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# Ultrastructural examination of broncho-alveolar lavage for diagnosis of pulmonary histiocytosis X:

## Preliminary report on 4 cases

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**Basset, F., Soler, P., Jaurand, M. C., and Bignon, J. (1977).** *Thorax*, **32**, 303–306. **Ultrastructural examination of broncho-alveolar lavage for diagnosis of pulmonary histiocytosis X.** Fibreoptic broncho-alveolar lavage was used in four patients; the diagnosis of histiocytosis X had been established by lung biopsy in three and was suggested on clinical grounds in the remaining patient. Characteristic cells with an ultrastructural cytoplasmic marker (X body) were found in the washes of all four patients. In the patient without biopsy confirmation, the findings in the broncho-alveolar washes supplied the corroborating evidence for the diagnosis.

From this preliminary study the technique seems able to provide a diagnosis in pulmonary histiocytosis X without the need for an open lung biopsy.

This communication concerns the diagnosis of pulmonary histiocytosis X by means of the ultrastructural examination of sediments of washes obtained by broncho-alveolar lavage.

In previous studies extending over a 10-year period (Basset and Nézelof, 1966, 1969; Basset *et al.*, 1976), which comprised a series of 25 patients with pulmonary histiocytosis X (HX), we have described and indicated the diagnostic usefulness of identifying in biopsy specimens the telltale cytoplasmic structures (X bodies) within the cells making up the main part of the granulomata in this disorder.

Lung biopsies from these cases often exhibited the characteristic HX cells within the alveolar spaces and bronchioles adjacent to the granulomata, possibly through migration of these cells by diapedesis. With the improved techniques of broncho-alveolar lavage which have become available (Finley *et al.*, 1967; Jaubert *et al.*, 1974; Yeager *et al.*, 1974), it was considered likely that sediments of washes from the lungs might contain sufficient numbers of recognisable HX cells to establish the diagnosis, avoiding open lung biopsy which is now the accepted means of diagnosis in the disease.

Fibreoptic bronchoscopy is well tolerated by patients, and the lavage can be carried out on outpatients.

The preliminary results obtained from a study of four cases of pulmonary histiocytosis X, with ultrastructural findings of the cells obtained by broncho-alveolar lavage, are presented in this study.

### Methods

#### TECHNIQUE OF LAVAGE

Broncho-alveolar lavage of a lung subsegment was performed with the fibreoptic bronchoscope in all four patients. The technique of lavage used was adapted from that of Yeager *et al.* (1974). The oral route for the bronchoscope was used in all but one patient, in whom the nasal route was followed. The lavage fluid was recovered by gravity instead of by aspiration. As in a previous study with a Metras catheter (Jaubert *et al.*, 1974), the gravity method of collecting the sample was preferred, in order to avoid an undesirable admixture of bronchial secretions, bronchial epithelium, red cells, and dead cells which tend to contaminate the lavage specimen when active suction is used to aspirate the lavage fluid (see below).

After wedging the catheter into the smallest possible bronchial branch, the lung was irrigated successively with two to four 50-ml aliquots of sterile physiological saline warmed to 37°C. The

saline reservoir was held at a height of 80 cm above the examination table. Through a three-way stopcock, the fluid returned was collected in a silicone-coated glass container kept on ice which had been held 80 cm below the examination table. Before removing the fibreoptic bronchoscope, some of the fluid remaining in the lung was aspirated by vacuum and kept in a separate tube for comparative cytological examination.

Cells were counted with a haemocytometer and then cell viability was estimated with the Nigrosin test: one volume of 1·5% Nigrosin solution (Merck Lab) was mixed with seven volumes of cell suspension and incubated seven minutes at 37°C; the cells were then counted with a haemocytometer. Uncoloured cells were considered viable.

The lavage fluid was centrifuged, and cells and supernatant were used for metabolic and biochemical studies as well as for the electron microscopic (EM) examination which is the subject of the present study.

For EM processing, a sample of recovered fluid and/or an aliquot of resuspension, corresponding to at least $1\times10^6$ cells, was immediately fixed in 1·7% cacodylate-buffered cold glutaraldehyde at pH 7·4. It was centrifuged after 15 minutes and the pellet was processed according to the usual procedures. After selection on thick sections, ultrathin sections were contrasted with lead citrate and uranyl acetate and examined with a Siemens Elmiskop 101 EM.

PATIENTS

Two patients (cases 1 and 4), males aged 16 and 40 respectively, had had recent lung biopsies, confirming the diagnosis of pulmonary or multifocal histiocytosis X.

Case 3, a 28-year-old man, had had a lung biopsy three years previously, but the specimen had not been processed for EM. Up to the time of lavage, chest radiographs had shown progressive clearing, but a fine microreticular network was still visible and a mild diabetes insipidus had persisted.

The fourth patient (case 2) was a man aged 21 with discrete bilateral microreticular nodulation on a chest radiograph detected during routine ex-

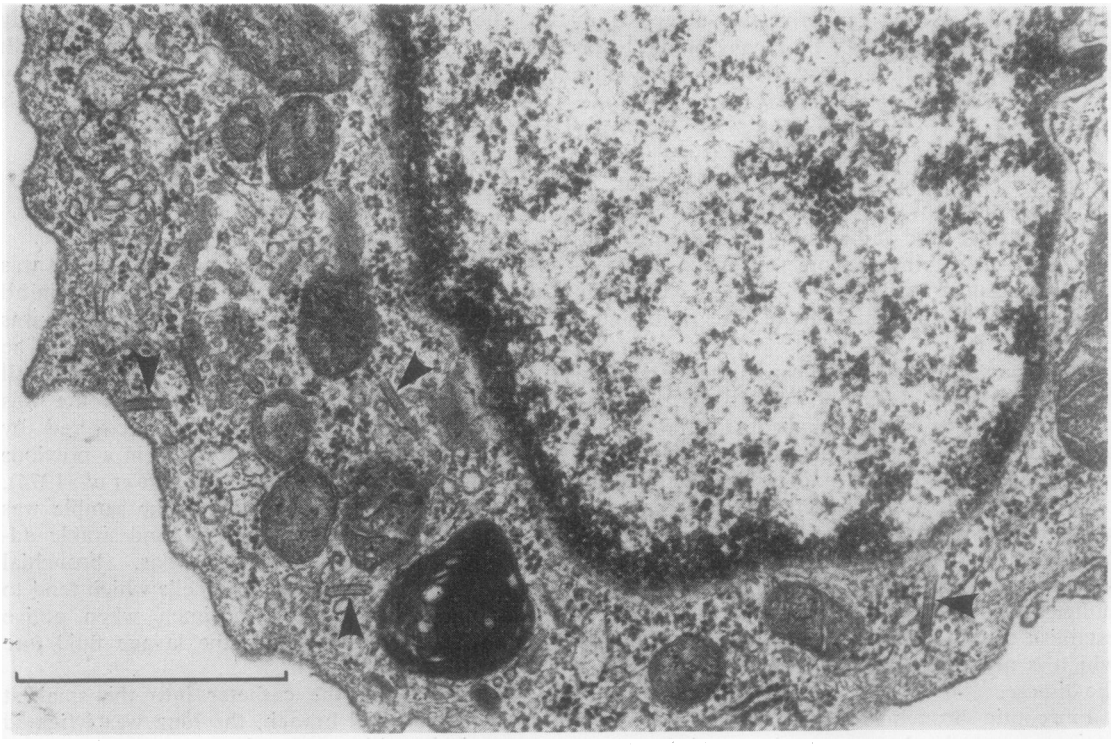

Fig. 1 *Broncho-alveolar lavage. Part of a typical HX cell with several intracytoplasmic X bodies (arrow heads).*

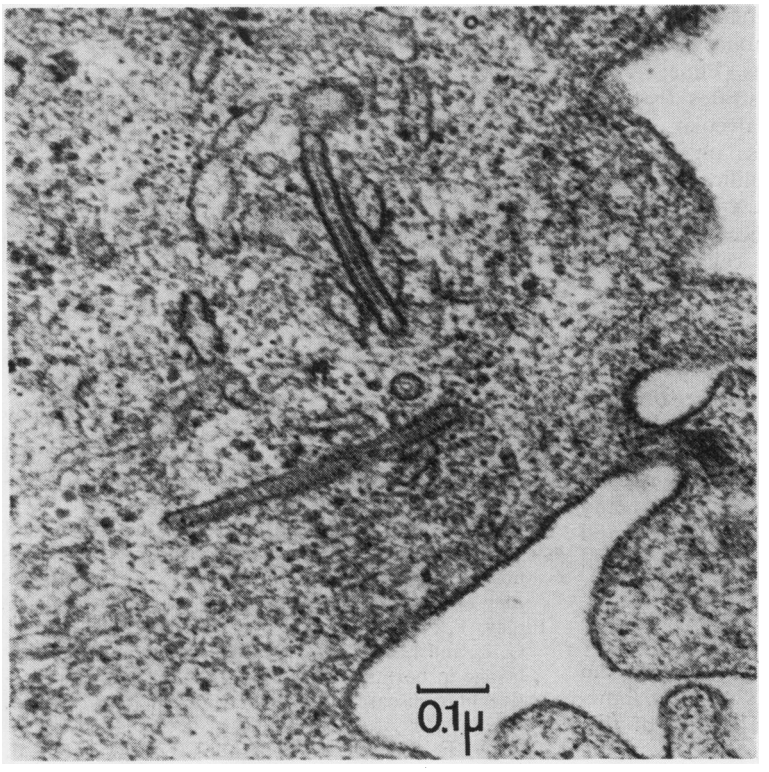


Fig. 2 *Broncho-alveolar lavage. Details of X bodies showing the periodic striation of the central layer.*

Table *Data obtained from broncho-alveolar saline lavage of four patients with histiocytosis X*

| *Case* | *HX cells* | *Volume instilled (ml)* | *Volume removed (ml)* | *No. of cells* | *No./ml* | *% viability* |
|---|---|---|---|---|---|---|
| 1 | ++ (7%) | 100 | 47 | $7{\cdot}6\times10^{6}$ | $1{\cdot}6\times10^{5}$ | 90 |
| 2 | + | 150 | 75 | $11\times10^{6}$ | $1{\cdot}4\times10^{5}$ | 78 |
| 3 | + | 150 | 60 | $8{\cdot}4\times10^{5}$ | $1{\cdot}4\times10^{5}$ | 94 |
| 4 | + | 170 | 62 | $42\times10^{6}$ | $6{\cdot}7\times10^{5}$ | 85 |

amination. There was also an osseous defect of the skull, pointing to the likelihood of the presence of histiocytosis X.

## Results

Ultrastructural examination revealed typical HX cells (Figs. 1 and 2) in great numbers in case 1, amounting to 7% of all cells examined. In the three other patients, HX cells were much less numerous, but their presence was still diagnostic.

In all four cases, the volume of fluid recovered was approximately half or less of the instilled volume. This fact, illustrated in the Table, is in agreement with the results obtained by others (Reynolds and Newball, 1974; Davis *et al.*, 1976). The total number of cells yielded varied between $7{\cdot}6\times10^{6}$ (case 1) and $42\times10^{6}$ (case 4). However, the concentration of cells per millilitre of removed fluid was less variable, approximately $1{\cdot}5\times10^{5}$/ml in three cases, and $6{\cdot}5\times10^{5}$/ml in one case.

The specimens from case 1 consisted of three pellets of fluid collected by gravity and three more pellets of fluid obtained by aspiration at the end of the lavage. Under EM over 300 cell profiles on each grid were examined, yielding a total of 950 cells for each of the two fluids.

In addition to macrophages and HX cells found in specimens obtained by gravity and aspiration, the proportion of bronchial and non-living cells was significantly higher in the aspirated fluid.

## Discussion

Alveolar lavage has been used effectively for diagnosis in bronchiolo-alveolar carcinoma

(Dobbins *et al.*, 1973), in alveolar proteinosis (Costello *et al.*, 1975), and in pulmonary infections caused by opportunistic organisms (Finley *et al.*, 1967). The present study demonstrates that HX cells can be identified among alveolar macrophages and inflammatory cells obtained by broncho-alveolar lavage. The significance of the characteristic cells in histiocytosis X is not understood. It is known that HX cells bear a very close resemblance to the Langerhans cells found in normal epidermis, which contain similar if not identical cytoplasmic 'granules'. These 'granules' were first described with the EM by Birbeck *et al.* (1961) in normal skin. But certain morphological differences do exist between the Langerhans cells and HX cells (Wolff, 1972). Moreover Langerhans cells have rarely been found in the human lung, according to a systematic study of a large series of biopsies obtained from patients with many pathological conditions (Basset *et al.*, 1976), and there is no report of their ever having been found in normal human lung or in animal lungs. The relationship, if any, between Langerhans cells and HX cells and the true nature of HX still have to be discovered. The diagnostic value of the presence in tissues of typical HX cells is, however, unquestionable in all clinical forms of this disease (Wolff, 1972; Basset *et al.*, 1976).

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