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Case report

Measles associated with coronary arteritis

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Summary. A two-year-old girl with measles virus (MV) and chronic Epstein-Barr virus (EBV) infection developed lethal coronary aneurysmal arteritis accompanied by giant cell pneumonia, systemic lymphadenitis and hepatosplenomegaly. In her coronary arteries, lungs and aorta, cells containing intranuclear and intracytoplasmic inclusions, including syncytial giant cells, were detected, the presence of MV in the organs being proved by electron microscopic and immunofluorescent studies. Immunopathology further demonstrated MV to be disseminated in almost all organs other than lymph nodes. Clinical diagnosis of chronic EBV infection was established on the basis of persistent high titers of antibodies against capsid and early antigens of EBV and viral presence was confirmed by Southern blot hybridization in a mesenterial lymph node obtained at autopsy. To the best of our knowledge, this is the first description of MV association with coronary aneurysmal arteritis, raising the possibility that measles infection can cause severe vasculitis under immuno-suppressive states, such as that caused by chronic EBV infection.

Key words: Measles – Coronary arteritis – Epstein-Barr virus

Introduction

Measles is a highly communicable disease characterized by fever, cough, coryza, conjunctivitis and erythematous cutaneous eruption. Although most measles patients are cured in less than two weeks from the onset, a few exceptional cases will advance to measles giant cell pneumonia or subacute sclerosing panencephalitis. The measles virus (MV) is one of the paramyxovirus group demonstrating some pleomorphism, but the particle is usually spherical, ranging from 120 to 250 nm in diameter. It has haemagglutinin glycoprotein on its envelope surface but no neuraminidase and MV-infected cells bear intranuclear and intracytoplasmic inclusions, a characteristic feature which is used to differentiate them from cells infected with other viruses (Sata et al. 1985). There has been no previous report describing that MV causes coronary arteritis or systemic vasculitis, although a description has been made of MV inclusions in endothelial cells (Akhtar et al. 1973).

Epstein-Barr virus (EBV) induces a broad spectrum of illness including infectious mononucleosis which is an acute disease characterized by sore throat, fever, lymphadenopathy and leukocytosis, composed in part of atypical lymphocytes. Chronic EBV infection is characterized by persistent or recurrent fatigue and some degree of immuno-suppression (Tobi et al. 1988). However, EBV infection in the young is often asymptomatic. One of the herpes group, ranging from 120 to 130 nm in diameter and consisting of envelope, tegument, capsid and core, EBV has a unique tropism for B lymphocytes (Epstein and Achong 1973) and its presence can be confirmed by virus isolation or by genetic methods (Kikuta et al. 1988; Dambaugh et al. 1980).

We recently experienced an infant mortality demonstrating serious aneurysmal arteritis associated with MV and EBV infection which suggested the possibility that MV can be a causative agent of severe vasculitis under specific conditions.

Case report

A one-year and ten-months-old girl was admitted to the Department of Paediatrics, Odawara Municipal Hospital in August, 1988, because of prolonged high fever with hepatomegaly and lymphadenopathy. The total white blood cell count was 15.9×10^9/L, differentiated into neutrophils, 21% and lymphocytes, 79% respectively. The titers of S-GOT, S-GPT and LDH were 309 IU (normal range; 5–30), 243 IU (normal range; 2–25) and 1204 IU (normal range; 220–430), respectively. Intensive care with antibiotic and adrenocortical steroid hormone therapy resulted in some improvement. However, two months later, liver function worsened with S-GOT 1051 IU, S-GPT 586 IU and LDH 2315 IU and this dysfunction persisted until her death. Three months after admission, titers of antibodies to EBV capsid antigen (VCA), early antigens (EA) and nuclear antigen (EBNA) were assessed to be high (VCA-IgG: 1280, VCA-IgM: 10, EA-Dr-IgG: 320, EA-DR-IGA: <10, EBNA: 160), these levels persisting thereafter. No atypical lymphocytes were noticed during the whole course of the disease. The levels of CD3 and CD4 in peripheral lymphocytes gradually decreased from 84% and 78.6% to 57.6% and 45.8%, respectively, and the content of CD8 rose gradually from 5.8% to 12.6% during the five month period of observation. The CD4/CD8 ratio changed from 13.6 to 3.6 during that time. Echo cardiogram studies revealed aneurysmal dilatation of the base of the right coronary arteries in the beginning of her illness. Diagnosis of mucocutaneous lymph node syndrom (MCLS) could be excluded, because appropriate symptoms were lacking. Erythematous cutaneous eruptions or Koplik spots were also absent at all times. She died of severe pneumonia eight months after admission.

Results

At autopsy, aneurysmal dilatation of the bilateral coronary artery bases, up to 5 mm diameter in the left and up to 4 mm diameter in the right, eccentric cardiac hypertrophy (Heart: 85 g/expected 37 g), generalized lymphadenopathy, hepatomegaly (555 g/expected 260 g) and splenomegaly (155 g/expected 20 g) were identified. The lungs (Left: 127 g/expected 47 g, Right: 160 g/expected 53 g) were firm, meaty, and hypocrepitant, and showed a dull-red to yellow-tan cut surface.

On histological examination, marked thickening of the intima with infiltration of lymphocytes, plasma cells and histiocytes accompanied by syncytial giant cells was noted. The giant cells demonstrated characteristic intranuclear eosinophilic inclusions surrounded by a halo. Disruption of the internal elastic lamina of coronary arteries was also noted (Fig. 1). Hypertrophy of the myocardial fibers and myocardial oedema were present. In the aorta, common iliac, splenic and gastroduodenal arteries, mild to moderate vasculitis, including the sporadic presence of syncytial giant cells, was observed.

Moderate numbers of lymphocytes, plasma cells and histiocytes were found infiltrating the small to medium sized bronchi, with necrotic masses deposited in their lumens. Scattered syncytial giant cells were seen in the bronchi, and diffuse haemorrhage in the alveoli and fibrotic alveolar septa were found.

In the lymph nodes, proliferation of lymphocytes in the T cell zone was marked, with small necroses observed sporadically. In the liver, moderate infiltration of lymphocytes into Glisson's sheaths, moderate amounts of small hepatic lipid, small foci of necrosis and severe congestion were seen. In the spleen, multiple necrosis and increased numbers of lymphocytes were noted. In the lymph nodes, spleen and liver, syncytial giant cells were rarely present.

Cerebral autopsy was not permitted.

For electron microscopy tissues were fixed in a phosphate buffered neutral solution containing 20% v/v formalin. They were rinsed with 0.1 M phosphate buffer solution (pH 7.4), postfixed in osmium tetroxide, and then embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and then examined for inclusion-bearing cells under a transmission electron microscope (Hitachi H-7000). Inclusions were observed in both the nuclear and cytoplasmic compartments which could easily be seen in tissues of the coronary arteries and aorta. A typical inclusion consisted of aggregates of numerous tubular structures, 16 to 20 nm in diameter. In longitudinal sections, these tubules showed faint cross striations, with a periodicity of 5 to 7 nm (Fig. 2).

Tubular aggregates (nucleocapsides) are a well-recognized ultrastructural morphological feature of MV and several other paramyxoviruses, and correspond to the intranuclear and/or intracytoplasmic inclusions which are observed by light microscopy in both the nucleus and cytoplasm of cells infected with measles. This dual location is characteristic of MV (Rain et al. 1969) and therefore, the nucleocapsids in this case were interpreted as indicating presence of this virus.

For immunofluorescence tissues were fixed in 20% phosphate buffered neutral formalin solution and embedded in paraffin five μm tissue sections were examined by indirect immunofluorescence microscopy. Anti-measles antibody (Transformation Research, Inc., Framingham, USA), anti-EBV VCA and anti-EBV VMA antibodies (Clonatec, Paris, France), anti-cytomegalovirus (CMV) antibody (BioGenex Laboratories, Dublin, USA) anti-herpes simplex virus (HSV) type I (BioGenex Laboratories, Dublin, USA) antibody, and anti-HSV type II antibody (BioGenex Laboratories, Dublin, USA) were used.

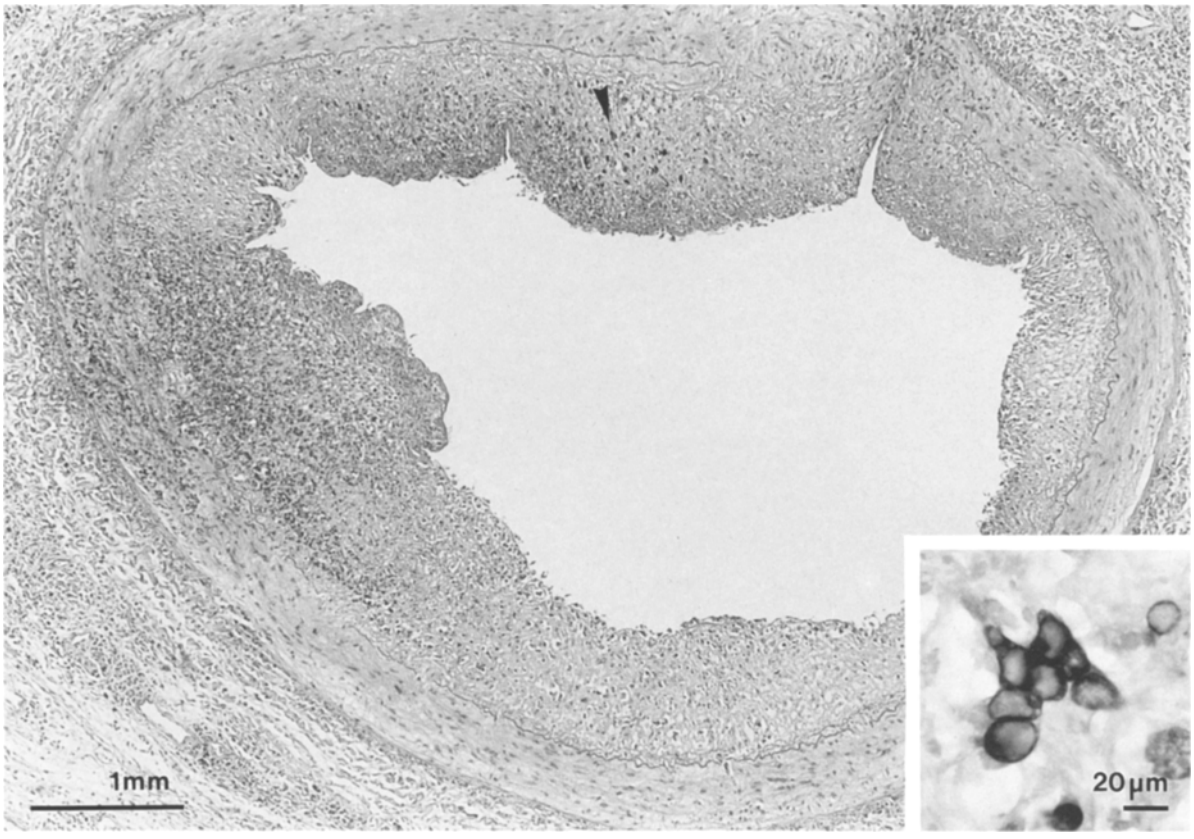

Fig. 1. Left slightly dilated coronary artery. The thickening of the intima with marked infiltration of inflammatory cells are observed. The internal elastic lumina is partly disrupted (× 20). Scattered inclusion-bearing syncytial giant cells are evident, one of which (*arrowhead*) is shown in the inset (× 300)

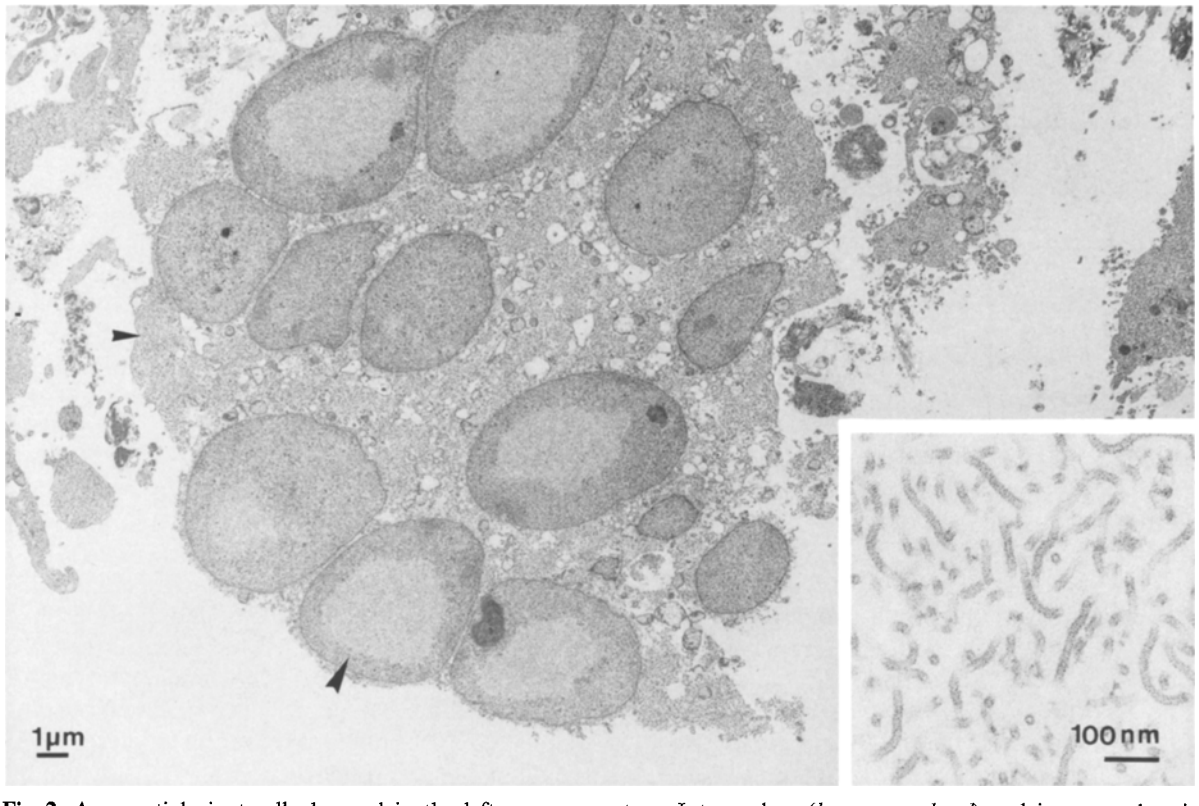

Fig. 2. A syncytial giant cell observed in the left coronary artery. Intranuclear (*large arrowhead*) and intracytoplasmic tubular aggregates (nucleocapsids, *small arrowhead*) are evident (× 4,000). Tubular aggregates are shown at higher magnification in the *inset* (80,000)

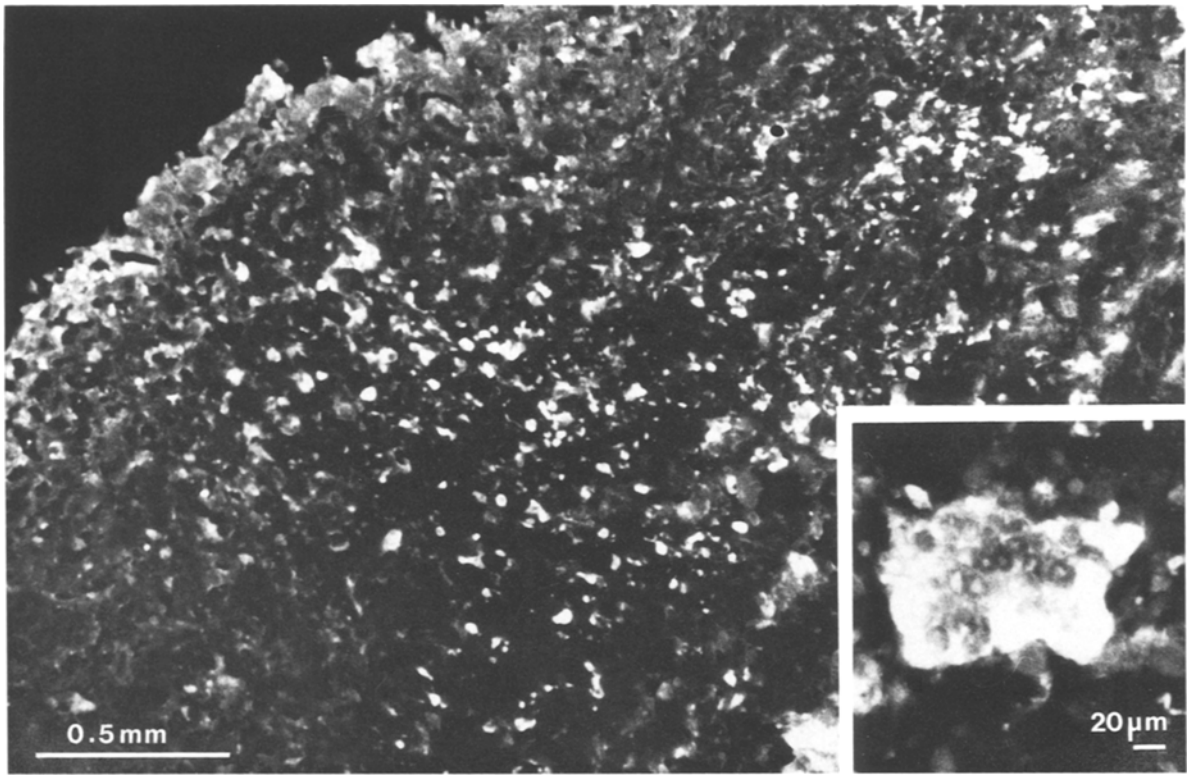

Fig. 3. Immunofluorescence of the left coronary artery. Many fluorescence emitting cells are observed in the internal tunica (×50). The *inset* illustrates a fluorescing syncytial giant cell (×300)

In the coronary arteries, fluorescence emission with anti-measles antibody was accumulated mainly in cells in the intima and outer region of the adventitia (Fig. 3). Similar fluorescence was also found in cells in the aorta, lungs, spleen, liver, heart, kidneys, pancreas, adrenal glands, thyroid glands, uterus, urinary bladder, common iliac artery, splenic artery and gastroduodenal artery, although in these tissues the numbers of cells were appreciably less than in the coronary arteries. However, emitting cells were never detected in the lymph nodes.

With anti-VCA and anti-VMA antibodies against EBV, scattered fluorescent emission was apparent only in the lymph nodes of all examined tissues. With anti-CMV, anti-HSV I and anti-HSV II antibodies, no emitting cells were noticed and any of the organs investigated.

To clarify the origin of the inclusion-bearing cells, antibodies against Factor VIII related antigen, alpha 1 anti-trypsin antigen, S 100 protein and several lymphocyte antigens, including leukocyte common antigen were applied, inclusion-bearing cells reacted to antibodies against antigens characteristic for histiocytes or lymphocytes. However, they never reacted to Factor VIII related antibody, for which endothelial cells are known to be positive.

Detection of EBV genome

A DNA sample from the mesenteric lymph nodes obtained at autopsy and several reference DNAs were analysed by the Southern-blot hybridization technique. A recombinant plasmid containing one of the EBV-W fragments of the EBV genome was kindly provided by Dr. T. Yokochi, Fukui Medical College, a 2.9 kbp insert purified after digestion with the restriction enzyme, BamHI and subsequent ^{32}-P labeling, used as a probe.

An approximately 3.5 kbp BamHI fragment of DNA (Fig. 4B, lane b) hybridized to the EBV probe but not to BamHI fragments of any of the following six DNAs used for reference, i.e., herpes simplex virus type 1 (HSV-1) strain HF, HSV-2 strain 112, varicella-zoster virus (VZV) strain H-N3, human cytomegalovirus (HCMV) strain KH, a normal human embryonic lung fibroblast (HEL) DNA, and a reference human tonsil tissue DNA (Fig. 4B, lanes a, f, g, h, i, j). The viral DNAs and HEL DNA were generous gifts of Dr. R. Hondo, Institute of Medical Science, University of Tokyo. This EBV probe hybridized to an approximately 3.2 kbp BamHI fragment of Raji cell DNA as a positive reference (Fig. 4B, lane C) as well as the expected 2.9 kbp EBV HI DNA used as controls (Fig. 4B, lanes d and c).

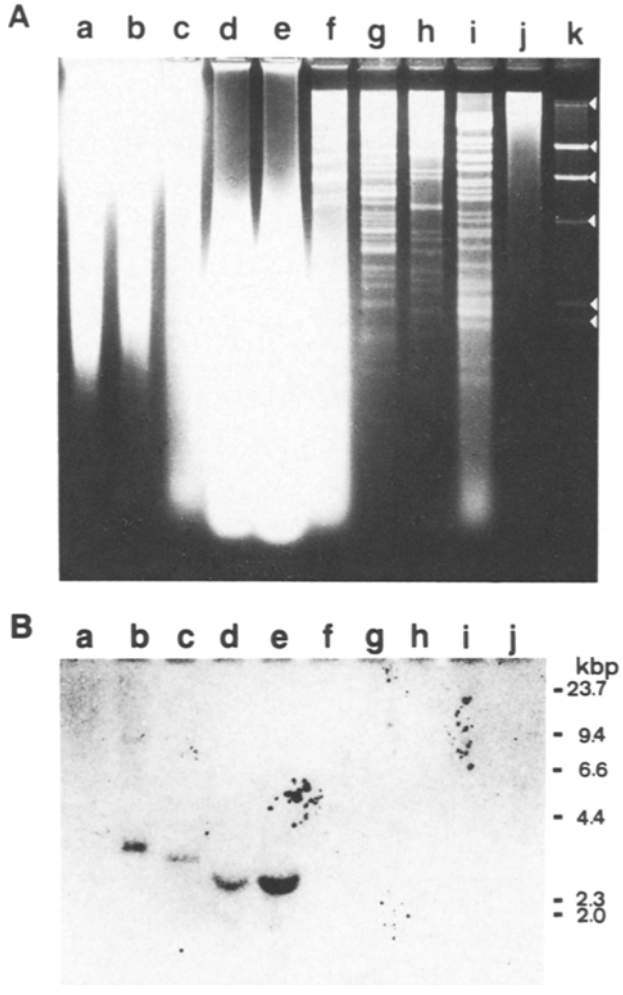

Fig. 4A and B. Electrophoretic restriction patterns of the patients DNA and reference DNA sampes (**A**), and autoradiographic demonstration of EBV DNA in the patient DNA (**B**). BamHI digested – and agarose gel resolved-DNAs were stained with ethidium bromide (**A**). The resolved DNAs were transferred, hybridized with labeled probe DNA, and were autoradiographed for 7 days (**B**). Samples illustrated are reference tonsil DNA, 20 μg (a); patient lymph node DNA, 20 μg (b); Raji cell DNA, 6.3 μg (c); 2.9 kbp EBV BamHI-W fragment DNA: 10 pg (d), 20 pg (e); HSV-1/HF DNA (f); HSV-2/112 DNA (g); VZV/H-N3 DNA (h); MCMV/KH DNA (i); and HEL/K8 DNA (j). Approximately 0.6 μg of reference viral DNA samples were applied per lane (f to j). DNA fragments obtained by HindIII digestion of Lambda phage DNA were used as molecular size markers (k), and the fragment sizes in kilobase pairs corresponding to the bands in (k) are shown on the right of (**B**)

We confirmed that the BamHI-generated restriction patterns of the reference virus DNAs are reproducible and comparable with those of published results (Hondo et al. 1987), that the amount of DNA fragments loaded on the gel were sufficient (Fig. 4A), and that the probe could detect as low as 10 pg of the 2.9 kbp EBV BamHI fragment. Restriction fragment length polymorphism (RFLP) was noted in the detected bands of Raji cell DNA and patient DNA (Fig. 4B, lanes b, c). Similar results of DNA size variation have been noted among the reiterated BamHI fragments of EBV from different cell lines, or from different organs of a patient (Andiman et al. 1985). With this level of sensitivity and reliability, the results demonstrated the presence of EBV DNA in the patient's lymph node but none of the other four herpes family DNAs examined.

Discussion

The results of the present electron microscopic, immunofluorescent and histopathologic study at autopsy clearly revealed the case to be one of generalized measles' infection excluding the lymph nodes. The finding of associated coronary aneurysmal arteritis is of interest, since to our knowledge, there has been no previous description of coronary arteritis caused by MV. A causal relationship was evidenced by characteristic MV intranuclear and intracytoplasmic inclusions (nucleocapsids) in the cells of coronary arteries, lungs and aorta. Although a previous report mentioned the presence of inclusions in endothelial cells (Akhtar and Young 1973), the present electron microscopic and immunofluorescent results suggested that the inclusion-bearing cells were not of endothelial origin but rather were derived from histiocytes or lymphocytes.

Despite an intensive search for other viral and/or bacterial infections in addition to MV in this case, only EBV was shown to be present persistently throughout disease course. Although only a limited DNA copy number of EBV was indicated, and cells demonstrating positive binding of EBV VCA and EBV VMA antibodies were relatively few, the conclusion that EBV infection in this case was an incidential, opportunistic and therefore unimportant infection is unlikely in view of the observed prolonged high titers of antibodies to EBV, the presence of both IgG anti-viral capsid antigen and anti-early antigen. These findings further suggest that the patient was suffering chronic active EBV infection in the lathal course (Jones et al. 1985; Straus et al. 1985). The RFLP we detected may suggest that EBV genome modifications had occurred before or after development of the disease, since similar RFLPs were also observed when DNA from Raji cells was digested with BamHI, and probed with the EBV EcoRI B fragment. Equivalent RFLPs were found for DNAs purified from different organs in a patient reported earlier (Andiman et al. 1985). Although the progressive decrease of helper T lymphocytes and the relative increase of suppressor T lympho-

cytes found in the present case may be considered to be directly related to the MV infection (Rima 1983), a second possibility is that the shift was due to EBV, since recent reports have shown that this virus attacks T lymphocytes as well as B lymphocytes (Kikuta et al. 1988; Tosato et al. 1985). It is quite likely that the persistent hepatitis and necrotizing lymphadenitis were also caused by EBV indirectly (Nelson and Darragh 1956) and that a defect in immune function due to chronic active EBV infection (Kibler et al. 1985) was the basic trigger for the severity of the MV infection in this case.

Attention should be drawn here to acute mucocutaneous lymph node syndrome (MCLS), because the histological features of the coronary arteritis of this case resemble those of MCLS, in which the most major and often lethal lesion is a coronary aneurysm based on coronary arteritis, with proliferative or granulomatous arteritis (Naoe et al. 1983; Tanaka et al. 1983) accompanied by systemic vasculitis. With regard to its aetiology, a number of viruses including EBV (Iwanaga et al. 1981; Kikuta et al. 1984), parainfluenza type 3 (Schnaar et al. 1982) and some retroviruses (Shulman and Rowley 1986) have been implicated. Since MV is one of the paramyxovirus group which readily proliferative in endothelial cells in culture (Friedman et al. 1981), it might be reasonable to suspect that it could also be a cause of MCLS. However, since suppressor T lymphocytes decrease in MCLS cases (Leung et al. 1983) but, in the present patient, it was the helper T lymphocytes which were decreased and no clinical symptoms suggestive of MCLS were observed it was thought unlikely that this disease was involved.

Cerebral autopsy was not permitted and therefore, the question of whether subacute sclerosing panencephalitis (SSPE) was present could not be answered. Nevertheless, the results did demonstrate, that under particular conditions coronary arteritis can occur in association with measles. Further cases are required to clarify the relationships between MV infection and MCLS.

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