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Analysis of viral antigens in giant cells of measles pneumonia by immunoperoxidase method

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Summary. The localization of measles virus proteins was analyzed by immunoperoxidase method using both monospecific and monoclonal antibodies. In Vero cells infected with the Edmonston or EB–L strain, the former being a laboratory strain and the latter a fresh isolate from a measles patient, nucleocapsid protein was located in the nuclei, and matrix protein, phosphoprotein, haemagglutinin and fusion protein were located in the cytoplasm. In the lung tissues of eight cases with measles giant cell pneumonia, the similar findings were obtained. The presence of haemagglutinin on the surface of giant cells at the luminal side was also noticed. Histopathologically, measles giant cells had nuclear and cytoplasmic eosinophilic inclusion bodies with some differences in appearance. The significance of localization of viral proteins is discussed in comparison with histopathological findings in measles giant cells.

Key words: Measles virus – Measles giant cell – Monospecific antibody – Inclusion body – Immunohistochemistry

Introduction

Giant cell pneumonia, which occurs frequently in immunocompromised hosts is caused by viral infections such as measles (Enders et al. 1959), parainfluenza type 2 (Karp et al. 1974) and type 3 (Jarvis et al. 1979; Delage et al. 1979; Little et al. 1981), and respiratory syncytial (Delage et al. 1984) viruses. Histopathologically giant cell pneumonia is characterized by the formation of multinucleated giant cells mostly originating from alveolar epithelia (Becroft and Osborne 1980; Spencer 1985). Among these viruses, only measles virus forms the giant cell with both intranuclear and intracytoplasmic eosinophilic inclusion bodies (Becroft and Osborne 1980). The nature of the intracytoplasmic inclusion bodies has been controversial due to the difficulties in electronmicroscopic observation of the inclusions in giant cells (Archibald et al. 1971; Joliat et al. 1973; Breitfeld et al. 1973).

Monoclonal antibodies against structural proteins of measles virus have been applied to study the localization of viral proteins in in vitro by immunofluorescence (Norrby et al. 1982; Giraudon and Wild 1984). To the best of our knowledge, however, the localization of measles virus proteins in the human lung tissues with measles giant cell pneumonia has not been reported.

In this paper, the localization of measles virus proteins was analysed in the giant cells using both monospecific and monoclonal antibodies by immunoperoxidase method, and the significance of the expression of viral antigens in the measles giant cells is discussed.

Materials and methods

Autopsied cases of measles giant cell pneumonia. The lung tissues of eight cases with measles giant cell pneumonia which had been fixed in formalin and embedded in paraffin, were collected from various hospitals according to the record files of our institute during the last 15 years. They consisted of one male and seven females, aged from 7 months to 8 years, all were immunocompromised due to various underlying diseases.

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Measles virus infected Vero cells. The Edmonston, a laboratory strain of measles virus, and the EB–L strain, which was isolated from peripheral lymphocytes of a measles patient and passaged four times in Vero cells, were used. The cytopathic effects (CPE) appeared 24 to 33 h after inoculation with the virus at m.o.i. of 0.1 into Vero cell culture. When the CPE was developed, monolayer of the cultured cells in chamber slide (Lab-Tek, Miles Labo, Naperville, USA) were fixed in aceton for 5 min. The cell pellets of Vero cells infected with measles virus were fixed in 10% buffered formalin and embedded in paraffin.

Antibodies to viral proteins. Monospecific antibodies to nucleocapsid protein (N), matrix protein (M) and haemagglutinin (H) were raised in rabbits (Uchiyama et al. 1980). Monoclonal antibodies against phosphoprotein (P), fusion protein (named as F–1 and F–2) and haemagglutinin (named as H–1, H–2 and H–3) were produced in mouse system (Sakaguchi et al. 1985; Uchiyama et al. 1985). The specificity were defined by immunoprecipitation and Western blotting method using Vero cells infected with the Edmonston strain.

Immunoperoxidase method. The infected cells were stained by indirect immunoperoxidase method without step of the inactivation by H_2O_2 in methanol, because Vero cells have no endogenous peroxidase activity. The infected cells were reacted with the monospecific or monoclonal antibodies and then with anti-rabbit or mouse IgG serum conjugated with horseradish peroxidase (MBL, Nagoya, Japan) for 60 min at 37° C for each step. The staining was performed by 0.02% 3,3′-diaminobenzidine tetrahydrochloride (Dohjin Chemical Labo, Kumamoto, Japan) in Tris-HCl buffer (0.05 M, pH 7.6) and 0.015% H_2O_2 for 4 min at room temperature. The nuclei were stained by haematoxylin.

ABC immunoperoxidase method was applied to the sections of cell pellets and autopsied human lung tissues. After deparaffinization, the sections were digested by 0.25% trypsin (Kurata et al. 1983) for 2 h at 37° C in moist chambers and immersed in 0.3% H_2O_2-methanol for 30 min at room temperature to inactivate endogenous peroxidase activities. Prior to the application of the primary rabbit or mouse antibody, the normal goat or horse serum was overlaid for 20 min at room temperature, respectively, to reduce the background staining. Then anti-measles antibodies at desired dilution were applied overnight at 4° C, followed by biotinylated anti-rabbit or mouse IgG serum (Vector Labo, Burlingame, USA) for 30 min at 37° C and ABC solution (Vectastain ABC Kits, Vector Labo, Burlingame, USA) for 60 min at 37° C. Peroxidase activity was developed by 0.02% 3,3′-diaminobenzidine tetrahydrochloride in Tris-HCl buffer with 0.015% H_2O_2 for 8 min at room temperature. The counterstaining was made by 2% methylgreen (Chroma, Stuttgart, FRG) in Veronal acetate buffer.

As a control for the primary antibodies, normal rabbit and mouse sera were used. Autopsied human lung tissues without measles infection were prepared as a normal control.

Histopathological examination. The lung sections were stained by haematoxylin and eosin (H.E.) for histopathological examination of giant cells with inclusion bodies.

Results

Localization of viral antigens in infected Vero cells

The N antigen was located in both nuclei and cytoplasm of Vero cells infected with the EB–L strain (Fig. 1), but only in the cytoplasm of cells infected with the Edmonston strain. The M, P, H and F antigens were observed as intracytoplasmic diffuse staining without nuclear staining in the cells infected with either virus (Fig. 2) (Table 1).

The antigens corresponding to anti-F monoclonal antibodies (F–1 and F–2) were lost during formalin fixation. The other antibodies were able to detect viral antigens in the cells fixed in formalin. To examine the precise localization in the monolayer cells, the sections of pellets of the virus-infected cells were subjected to ABC immunoperoxidase method. The similar findings were obtained between two strains except for different intensity and frequency of nuclear staining by anti-N antibody. The location of the antigens corre-

Table 1. Localization of measles virus antigens in Vero cells (indirect immunoperoxidase method)

	Reactions with								
	monospecific antibodies				monoclonal antibodies				
	N	M	P	H	H–1	H–2	H–3	F–1	F–2
Edmonston strain									
Nucleus[a]	–[c]	–	–	–	–	–	–	–	–
Cytoplasm[a]	+	+	+	+	+	+	+	+	+
Cell surface[b]	–	–	–	+	+	+	+	ND	ND
EB–L strain									
Nucleus[a]	+	–	–	–	–	–	–	–	–
Cytoplasm[a]	+	+	+	+	+	+	+	+	+
Cell surface[b]	–	–	–	+	+	+	+	ND	ND

[a] Detection of antigen was tried with cultured monolayer cells
[b] Antigen on the cell surface was examined by ABC method using formalin-fixed and paraffin-embedded section
[c] N antigen in the nuclei was detected partially in paraffin sections

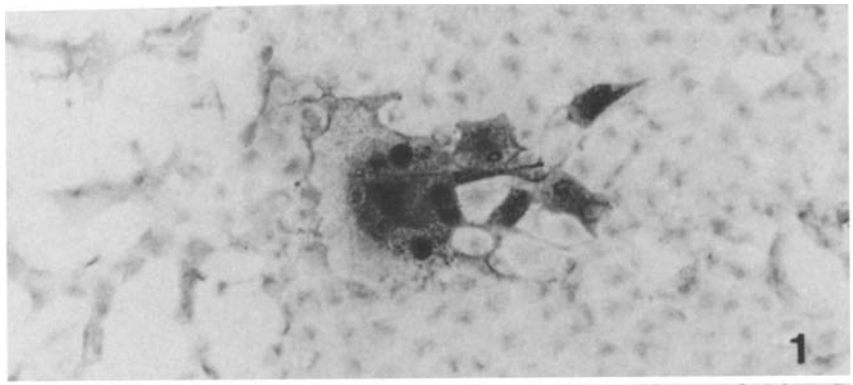

Fig. 1. N antigen by indirect immunoperoxidase method showing dotlike and diffuse staining in the nuclei and cytoplasm, respectively, in Vero cells infected with the EB–L strain of measles virus. × 200

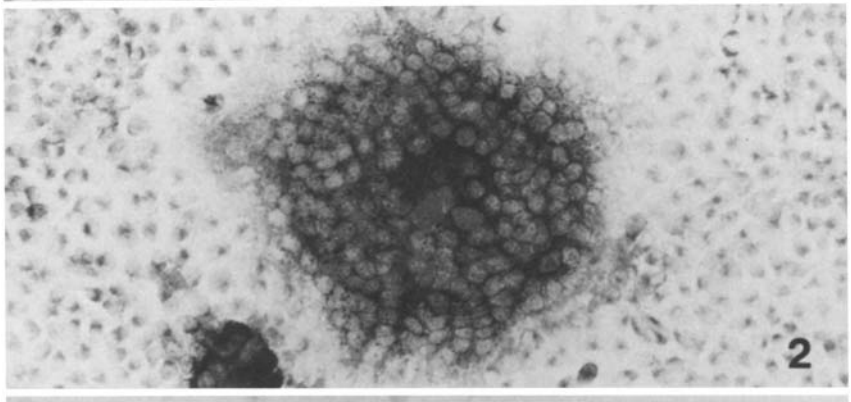

Fig. 2. H antigen by indirect immunoperoxidase method using H–3 monoclonal antibody showing diffuse staining in the cytoplasm without nuclear staining in Vero cells infected with the Edmonston strain. × 200

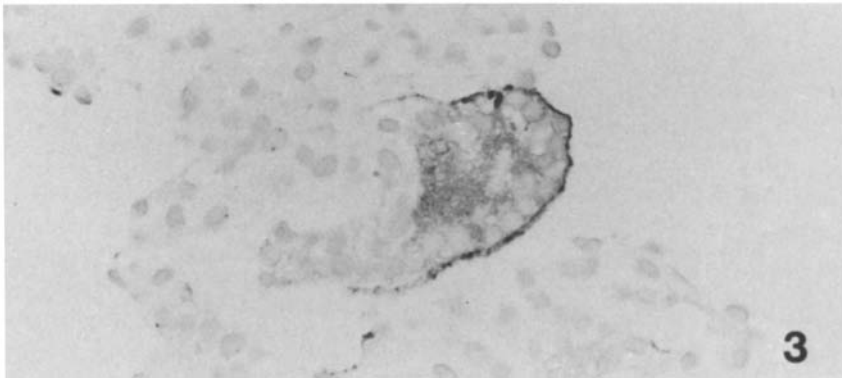

Fig. 3. Intensive staining of H antigen by ABC immunoperoxidase method using H–3 monoclonal antibody at the cell surface of multinucleated giant cells and positive reaction in the cytoplasm in the section of Vero cells infected with the Edmonston strain of measles virus. × 200

sponded with that found in the monolayer cells, except intense staining on the cell surface by anti-H antibody and three anti-H monoclonal antibodies (H–1, H–2 and H–3) (Fig. 3).

Localization of viral antigens in the lung of measles giant cell pneumonia

Serial sections of the lung were prepared from the eight cases with measles giant cell pneumonia and examined by ABC immunoperoxidase method using anti-N, M, H monospecific antibodies and anti-H(H–3) and P monoclonal antibodies. The results were summarized in Table 2. N antigen was

Table 2. Localization of measles virus antigens in giant cells in measles giant cell pneumonia (ABC immunoperoxidase method)

	Monospecific and monoclonal anti-measles virus antibodies			
	N	M	H & H–3	P
Nuclear inclusion	+++	−	−	+[a]
Cytoplasmic inclusion	+++	+	+	++
Cytoplasm	++	++	++	+
Cell surface	−	−	+++	−

[a] P antigen was observed only in degenerative measles giant cells

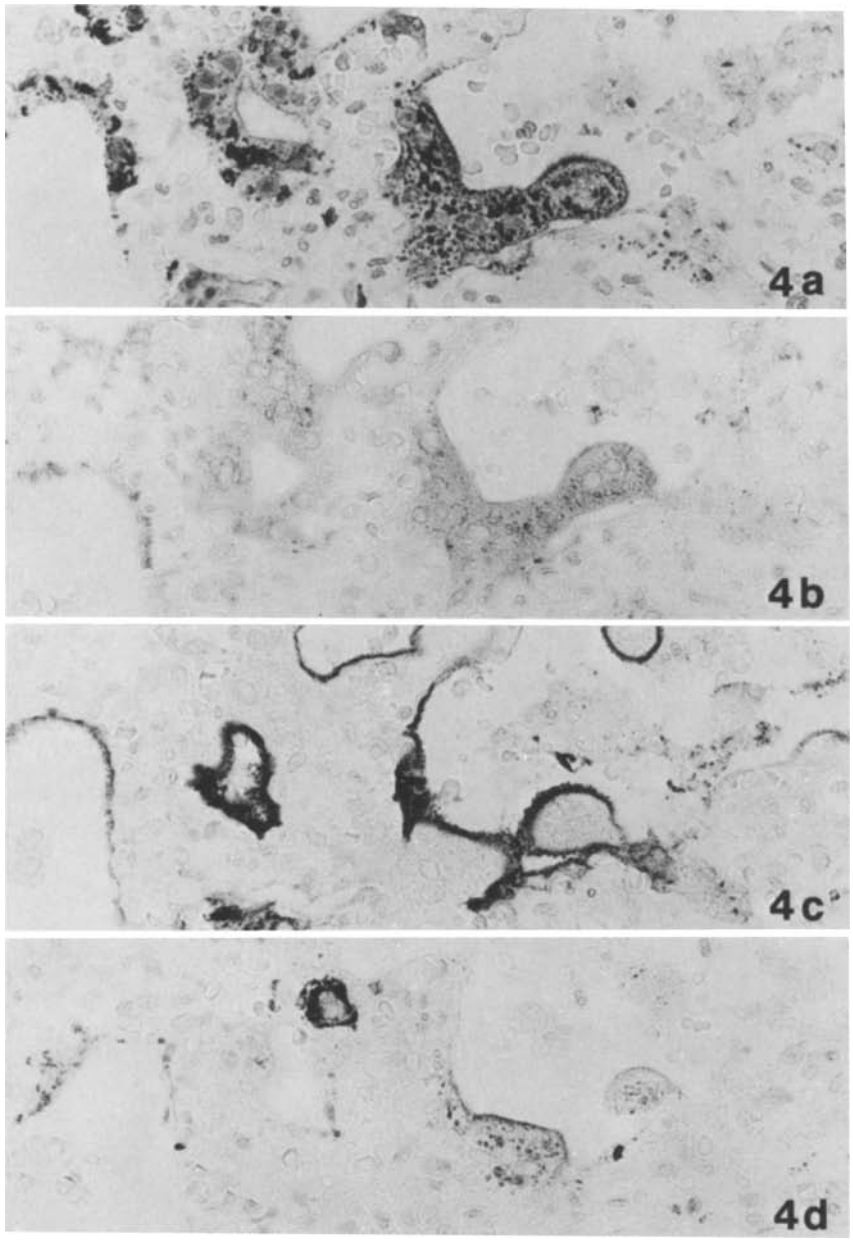

Fig. 4. The staining of serial lung sections by ABC immunoperoxidase method showing **a** N antigen in the alveolar giant cell of measles giant cells pneumonia in the nuclei and cytoplasm with the alignment of positive reaction under the cell membrane. **b** M antigen in the cytoplasm of alveolar giant cell except for nuclei. **c** Intense staining of H antigen by H–3 monoclonal antibody in the cell surface of alveolar giant cells. **d** P antigen with various-sized granules in the cytoplasm and cytoplasmic inclusions. × 400

localized in the nucleus involving the nuclear inclusions and in the cytoplasm involving the cytoplasmic inclusions with coarse granular pattern. The alignment of N antigen under the cell membrane was also seen (Fig. 4a). M antigen was observed in the cytoplasm but not in the nuclei (Fig. 4b). H antigen was localized at the cell surface of the free side of the giant cell and weakly in the cytoplasm including inclusions. H antigen stained by H–3 monoclonal antibody was located mainly on the cell surface (Fig. 4c). P antigen was detected as various-sized granules in the cytoplasm and cytoplasmic inclusions (Fig. 4d), and sometimes in the nuclei of degenerated giant cells.

Histopathological findings of the lung with giant cell pneumonia

Giant cells were mainly found in the area of alveoli in the lung. These cells had eosinophilic inclusions in the nuclei and cytoplasm. Nuclear inclusions with halo were uniform in size, and stained homogeneously and were faintly eosinophilic. In contrast, cytoplasmic inclusions were intensely eosino-

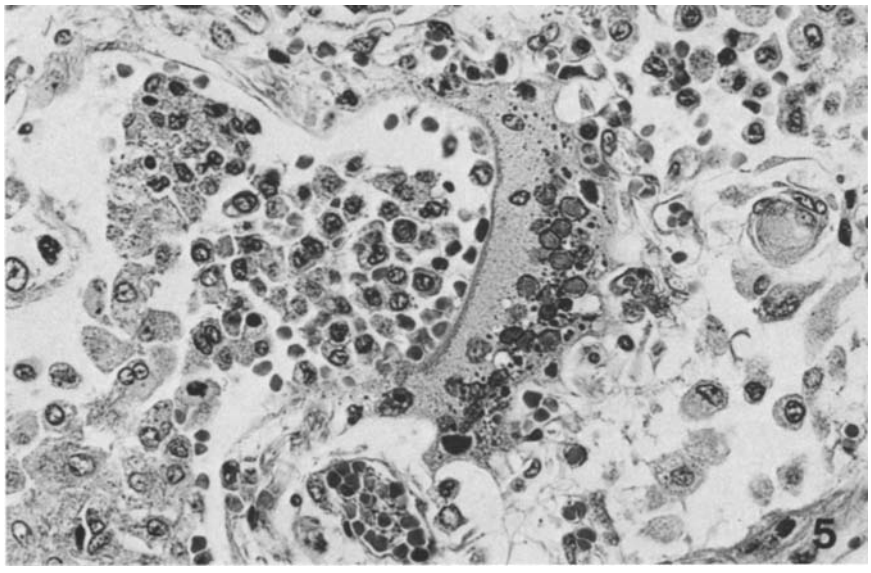

Fig. 5. Multinucleated giant cell in the measles giant cell pneumonia with intranuclear and intracytoplasmic eosinophilic inclusions and thickened cell membrane at the luminal side of the cell. (H.E. ×400)

philic with various-sized granular appearance. They showed occasionally crystal-like appearance. Nuclei with N antigen in the multinucleated giant cells sometimes lacked obvious eosinophilic inclusions. The cell membrane at the luminal side of the cells was thickened and intensively eosinophilic (Fig. 5).

Discussion

Measles virus is composed of six structural proteins such as nucleocapsid protein, matrix protein, phosphoprotein, haemagglutinin, fusion protein and large protein (L). The function of these virion proteins has been extensively studied (Wechsler and Meissner 1982; Rima 1983). However, there have been no reports studying the localization of measles viral proteins in the lung with measles giant cell pneumonia. In this paper, immunohistochemical studies on the lung with measles giant cell pneumonia using monospecific and monoclonal antibodies against measles virus proteins revealed the antigens of N and partly P in the nuclei, and N, M, P and H in the cytoplasm with intense staining by anti-H monospecific and monoclonal antibodies at the luminal side of giant cells.

In several studies on the localization of measles virus proteins in in vitro cells using monoclonal antibodies, it was revealed that only N antigen was located in the nuclei and M, H, F, P and L antigens in the cytoplasm (Tyrrell et al. 1980; Giraudon and Wild 1981; Norrby et al. 1982; Giraudon and Wild 1984; Sheshberadaran et al. 1985; Sato et al. 1985). Our data on Vero cells infected with the Edmonston and the EB–L strain of measles virus in vitro and on the lung with measles giant cell pneumonia in vivo were consistent with these observations. The apparent difference between the Edmonston and the EB–L strain in the frequency and intensity of nuclear staining by anti-N antibody, suggest that the mode of production of measles viral proteins may change by passages in in vitro cells.

Histopathologically, the findings of nuclear inclusions were different from that of cytoplasmic inclusions. Electron microscopically, two forms of nucleocapsids of measles virus, smooth form in the nuclei and fuzzy form in the cytoplasm, were observed in in vitro studies (Nakai and Imagawa 1969; Oyanagi et al. 1971). The fuzzy cytoplasmic nucleocapsid is larger than the smooth intranuclear one and viral proteins are more abundant in the fuzzy nucleocapsids than in the smooth ones (Robbins et al. 1980). The electronmicroscopic findings of the nucleocapsids in in vivo were essentially similar to the in vitro study, although it was difficult to identify the fuzzy nucleocapsids due to their intense aggregation (Breitfeld et al. 1973). The detection of viral antigens in the nuclei and cytoplasm of measles giant cells in the lung by immunofluorescence using convalescent sera (Koffler 1964) have suggested that the nature of the intracytoplasmic inclusion bodies are originated from measles virus itself. Our immunohistochemical analysis of the localization of measles virus proteins in the lung, therefore, suggests that the difference of proteins between in the nuclei and cytoplasm of measles giant cells implicates the morphological differences of these inclusions as observed by histopathological and electron microscopical examinations.

Moreover the intense staining by anti-H monospecific and monoclonal antibodies at the luminal side of the giant cells may indicate that the direction of virus budding occurs at the luminal side of the epithelium and that airborn infection serves as a major route of measles virus spread. Systemic pathological and immunohistochemical examination of all tissues from the other organs will be published elsewhere.

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