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Histology and Histopathology

Hyaline-vascular type of Castleman's disease (angiofollicular lymph node hyperplasia) with monotypic plasma cells. An immunohistochemical study with monoclonal antibodies*

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Summary. A case of angiofollicular lymph node hyperplasia (Castleman's disease) characterized by monotypic (IgG+, lambda+) plasmacytosis is described. Fresh tissue was available and a thorough imunohistochemical analysis of lymphoid and non-lymphoid cells was performed on cryostat sections. Although lymphoid follicles were numerous and exhibited some abnormal features they did not appear part of the monoclonal cell proliferation. Follicular lymphocytes were mixtures of Kappa+ and lambda+ cells. Vessels penetrating within these abnormal follicles expressed reduced levels of FVIII and Leu-M5 antigens and exhibited thicker layer of collagen type IV. The analysis of T-cell subsets showed a normal (3:1) T4/T8 ratio. This case extends to the mixed variant of hyaline-vascular Castleman's disease, the neoplastic potential previously associated to the plasma cell variant of the disease.

Key words: Castleman's disease - Monotypic plasma cells - Immunohistochemistry

Introduction

Castleman's disease (CD), (angiofollicular lymph node hyperplasia, giant lymph node hyperplasia), is defined as a non-neoplastic lymphoproliferative disorder, although its precise nature is presently just a matter for speculation et al., 1978; Frizzera et al., 1983; Frizzera, 1985; Lachant et al., 1985). Different clinical forms of CD have been recognized, including localized and multicentric cases.

On the basis of the clinical heterogeneity and the lack of specificity of histological features characterizing CD, its own existence as unique pathological entity has been questioned (Frizzera, 1985). Different hypothesis have been formulated on the nature of CD, including either a vascular hamartoma, a reactive-hyperplastic process or an atypical non-neoplastic lymphoproliferation. The reactive, non-lymphomatous nature of the lesion was also confirmed by the many studies demonstrating a polytypic immunoglobulin expression in the plasma cells as well as (in the few cases where fresh tissue was available) in the B lymphocytes forming the lymphoid follicles (Diamond and Braylan, 1980; Tanda et al., 1983; Jones et al., 1984; Miller et al., 1984; Kessler, 1985; Martin et al., 1985).

A number of cases of CD have been reported in which plasma cells exhibited a monoclonal pattern on immunohistochemical analysis of light chains (York et al., 1981; Clark and Keren, 1982; Hineman et al., 1982; Scholosnagle et al., 1982; Chan et al., 1984). However, since these cases were studied only on paraffin-embedded material no data were available on the membrane phenotype of lymphocyte populations.

In this paper we describe the case of a young woman who had a lesion in her neck, microscopically characterized as angiofollicular lymph node hyperplasia (CD) intermediate type. The lesion, which locally recurred after excision, was characterized by monotypic lambda-type plasma cells. Fresh samples were available and a thorough immunohistochemical characterization of lymphoid and non-lymphoid cells was performed on cryostat sections.

Materials and methods

Case report

A 34 year old white woman was admitted to hospital

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in 1984 for an enlarged laterocervical lymph node, in absence of any other clinicasl signs.

Serum electrolite and routine blood chemistries were within normal limits, Bence-Jones proteinuria was absent and the levels of beta-2 microglobulin and ESR were normal.

No therapy, excluding lymph node excision, was performed. Lymph node biopsy showed an histopathological pattern compatible with the diagnosis of angiofollicular lymph node hyperplasia. Two months later an identical lesion was excised from the same site.

After two years follow up the patient does not present evidence of disease.

Immunohistochemistry

Fresh biopsy fragments of the laterocervical lymph node were available. The specimens were snap-frozen in liquid nitrogen in O.C.T. medium (Ames) and cut in a cryostat, obtaining 5 μm thick frozen sections which were stuck onto polylisine - coated glass slides (Sigma) and air-dried with a cold fan. The sections were then fixed in chloroform-acetone (1:1) mixture, dried again, and immunostained using heterologous antisera or monoclonal antibodies (listed in Table 1) with peroxidase anti-peroxidase or indirect immunoperoxidase techniques. Sections for enzyme histochemistry were fixed in 10% buffered formol and stained for nonspecific esterase. The methods used have been previously described with more details (Chilosi et al., 1983).

Formalin-fixed, paraffin sections were stained for haematoxylin-eosin or immunostained for cytoplasmic immunoglobulins (different heavy and light chains) using the peroxidase anti-peroxidase method after mild trypsin digestion of xylene-deparaffinized sections.

Results

Histology

The latero-cervical lymph node presented as a well-encapsulated, roughly spherical mass measuring 3X2 cm in diameter approximately. On microscopic examination the lymph node appeared without distinct separation between cortical and medullary zones and was characterized by a large number of medium sized lymphoid follicles and richly vascularized interfollicular areas. Non-confluent sheets of plasma cells were found in these areas. The plasma cells did not show morphological abnormalities.

The follicles were mainly formed by small mantle-zone lymphocytes concentrically arranged in layers giving the typical "onion skin" appearance observed in the hyaline-vascular variant of Castleman's disease. Well formed germinal centers were rarely recognized. The blood vessels entering the follicle centers showed thick hyalinized walls whereas interfollicular vessels did not show major abnormal features.

Aggregates of large, mononuclear cells with blastic appearance and slightly basophilic cytoplasm were found close to some follicles. Morphologically these clusters were similar to those previously described as "reticular lymphoblasts" by Keller et al. (1972) or as plasmacytoid T-cells by Vollenweider and Lennert (1983).

The recurred lesion was also analysed and showed the same histological pattern.

Immunohistochemistry

Immunoglobulin content and phenotype of plasma cells and follicular lymphocytes: The first relevant immunohistochemical finding was the monotypic , lambda-type, Ig expression in the cytoplasm of the plasma cells forming the numerous sheets observed in interfollicular areas (Fig. 1a and 2a). The kappa chain containing plasma cells was in fact extremely rare. Cytoplasmic gamma-type heavy chains were also present in plasma cells. The lymphocytes forming the "hyaline-vascular" lymphoid follicles were, on the other hand, composed of cells exhibiting polytypic (kappa or lambda) staining for surface immunoglobul ins as revealed on cryostat sections (Fig. 2). In addition, these lymphocytes normally expressed on their membranes IgM, and IgD, as well as mantle-B cell-related antigens revealed by the monoclonal antibodies BA1 and Leu8. In most follicles well-formed germinal centers were absent, with only very small clusters of IgD-negative, BA1-negative cells present in few follicles.

Follicle microenvironment: When immunostained with the specific antibodies RFD3 and DRC follicular dendritic cells appeared regularly arranged in a coil responsible of the "onion skin" appearance of the hyaline-vascular variant of CD (Fig. 3). In the few follicles where small germinal centers were present the follicular dendritic cells regularly expressed IgM, kappa and lambda chains, but lacked IgD.

Other cell types normally present within the follicular germinal center were extremely rare or absent within the modified follicles of CD. Macrophages (detected by leu-M5 antibody and nonspecific esterase histochemistry) and Leu7-positive T cells were barely detectable in a very small minority of follicles.

Abnormalities could be observed in the vascular component of the follicle. The hyalinized capillaries penetrating the follicle centers showed decreased expression of Factor VIII r.a. and OKM5. In addition, a thicker band of staining was evident in the sections tested with anti-collagen type IV antibody.

Interfollicular areas: In interfollicular areas aggregates of T lymphocytes were found beside sheets of plasma cells. The T-cells were unevenly distributed forming dense, packed nodules or loose collections of cells. The T-cell subset ratio evaluated on consecutive cryostat sections immunostained with OKT4 and OKT8 monoclonal antibodies was comparable to normal lymph node (3/1). Blood vessels were numerous in these areas and exhibited normal staining for FVIII, OKM5 and collagen type IV.

The regular aggregates of "reticular lymphoblasts" (plasmacytoid T cells), when analyzed on serial sections

showed positive staining for common-leukocyte antigens (2D1 and LC), HLA-DR, and the "helper" related antigen CD4, but lacked other mature and immature T- and B-cell antigens such as T1 (CD5, Leu-9 (CD7 and Leu-4 (CD3). Activation (IL2R) or macrophage (Leu-M5) related antigens were absent.

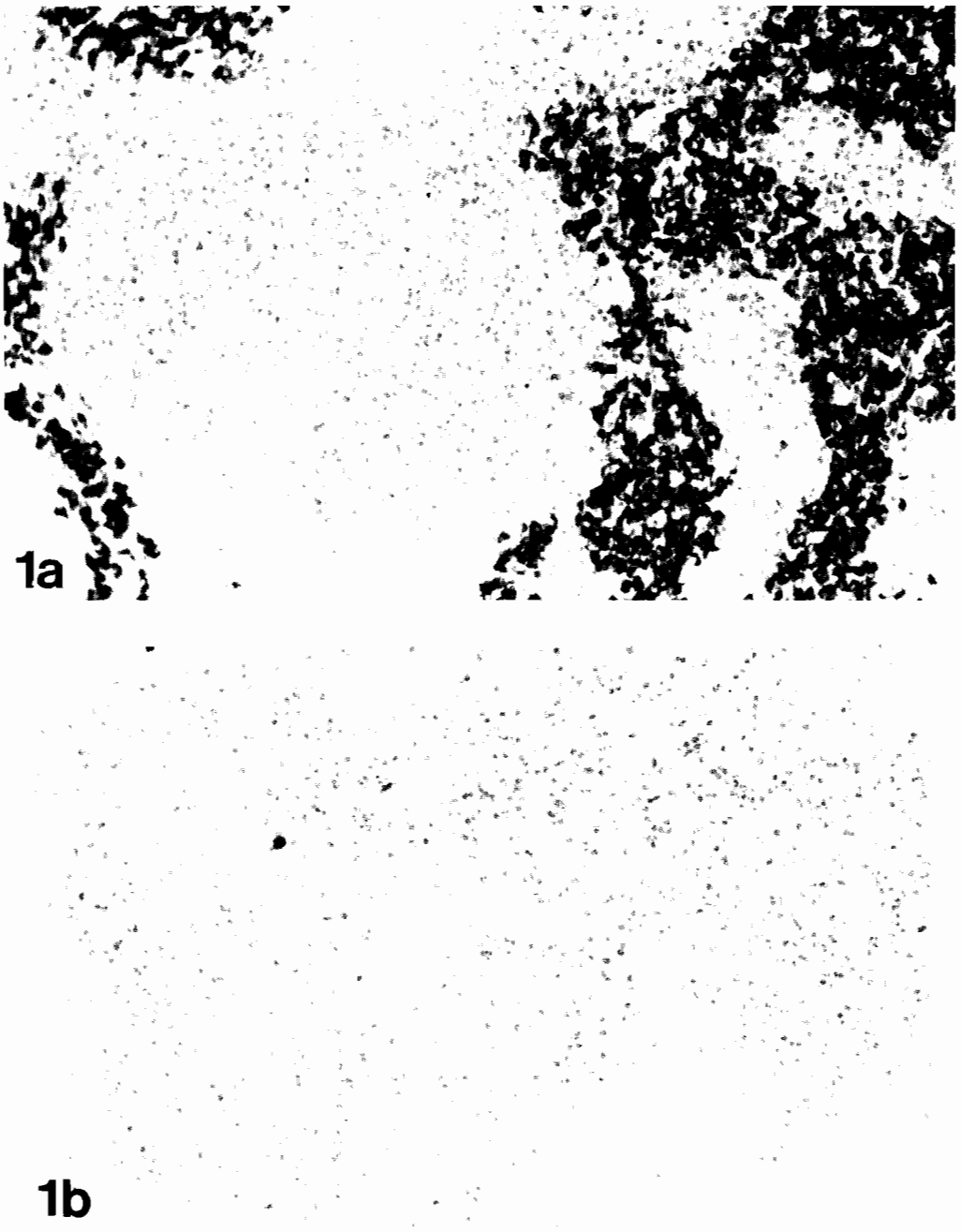
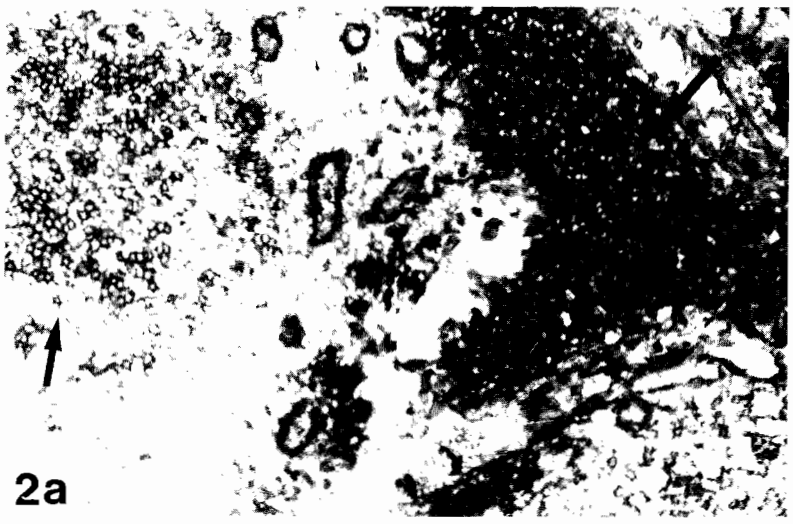


Fig. 1. Lymph node paraffin sections immunostained for cytoplasmic immunoglobulin light chains. All plasma cells forming interfollicular cords express lambda type chain (a), but lack kappa type chain (b). Immunoperoxidase PAP technique.



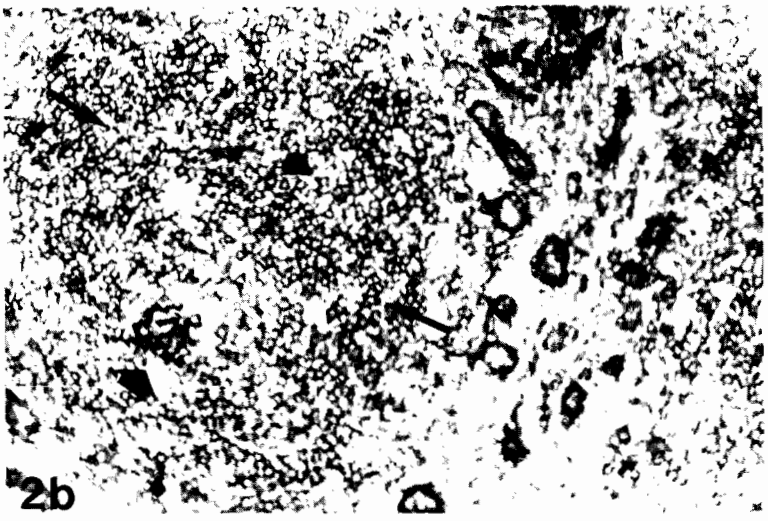
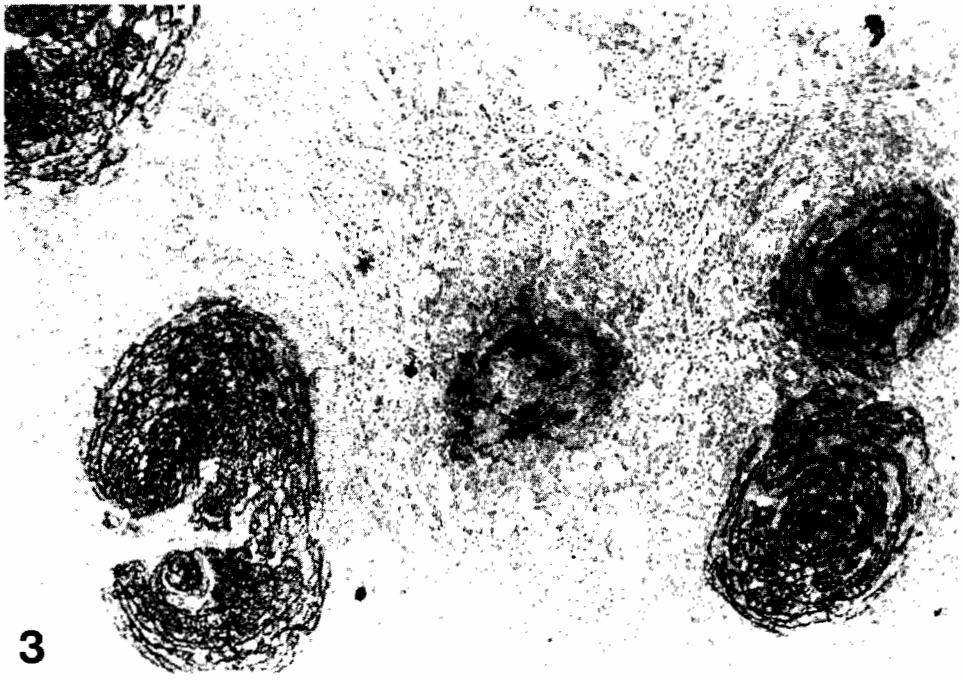


Fig. 2. Lymph node cryostat sections immunostained for immnunoglobulin light chains. B lymphocytes forming the enlarged follicular mantles express either lambda (a) or kappa (b) surface immunoglobulins (arrows). A large cord of lambda positive plasma cells is shown in a) (long arrow). A small cluster of follicular dendritic cells is evidenced by its kappa positive network (b, large arrow).

Fig. 3. A lymph node cryostat section immunostained with RFD3 monoclonal antibody (specific for follicular dendritic cells) using indirect immunoperoxidase technique. The dendritic network within the follicle emphasizes the characteristic features observed in Castleman's disease including germinal center atrophy, vessel penetration and follicle splitting.



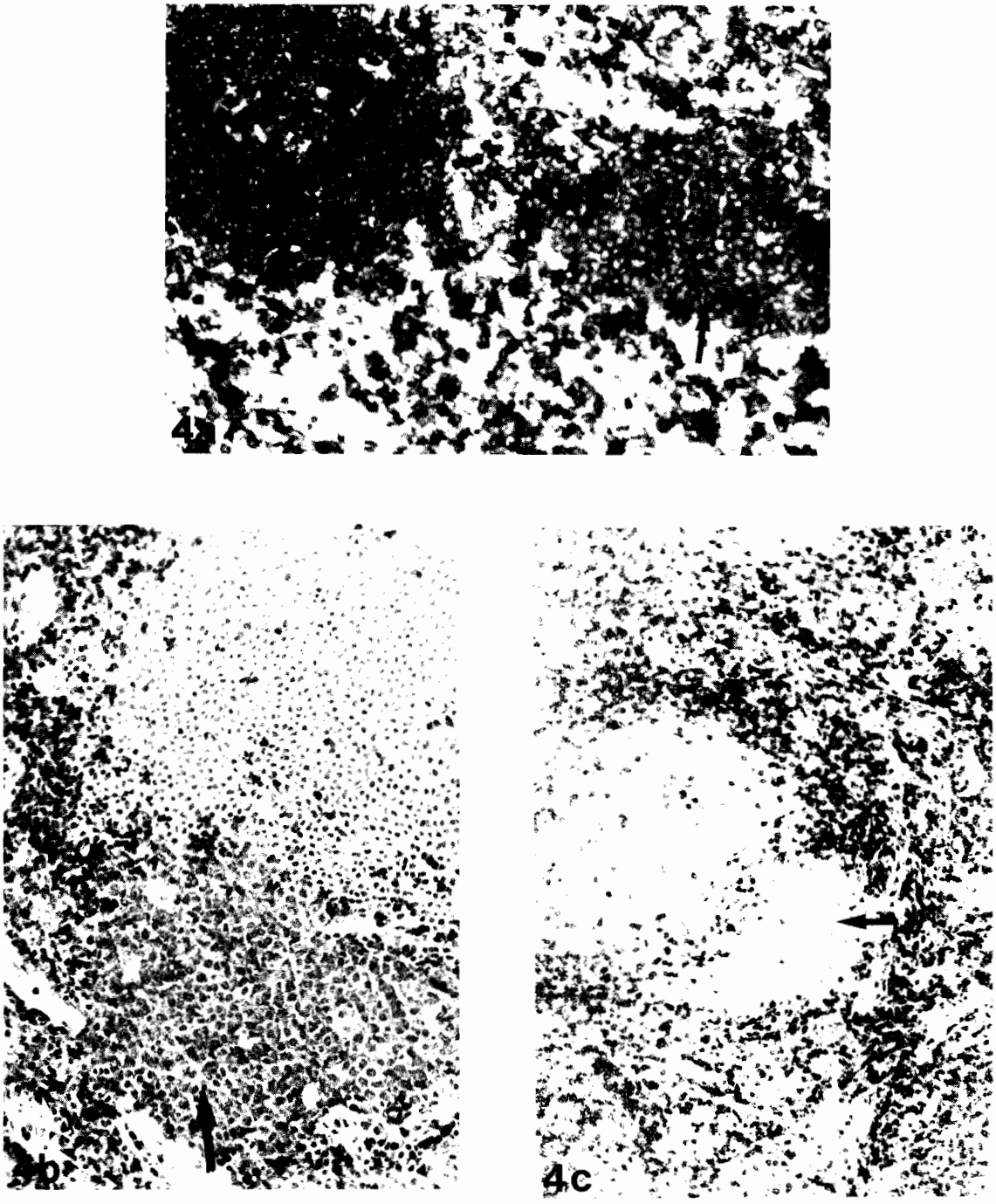


Fig. 4. Consecutive cryostat sections stained to characterize the phenotype of a cluster of so called "plasmacytoid T cells". These cells show positive staining for HLA-DR (a), and OKT4 (b), but lack OKT1 reactivity (c), arrows.

Table 1.

monoclonal Ab	specificity	source
2D1	pan-leuk.	B&D
LC	pan-leuk.	Dako
DRC	foll. dendr. cells	Dako
RFD3	foll. dendr. cells	RFH
RFB4	CD22 pan-B	RFH
RFB6	CD21 C3dR	RFH
CALLA	CD10	B&D
OKT4	CD4 Th	Ortho
OKT8	CD8 Ts	Ortho
RFT1	CD5 pan-T	RFH
Leu-4	CD3 pan-T	B&D
Leu-9	CD7 pan-T	B&D
OKM5	endothelia	Ortho
IL2R	activated T-cells	B&D
Leu-M5	macrophages	B&D
IgM	B-cells	B&D
IgD	B-cells	B&D
kappa	B-cells	B&D
lambda	B-cells	B&D
Leu-8	B and T cells	B&D
BA1	B-cells	Hybritech
Leu-7	K/NK cells	B&D

CD: cluster of differentiation
RFH: a gift from Prof. G. Janossy, Dept. Immunology, Royal Free Hospital School of Medicine, London.

Discussion

In this report we describe the case of a female patient affected by the localized form of CD, histologically characterized by the features of the so-called intermediate or mixed variant of the disease. Interestingly, the plasma cell component of the lesion was monotypic (all plasma cells exhibiting IgG/ lambda chains) on immunohistochemical analysis on paraffin sections.

The presence of monotypic plasma cells in CD has been reported in several other cases (York et al., 1981; Clark and keren, 1982; Hineman et al., 1982; Scholosnagle et al., 1982; Chan et al., 1984), and has been considered as evidence of neoplastic potential of the disease. This prompted us to test in our case whether the lymphocytes forming the abnormal follicles were also exhibiting an abnormal phenotype as found in some cases of B-cell lymphoma, where both lymphocytes and plasma cells are part of then same neoplastic proliferation (Keith et al., 1985). A thorough immunohistochemical analysis of the lesion was then performed on cryostat sections using a large panel of antibodies.

The follicular lymphocytes showed the immunological phenotype of normal mantle-zone lymphocytes. They were in fact characterized by heterogeneous kappa/ lambda expression, and also exhibited on their membrane IgD, IgM, and the antigens revealed by RFB4, Leu-8, BA1 and RFB6 monoclonal antibodies.

The monotypic plasma cells in our case did not show morphological atypias. In addition they did not exhibit the enhanced expression of the CALL-antigen observed in aggressive cases of plasma cell neoplasia (Caligaris-Cappio et al., 1985).

The immunohistochemical analysis of follicular microenvironment showed in more detail some abnormal features. In particular, blood vessels were characterized by decreased levels of F-VIII and OKM5 and thicker deposits of collagen. These abnormalities of vascular walls might be related to the depletion of germinal center cells, including B-cells, T-cells, macrophages and cells with natural killer phenotype (Pizzolo et al., 1984).

The T4/T8 ratio was similar to that usually observed in nonspecific lymphadenitis, with prevalence of T4+ lymphocytes. Similar data have been reported in three cases of CD described by Martin et al., (1985). In a single case of CD van der Oord et al. (1984) found that the T-cell subset distribution was regular in the interfollicular areas, but abnormal within the follicles. Jones et al. (1984) found an altered T4/T8 ratio in another two cases of the hyaline-vascular type of CD.

We could immunohistochemically characterize on serial sections the cellular aggregates of "reticular lymphoblasts" observed close to some follicles. The peculiar phenotype of these cells (T4+, HLA-DR+, 2D1+, T1-, T3-, Leu9-) is very similar to that found in the so called "plasmacytoid T cells" described by Vollenweider and Lennert (1983) in non-specific lymphadenitis and also in cases of Castlemans's disease. The nature of these cells is presently unknown, although a T-lineage and "secretory" function

have been proposed (Muller-Hermelink et al., 1983).

The case here described of CD with hyaline-vascular follicles and monoclonal plasma cell component gives some support to the view that the different forms of CD are somehow correlated. Our case in fact, together with the similar case described by Schosnagle et al. (1982) extends to the hyaline-vascular variant of Castleman's disease, the neoplastic potential (presence of monotypic plasmocytosis) which had been so far only associated to the plasma cell type. In line with this view is the observation of cases of the hyaline-vascular or intermediate variants of CD complicated by constitutional symptoms and multicentric involvement (Gaba et al., 1978; Summerfield et al., 1983; Dickson et al., 1985; Weisenburger et al., 1985).

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