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Increased Apoptosis in the Alveolar Microenvironment of the Healthy Human Lung

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Background. **Apoptosis represents a physiological clearance mechanism in human tissues. The role of apoptosis has not been examined in normal lung cell populations, such as alveolar macrophages and polymorphonuclear cells. What is the percentage, as well as the role, of apoptosis in the alveolar microenvironment of the healthy human lung?**

Patients and methods. **Bronchoalveolar lavage was obtained from 21 volunteers without lung disease. The specimens were analyzed using: Annexin V binding, DNA laddering, light microscopy and immunohistochemistry for bcl-2 expression.**

Results. **Apoptosis of the total bronchoalveolar lavage cell population was 51.2%. Both alveolar macrophages and polymorphonuclear cells had a high apoptotic rate (62.1% and 48.3%, respectively) as determined by Annexin V binding. These findings were further confirmed using morphological criteria for apoptosis and gel electrophoresis for DNA fragmentation. In the majority of the individuals examined, (8 out of 21), the bcl-2 gene was expressed in the lymphocyte population mainly.**

Conclusions. **The percentage of apoptosis in lung cells of healthy humans is high. Apoptosis plays a key role in normal lung cell death. It appears to be the mechanism that opposes cell proliferation by eliminating, aged or damaged cells thus facilitating the process of lung remodeling.**

Key Words: **bronchoalveolar lavage; alveolar macrophages; polymorphonuclear cells; Annexin V; DNA ladder.**

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INTRODUCTION

The main function of the alveolar epithelium is to provide an extensive and thin surface for gas exchange. The pulmonary epithelium serves a number of additional functions that act to preserve the capacity for such gas exchange. It provides a host barrier from the outside environment while it contributes to the maintenance of lung fluid balance. The lung epithelium also plays an active role in the metabolism of endogenous mediators and xenobiotic agents [1]. These functions are carried out by millions of cells of the lung parenchyma, which are mounted in a specific architecture.

Bronchial secretions and the recovered bronchoalveolar lavage (BAL) are rich in many structural and immunological components of the alveolar network of the lungs. In particular, bronchial secretions are made up mainly by T and B-lymphocytes, macrophages, polymorphonuclear cells (PMNs), and some epithelial cells (type 1 and type 2 pneumocytes). Both T and B-lymphocytes are found in bronchoalveolar lavage fluid from normal lung in similar proportions to those found in peripheral blood. The alveolar macrophages are the main phagocytic cells of the acinus and the probable explanation for the usual sterility of the alveolar surface [1a]. They comprise the majority (>90%) of the alveolar cell population, with greater numbers being obtained from smokers' lungs. Few PMNs are usually observed in the BAL fluid of normal lungs since these cells are chemotactically attracted to specific sites when and where inflammation occurs. Type 1 pneumocytes are the pavement epithelium of alveoli, specialized for the diffusion of gas from alveolus to capillary. Type 2 pneumocytes are more numerous than

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type 1 cells and are secreted into the alveolar lumen as surfactant; both type 1 and type 2 pneumocytes are usually present in very small numbers in the bronchoalveolar lavage fluid recovered from normal lung.

Over the last few years compelling evidence has established an important physiological role for apoptotic cell death in maintaining optimal cell number in multicellular organisms [2]. The regulation of cell number is a crucial property of complex multicellular eukaryotes, and evolution has selected for the development of elegant mechanisms to modulate the rates of both cell birth and cell death (apoptosis). Individual organ systems can tolerate impressive levels of flux in cell number in response to physiological cues, but they retain the capacity to maintain numerical equilibrium [3]. Furthermore, the perturbation of normal rates of apoptosis in adult tissues has been associated with a number of malignancies, suggesting an important role for apoptosis in homeostasis in adult tissues [4]. Apoptosis has been described in the mature lung in response to acute lung injury and other lung diseases, but its role has not been implicated, thus far, in the maintenance of the normal lung tissue. In the lung, several studies have shown the existence of apoptosis in lung parenchymal cells either in normal development [5, 6] or pathological states [7–9].

PATIENTS AND METHODS

Study Subjects

BAL was obtained with consent from 21 non-smoking volunteers with no concomitant lung disease. Nineteen underwent elective laparoscopic cholecystectomy for cholelithiasis and two underwent bronchoscopy for diagnostic purposes. Patients were not included in the study if they met at least one of the following exclusion criteria: if they had primary lung pathology, if they had previously received immunosuppressive agents, if they had acquired immunodeficiency syndrome, were transfused with more than two units of blood in the 12 h preceding the study enrollment, were younger than 18 and older than 85 years of age and/or had a history of malignancy. Their mean age was 55.4 years (SE ± 3.06, median, 56.5). Seven were males (33.3%) and 14 were females (66.7%) (M/F: 0.5). Data, including the white blood cell count erythrocyte sedimentation rate and transferin were within normal values (Table 1).

The samples from patients with cholelithiasis ($n = 19$) were taken immediately after intubation. The other two patients underwent bronchoscopy under light sedation for diagnostic reasons. There were no postoperative complications. Patients that were operated were hospitalized for an average of 3.7 days (SE ± 0.36, median, 4), while patients who underwent diagnostic bronchoscopy were discharged 6 h afterward.

All of the procedures followed were in accordance with the ethical standards on human experimentation approved by the institutional review board for research. Informed consent was obtained from the patient or a surrogate.

Study Design

BAL was obtained from 21 healthy, non-smoking volunteers. The specimens were analyzed using: Annexin V binding to measure membrane changes associated with apoptosis, DNA laddering, light microscopy to assess and confirm morphological characteristics of apoptosis, and immunohistochemistry for the detection of bcl-2 expression.

Methods

BAL Protocol

The same physician performed all BALs. Briefly, a fiberoptic bronchoscope (Pentax 15p, Tokyo, Japan) was inserted into the endotracheal tube (≥8.0-mm i.d.) through an adapter. During BAL, the fractional inspired oxygen concentration was increased to 100%. The tip of the bronchoscope was wedged in the right middle lobe or the lingula and initially 20 mL of sterile isotonic saline (0.9% NaCl) at 37°C were infused and the fluid recovered was discarded, reflecting a bronchial sample. Subsequently, three aliquots of 60 mL 0.9% NaCl at 37°C were instilled and recovered using low-pressure suction. The volume of fluid recovered from the 180 mL of total fluid instilled ranged from 50 to 140 mL (30–80%), with a mean volume of 95 mL (55%). The BAL aliquots were then transported to the laboratory. The fluid was filtered through gauze and moistened with 0.9% NaCl to remove mucus.

Total Viable Cell Yield

Total viable cell yield was determined by trypan blue exclusion. Cell counts were then adjusted to 1×10^6/mL using phosphate-buffered saline (PBS), pH 7.4.

Morphological Criteria for Apoptosis

The morphological identification of apoptotic nuclei was assessed by two independent investigators. The cells were considered apoptotic if they showed dense condensation of chromatin in the form of either a single nucleus or nuclear fragments not connected by strands [10, 11].

Flow Cytometry

Flow cytometric measurements were performed using the Coulter Epics XL-MCL flow cytometer, with corresponding software System II and EXPO 32 (Coulter Corp., Palo Alto, CA).

Annexin V Binding and Propidium Iodide

To measure membrane changes associated with apoptosis, the BAL cells were labeled with Annexin V-FITC [12, 13] (Annexin V

TABLE 1

Clinical and Laboratory Data of the Study Group

Systolic BP (mmHg)	Mean 129.58 SE ± 4.62	Median 122.5 (115–170)
Diastolic BP (mmHg)	Mean 71.60 ± 2.78	Median 72.5 (55–90)
Heart rate (/min)	Mean 72.80 SE ± 2.29	Median 72 (62–90)
Breath rate (/min)	Mean 14.33 SE ± 0.60	Median 14 (12–20)
GCS 15 · 15		
White blood cell (/mm^3)	Mean 7966 SE ± 494.1	Median 7725 (5500–11000)
Hematocrit (%)	Mean 41.4 SE ± 0.75	Median 41 (38–47)
Platelets (/mm^3)	Mean 286833 SE ± 16774	Median 280000 (190–360000)