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Case report

Herpes simplex virus lymphadenitis mimicking tumoral relapse in a patient with Hodgkin's disease in remission

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Summary. A patient treated for Hodgkin's disease and presenting 12 years later with a left inguinal lymphadenopathy mimicking a relapse is reported. Histopathological study disclosed large histiocytic granulomas in the sinuses. Some of these granulomas showed necrotic areas with numerous neutrophils. At the edge of the necrotic zones, cells of undetermined origin exhibited intra-nuclear inclusions typical of Herpes simplex virus. The diagnosis was confirmed by immunolabelling, revealing Herpes simplex viral antigens in frozen and paraffin sections, and by ultrastructural studies. The diagnostic value of the histological methodology and pathological changes and the significance of the disease, appearing in a patient treated for Hodgkin's disease are discussed.

Key words: Herpes simplex lymphadenitis – Viral particles – Ultrastructure – Immunolabelling – Histopathology – Intra cellular viral antigen

The discovery of an enlarged lymph node in a patient in remission for Hodgkin's disease (HD), raises the possibility of recurrence. We report such a case, in which histopathological study of the lymph node biopsy allowed the diagnosis of a viral lymphadenitis due to Herpes simplex virus (HSV). Immunohistochemical detection of HSV antigen on frozen and paraffin sections on the one hand, and electron microscopic studies on the other, are both important in the diagnosis of HSV lymphadenitis.

Case report

A male patient, born in 1947, was seen every year at the department of Hematology (Hôtel-Dieu de Paris) for the follow-up of Hodgkin's disease (HD). In October 1971, HD, nodular sclerosing type, clinical stage II A (bilateral cervical, and axillary lymph nodes, with mediastinal involvement) was detected and treated by radiotherapy. In January 1972, he relapsed. A laparotomy with splenectomy disclosed a normal spleen with HD in the splenic hilar lymph node without

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involvement of the liver. Additional radiotherapy was undertaken. In January 1975, a right axillary lymph node was discovered, biopsy of which showed nodular sclerosing HD. The patient was given chemotherapy (6 MOPP). From 1976, the patient was in complete remission. In August 1983, he developed dorsal Herpes Zoster. In December of the same year, systematic clinical examination disclosed a large left inguinal lymph node (3 cm in diameter) without any other clinical symptoms. The patient was well, without fever or weight loss. It was decided to perform a biopsy because of the risk of HD relapse. After the results of the histopathological study, the patient explained to the physician that 3 weeks before the discovery of the inguinal adenopathy he had a recurrent genital Herpes simplex, which had occured for many years. In October 1984 the patient was well.

Methods

The lymph node sections were fixed in aqueous Bouin's solution, embedded in paraplast® and piccolyte®. The 4 μm sections were stained with haematein eosin, Giemsa, APS and silver impregnation, according to the technique of Gordon and Sweet.

One part of the specimen was immediately frozen in acetone and dry ice and stored at −80° C. Five micrometer cryostat sections of the lymph node specimen were cut, air-dried for 2 h, and fixed in cold acetone. Immunohistological study of reactive lymphoid tissue was performed with mouse monoclonal antibodies which included CD1 (IOT6[1]), CD2 (T11[2]), CD4 (Leu 3a[3]), CD8 (IOT8[1]), CD9 (ALB6[4]), CD10 (ALB2[4]), CD11 (OKM1[5]), CD22 (Leu 14[3]), CD24 (ALB9[4]), anti RC3b (J_3D_3[6]), anti mu[7], anti delta[7], anti kappa[2], and anti lambda[2]. Sections were incubated with monoclonal antibodies at optimal dilution (1/20 to 1/200) for 30 min at room temperature in a moist chamber, and then washed with 0.05 M Tris buffered saline, pH 7.6 (TBS). The ABC peroxidase labelling procedure was performed with the Vectastain ABC kit, VPK 4002, obtained from Vector Lab., Burlingame, USA. The reaction was revealed with aminoethylcarbazole (AEC) and H_2O_2. *HSV antigens* were identified on frozen and paraffin embedded sections using Rabbit immunoglobulins against HSV (Dakopatts[7] and Ortho Pharmaceutical[5]) with the ABC procedure (Vectastain, VPK 4001) or the PAP method (Histoset[5]) according to the manufacturer's instructions. The reactions are revealed with AEC. Non immune rabbit immunoglobulins were used as control experiments.

An electron microscopic study was performed on lymph node tissue fixed in two different ways:

1. Six of the fresh tissue fragments were immediately fixed in 1.5% glutaraldehyde, then in 2% osmium tetroxide, and finally included in epoxy resin (LX 112).

2. One of the fragments fixed in aqueous Bouin's solution and included in paraffin, was deparaffinized. Six fragments from the necrotic areas were reincluded in Epoxy resin. The semi-thin sections taken from 12 blocks and stained with toluidine blue, determined the choice of areas to be studied using ultrathin sections; these were stained by uranyl acetate and lead citrate. These sections were observed using a Siemens Elmiskop 101 electron microscope.

Results

The lymph node measured $3 \times 2 \times 1.5$ cm. Cutting showed a nodular arrangement with a small yellow area of necrosis.

Some follicles or remnants of follicles could be recognized. The germinal centres were rich in centroblasts, immunoblasts, plasma cells and macro-

1 Immunotech. Marseille, France
2 Coulter Electronics. Hialeth. Florida, USA
3 Beckton-Dickinson. Sunnyvale. California, USA
4 Gift from Dr. Claude Boucheix. Paris, France
5 Ortho Pharmaceutical Cie. Mamitan New Jersey, USA
6 Gift from Dr. Claude Boucheix and Dr. Michel Kazatchkine. Paris, France
7 Dakopatts Laboratories. Copenhagen, Denmark

phages with tingible bodies. Some were infiltrated by clusters of small lymphoid cells. The peripheral borders of the germinal centres were irregular, but the mantle zone was large and well developed. Rare small clusters of epithelioid cells were found near the follicles (Fig. 1).

Large areas of the lymph node were infiltrated by sheets of histiocytes, some of them with marked phagocytic activity. These sheets infiltrated interfollicular and deep cortical zones. Some seemed to develop inside sinuses filled with erythrophagocytic histiocytes and also around them (Fig. 2). Within some of these histiocytic areas, suppurative necrosis developed with large numbers of neutrophils and with thrombosis of some small venules (Fig. 3).

In other parts of the lymph node, it was possible to recognize deep cortical hyperplasia with epithelioid venule hyperplasia and interdigitating cell hyperplasia (Fig. 1). There was also extensive immunoblastic hyperplasia and severe plasmacytosis producing a diffuse polymorphic hyperplasia (Fig. 2). The sinuses exhibited an erythrophagocytic histiocytosis or in some places a B lymphocytosis (previously called "immature histiocytosis"). There was also heavy periadenitis with immunoblasts, a large number of plasma cells and some histiocytic granulomas. There was no tumor.

Lengthy and careful examination revealed cells exhibiting intranuclear inclusion bodies. Most of these were pale homogeneous amphophilic or slightly acidophilic hyalin bodies filling an enlarged nucleus. They were circumscribed by a thin rim of marginated nuclear chromatin, but without a clear halo (Fig. 3). In a very small number of cells, the inclusions had the morphology of so-called Cowdry's type A nuclear inclusion (Cowdry 1940). They had the appearance of fairly dense, granular or hyalin acidophilic masses with a somewhat irregular outline, within a pale relatively clear halo circumscribed by a basophilic rim of marginated nuclear material. Both inclusions were found in only a few cells around the areas of suppurative necrosis. It was not possible to recognize precisely which type of cells exhibited nuclear inclusions. Some of the large cells resembled immunoblasts.

Using our immunolabeling technique, the presence of HSV antigens was shown as red precipitates. The intranuclear inclusions were heavily stained (Fig. 4A). In many cells, these reaction products were also found in the cytoplasm with a variable intensity (Fig. 4B).

The ultrastructural study of the 6 fragments which were taken following systematic procedure from the fresh lymph node and correctly fixed in glutaraldehyde, only concerned the interfollicular and deep cortical areas. No necrotic area was observed, nor any intra- or extracellular viral structure. For this reason, fragments of tissue fixed in aqueous Bouin's solution and included in paraffin, were subsequently embedded in Epon, after the necrotic zones had been denoted, since the viral structures are resistant to the fixing solutions. Several viral particles, grouped in small masses, could then be identified (Fig. 5A and B). They were intracytoplasmic or intranuclear, in the form of either nucleocapside with a dense core separated from the capsid by a clear halo, or of empty capsids (Fig. 6A and B). Their average diameter

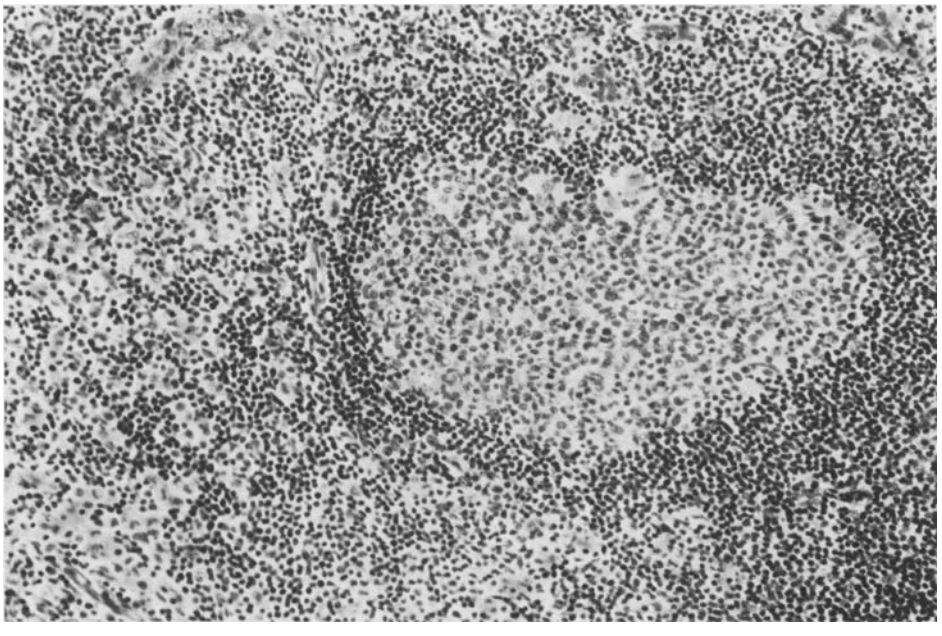

Fig. 1. Follicular hyperplasia with a large and well developed mantle zone. Notice the small clusters of histiocytes and epithelioid cells at the peripheral borders of the germinal centre. There is deep cortical area hyperplasia with clusters of pale interdigitating cells and proeminent venules with high endothelial cells. (HES, $G = \times 100$)

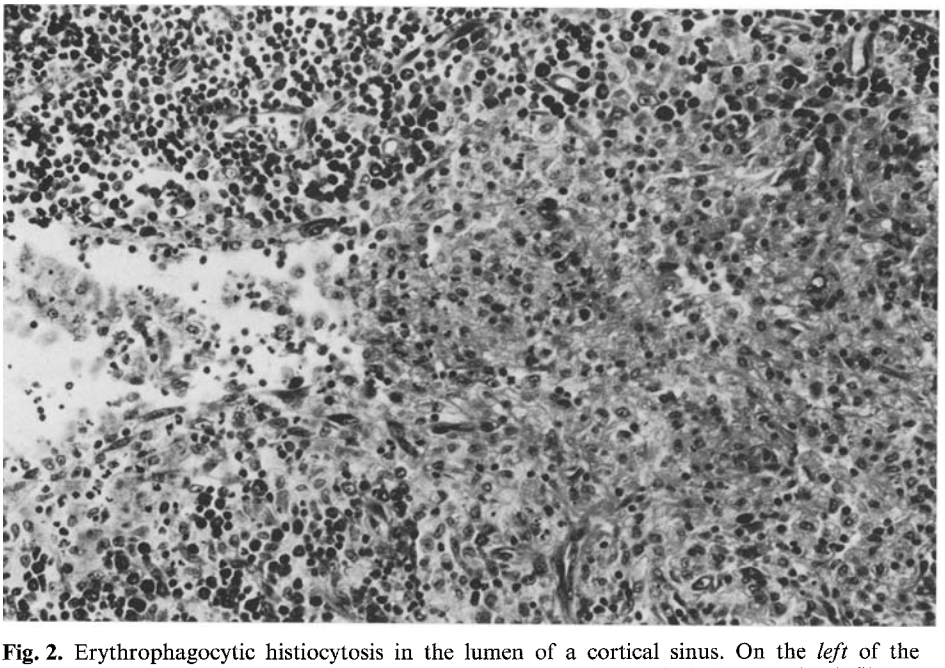

Fig. 2. Erythrophagocytic histiocytosis in the lumen of a cortical sinus. On the *left* of the figure, the sinus is filled with densely packed histiocytes. Sheets of histiocytes also infiltrate the pulp on both sides of the sinus. Large numbers of plasma cells and immunoblasts around the granuloma and along the sinus. (Giemsa, $G = \times 190$)

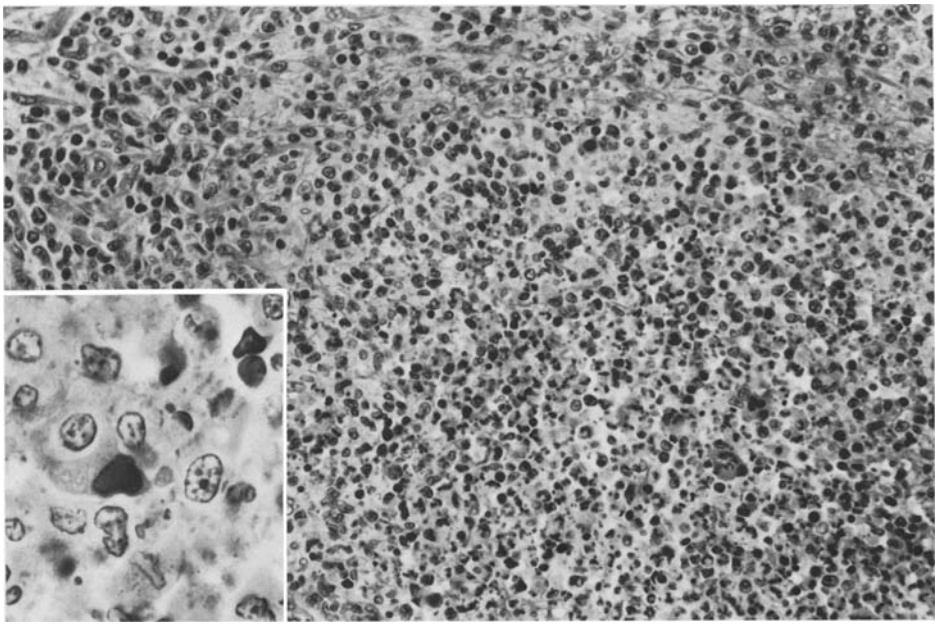

Fig. 3. Large area of histiocytes with macrophagic activity associated with neutrophil granulocytes and foci of necrosis. (HES, $G = \times 250$). *Inset:* At the edge of a necrotic area, a dense intra nuclear inclusion body without a clear halo can be recognized. (HES, $G = \times 945$)

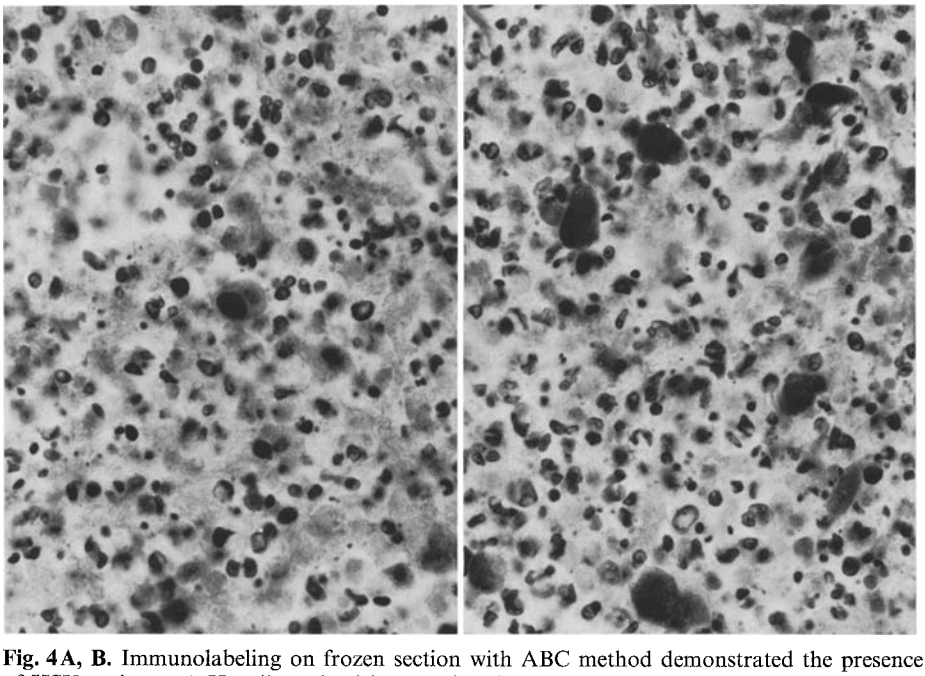

Fig. 4A, B. Immunolabeling on frozen section with ABC method demonstrated the presence of HSV antigens. **A** Heavily stained intranuclear inclusion body. ($G = \times 570$). **B** In addition, presence of antigens in the cytoplasm. ($G = \times 570$)

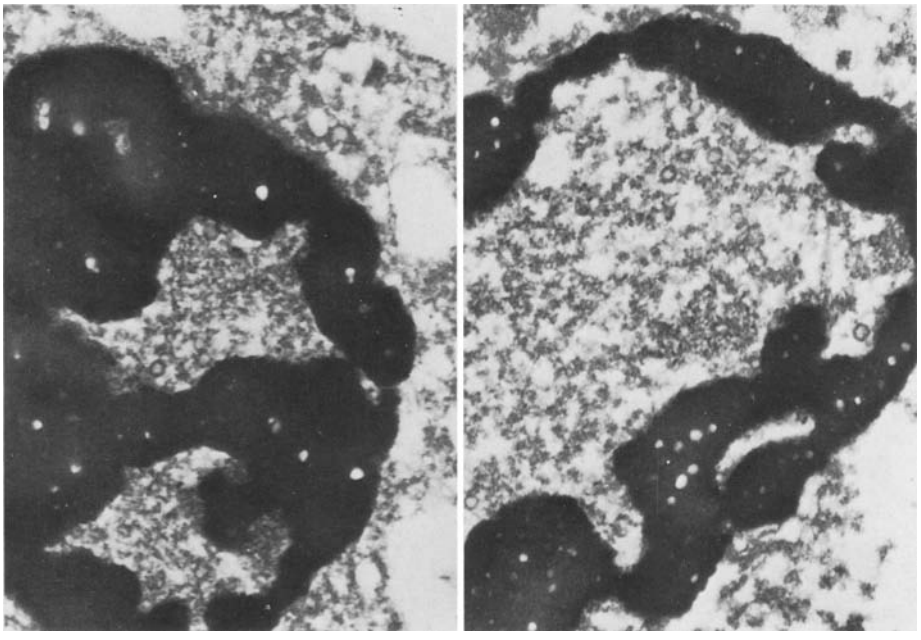

Fig. 5A, B. Despite the bad quality of the fixation, ultrastructural study of necrotic zones in tissue fixed in Bouin's solution and reembedded in Epon disclosed intranuclear inclusions characterized by the presence of several viral particles grouped in small nests. **A, B** ($G = \times 2{,}800$)

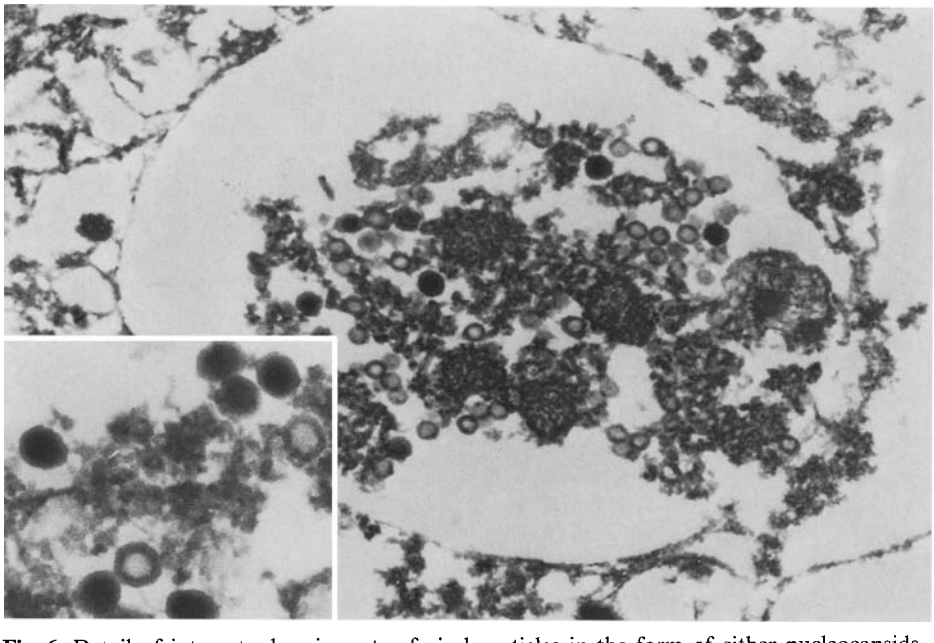

Fig. 6. Detail of intracytoplasmic nests of viral particles in the form of either nucleocapsids with a dense core separated from the capsid by a clear halo, or of empty capsids. ($G = \times 30{,}000$; *inset* $G = \times 50{,}000$)

was 100 nm. The morphology and the size of these structures were very characteristic of the viruses of the Herpes group. Unfortunately, the bad quality of the fixation and the necrotic character of the cells, did not allow precise identification of the cell type (or types) containing these viral structures.

The reactive lymphoid lesions were studied with monoclonal antibodies immunolabelling on frozen sections. The follicles could be easily recognized (RC3b, mu). Delta heavy chain antibody showed the presence of a large mantle zone; the anti C3b receptor revealed, in the germinal centres, a dense network according to dendritic reticular cells. In some areas, this network was broken and disrupted, and these ruptured areas were occupated with nests of delta lymphocytes. Many T cells with the helper phenotype (CD4) were seen in the follicles but suppressor T cells (CD8) were very rare.

In the interfollicular and deep cortical areas, the pan T markers (CD2) demonstrated a high number of T cells, most having the helper/inducer phenotype (CD4 positive). The number of T cells with suppressor/cytotoxic phenotype (CD8 positive) was lower. However, these suppressor/cytotoxic T cells appeared to be concentrated around the necrotic area. Numerous clusters of polyclonal mu positive plasma cells were found near the necrotic area. Numerous histiocytes and macrophages were also detected, using the OKM1 antibody (CD11), which were disseminated between the T cells and also concentrated around the necrotic area.

Discussion

Lennert et al. (1981) have summarized the histological changes in lymphoid tissue in viral disease. To our knowledge, the histopathological findings in lymph nodes have not previously been shown in detail in HSV infections, despite the fact that adenopathies have been described as a clinical symptom of cutaneous or mucous membrane localisation (Lennert 1981). Biopsy is seldom necessary and a very small number of HSV lymphadenitis have been described. Tindle (1978) briefly described herpetic lymphadenitis as being characterized, in general, by immunoblastic proliferation. Lennert et al. (1981) stressed that lymph node lesions have not been well studied in HSV and quoted a case from Noel which exhibited follicular hyperplasia, polymorphic hyperplasia of the pulp and so-called "immature histiocytosis" with foci of necrosis. In the necrotic area, some large cells had viral inclusions in the nucleus and in the cytoplasm. However in this case it is not certain that HSV was the aetiologic agent. In the case reported by Lapsley et al. (1984), there was pronounced expansion of the paracortical regions with abundant immunoblasts, many lymphoplasmacytoid cells and macrophages. Several atrophic germinal centres remained and the sinuses were preserved. There was neither necrosis nor periadenitis. The authors did not mention the presence of sinusal histiocytosis, and no intranuclear or intracytoplasmic inclusions were found. In some instances histopathological lesions

of viral lymphadenitis can mimick malignant lymphoid proliferations (Doerr and Drings 1975).

In viral infections, the only specific lesions are the *virus inclusions*. HSV forms nuclear inclusions which are in the first type, amphophilic or slightly acidophilic, pale, hyalin, homogeneous and are not circumscribed by a pale halo. Inclusions of the second type, which were very rare in our case, resemble the CMV inclusion, but the inclusion is eosinophilic rather than basophilic and the peripheral halo is smaller.

The most salient histopathological lesion in our case was the severe histiocytosis which begins in the cortical area, around foci of sinusal histiocytosis with erythrophagocytosis (Risdall et al. 1979), and inside the sinuses. The other lesion which should be emphasized is suppurative necrosis.

Immunohistochemistry can reveal viral antigen in the nuclei and cytoplasm of infected cells on frozen sections (Balchandran et al. 1982; Leloup et al. 1983; Schmidt 1980). The distinction between Herpes simplex virus type 1 or 2 could not be made. In our case, the notion of a recurrent genital herpes infection was enough to suggest the diagnosis of HSV type 2 lymphadenitis.

The detection of viral particles in the nuclei and the cytoplasm by electron microscopy can also be helpful. However, the technique is time consuming and expensive, offering little advantage over immunolabelling. However, the viral particles are resistant to the fixative and paraffin embedding. Thus, an ultrastructural study can be performed on paraffin embedded tissue after re-embedding in Epon (Leloup et al. 1983).

Our patient was treated for Hodgkin's disease and was in complete remission. He was in good health without any symptom of immunodepression, but no sophisticated explorations of his immune status was carried out. The viral infection he was known to have was common recurrent genital Herpes (most frequently due to HSV 2). This viral infection begins many years before the recent attacks and differs from the generalized HSV infection described in most immunosuppressed patients, for example after renal graft (Montgomerie et al. 1969) or various malignancies (Aston et al. 1972; Feldman and Cox 1976; Lam et al. 1981; Muller et al. 1972; Wong and Hirsch 1984). Despite the symptomatology and recognising that he had developed a dorsal Herpes Zoster a few months beforehand, it seems possible that our patient had immunodeficiency due to therapy and/or Hodgkin's disease.

In conclusion, different patterns of lymphoid reactions may suggest a viral aetiology for an adenopathy. Some association of criteria may be sufficiently characteristic to propose a precise diagnosis, for example: post vaccinial lymphadenopathy, infectious mononucleosis, or measles. The association of large histiocytic infiltrates, beginning around foci of sinusal histiocytosis with erythrophagocytosis, then evolving into areas of suppurative necrosis, may suggest HSV infection, although this may be associated with various kinds of lymphoid hyperplasia. Typical intranuclear inclusions should be looked for. Immunohistochemistry and electron microscopy are

of the greatest importance for the demonstration of the specific viral origin of the disease.

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