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Measles Giant Cell Pneumonia without Rash in a Case of Lymphocytic Lymphosarcoma

An Electron Microscopic Study

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Summary. A case of measles pneumonia with no rash is reported in an adult woman with lymphocytic lymphosarcoma. It was characterized by the presence of inclusion-bearing giant cells lining the alveoli. Electron microscopic studies of the lung showed that the giant cells were formed by fusion of type II alveolar epithelial cells. Inclusion bodies were filled with tubular and rod-shaped filaments similar to those observed in cell cultures infected with measles virus. The progression of the giant cell pneumonia may have been facilitated by depressed cell-mediated immunity secondary to lymphocytic lymphosarcoma.

Introduction

Hecht's pneumonia (Hecht, 1910) is characterized by the presence of multinucleated inclusion-bearing giant cells lining the alveoli, alveolar ducts, and bronchioles. The cultivation of measles virus from the lungs of patients with Hecht's pneumonia confirmed the opinion that this disease represents a peculiar form of measles pneumonia (Enders *et al.*, 1959). Recently Archibald *et al.* (1971) described electron microscopic features of these inclusion-bearing giant cells and showed that intranuclear inclusions were filled with filaments closely resembling measles nucleocapsids observed in cultured cells infected with measles virus (Waterson *et al.*, 1961; Nakai *et al.*, 1969).

Measles, a common disease of man, is very rarely fatal unless secondary infection occurs (Roberts *et al.*, 1958). However death from measles infection alone has been reported in 12 children with immunologic defects (Enders *et al.*, 1959; Mitus *et al.*, 1959; Lipsey *et al.*, 1967; Nahmias *et al.*, 1967; Meadow *et al.*, 1969; Archibald *et al.*, 1971); four of these had malignant disease of lymphoid tissue. Cases have also been reported in an adult with an undetermined hematologic disorder (Koffler, 1964), an adult with malignant reticulosis (McConnel, 1961), and in two adults without apparent predisposing disease (Milles, 1945).

The present report concerns an adult woman with lymphocytic lymphosarcoma who died from Hecht's giant cell pneumonia. Because of optimal fixation for electron microscopy (EM), studies of the fine structures of the inclusion bodies and of the cells containing them were possible. The reasons why the patient did not develop a rash shall be briefly discussed; certain aspects of the interplay

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between measles and the immune deficiencies associated with lymphocytic lymphosarcoma will be reviewed.

Case History

This 69-year old woman noticed small subcutaneous lumps on the face and thighs 7 months before her final admission. Biopsy of one nodule revealed a non-necrotizing vasculitis with infiltration of the subcutaneous blood vessel walls with plasma cells and lymphocytes. At that time the diagnosis was thought to be allergic vasculitis so the patient received betametazone 1 mg/day. She had experienced intercostal herpes zoster one year prior to her final admission and still complained of residual chest pains.

Physical examination on admission revealed an elderly woman in satisfactory general condition with erythematous, indurated, cutaneous lesions on the external surfaces of the thighs. Her temperature was 38° C. Dyspnea was slight and there was no cyanosis. Chest X-ray film showed fine, diffuse reticular, and micronodular densities in both lung fields. Her condition worsened suddenly the day following admission. In spite of antibiotic therapy she developed acute respiratory distress with cyanosis and was admitted to the intensive care unit on her third hospital day. Her temperature was 38.4° C; pulse 180/min and irregular; blood pressure 120/50; central venous pressure 19.5 cm H_2O.

The heart sounds were normal. Her respiratory rate was 55/min with a minute volume of 18 l/min determined by spirometry. Fine rales were audible on the right side of the chest. The liver was at the right costal margin and the spleen was not palpable. Chest X-ray showed reticular and nodular shadows which were denser and more confluent than on admission, and opacification of the base of the left lung. The respiratory insufficiency worsened progressively and the patient died 3 days after admission to the intensive care unit.

Pertinent laboratory studies performed the day before death were as follows: Sputum revealed giant cells but no bacteria nor Pneumocystis carinii on PAS stain; bacteriological culture were sterile. The electrocardiogram showed atrial fibrillation and ventricular extrasystoles. The hemoglobin was 10.9 g/100 ml and the white cell count 8.830/mm^3 with 5% lymphocytes; no atypical white cells were noted. Total serum protein concentration was 1.5 g/100 ml with 5.8% gamma globulin. Immunoelectrophoresis showed a marked decrease of IgG and IgM, and absence of IgA. Her serum did not contain complement-fixing antibodies against Q-fever, psittacosis or mycoplasma pneumoniae, or against the following viruses: influenza A and B, cytomegalovirus, herpes simplex, chickenpox, respiratory syncitial virus, adenovirus, mumps, distemper, or against measles. Neutralizing antibodies inhibiting 100 $TCID_{50}$ (Tissue Culture Infective Dose) were below 1:8 for measles.

Post Mortem Findings

Necropsy (A: 398/71) performed within an hour of death revealed tumor masses in the retroperitoneal, mediastinal, axillary and laterocervical lymph nodes. The largest masses were retroperitoneal and measured $10 \times 4 \times 2$ cm; they were lobulated, not adherent to bone or other parenchyma, and appeared pearly-gray, smooth and moist on cut surfaces. The liver weighed 1320 g, and was pale but of normal consistency. The spleen weighed 390 g and was firm; there was an infarct at the upper lobe. The heart weighed 235 g. Coronary arteries were patent, free from atherosclerosis. The bone marrow was pale with some hemorrhagic foci.

Histologic examination of the tumors revealed lymphocytic lymphosarcoma in lymph nodes containing well-differentiated lymphocytes. Many phagocytic histiocytes were scattered among these lymphocytes giving the lymph nodes a starry sky appearance. Significant lymphocytic infiltration was also found in the portal spaces, the spleen and the bone marrow. Similar infiltration was found focally around blood vessels and in the interstitium of the subcutaneous tissue.

There was no pleural effusion. The lungs were uniformly consolidated and together weighed 980 g. Their cut surface was pale and fleshy, and showed small focal hemorrhages. The tracheal mucosa was hyperemic and covered with mucus. Tissue samples from one lung were either fixed in formalin and Helly solutions, or deep frozen at −30° C. The other lung

was fixed by intratracheal instillation (at 30 cm water pressure) of phosphate-buffered glutaraldehyde and post-fixed with 2% OsO_4, adjusted to 330 mosm and pH 7.3 with s-collidine. The details of these fixation techniques for EM are given elsewhere (Kapanci *et al.*, 1969). Sections for EM were chosen to include both intracytoplasmic and intranuclear inclusion bodies.

Light Microscopic Examination of the Lung

A diffuse giant cell pneumonia was found. This was characterized by giant cells bearing from 2 to 60 nuclei which were distributed unevenly in a roughly granular eosinophilic cytoplasm. These cells lined alveolar septa, many alveolar ducts and occasional respiratory bronchioles (Fig. 1a und b). They contained intranuclear eosinophilic inclusion bodies which stained red with tartrazine phloxine, red with methyl green pyronin, and orange with acridine orange; they did not stain with Feulgen's reaction. A tiny clear space was occasionally visible around these inclusions. Eosinophilic inclusion bodies were also observed in the cytoplasm of such giant cells; these cytoplasmic inclusion bodies resembled those in the nuclei, but were more irregular in size (Fig. 3a). In areas where the septa were not lined by multinucleated cells, alveolar cells showing the same type of inclusion body were found (Fig. 1c). In the alveolar septa and spaces, polymorphonuclear leucocytes and some macrophages were seen; lymphocytes and plasma cells were rare. The alveoli contained edema fluid and a few inclusion-bearing giant cells; in many areas there were typical hyaline membranes (Fig. 1a). In the bronchial and tracheal mucosa, giant cell with inclusion bodies were also observed. The epithelium was often hyperplastic and showed squamous metaplasia.

Electron Microscopic Examination of the Lung

The alveolar septa were lined by cells interpreted as modified granular pneumocytes. Most of these cells had many nuclei; their surfaces were covered by regular microvilli and there were junctional complexes between individual cells (Fig. 2a and b). Rough endoplasmic reticulum, free ribosomes were abundant. They contained few osmiophilic lamellated bodies specific for type II alveolar epithelial cells (Fig. 2c).

Intranuclear and "intracytoplasmic" inclusion bodies were the prominent characteristic of these cells (Fig. 3a). The normal chromatin pattern of the nuclei was partially or completely replaced by a lighter staining filamentous structure which contrasted well with the nucleolus and heterochromatin (Fig. 3a and b). In some areas these filamentous structures filled the nucleus but for a thin and irregular chromatin crown at the periphery. At high magnification they appeared as tubules or tiny rod-shaped particles with blunt tips and measured about 150 Å in width (Fig. 4). Some of the rod-shaped particles showed fine striations with a periodicity of 60 Å (Fig. 4 inset). On cross section the tubular and rod-shaped particles appeared as circles and granules of about the same size as ribosomes.

The "cytoplasmic" inclusion bodies seen by the light microscope were surrounded by a double membrane (Fig. 3c), of which the internal leaflet was thicker than the external one. They were interpreted as nuclei completely filled with viral nucleocapsids. Occasionally some bits of tubular material were seen passing through pores of the nuclear membranes, and spreading out into the cell matrix.

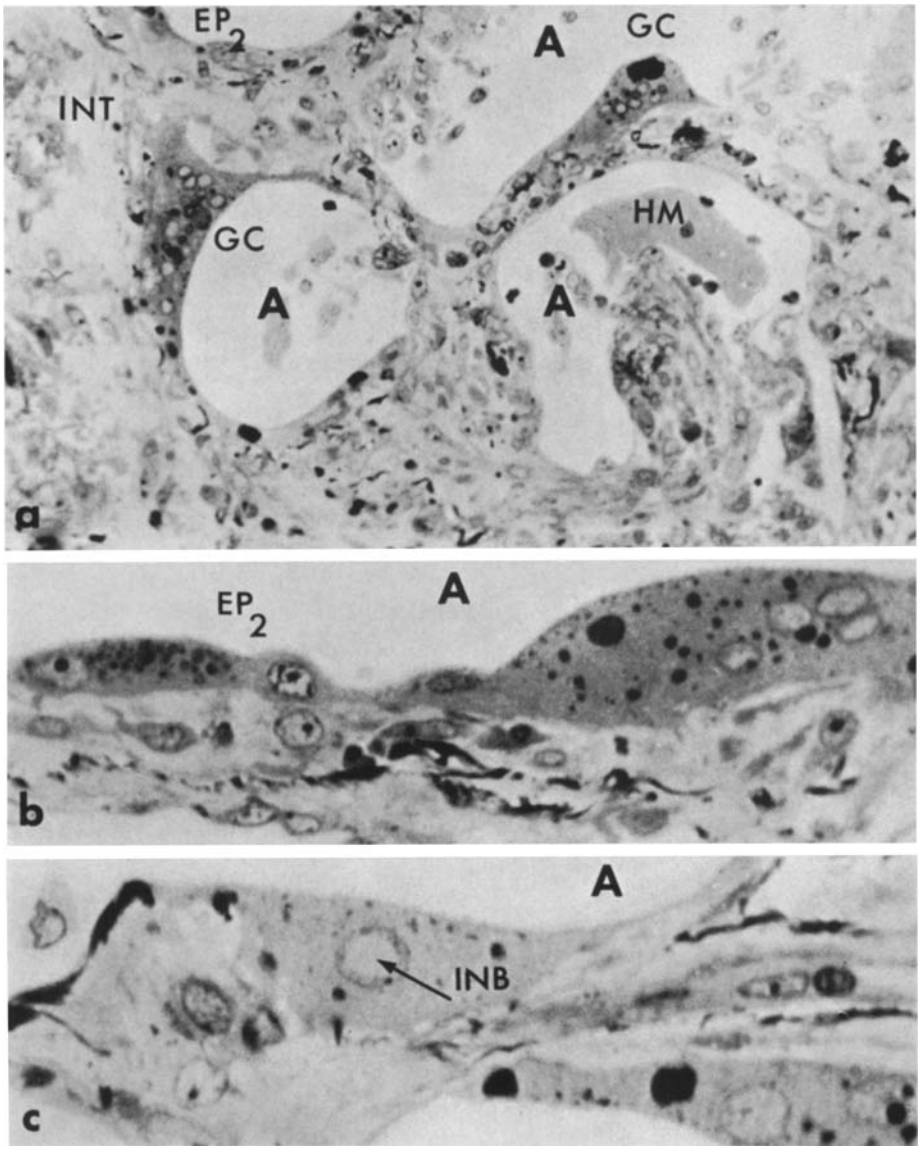

Fig. 1a—c. Hecht's pneumonia with proliferation of type II alveolar epithelial cells and inclusion-bearing giant cells lining the alveoli (epon embedded thick section stained with toluidine blue). a Alveoli (*A*) lined by giant cells (*GC*) and mononucleated type II epithelial cells (EP_2): the alveoli contain hyaline membranes (*HM*) and the interstitium (*INT*) is oedematous (×120). b Alveolus lined by type II epithelial cells and inclusion-bearing giant cell forming a continuous layer (×300). c Mononucleated type II cell with intranuclear inclusion (×300)

However, in the cytoplasm, free particles were rarely seen while at the cell surface there were some tiny buds measuring about 1550 Å in diameter and containing tubular and rod-shaped structures surrounded by a double membrane (Fig. 3d).

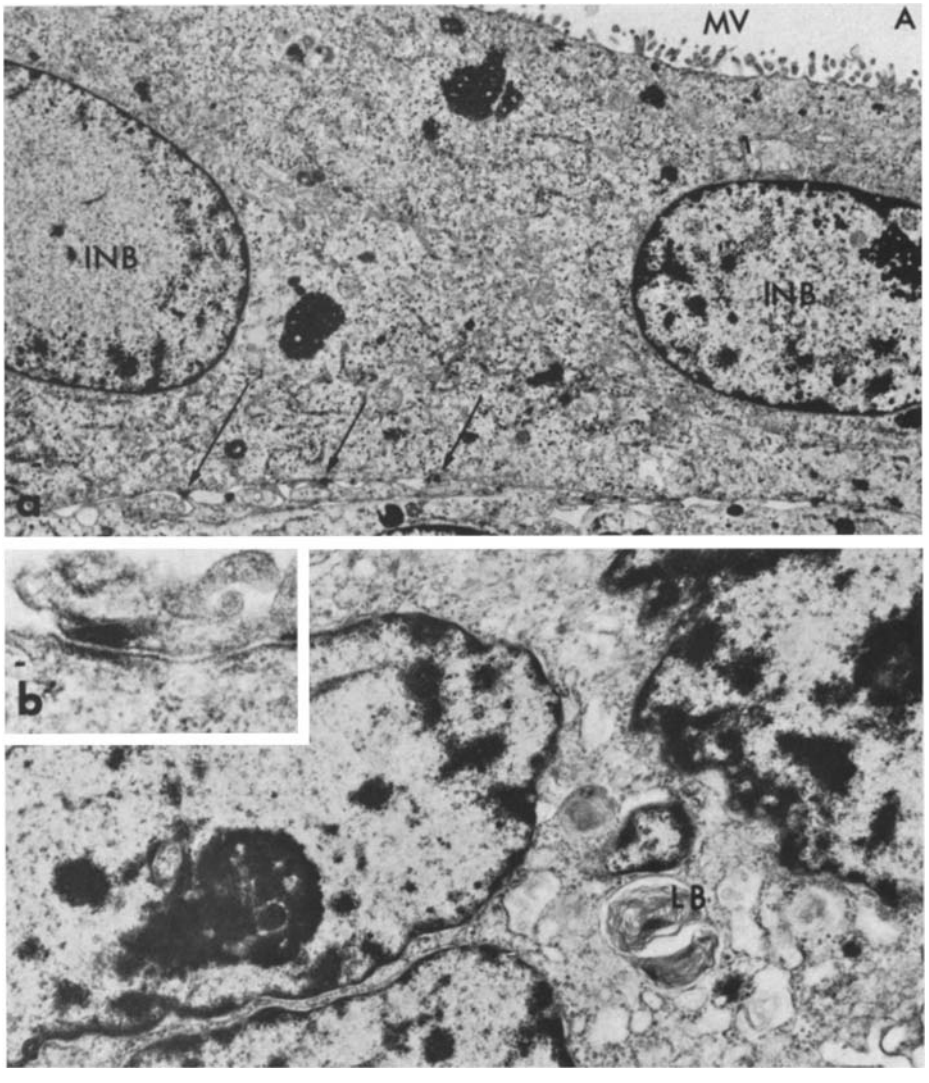

Fig. 2a—c. Multinucleated cells showing feature typical of type II alveolar epithelial cells. a Multinucleated cell showing microvilli (*MV*) and junctional complexes (arrows). Also note intranuclear inclusions (*INB*) (×5423). b Junctional complex between adjacent type II cells (×24270). c Multinucleated cell showing lamellated bodies (*LB*) (×11180)

Discussion

Giant cell pneumonia with both intranuclear and intracytoplasmic inclusion bodies was demonstrated in this 69-year old woman with lymphocytic lymphosarcoma. Histologically the staining properties of the inclusion bodies were those of an RNA virus highly suggestive of measles (Llanes-Rodas, 1965; Fenner, 1968a). Electron microscopic studies showed that the inclusion bodies were tubular and rod-shaped particles similar to those described *in vitro* in BSC-1 and HEp-2

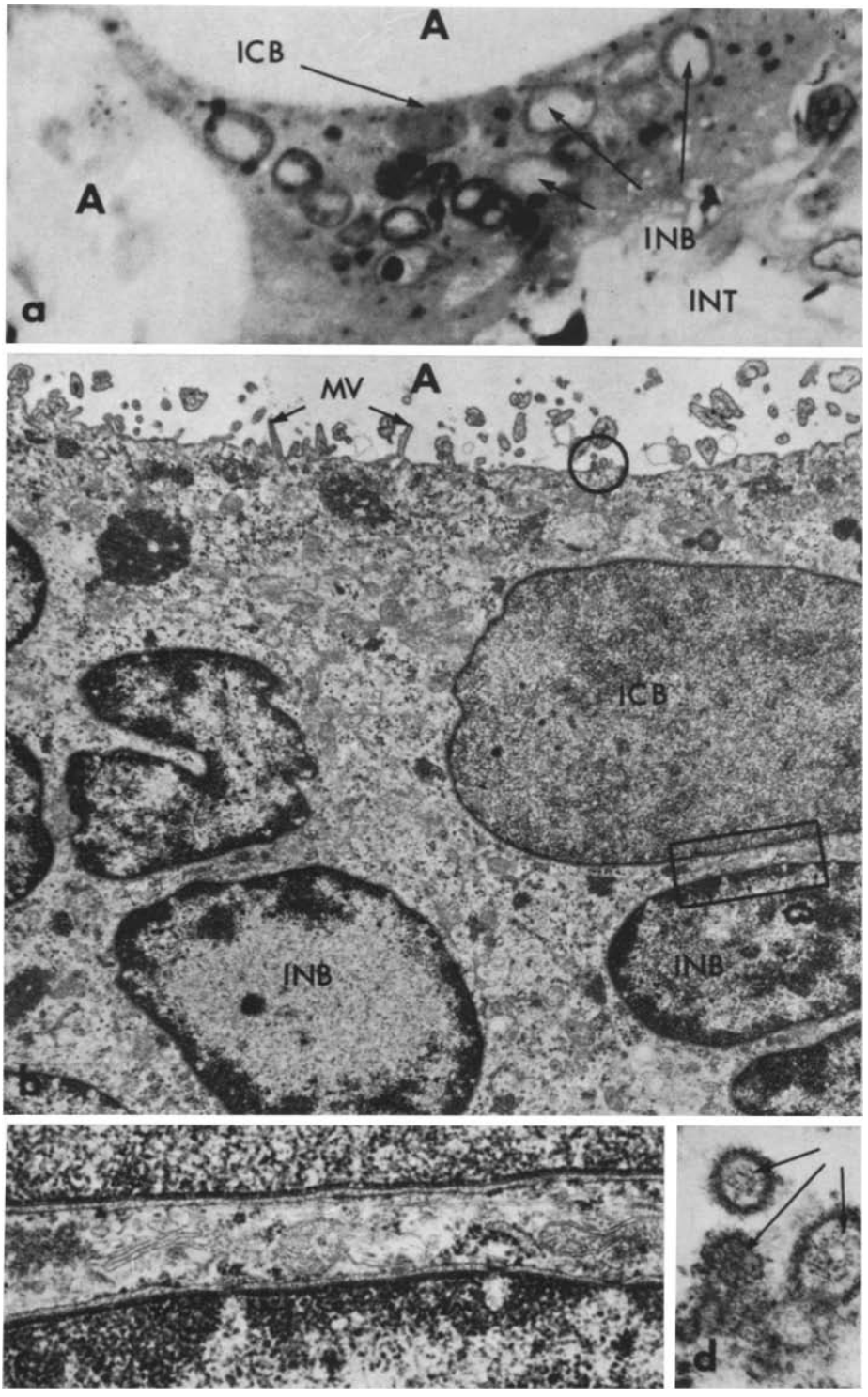

Fig. 3a—d

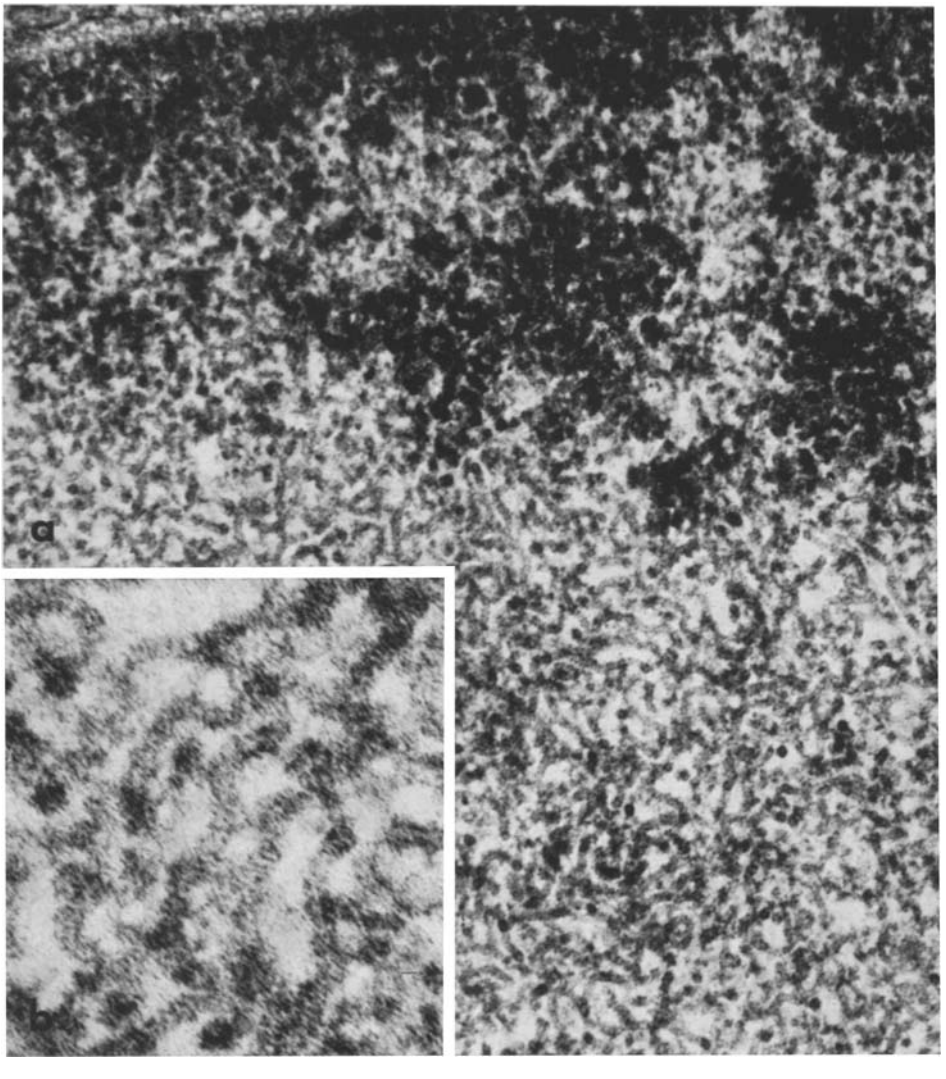

Fig. 4. Detail of intranuclear inclusion: these are composed of rod-shaped particles, presumably corresponding to measles nucleocapsids (×95760); inset (×287280)

Fig. 3a—d. Light and electron microscopic appearance of an inclusion-bearing giant cell. a Light microscopy of an epon embedded section of a giant cell showing both intranuclear (*INB*) and intracytoplasmic (*ICB*) inclusions (toluidine blue; ×300). b Electron microscopy of the same cell showing that the inclusion appearing as intracytoplasmic (*ICB*) on Fig. 3a corresponds to a nucleus filled with filamentous particles. The detail of the area in the rectangle is shown on Fig. 3c, and that in the circle on Fig. 3d (×8300). c Detail of so-called *ICB* and adjacent nucleus with *INB*. Both are surrounded by a double membrane (×33200). d Detail of viral budding at the cell surface. Arrows indicate rod-shaped bodies (×75240)

cultured cells infected with measles virus (Waterson *et al.*, 1961; Nakai *et al.*, 1969). Correlation between thick sections and EM findings shows that intracytoplasmic inclusion bodies were surrounded by a double membrane and looked rather like nuclei whose chromatin pattern had been totally replaced by viral nucleocapsids. This concept has already been suggested by authors (Archibald *et al.*, 1971) who performed EM studies on two human cases of measles giant cell pneumonia; but without concurrent light and EM studies this suggestion could not be confirmed. We also observed some structures suggestive of viral budding at the cell surface which were similar to those described *in vitro* in human amnion cells infected with measles virus (Baker *et al.*, 1960). According to our observations the giant cells appear to be derived by fusion of type II alveolar epithelial cells. These cells contain very few lamellated bodies which are considered to be the most prominent feature of type II alveolar epithelial cells (Kapanci *et al.*, 1969). However it has been shown that in actively growing type II cells the lamellated bodies may be few or even lacking (Kapanci *et al.*, 1969). Thus the cells without lamellated bodies but lining the alveolar walls and having typical junctional complexes and microvilli were considered to be type II epithelial cells.

These morphologic observations point to measles as being the cause of the giant cell pneumonia in this case. However, the patient developed neither a rash nor circulating antibodies against measles. These manifestations are probably lacking because of the immunologic state induced by the lymphocytic lymphosarcoma and the steroid therapy: two conditions associated with immunologic impairment (Miller, 1968; Woodruff, 1969). It has been shown that patients with measles develop a subclinical interstitial pneumonia (Kohn *et al.*, 1933) which heals when the rash and antibodies appear (Robbins, 1962). However, unimpeded progression of giant cell pneumonia tends to occur in patients with congenital (Lipsey *et al.*, 1967; Nahmias *et al.*, 1967; Archibald *et al.*, 1971) or acquired (Enders *et al.*, 1959; Mitus *et al.*, 1959; McConnell, 1961; Meadow *et al.*, 1969) immunologic defects. This has been explained recently (Nahmias *et al.*, 1967; Burnet, 1968) on the basis of a deficient cell-mediated immune reaction. Burnet (1968) summarized the pathogenesis of measles and the interactions between the host's immune mechanisms and the measles virus. He emphasized that cell-mediated immunity was more important than humoral immunity in protecting man from the disease. In fact hypogammaglobulinemic children with normal cell-mediated immunity recover normally from measles and develop a rash. Furthermore some of the reported cases of measles giant cell pneumonia in which hypogammaglobulinemia was assumed to be responsible for the progression of the disease had also received cancer chemotherapy (Enders *et al.*, 1959; Mitus *et al.*, 1959; McConnell, 1961; Meadow *et al.*, 1969) or steroids (Meadow *et al.*, 1969); others showed malabsorption or malnutrition (Enders *et al.*, 1959) or had various thymic anomalies (Lipsey *et al.*, 1967; Archibald *et al.*, 1971). All these conditions can depress cell-mediated immunity (Smythe *et al.*, 1971; Woodruff, 1969; Good, 1968). Most of these patients did not develop a rash (Enders *et al.*, 1959; McConnell, 1961; Lipsey *et al.*, 1967; Archibald *et al.*, 1971) which is an expression of the delayed hypersensitivity reaction (Mims, 1966; Fenner, 1968 b).

Thus our patient, because of an impaired cell-mediated immunity secondary to both lymphocytic lymphosarcoma and steroid therapy (Miller, 1968; Woodruff,

1969), was in a state that favoured the development of a giant cell pneumonia (Burnet, 1968). This same state meant that she could not develop a rash. Furthermore she was hypogammaglobulinemic with a low level of immunoglobulins (Miller, 1968) and could not produce circulating antibodies against measles. This condition, characterized by defects in both cell-mediated and humoral immunity, seems to have predisposed her to the progression of any viral disease and particularly measles (Glasgow, 1970).

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