Virchows Arch. A Path. Anat. and Histol. 378, 321–328 (1978)

Virchows Archiv A

B-Cell Nature Malignant Lymphoma Probably Caused by EB-Virus Infection

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Summary. In cells in a haemopericardium associated with a B-cell malignant lymphoma, immature herpes type virus particles were found by electron microscopy. Epstein-Barr virus associated nuclear antigen (EBNA) and virus capsid antigen (VCA) were also found, both in the tumor cells, in the bloody pericardial fluid and in cultivated cells. Serological studies revealed high anti-toxoplasma antibody levels both in the pericardial fluid and in serum. Both EB virus and toxoplasma infections are assumed to have played an important role on the pathogenesis of the present case.

Key words: EB virus – EBNA – VCA – B-cell nature malignant lymphoma – Malignant reticulosis.

Introduction

No definite causative factors have been reported for lymphoma although EB virus infection has been considered to be important in the pathogenesis of Burkitt's lymphoma. We report here an autopsy case of malignant lymphoma that presented with intramuscular tumors and died of circulatory disturbance due to hemopericardium. As a result of electron microscopic and immunofluorescent studies of tumor cells in the bloody pericardial fluid, we have assumed that EB virus infection played an important role in the pathogenesis of the present case.

Clinical Findings

A 58 year old teacher complained of pain on movement associated with intramuscular tumors of the right shoulder and right buttock. A diagnosis of sarcoma unknown origin was made by biopsy, and both tumors were resected two months after the onset of the illness. He developed an intermittent fever with cough and dyspnoea. Marked hepatomegaly and slight superficial lymph node swelling were observed, but splenomegaly was not found. Three days before his death, pericardial punctures

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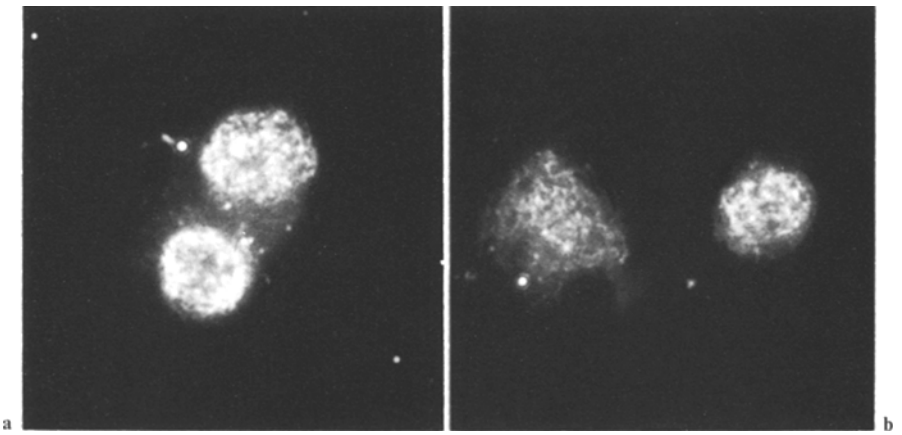


Fig. 1a and b. EBNA positive cells. **a** Tumor cells in pericardial fluid. ×400. **b** Cultivated cells from tumor cells in hemopericardial fluid. ×400

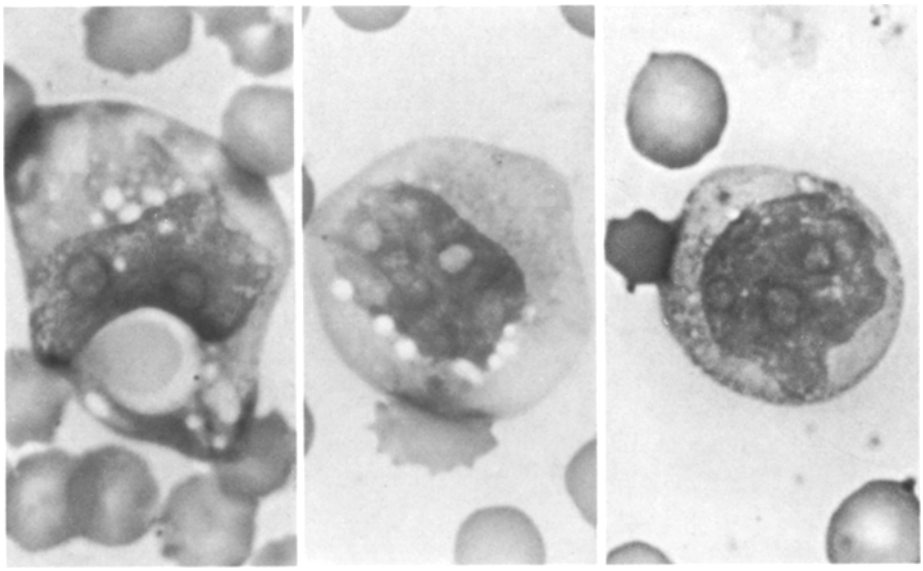
were performed to relieve dyspnoea, and bloody pericardial fluid was obtained on two occasions. He had received only 40 mg of adriamycin soon after the operation and 1 mg of vincristin two days before his death. The entire clinical course was of 4 months duration.

The right buttock tumor and cells from the pericardial fluid were cultured and two new lymphoblastoid cell lines were directly established. Details will be described in another paper (S. Oboshi), but, briefly, tumor cells in the pericardial fluid and both established cell lines showed the characteristics of B-cell lymphocytes. Toxoplasma was observed in the cultivated cells from the tumor cells in the pericardial fluid. EBNA and VCA were found on the tumor cells in the pericardial fluid and on both newly established cell lines (Fig. 1).

Other laboratory findings included a reduction of serum albumin and an increase of IgG (over 1527 mg/dl) at the time of his admission. Immunologically, anti-virus capsid antigen antibody (VCA-Ab) was at a titre of 1:160 and anti-early antigen antibody (EA-Ab) level was less than 1:10. Antitoxoplasma antibody level was 1:8190, the bloody pericardial fluid showed the same titre as serum for this antibody.

Numerous tumor cells, not observed in the peripheral blood smear, were found in the pericardial fluid. These cells were three or four times as large as red blood cells and they had a large nucleus, a few azurophilic granules and some vacuoles in the cytoplasm, shown on May-Giemsa stain (Fig. 2a). Small numbers of binuclear cells were also observed. The nuclear membrane was thin, chromatin was scattered diffusely in the nucleus and there were always 3–4 nucleoli. A pale round or oval area was observed in the nucleus of a very few pericardial tumor cells (Fig. 2b). Some tumor cells had red blood cells and/or nuclear debris in the cytoplasm (Fig. 2c). This cell population showed strong cytoplasmic brilliance with anti IgG and anti IgA serum.

Both the right buttock and the right shoulder tumors showed sarcomatous proliferation in the intramuscular tissue (Fig. 3). Numerous spotty necrotic areas accompanied by infiltration of histiocytes and granulocytes were observed in these tumors, together with a few mitoses. The tumor cells were about 10 to 20μ in diameter and had a large nucleus with a thin nuclear membrane. Notched nuclei were frequently observed as were some binucleated cells. There was abundant chromatin and a few nucleoli were also observed. Histochemically, tumor cells were stained faintly positive with PAS, were pyroninophilic with methyl green pyronine stain, and were negative with both naphtol AS-D chloroacetate esterase and the peroxidase reaction. Tartrate sensitive acid phosphatase was demonstrated in the tumor cells.



a b c

Fig. 2. **a** The morphology of the pathologic cells in the hemopericardial fluid. ×1000. **b** Note clear area (*arrowed*) in the nucleus. ×1000. **c** Pathologic cells have a red blood cell in the protoplasma. May-Giemsa stain. ×1000

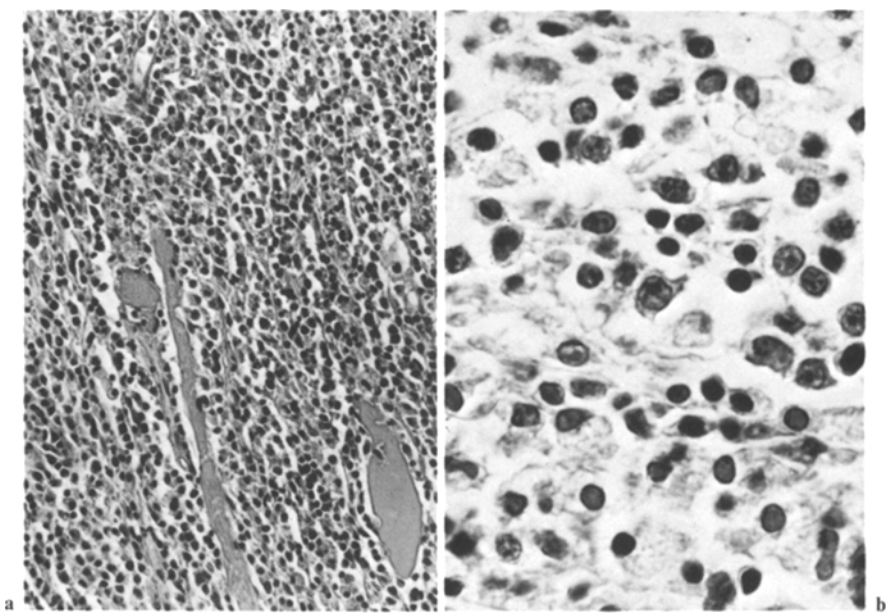


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Fig. 3a and b. Histologic finding of biopsy specimen from the right buttock tumor. **a** show severe intramuscular sarcomatous infiltration. ×50. **b** is the high magnification in the same specimen. ×400

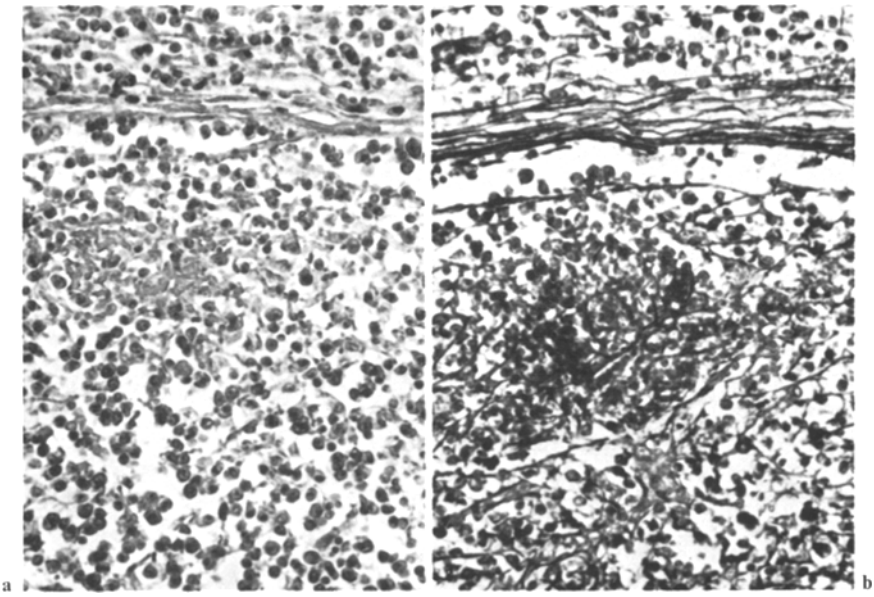

Fig. 4a and b. Marked infiltration of tumor cells accompanied with small necrotic foci retaining original architecture. **a** HE stain. × 50. **b** Silver impregnation. × 50

Autopsy Findings

The cadaver showed generalized lymph node enlargement, with formation of fused masses at the hilum of the lung. Each lymph node was up to 2 × 1.8 × 1.5 cm in size and was soft in consistency. The hilar mass extended into epicardial fatty tissue, replacing it with thick whitish tumor. Infiltration extended to the endocardium of the right atrium and to the outer half of the left ventricular myocardium. Many whitish small tumor nodules were also observed on the inner surface of pericardium, and ca. 700 ml of bloody fluid had accumulated in the pericardial sac. Tumor deposits, measuring up to 5 × 5 × 6 cm, were observed in the heart (700 g), the pericardium, the liver (1430 g), the spleen (85 g) and both kidneys (lt: 150 g, rt: 120 g).

Histologically, tumor cells with the same histochemical reactions were observed in almost all organs except for the brain, pancreas, and testes. Spotty necrosis was scattered in the various organs into which tumor cells had infiltrated. These areas of necrosis contained nuclear debris, neutrophils and reactive histiocytes and a few of the tumor cells seemed to have taken nuclear debris into their cytoplasm by phagocytosis.

In many organs, the capillary endothelium was swollen. Kupffer cells in the liver and splenic macrophages contained many red cells in their cytoplasm, but showed no neoplastic features. The original architecture of infilrated tissues was presented and a tendency to expansive growth was not seen (Fig. 4). A diagnosis of a sarcomatous type of malignant reticulosis was made and this appeared to be a B-cell lymphoma, from the findings of the immunological studies and the characteristics of membrane receptors.

Electron Microscopic Findings

We examined the tumor of the right buttock and tumor cells from the pericardial fluid during life and tumor from the heart, liver, spleen, kidneys and lymph nodes at autopsy. Cultivated pericardial cells were also examined. All materials

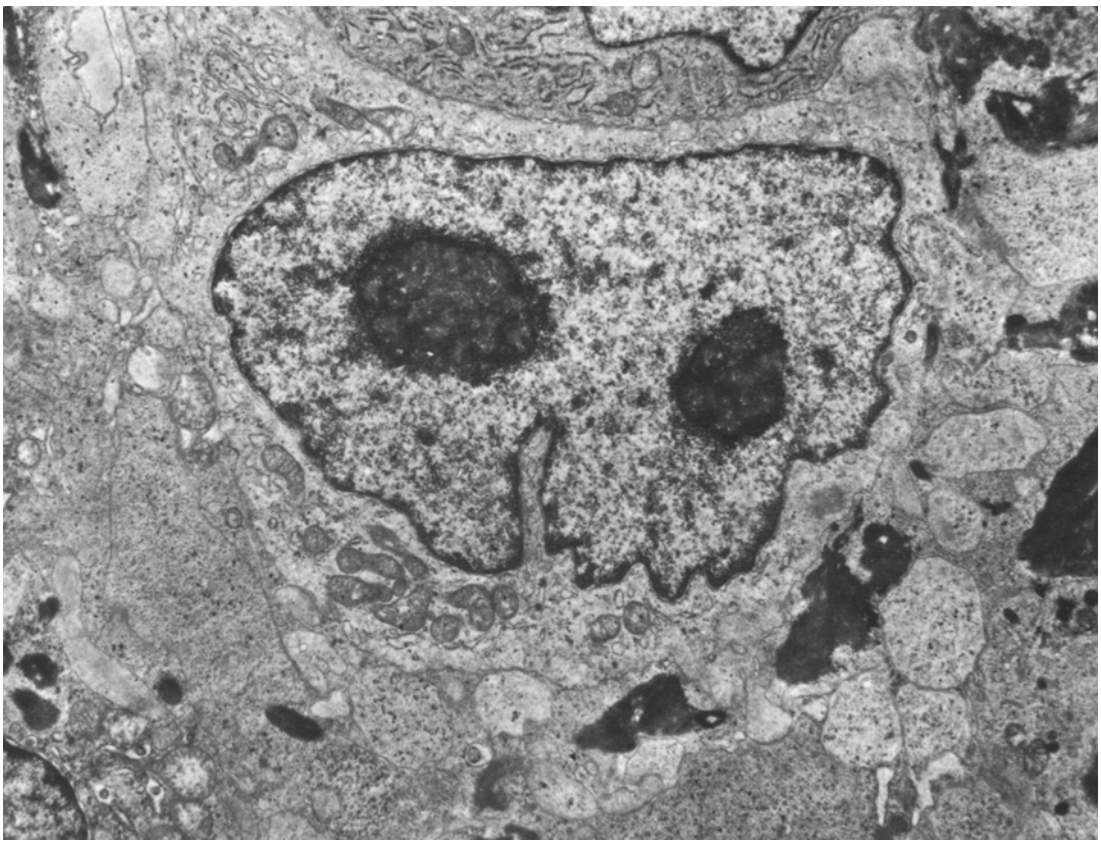

Fig. 5. Electronmicroscopic picture of tumor cells in the right buttock tumor. Notice the large nucleus which contains a few prominent nucleolus and many extoplasma. ×7600

were fixed by the ordinary method with glutaraldehyde and osmium (VIII) oxide. In all tissues the tumor cells manifested similar characteristics. The relatively narrow cytoplasm of the cells showed a low electron density, free ribosomes were scattered diffusely. The relatively small oval mitochondria had a tendency to gather at one side of the cytoplasm and moderately developed Golgi apparatus was observed. Rough surface endoplasmic reticulum was usually sparse. In a very few tumor cells, well developed dilated rough surface endoplasmic reticulum was seen, like that of plasma cells. Small numbers of tonofibrils were present. A few granules were also observed in the cytoplasma, without any particular structure. Almost all nuclei were round or oval in shape, sometimes deep notches were observed. Chromatin was diffuse and aggregated slightly around nuclear membranes. A few prominent nucleoli were also observed. Sometimes several cytoplasmic projections with only a few free ribosomes, were observed around the tumor cells of the tissue specimen (Fig. 5).

Numerous microtubular structures were seen in tumor cells and in the capillary endothelium. Many of these were in the smooth surface endoplasmic reticu-

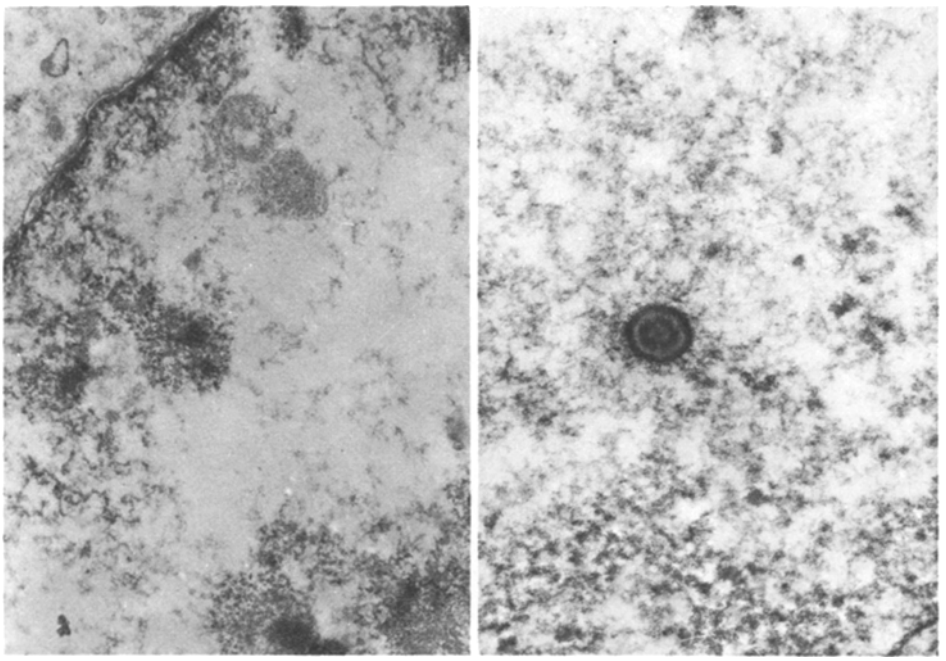

Fig. 6. a Tow round bodies near the nuclear membrane of the tumor cells which show clear area like that of virus containing cells. × 19,200. **b** Herpes type virus particle, showing double ring structure. Numerous needle-like structure was observed around the particle. × 100,000

lum and looked like tangled yarn. Multivesicular bodies, composed of many small round vesicles in a capsule of unit membrane, and many autophagosomes were also seen.

Herpes type virus particles were found in the nuclei of tumor cells in the pericardial fluid. These virus particles had round or oval double ring structures, and were about 97.5 mμ in diameter. As the inner cores were still immature, they resembled doughnuts. At high magnification, we found needlelike structures which thrust through the double ring structures (Fig. 6) and suggested that virus DNA was transferring from the outer to the inner core. Tumor cells in which virus particles assembled, showed at first a few round or oval clear areas in their nuclei. We thought that these clear areas corresponded to the whitish round or oval areas in the nuclei of the tumor cells shown by May-Giemsa stain (Fig. 2b). Virus particles were found only in such clear areas, and they apparently spread gradually, finally fused and covered the nuclei. At this point chromatin was gathered irregularly around the nuclear membrane and viral particles were found diffusely. We also observed a few round bodies in these areas but they did not accompany the virus particles.

In spite of great efforts viral particles were found only in the tumor cells in the pericardial fluid. Furthermore virus particles were found in tumor cells at the ratio of only one per 2000–3000 cells. Mature infectious virus particles were not observed, nor were they seen in the cytoplasm of tumor cells or in the extracellular space. In the cultivated pericardial cells, numerous viral

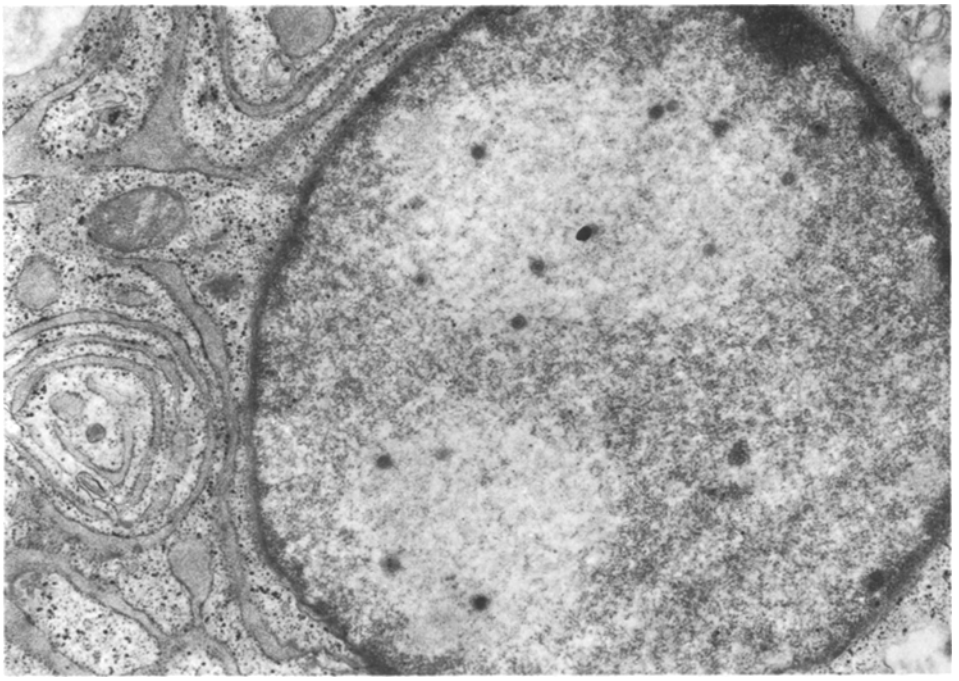

Fig. 7. Virus containg cell. Herpes type virus particles are found only in the clear area in the nucleus of the tumor cell. ×20,000

particles were observed in the nucleus and cytoplasm. Some particles seemed to be mature, sometimes the nuclei were destroyed.

Discussion

We have demonstrated the presence of herpes type virus particles in the nuclei of tumor cells in the pericardial fluid. We concluded that the virus was EB virus because EBNA was demonstrated in more than 80% of the tumor cells in the pericardial fluid. Epstein and Barr have shown that EB virus is present in the cultivated cells of the Burkitt lymphoma and other authors have discussed the oncogenicity of the EB virus (Shope et al., 1973; Zur Hausen et al., 1970; Henle et al., 1969; Miller et al., 1971; Klein et al., 1969). Epstein and Achong have discussed the aetiological relationship between EB virus and Burkitt lymphoma. They suggested that herpes virus infection might exist in three modes. (1) Virus productive, i. e. virus replication leading to cell death. (2) Non-productive, with the virus genome present. This type was divided into two subtypes. (a) Unexpressed viral genome, often activated to a productive cycle, as occurs with herpes simplex and varicella zoster. (b) Expressed viral genome manifest as malignant transformation, activated to a proliferative cycle (as in Marek's disease). Furthermore, they considered that malaria infection played an important role in the endemic manifestation of Burkitt lymphoma in Africa, by its effects on stimulating cell proliferation in the RES. In this case, virus particles

were only found in the tumor cells in the pericardial fluid and in cultivated cells, and they were never observed in the solid tumor. Mature infectious particles were not found in the cells of the pericardial fluid. We assume that the infectious mode of our cases belongs to type 2b) and that the virus was probably activated to a productive cycle and assembled only in the nuclei of the tumor cells in this fluid, because of suitable conditions found there.

The authors also suppose that toxoplasma infection might have played a role in the present case. Toxoplasma always assaults the cells of the RES, and might provide an altered "cellular soil" where EB virus could readily bring about malignant transformation.

The origins of tumor cells in malignant reticulosis remain unknown. From the data obtained in the present case, it is reasonable to assume that some malignant reticuloses are B-cell malignant lymphoma caused by EB-virus infection. In our case numerous scattered necrotic areas were observed in each organ in which tumor cells had infiltrated. They apparently begin as necrosis of a very small number of tumor cells follwed by infiltration of many kinds of inflammatory cells such as neutrophils and histiocytes. Such foci expanded with time and small areas fused with each other. These foci are thought to be an important histopathological finding in the present case. We speculate that these nercotic lesions were the results of an immunological reaction, since, if our case is assumed to be induced by EB virus infection, the virus induced tumor cells contained tumor specific antigen.

Acknowledgement. The authors thank Prof. Y. Hinuma for his immunological examination about EB virus, and to Dr. K. Miyamoto for his advice and criticism on electron microscopic findings. The case was reported at the 16th International Congress of Haematology in Kyoto, Japan.

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Received October 22, 1977