Alveolar Lavage Cytology as a Method for Diagnosis of Early Rejection of Transplanted Lungs

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Abstract. Alveolar lavages were performed repetitively on the normal and transplanted lungs of dogs that had received autografts or allografts without immunosuppression. One-half of the lavage returns was fixed as a cytologic smear; the other half was subjected to semi-thin section or electron microscopic examination. Of the staining methods used on the smears, the periodic acid-Schiff and Giemsa techniques were best for differentiating and counting cells. The Ladewig technique was best for evaluating the presence and location of fibrin.

After autotransplantation, the proportion of so-called alveolar macrophages increased, reached a peak in 4-7 days, and then returned to normal. Phagocytized fibrin increased for the first postoperative week, but no extracellular fibrin was ever observed. After allotransplantation, a progressive decrease in the proportion, size, and vacuolization of so-called alveolar macrophages was noted along with increasing amounts of extracellular fibrin. Intracellular fibrin could only be detected up to the third day.

These findings define adequate methods for preparing and staining material obtained from diagnostic alveolar lavages, and they suggest that the procedure may serve as an index of lung allograft rejection.

Keywords: Lung Transplantation-Alveolar Lavage-Cytology-Rejection.

The diagnosis of lung allograft rejection can presently be made with reasonable accuracy on the basis of clinical symptomatology, plain chest roentgenography, and the exclusion of pulmonary infection by bacteriologic examination of the sputum [1-4]. However, the information...
provided by these modalities is indirect and may not always distinguish rejection from pneumonia [5]. Moreover, better methods are needed to provide an earlier diagnosis of lung allograft rejection which would facilitate more effective prevention or reversal.

The present communication describes the methods that we have evolved for collecting and staining lavage samples and reports our results with the examination of alveolar lavages performed serially on dogs that have undergone lung autotransplantation and allotransplantation without immunosuppression.

METHODS

Five healthy mongrel dogs underwent autotransplantation of the left lung and five other dogs received left lung allografts. Previously described techniques of transplantation were employed [7, 8]. The right pulmonary artery was not ligated and no immunosuppressive treatment was administered.

Before and every 2-4 days after operation, the animals were lightly anesthetized with sodium pentobarbital and their tracheas were intubated. Under fluoroscopy, a Kifa Gray radio-opaque catheter with an external diameter of 2.5 mm was passed into the bronchus of first the right lower lobe and then the left lower lobe. In each instance the catheter was tightly wedged into a small branch bronchus, and 20 ml of sterile 0.9% NaCl solution at 20°C was instilled from a syringe. After 30 seconds, the saline was aspirated and divided into 2 aliquots. In preliminary studies using radio-opaque lavage fluid (50% sodium diatrizoate), we were able to show that with proper positioning of the catheter the alveoli of a subsegment could be filled, that spill of the lavage fluid into other areas of the lung and bronchial tree was minimal, and that volumes equal to 25-50% of the instilled lavage fluid could be recovered.

Material for light microscopic examination was prepared by placing one half of the recovered lavage fluid in a centrifuge tube, adding an equal volume of 95% ethanol, and centrifuging at 2500 rpm for 10 minutes. A set of 5 smears was prepared on clean grease-free slides from the resulting cell button. These wet smears were then fixed in 95% ethanol for 24 hours and allowed to air dry before staining. One slide from each set of smears was stained by each of the following methods: Hematoxylin and eosin (H & E), Papanicolau, Giemsa, periodic acid-Schiff (PAS) or Ladewig [9]. The H & E and Papanicolau techniques used were those employed in routine histological and cytological examinations.

A modified Giemsa method was used in which 1 drop of Giemsa stain was added to 2 ml of distilled water. The smears were stained with this solution for one hour at 60°C. Differentiation was then carried out by two 5 second rinses of 95% ethanol. The PAS staining times were 15 minutes in Schiff’s reagent and 6 minutes in hematoxylin. The Ladewig staining was performed according to the original method with microscopically controlled differentiation [9].

The remaining half of the lavage fluid material was aspirated into a