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Case report

Do measles early giant cells result from fusion of non-infected cells? An immunohistochemical and in situ hybridization study in a case of morbillous appendicitis

A. Gaulier[1], P. Sabatier[1], S. Prevot[2], and J.G. Fournier[3]

Service d'Anatomie Cytologie Pathologiques, [1] Hospital V. Dupouy, F-95107 Argenteuil Cedex, France
[2] Hotel-Dieu, F-75181 Paris Cedex 04, France
[3] U.43, Inserm, Hopital Saint-Vincent de Paul, F-75674 Paris Cedex 14, France

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Summary. A case of acute appendicitis with features of measles inflammatory reaction is studied. Two types of lymphoid polykaryons are seen: Warthin Finkeldey cells inside germinal centres (LN1 ++, LN2 +, L26 +, MB1 +, MB2 ±) and multinucleate plasma cells in the lamina propria (μ +, α ++, κ +, λ +). Both types of polykaryon are devoid of inclusions. The search for viral genetic information by in situ hybridization was negative in these cells. A positive signal was observed in interfollicular mononuclear cells and rare enterocytes. A possible mechanism of fusion from without, acting at the beginning of the disease to induce the appearance of polykaryons, is discussed.

Key words: Measles – Cell fusion – Giant cell (viral)

Introduction

Human and experimental monkey measles are considered to develop in four stages according to Fenner's model: primary invasion, multiplication within lymphoïd tissue, secondary viraemia and florid disease (Fraser and Martin 1978). Three types of polykaryons originating from lymphoid cells have been described (Nii and Kamahora 1964). The typical Warthin Finkeldey cells (WF) containing small lymphocyte nuclei are located in germinal centres. They bear no inclusions and predominate from the 5th to the 12th day following inoculation in experimental disease. The so-called "reticular" plasma cells appear later on, from the 9th until, the 16th day. They contain larger nuclei and nucleocytoplasmic inclusions. Plasma cell polykaryons are found incidentally in lymph nodes and in the spleen. Since early measles observations are rare and knowledge of virus diffusion limited, two kinds of lymphoid giant cells were studied.

Offprint requests to: A. Gaulier

Case report

A 7-year-old Caucasian boy was operated on for acute appendicitis 6 h following a complaint of pain in the right iliac fossa with mild hyperthermia and neutrophilic granulocytosis. Three days later a histological diagnosis of appendicular measles was corroborated by the emergence of Koplik's spots and, on the 4th day, typical exanthemata. A contact 9 to 7 days before appendicectomy was found retrospectively. The patient recovered without complication within 2 weeks.

Materials and methods

The appendix was fixed in alcoholic Bouin's fixative for 12 h and studied with haematein eosin safranin, May Grunwald Giemsa and periodic acid Schiff (PAS). Immunohistology was performed on the same paraffin-embedded material by a three-step method, the second and the third being performed with a Biolyon Kit (69572 Dardilly, France). First antibodies and sources are given in Table 1.

In situ hybridization was carried out on 5-μm paraffin sections as previously described (Fournier et al. 1986). Briefly, sections were deparaffinized in xylène, dehydrated, incubated in lithium carbonate and treated with 0.2 M HCl and proteinase K (25 mg/l). A DNA tritiated probe (0.2 mg/l) specific for the measles virus nucleocapsid gene was used with autoradiography after 4–8 weeks of exposure. The reaction medium contained salmon sperm DNA (0.2 g/l), yeast RNA (0.5 g/l) and *Escherichia coli* RNA (1 g/l). Control tests were performed on a similarly processed appendix from a sudden unexpected death in a 4-month-old child with no past history of measles or vaccination. A control test was also carried out with a DNA tritiated HIV probe.

A comparative in situ hybridization of measles-infected Vero cells was carried out as previously described (Fournier et al. 1983; 1985).

Results

Acute appendicitis with superficial ulceration and mild polymorphonuclear infiltrates is associated with diffuse hyperplasia of lymphoid tissue. A few immunoblastic cells with prominant nucleoli without inclusions are present inside interfollicular areas. Germinal centres contain WF giant cells, with 5–30 nuclei. These cells

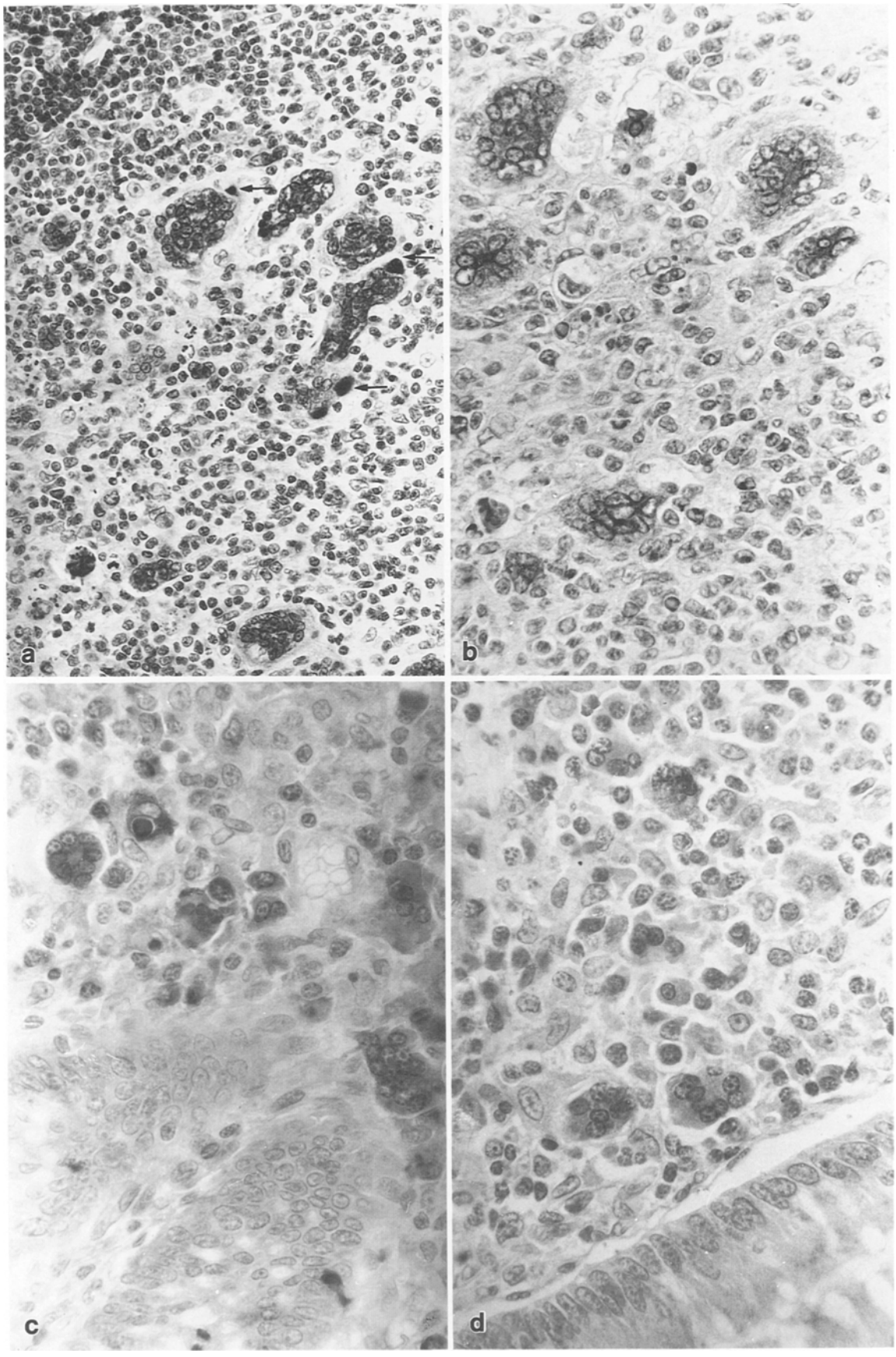

Fig. 1a–d. Polykaryon immunolabelling. **a** Strong MB1 peripheral "dot like" labelling of centrofollicular Warthin Finkeldey cells (*arrows*). Original magnification, ×200. **b** Weaker LN2 intracytoplasmic labelling. Original magnification, ×300. **c** Heavy chain α plasma cell labelling inside lamina propria. Original magnification, ×400. **d** Light chain K plasmodial plasma cell labelling. Original magnification, ×400

Table 1. Antibodies used in the study and results of immunostaining

Antibody	UCHL1	L26	LN1	LN2	MB1	MB2	S100	LeuM1	Lysozyme	IgG	IgA	IgM	K	L
Source	Dako		Miles		Eurobio		Dako	Becton	Dako	Dako			Biolyon	
Dilution	1/200	1/100	Prediluted		1/20		1/200	I/10	1/100	1/200			Prediluted	
Warthin Finkeldey lymphoid cells	0	+ Periph. Focal	++	++	+ Periph.	+/−	0	0	0	0	0	0	0	0
Plasma cell polykaryons	0	0	0	0	0	0	0	0	0	0	++	+	+	+

++, Intense staining; +, moderate staining; ±, weak staining; 0, no staining

contain no inclusions. They are labelled by LN1 (++), LN2 (+), L26 (+), MB1 (+), MB 2 (±). (Fig. 1a, b). Multinucleate plasmocytes with 2–10 nuclei labelled by IgA (++), IgM (+), K (+), L (+) are seen in the upper part of the lamina propria, near the epithelium, without inclusions (Fig. 1c, d). A few necrotic epithelial or crypt cells are seen without typical Masugi Minami epithelial cells.

On slides incubated with the specific labelled probe, an intranuclear and intracytoplasmic signal of hybridization is seen in perifollicular mononuclear cells (Fig. 2b, c) and in rare epithelial crypt cells (Fig. 2a). The WF giant cells and plasmodial plasma cells are unhybridized. A background is seen on slides from Bouin-fixed material, which does not preclude a clear distinction between hybridized slides (Fig. 2a–c) and control slides (Fig. 3a, b).

In Vero infected cells, several unhybridized plasmodes are seen mixed with others, intensely or moderately hybridized, regardless of their size (Fig. 3c).

Discussion

At an early stage of measles, the B lymphocyte nature of intrafollicular WF cells is demonstrated in this observation. The LN1, LN2, L26, MB1, MB2 labelling is in accordance with predominance of centrofollicular B lymphocytes in such polykaryons (Poppema et al. 1987; Norton and Isaacson 1989).

The plasma cell nature of the lamina propria plasmodes is shown by the labelling of intracytoplasmic immunoglobulins (intense, moderate, rarely null). It favours the coexistence of different light chains (κ and λ) and heavy chains (α and μ) in the same plasmodes, showing an origin from polytypic plasma cell fusion.

In vitro, the F viral protein induces multinucleated cells in monolayer cultures (Scheid and Choppin 1974, 1977; Harrowe et al. 1990). This protein is located on the external part of the viral envelope as well as on the surface of infected cells (Stallcup et al. 1979). In vivo, an indirect role of F protein in giant cell formations is suggested by the moderate abundance of such protein in subacute sclerosing pan-encephalitis devoid of multinuclear cells (Norrby et al. 1985) and by the lack of protein F in measle inclusion encephalitis without multinuclear cells (Baczko et al. 1988).

Lack of inclusions in cells in the early stages of the disease is classical. (Nii and Kamahora 1964; White and Boyd 1973).

During the third and fourth stages of the disease, giant cells are considered to result from infected cell fusion, a conclusion based on the electron microscopic observation of epithelial cells (Archibald et al. 1971; Sata and et al. 1986), the same features being observed in vitro (Rentier et al. 1978). In situ hybridization and fluorescence studies show identical results in acute fatal measles (Moench et al. 1988). During all stages of measles, whether common or complicated, mononucleated lymphoïd infected cells are present (Nii and Kamahora 1964; Fournier et al. 1985; Fournier et al. 1986; Moench et al. 1988). Such cells are demonstrated in this observation, by hybridization, located around germinal centres. The lack of hybridization for the measles DNA probe in early WF cells inside germinal centres raises different hypotheses. First, the lack of hybridization may be related to unspecific fixative in this case. Bouin's fixative favours background staining but does not preclude measle DNA probe use (Fournier et al. 1986) or use of other probes (Sue and Griffin 1987) providing that the fixation is not too long. We observed a clear discrepancy between the lymphoid and rare epithelial mononuclear hybridized cells, which are different from WF and plasma cells devoid of such a signal.

Second, the non-labelling of plasmodial cells may be due to a poor viral production, inversely related to the size of giant cells as described in vitro (Rentier et al. 1978). It does not explain the lack of hybridization in the smallest multinucleate cells in this observation. Our results from the study of in vitro Vero infected cells clearly reveal heterogeneity of the hybridization signal, unrelated to the polykaryon size. Hybridized cell localization in this case is strikingly similar to the interfollicular situation of Epstein Barr virus-infected lymphoid cells studied in acute infectious mononucleosis tonsillitis (Paellsen et al. 1990).

A third hypothesis is that a mechanism of fusion "from without" may occur during the two first stages

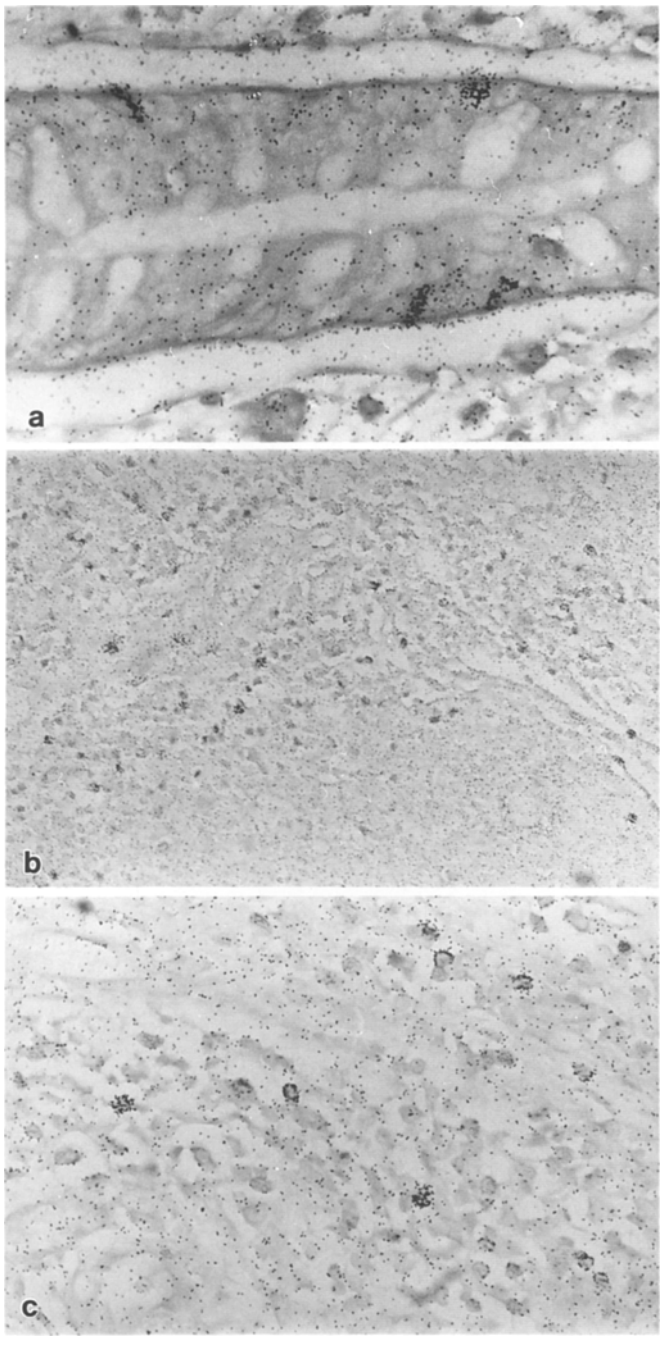

Fig. 2a–c. In situ hybridization. **a** Rare hybridized epithelial cells. Original magnification, ×800. **b** Interfollicular mononuclear hybridized cells Lack of centrofollicular hybridization. Original magnification, ×100. **c** Perivascular distribution in interfollicular area. Original magnification, ×300

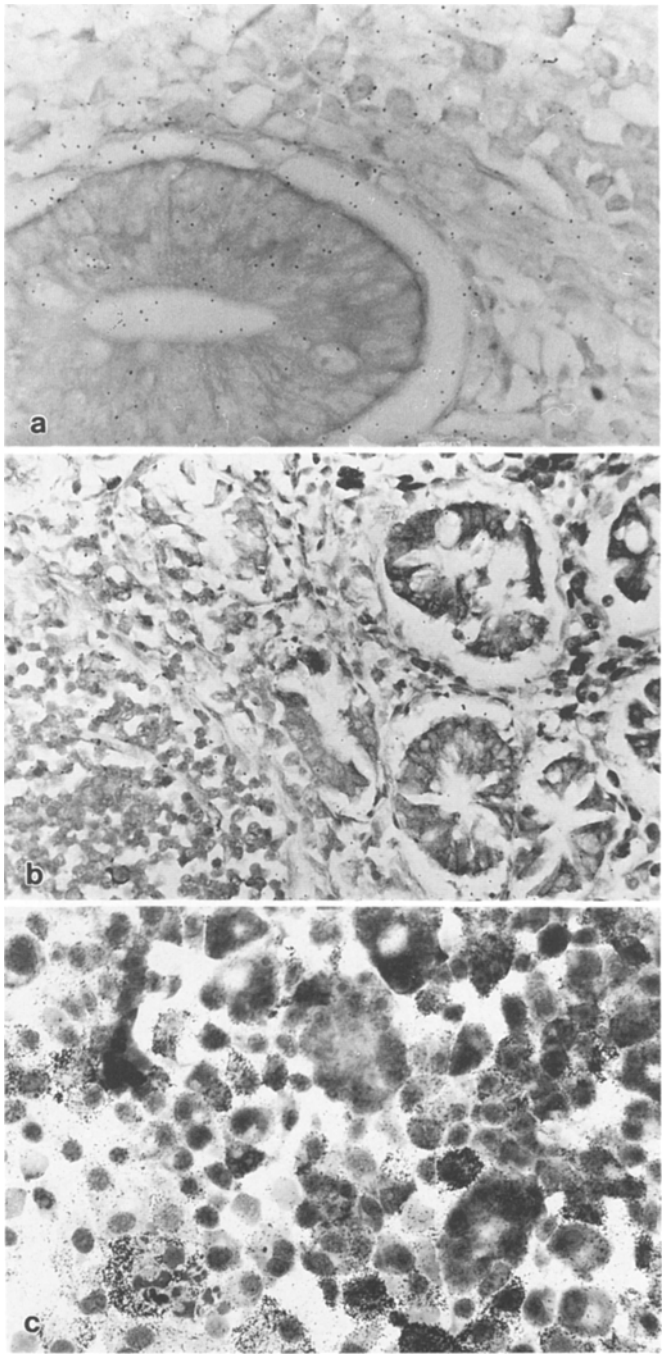

Fig. 3a–c. Control hybridization. **a** Lack of hybridization in measles appendix by using a DNA tritiated HIV probe. Original magnification, ×500. **b** Lack of hybridization in control appendix by using the DNA tritiated measle probe. Original magnification, ×200. **c** Heterogeneous hybridization in measles infected Vero cells (mono and multinucleated). Original magnification, ×300

of measles infection. Lysis of infected cells inside or near the germinal centres releases numerous extracellular virions containing the F surface protein, which may induce poorly or non-infected B cell fusion inside germinal centres. Such a model of fusion "from without" is established in vitro by using high quantities of inactivated virus (for review, see Poste 1972). It was first hypothesized during the measles inflammatory process by Nii and Kamahora (1964) from morphological observations. A fusion from without must also be considered for plasma cell polykaryons inside the lamina propria. The viral material may result from lymphoid or epithelial infected cell lysis, with possible involvement of specific antibodies on the outer membrane of plasma cells.

This observation underlines the fact that an optimal study of viral inflammatory processes requires the use of different fixatives for optical and electron microscopy just as the sampling of fresh frozen material. Such an

opportunity is rarely encountered in practice, especially during early measles infection, which exceptionally leads to surgery.

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