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The search for stem cells of the epithelium in pulmonary alveoli

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In recent years significant progress has been witnessed in the identification of stem cells, which have now also been identified in the lungs. The aim of this was to induce post-pneumonia alveolar regeneration to facilitate the identification of stem cells. The studies were performed on Buffalo strain rats. Pneumonia was induced in the animals by a sub-pleural injection of carragenin. On days 4, 5 and 10 of the experiment both the control and experimental animals received intraperitoneal injections of bromodeoxyuridine (BrdU). Twenty-four hours after the last BrdU injection the rats were sacrificed and samples of the lungs were taken for examination. In order to detect proliferating cells in the paraffin sections, BrdU incorporation was detected with monoclonal antibodies. In pilot experiments BrdU incorporation was demonstrated in individual alveolar cells of variable distribution and of variable intensity in the colour reaction. The results have confirmed the existence of stem cells in pulmonary alveoli but their closer characterisation requires further studies with other techniques to detect pulmonary stem cells.

Key words: stem cells, lungs

INTRODUCTION

A mature respiratory epithelium morphologically consists of flat Type I cells (Type I pneumocytes) and cuboid Type II cells (Type II pneumocytes). Type I pneumocytes of the alveolar epithelium form a thin lamina (alveolar lining), while Type II pneumocytes synthesise surfactant, the substance which controls surface tension in pulmonary alveoli. Type III pneumocytes are extremely rare and their free surface forms numerous short thick microvilli which provide the cells with a brush border (brush border cells). The cells of the respiratory epithelium referred to above form a single layer based on a basal membrane.

To date Type II pneumocytes have been thought to be responsible for the regeneration of Type I pneumocytes. However, it seems relatively improbable that fully differentiated, mature surfactant-producing cells could divide in specific conditions, providing a source of new Type I pneumocytes. Occasionally Type II pneumocytes are termed "stem cells of the alveolar surface". Nevertheless, the identity and number of the Type II pneumocytes which fulfil the criteria of stem cells remains unclear. Thus, the question arises as to whether a subpopulation of Type II pneumocytes exists which behave like stem cells [6].

Studies in recent years have indicated that stem cells play a key role in the renewal of injured Type I pneumocytes. However, there has been no proof of the presence of stem cells in alveolar epithelium [3].

In addition, no generally accepted definition of stem cells is available, even if the cells carry certain common characteristics including:

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— a capacity for self-renewal in the course of the entire life of an organism;
— the fact that their population cannot be depleted or used up;
— the fact that proliferation involves asymmetric divisions and that of the two daughter cells one remains in the stem cell line while the other acquires the potential for multidirectional differentiation (pluripotential cells);
— the fact that the cells share a simple structure and are small and free of differentiating traits [4].

In recent years significant progress has been achieved in distinguishing stem cells for organs such as the intestine, liver, pancreas, neural crest, testes and lens [3]. Identification of stem cells in the lungs remains an unsolved problem and for this reason we have attempted to distinguish these cells. The cells are capable of proliferating only in specific conditions (the development of the organ, trauma and inflammation), undergoing asymmetric divisions. The model of an inflammatory process applied by us leads to damage of the alveolar cells and their subsequent regeneration [1]. Thus the regeneration must be accompanied by augmented proliferative activity of the stem cells, which leads to the reconstruction of the normal morphological structure of the pulmonary alveoli. In order to demonstrate and to identify the stem cells, we have searched for cells of augmented proliferative activity using one of the techniques employed for the purpose of detecting bromodeoxyuridine (BrdU) incorporation [4, 5].

MATERIAL AND METHODS

The studies were performed on Buffalo strain rats, each weighing around 250 g. Pneumonia was induced in the experimental animals by intrapulmonary injection of 1.5% carragenin solution [1]. Subsequently, on the 4th, 5th or 10th day, the animals received an intraperitoneal dose of BrdU (50 mg/kb body weight). After 24 hours the animals were sacrificed and lung fragments were sampled for examination by light and electron microscopy. In order to visualise proliferating cells in the paraffin sections, immunocytochemical reactions were performed using monoclonal anti-BrdU antibodies (clone BU 33, SIGMA)(5). The control group consisted of rats in which no pneumonia was evoked but which were injected with the same dose of BrdU.

RESULTS AND DISCUSSION

Earlier studies on pneumonia ultrastructure enabled us to determine the points of time at which the immunocytochemical reactions should be performed [2]. On the 5th day, i.e. at the time of most advanced pneumonia (Fig. 1), a more intense colour reaction and the highest number of labelled cells (Figs. 2, 3) were noted. Among the labelled cells only

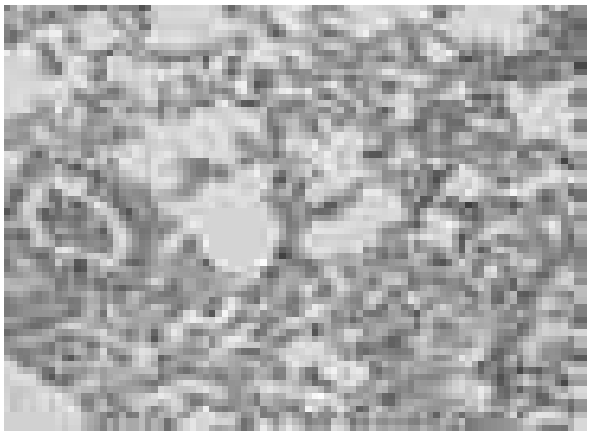

Figure 1. Massive imflammatory reaction in the lungs on the 5th day of the experiment; H+E staining, × 100.

Figure 2. Labelled cells of the alveolar surface. Immunocytochemical reaction with BrdU; × 200.

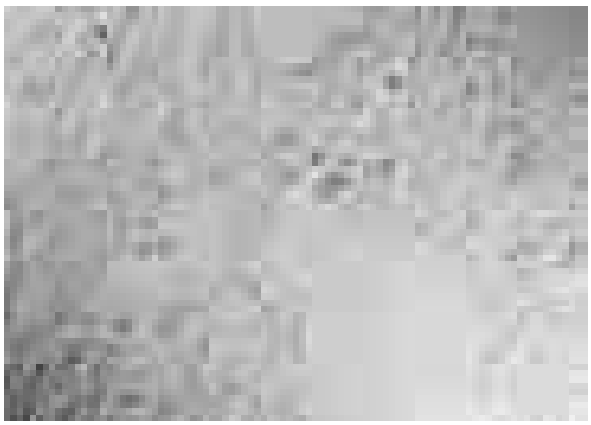

Figure 3. Labelled cells in the interstitial part of lungs. Immunocytochemical reaction with BrdU. × 200.

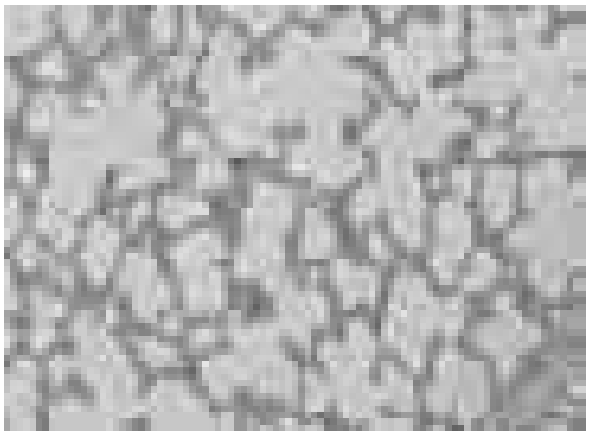

Figure 4. Complete regeneration of the pulmonary alveoli on the 10th day of experiment. H+E staining. × 100.

a few corresponded to alveolar surface cells, as shown by their localisation (Fig. 2). The clear majority of the cells were noted in the interstitial portion of the lungs, close to the blood vessels and interalveolar septa (Fig. 3). The labelled cells were distributed individually or in small groups. On the 10th day, the completed regeneration of pulmonary alveoli (Fig. 4) was accompanied by a negative immunocytochemical reaction and no labelled cells could be noted.

In numerous studies on the identification of stem cells several labelling techniques have been used to demonstrate the cells. To date it has not been possible to specify any single technique which could unequivocally identify stem cells in the lungs. This may reflect the heterogeneity of the cell population [4]. For example, the liver contains several types of stem cell which are engaged in the reparative processes and their activation reflects the nature of the injury [3]. Our experimental model of pneumonia and BrdU labelling enables the individual proliferating cells to be demonstrated within the pulmonary alveoli in the neighbourhood of blood vessels and the septa of connective tissue [4]. Which of the cells in fact represent stem cells? We intend to look for a solution to this in additional studies using an electron microscope, which will permit us to identify the types of labelled cell and their precise location.

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