be seen which cells exhaust criteria of stem cells for pneumocytes. Therefore, the question appears whether a subpopulation of cells exists in the respiratory epithelium which exhibits traits of the stem cells.

The present study was aimed at detecting and identifying the dividing cells which may correspond to stem cells of the pulmonary alveolar epithelium in the rat and which participate in post-inflammatory regenerative processes. The studies performed till now indicate that regenerative processes linked to augmented proliferative activity of cells in pulmonary parenchyma may occur in the 4th-5th d following induction of pneumonia with carrageenin (2, 1).

Material and Methods

The studies were performed on Buffalo strain rats, weighing each around 250 g. In the experimental animals pneumonia was induced by intrapulmonary injection of 1.5% carrageenin solution (2, 1). Then, on the 4th, 5th and 10th d bromodeoxyuridine (BrdU) was intraperitoneally injected at the dose of 50 mg/kg body weight. Twenty-four hours after the injection of BrdU the rats were sacrificed and lung samples were taken for light microscopy and electron microscopy. In order to visualise proliferating cells, immunocytochemical reactions were performed in paraffin sections using monoclonal anti-BrdU antibodies (clone BU 33, Sigma, product diluted 1:50) (6). BrdU was visualised using
biotinylated antibodies and streptavidin-peroxidase complex (LSAB2, Dako) and diaminobenzidine (DAB). In control rats no pneumonia was induced and BrdU was administered in the same dose. Moreover, immunocytochemical reactions were performed with colloidal gold and they were evaluated in an electron microscope. Material for electron microscope studies was fixed in 4% paraformaldehyde in 0.1 M cacodylate buffer and then dehydrated and embedded in Epon 812. The sections were incubated in 0.5% BSA and 0.05% Tween-20. Subsequently, the sections were incubated with anti-BrdU antibodies (product diluted 1:500, 24 h exposure at room temperature) which were visualised using anti-mouse IgG antibodies coated on colloidal gold (15 nm). All reagents used for electron microscopy originated from Sigma (7).

Results

Light microscopy. On the 5th d of the experiment pneumonia was most advanced; an intense colour reaction was observed as well as the highest number of BrdU-stained cells. Due to their location, only few of the cells corresponded to cells of pulmonary alveolar surface; most of the stained cells were positioned in the pulmonary interstitium, close to vessels, in their lumen and in inter-alveolar septa. The labelled cells were manifested individually or in small groups (Figs 1, 2). On the 10th d, on the other hand, the regeneration of the pulmonary epithelium was almost complete and the immunocytochemical reaction did not label any cell.

Electron microscope. Results of the immunocytochemical reactions examined under light microscope were confirmed in the electron microscope studies with colloidal gold. On the 5th d, definitely most numerous labelled cells and stronger labelling with colloidal gold were noted, pointing to the incorporation of BrdU to interstitial pulmonary compartment, in blood vessels and inter-alveolar septa. Few of the labelled cells corresponded to alveolar surface cells. A pronounced labelling could be noted in cells of the vascular endothelium (Figs 3, 4), in which repair processes used to be linked to the proliferation and exchange of cells damaged by the inflammatory process. In lumen of capillaries which surrounded pulmonary alveoli an intense reaction with colloidal gold was observed in the small, non-adherent oval cells (Figs 5, 6). Intensely labelled with gold were also the cells which migrated to the alveolar surface from connective tissue septa (Figs 7, 8). The labelling of cells which resembled traits of pneumocytes type II could never be detected.

![Fig. 1. The BrdU-stained cells of pulmonary alveolar surface manifested individually. x 400.](image1)

![Fig. 2. The BrdU-stained cells of pulmonary intestitium manifested in small groups. x 400.](image2)
Fig. 3. The reaction with colloidal gold in the cells of vascular endothelium. EM x 10000.

Fig. 4. The reaction with colloidal gold in the cells of vascular endothelium. EM x 30000.

Fig. 5. The reaction with colloidal gold in the small, non-adherent oval cell. EM x 8000.

Fig. 6. The reaction with colloidal gold in the small, non-adherent oval cell. EM x 40000.
Discussion

In numerous studies aimed at identification of stem cells various labelling techniques were used for the purpose. Until now, however, no single specific technique has been identified which would permit unequivocal detection of stem cells in the lungs. This might reflect heterogenous character of the cell population (3). For example, in the liver multiple types of stem cells are engaged in reparative processes and their activation depends on the nature of the damaging agent (4).

In our experimental model of pneumonia, post-inflammatory reparative processes were stimulated (2, 1). In such conditions, application of BrdU permitted to demonstrate individual proliferating cells, which might include stem cells. The labelled cells were observed within pulmonary alveoli, in the blood vessel lumen, close to vessels and in inter-alveolar septa. This seems to demonstrate that probably several types of stem cells are engaged in reparative processes and their activation depends on the nature of the damaging agent (4).

As mentioned above, no specific surface markers are at present available, which would permit the detection of individual types of tissue-oriented stem cells (8). Thus, at present stage we are unable to specify which of the labelled cells represent potential stem cells participating in repair processes.

Positive results of the immunocytochemical reactions in the light and electron microscopes point to the participation of a few heterogenous populations of proliferation cells in post-inflammatory processes of repair in the respiratory epithelium. On the other hand, a role and significance of differentiated, surfactant-producing pneumocytes of type II in the processes seems highly controversial and requires new approaches to the problem.

References
