Virchows Archiv A Pathol Anat (1993) 423:369–382

Virchows Archiv A
Pathological Anatomy and Histopathology

Morpho-immunophenotypic diversity of Castleman's disease, hyaline-vascular type: with emphasis on a stroma-rich variant and a new pathogenetic hypothesis

Ardis D. Danon, Jayashree Krishnan, Glauco Frizzera

Department of Hematopathology, Armed Forces Institute of Pathology, Washington, D.C., USA

Received July 20, 1993 / Received after revision and accepted August 20, 1993

Abstract. A histologic review of 102 cases of Castleman's disease of the hyaline-vascular type, with a detailed paraffin immunophenotypic study of 23 of them, was undertaken to evaluate the morphologic variability of this disorder and its immuno/cyto-architectural characteristics. All cases had features in common including: abnormal follicles, with increased vascularity, poorly formed germinal centers and predominance of the mantle zone; lack of sinuses; and hypervascular interfollicular tissue containing large numbers of KP1-positive plasmacytoid monocytes. Networks of actin-positive cells [fibroblastic reticulum cells (RCs) or "myoid cells"] and KP1-positive dendritic cells (histiocytic RCs) were seen. There were differences in the proportion of follicles to interfollicular tissue, which covered a continuum from a "follicular", through a "classic", to a "stroma-rich" variant. The last-mentioned was qualitatively different as it showed loss of HECA-452 and MECA-79 reactivity in the blood vessels, decreased plasmacytoid monocytes and increased myoid cells and histiocytic RCs. In 5 cases there was formation of distinct nodular growths which varied from spindle cell foci to angio-histiocytic-RC proliferations, all of which may be confused with vascular or follicular dendritic RC neoplasms. From our findings, data from the literature and the working hypothesis that plasmacytoid monocytes are the precursors of both follicular dendritic RCs and sinus lining cells (Parwaresch et al.), a pathogenetic theory is proposed for this type of Castleman's disease which postulates that a developmental block in plasmacytoid monocytes results in their accumulation with poor formation of germinal centers and sinuses under stimulation. The lack of sinuses would lead to impaired egress of circulating lymphocytes which, however, would continue to enter the node through functional high endothelial venules and to accumulate in the mantle zone. The factors responsible for angiogenesis and for the cellular growths that characterize the stroma-rich variant remain to be determined, as do the relationships between the three variants.

Key words: Castleman's disease – Angiofollicular lymphoid hyperplasia – Plasmacytoid monocytes – Reticulum cells

Correspondence to: G. Frizzera, Department of Pathology, New York University Medical Center, 560 First Avenue, New York, NY 10016, USA

The opinions expressed in this article are the personal views of the authors and are not to be construed as representing the views of the Department of the Army or the Department of Defense.

Introduction

Castleman's disease (CD) was first described in 1956 as a hyperplastic lymphoid process localized in the mediastinum and characterized by peculiar Hassal body-like germinal centers (GCs) and marked vascular proliferation (Castleman et al. 1956); it was soon reported in other anatomic locations. Descriptions of systemic effects associated with CD (Neerhout et al. 1969; Sethi and Kepes 1971), and the recognition of a nodal lesion characterized by hyperplastic GCs and an abundance of plasma cells (Flendrig 1970), led Keller and coworkers (1972) to add a plasma cell (PC) variant to their original type, now referred to as the hyaline-vascular (HV) type. More recently, a systemic form of CD has emerged (Diebold et al. 1980; Frizzera et al. 1983, 1985; Weisenburger et al. 1985) associated with multisystem involvement and graver prognosis. The spectrum of morphologic patterns to be included under the term of CD poses a problem to the general pathologist. Moreover while all would agree that the PC variant is a form of nodal immune reaction, the plethora of synonyms used for the HV type (angiofollicular lymph node hyperplasia, follicular lymphoreticuloma, angiomatous lymphoid hamartoma, benign giant lymphoma; Harrison and Bernatz 1953; Zettergren 1961; Tung and McCormack 1967; Flendrig 1970) is confusing and an indication of the uncertainty existing as to its pathogenesis.

Our study is aimed at the HV type of CD. This is the most common of the two localized forms described by Keller et al. (1972). Essential for its diagnosis is the combination of abnormal follicles and hypervascular in-

terfollicular tissue which, in its classical form, is easily recognized. The proportion of the two components, however, may vary from a striking predominance of the follicles to a predominantly vascular and fibrotic tissue; histopathologic extremes which, if unrecognized, may lead to serious misdiagnoses. In an attempt to reach a better understanding of this morphological variability, we have reviewed all cases of HV CD diagnosed at our institution over the past 22 years and have carried out a detailed immunohistochemical study of a selected group of these cases, looking for clues to the pathogenesis of this enigmatic disease.

Materials and methods

One hundred and sixty-two cases classified as CD or angiofollicular lymphoid hyperplasia over a 22-year period (1970–1991) were retrieved from the files of the Armed Forces Institute of Pathology and reviewed. After discarding cases in which the diagnosis was not confirmed, those of PC type and cases of HV type with insufficient or inadequate material, we were left with 102 cases of CD, HV type. Clinical data were obtained from the patients' records and through the courtesy of contributing pathologists and their clinicians.

In all cases hematoxylin and eosin (H&E) stained sections were available, often from several blocks, and in 23 cases, selected because optimal formalin-fixed material was available, paraffin blocks were recut and immunohistochemical studies were performed using an avidin-biotin-complex technique (Hsu et al. 1981). The panel of reagents employed (Table 1) included antibodies to lymphoid cells (L26, MB2, UCHL1, CD3, MT1, MT2, Leu7), dendritic and histiocytic cells (CD21, anti-S100 protein, KP1), endothelia (anti-factor-VIII-related antigen (anti-F8), CD34, HECA-452, MECA-79) and stromal elements (anti-vimentin, anti-smooth muscle actin, anti-desmin, anti-myosin). The H&E and immunoperoxidase-stained sections were evaluated for the follicular (F) and interfollicular (IF) characteristics listed below. For the follicles: the ratios of mantle zone (MZ) to GC, of HV to hyperplastic GCs, of GCs with round versus "geographic" shape and of single to multiple GCs within a single MZ; the number of B, T, and Leu7-positive cells; the staining pattern with CD21, KP1 and anti-vimentin in GCs and MZs; the degree of vascularity and the endothelial reactivity with anti-F8 and CD34 antibodies. As for IF characteristics: the number of B, T, and Leu7-positive cells; the number and type of S100-protein-positive cells (interdigitating reticulum cells and small lymphocytes) and of KP1-positive cells (plasmacytoid monocytes and cells with dendritic morphology); the number of actin-positive and vimentin-positive cells; and the degree of vascularity and endothelial reactivity with anti-F8, CD34, HECA-452 and MECA-79 antibodies. In addition to normal lymph nodes, as controls for staining patterns with specific monoclonal antibodies (HECA-452, MECA-79, anti-actin, anti-desmin, anti-myosin) we had internal controls in 5 cases, namely, uninvolved lymph nodes present in the section containing tissue involved by CD.

Table 1. Antibodies employed in this study

Antibody	CD	Main reactivities	Dilution	Source
L26	20	B cells	1:200	DAKO, Carpenteria, Calif., USA
MB2		B cells, epithelia, endothelia	1:50	Biotest, Denville, N.Y., USA
UCHL1	45RO	T cell subsets, macrophages	1:200	DAKO
CD3[a]	3	T cells	1:500	DAKO
MT1	43	T cells	1:50	Biotest
MT2	45RA	T cells, B cell subset	1:20	Biotest
Leu7	57	NK cell and T cell subsets	1:10	Becton-Dickinson, San Jose, Calif., USA
1F8	21	FDRCs, B cell subset	1:50	DAKO
Anti-S-100-protein[a]		IDRCs, macrophages	1:2000	Sigma, St. Louis, MO., USA
KP1	68	Macrophages	1:400	DAKO
Anti-F8[a]		Endothelia	1:40	DAKO
QBEND10	34	Hemopoietic progenitor cells, endothelia	1:100	AMAC, Westbrook, ME., USA
HECA-452		Endothelia of nodal high endothelial venules	1:10	Dr. E.C. Butcher (ECB), Department of Pathology, Stanford, Calif., USA
MECA-79		Endothelia of nodal high endothelial venules	1:10	ECB
V9 (Vimentin)		Endothelia, fibroblasts, vascular smooth muscle cells	1:80	DAKO
1A4 (Smooth muscle actin)		Smooth muscle cells, pericytes	1:1200	Sigma
D33 (Desmin)		Striated and smooth muscle cells	1:100	DAKO
Anti-myosin[a]		Skeletal muscle cells	1:40	Chemicon, Temecula, Calif., USA

F8, Factor-VIII-related antigen; NK, natural killer; FDRC, follicular dendritic reticulum cell; IDRC, interdigitating dendritic reticulum cell

[a] Polyclonal antiserum

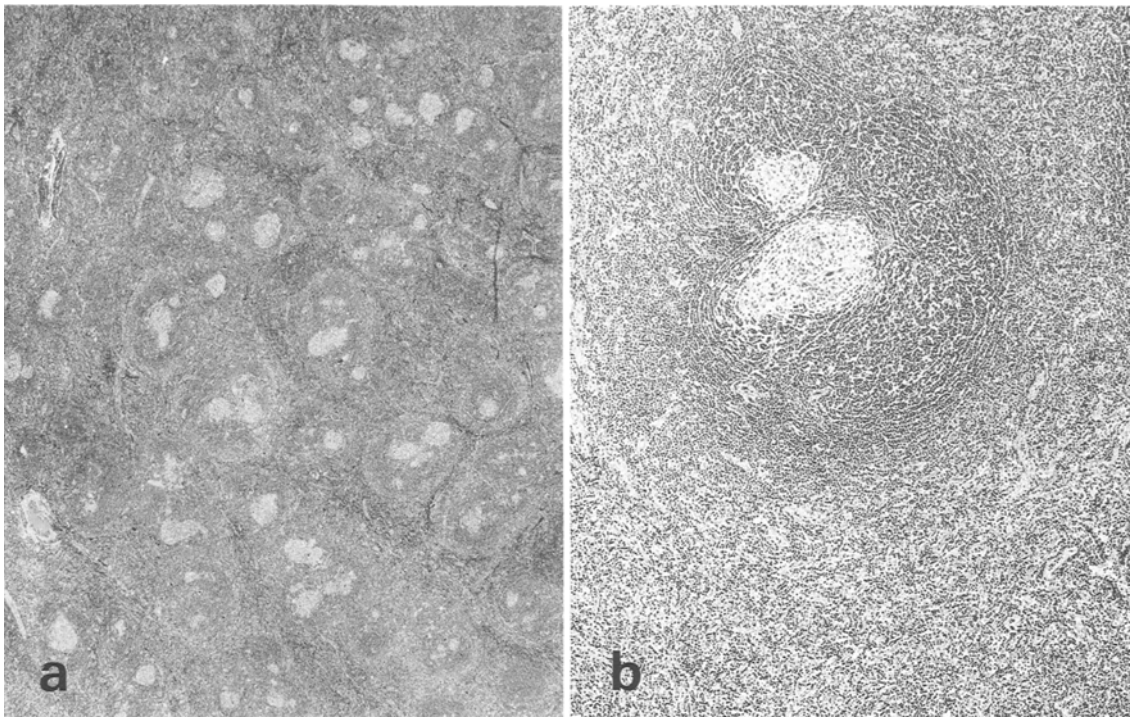

Fig. 1 a, b. Classic case of hyaline-vascular (HV) type of Castleman's disease (CD), showing abnormal follicles alternating with hypervascular areas (**a**, H&E, ×20) and the HV morphology of the germinal centers (**b**, H&E, ×75)

Results

By definition, all lesions consisted of abnormal lymphoid tissue featuring a combination of abnormal follicles and hypervascular IF areas (Fig. 1). In all cases but one (see below) lymph node sinuses were conspicuously lacking within the lesion; they could, however, be identified at the periphery of the lesion in 58 of 102 cases. Fibrosis was common, both peripherally and in large bands within the lesion.

Follicles varied widely in size among different cases, but much less so in each lesion; in most cases they were round in shape but in a significant minority they showed a "geographic" configuration (Abell 1957). Within the MZ, often strikingly predominant, one or frequently multiple GCs were seen; most of these had the classic HV morphology (Fig. 1b) and only a few were hyperplastic.

The vascularity of the GCs and MZs varied but was always in excess of normal. As a rule, CD34 detected blood vessels in greater number and of smaller caliber, and produced consistently more intense staining than the anti-F8 antibody. The anti-actin antibody, by decorating the pericytes, also highlighted the vascular network well. Staining with CD21 showed a network of follicular dendritic reticulum cells (FDRCs) which was intense and tight within the GCs but variable in the MZ where it might be intensely stained and tightly concentric (Fig. 2), intensely stained but broken apart, or loose and pale staining. With KP1, weakly reactive cells with dendritic morphology were detected often, but macrophages only rarely. At the periphery of some of the MZs, a dendritic network with a concentric pattern was identified uncommonly with KP1, but more often with the anti-vimentin antibody (which also stained the endothelia).

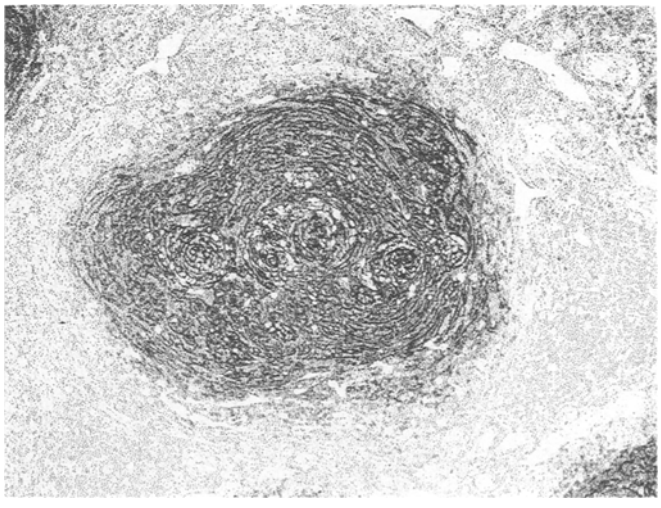

Fig. 2. Dense network of CD21-positive follicular dendritic reticulum cells in the mantle zone and, even denser, in the small germinal centers (immunoperoxidase, ×75)

The B cell reagents L26 and MB2 marked more strongly the GCs than the MZs: the cells were MT1- and MT2-negative in the former, MT1-negative and MT2-positive in the latter. Varying numbers of T cells

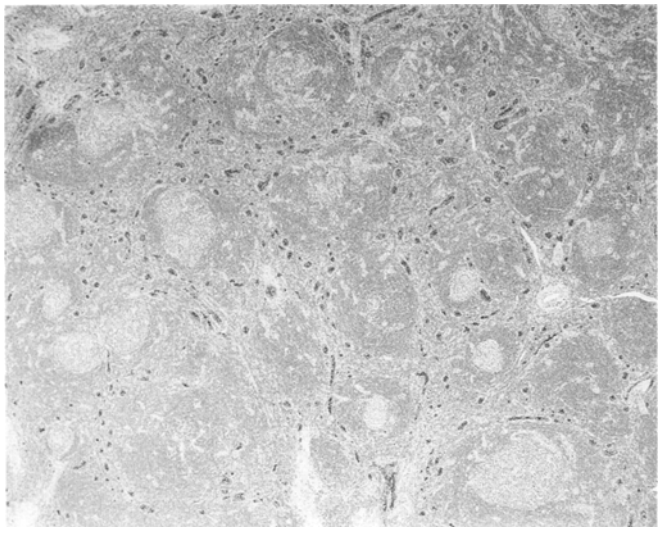

Fig. 3. High endothelial venules highlighted with MECA-79 (immunoperoxidase, ×30)

were identified using UCHL1 and polyclonal CD3, with a greater concentration in the hyperplastic than in the HV GCs. Leu7 detected small reactive lymphocytes in small numbers within the GCs of all cases, but rarely within the MZs. Large atypical cells with giant, bizarre, dystrophic nuclei and inconspicuous cytoplasm were seen in many cases, both within the follicles and occasionally in the IF zones. Their immunohistochemical reactivity and nature will be addressed separately in a future publication.

IF areas were highly vascular. A complex and ramified network of vessels was present, ranging from delicate high endothelial venules to vessels with fibrotic cuff and lined by flattened endothelium. The endothelia of the IF vessels, in contrast to those of the F vessels, expressed the F8 more strongly than the CD34 antigen. In all cases, a variable vascular component showed endothelial reactivity with HECA-452 (Duijvestijn et al. 1988) and, even more strongly, with MECA-79 (Streeter et al. 1988; Fig. 3). These characteristics identify the high endothelial venules of peripheral lymph nodes.

The cellular composition of the IF areas was somewhat variable, but always included: (1) small, regular lymphocytes, predominantly of T cell phenotype, with rare Leu7-positive cells; (2) an abundant population of plasmacytoid monocytes (Facchetti et al. 1988), which occurred in clusters or large sheets, easily identifiable on H&E-stained sections (Fig. 4), or were scattered diffusely throughout and were then only recognizable with KP1 staining (Fig. 5). These cells were also highlighted with HECA-452 (Facchetti et al. 1989b), but were unreactive with MECA-79. A population of cells with dendritic or, rarely, spindle shapes and ovoid vesicular nuclei, decorated with KP1, which will be referred to as histiocytic reticulum cells (HRCs), was seen (Mueller-Hermelink and Lennert 1978). There was a network of dendritic or spindle cells with plump, ovoid nuclei, reactive, like the pericytes of blood vessels, with anti-actin antibody (Fig. 6). These cells, corresponding to the fibroblastic reticulum cells of Mueller-Hermelink et al. (1981) or the myoid cells of Pinkus et al. (1986) Toccanier-Pelte et al. (1987), were also seen regularly underlying the sinuses of normal control lymph nodes, but showed

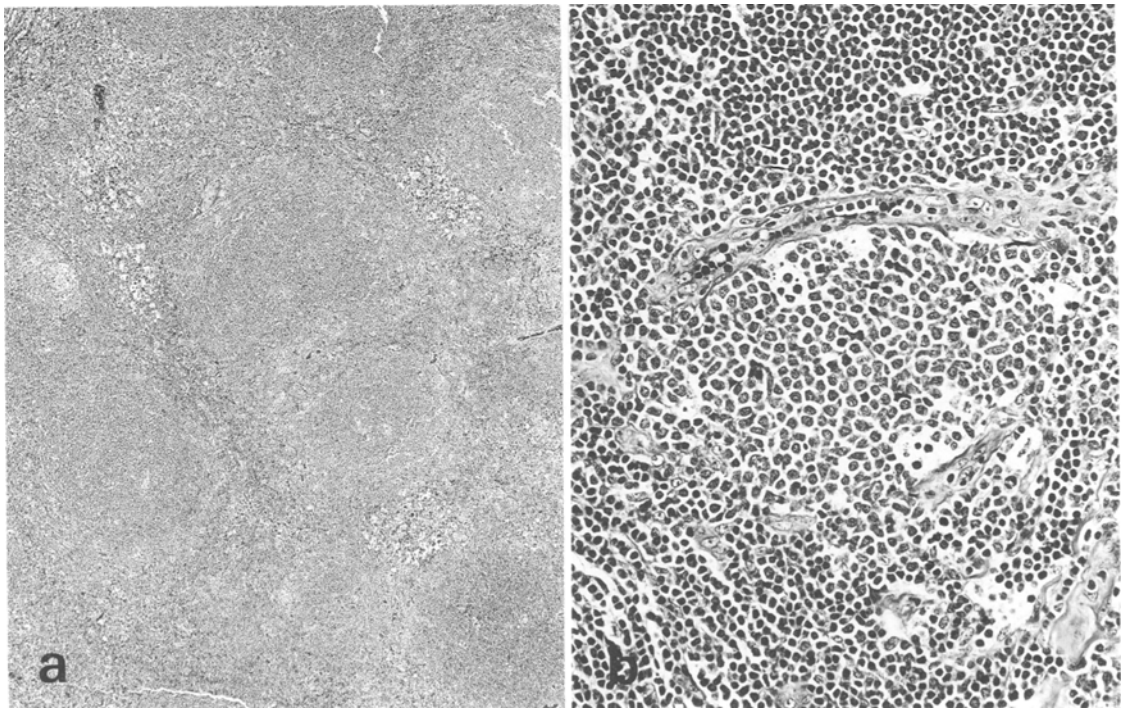

Fig. 4a, b. Interfollicular patches of plasmacytoid monocytes with the characteristic starry-sky pattern (**a**, H&E, ×48) and round, pale staining nuclei (**b**, H&E, ×300)

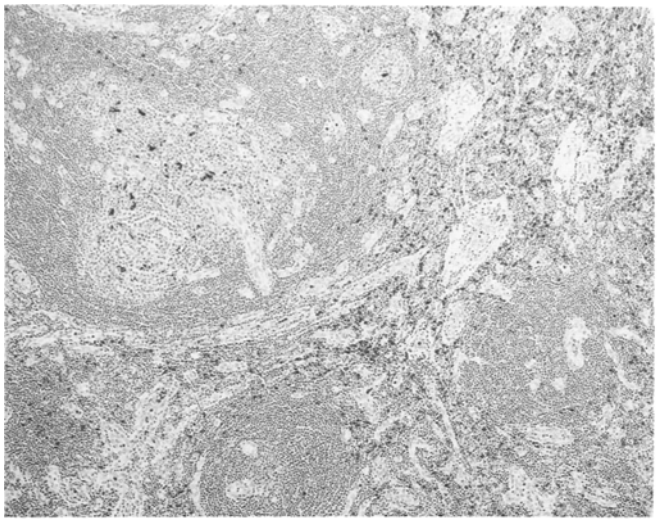

Fig. 5. Staining with KP-1 highlights the abundant plasmacytoid monocytes scattered in the interfollicular areas (immunoperoxidase, ×75)

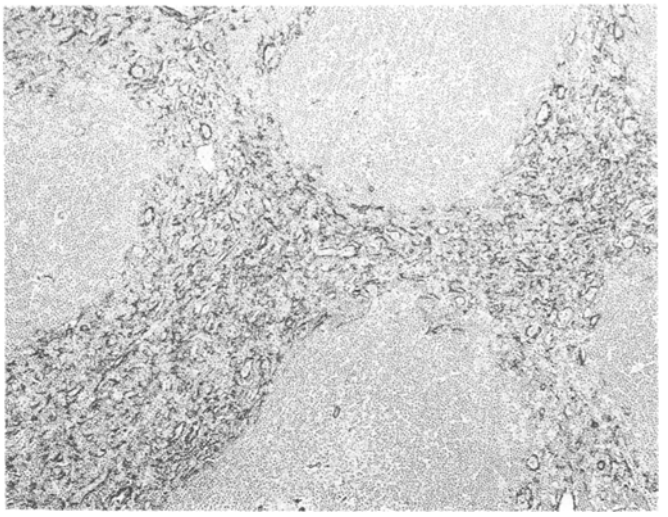

Fig. 6. The anti-actin antibody decorates the pericytes of blood vessels and the network of "myoid" (fibroblastic reticulum) cells (immunoperoxidase, ×75)

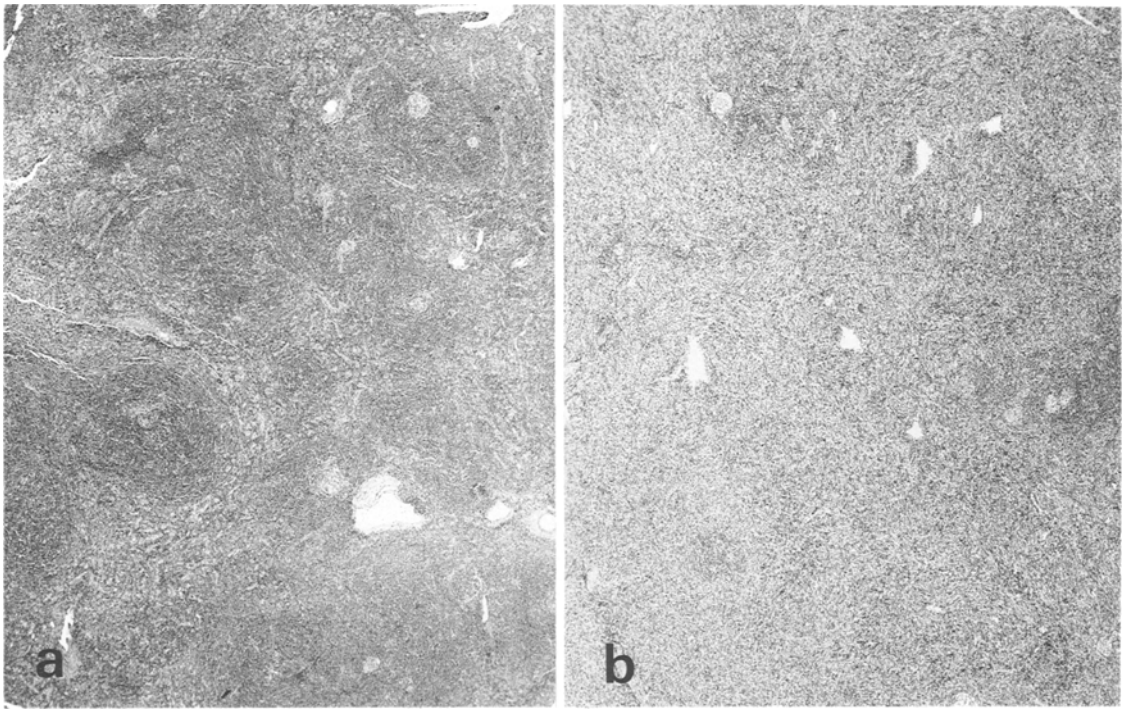

Fig. 7. The two extremes of the follicular (**a**) and stroma-rich variant (b), to be compared with the "classic" morphology of HV CD in Fig. 1 (both H&E, ×30)

retained sinuses in only one focal area of one case of CD. No stromal cells reacted with the anti-desmin and anti-myosin antibodies. A sparse and variable dendritic network of cells stained, like the endothelia of blood vessels, with anti-vimentin antibody. This population probably overlaps that of the actin-positive cells and represents fibroblastic reticulum cells (Gloghini et al. 1990). S100-protein-positive interdigitating reticulum cells (IDRCs) with large, delicate, contorted nuclei, as well as S100-protein-positive small/medium-sized lymphocytes (Takahashi et al. 1987) were seen in large numbers in 8 of 23 cases (35%) in our series.

Of note was a striking lack of "inflammatory" features within the IF areas: less than 5% of cases showed a relevant number of PCs, immunoblasts, eosinophils or granulomas, cell populations commonly seen in any reactive lymph node.

While all cases shared the above morpho-immunophenotypic features, there were quantitative differences, from case to case, in the proportion of the F to the

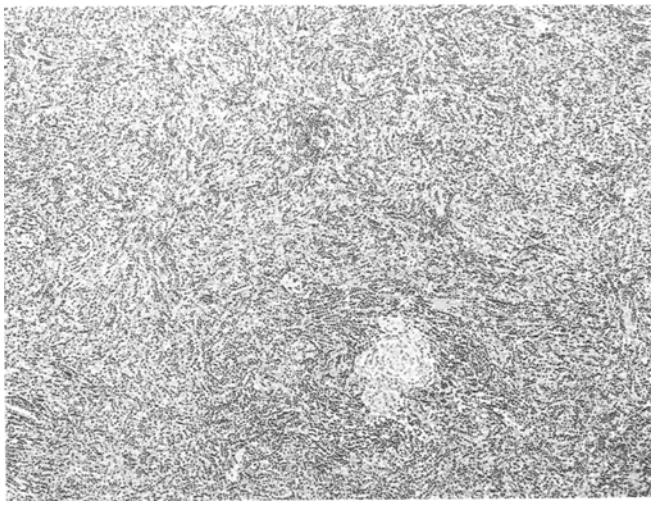

Fig. 8. Stroma-rich variant: rich cellular and vascular proliferation around a small residual follicle (H&E, ×75)

IF component. Based on this purely quantitative criterion, the cases could be assigned to one of three categories: "classic", with approximately equal proportion of F and IF components (52 cases); "follicular" (Fig. 7a), showing a predominance of follicles (together representing an area greater than 50% of the lymphoid tissue in the section) over the IF areas (30 cases); and "stroma-rich" (Figs. 7b, 8) showing reduced follicles, some with small, hyalinized GCs, and predominant (>50%) IF tissue (20 cases; in fact, in all of these, the extent of the IF component was greater than 75% of the section area).

We then compared these three groups histopathologically and, in the 23 selected cases (8 classic, 8 stroma-rich, and 7 follicular), immunophenotypically. Except for the amount of F and IF components, we found no substantial differences between the follicular and the classic subtypes in any of the morphologic or immunophenotypic characteristics evaluated. However, the least frequent stroma-rich variant emerged as distinct from either of the other in two regards: for some structural and cytologic features of both the F and IF components, and, in 5 cases, for the presence of IF foci of distinctly different cell composition.

In the stroma-rich variant, the IF areas (Table 2: see "diffuse interfollicular") were richer in both actin-positive myoid cells and KP1-positive HRCs. KP1-positive plasmacytoid monocytes were less numerous and more sparsely distributed and HECA/MECA-positive high endothelial venules were distinctly less numerous. The follicles, which had a much reduced MZ and minute HV GCs, featured more numerous and more intensely F8-positive blood vessels than either the classic or the follicular variant and, more frequently than either, a dendritic cell network reactive with KP1 and anti-vimentin antibody within the MZs.

On this background, distinct focal proliferatons were seen in 5 of the 8 cases studied by immunohistochemistry (Table 2). In 4 cases, single or multiple foci of storiform pattern were present, showing an increase in blood vessels and spindle cells, but a great reduction of the lymphoid component (Fig. 9). The blood vessels were crowded and much ramified and their endothelia expressed the F8 (Fig. 10a) and CD34 antigens strongly, but, in contrast with the adjacent diffuse IF areas, did not react with HECA-452 or MECA-79. The predominant cells were dendritic or spindle actin-positive cells (Fig. 10b) with a few cells of dendritic morphology reactive with KP1 or the anti-S100-protein antibody. Cohesive small aggregates of CD21-positive cells (HV GCs) could be seen entrapped within this proliferation. The foci in these 4 cases qualify histochemically as "angiomyoid" in type.

Table 2. Stromo-vascular patterns in the stroma-rich variant

	Diffuse inter--follicular	Focal angio-myoid cell	Focal angio--HRC	Focal FDRC
Blood vessels:				
number	++	++++	++++	+
morphology		crowded and ramified	crowded and ramified	spread out, stretched
number HECA positive	+	−	−	++
Spindle cells:				
number	−	++	−	++
phenotype		A++, V+/−		CD21++, A−, V++, KP1+
KP1-positive cells:				
plasmacytoid monocytes	+/−	+/−	+/−	+
dendritic	+	+	+++	++
Lymphocytes	++	+/−	+++	+++
S-100-positive cells:				
IDRC/small lymphocytes	+/+	+/+	+/−	+/+
CD21-positive network	−	−	−	++

A, actin; V, vimentin; IDRC, interdigitating reticulum cells; HRC, histiocytic reticulum cell; FDRC, follicular dendritic reticulum cell

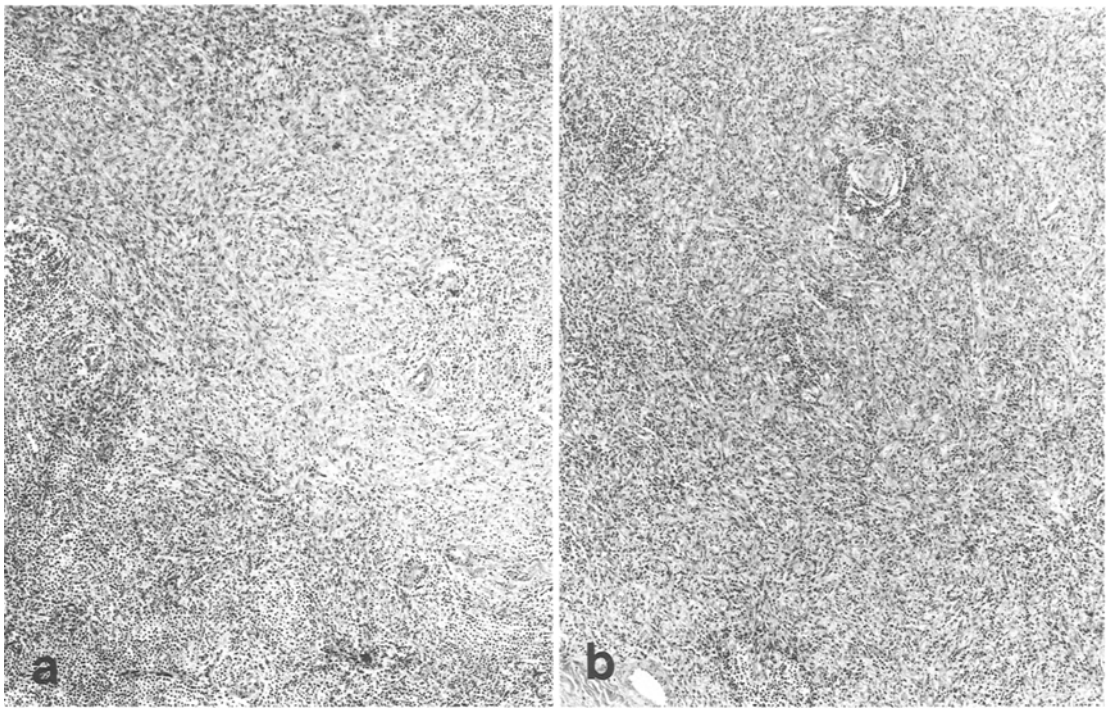

Fig. 9a, b. Angio-myoid cell foci from two patients. Vascular and spindle cell proliferations, forming a paler area adjacent to a more richly lymphoid interfollicular tissue (**a**) and surrounding a residual HV germinal center (**b**) (both H&E, ×75)

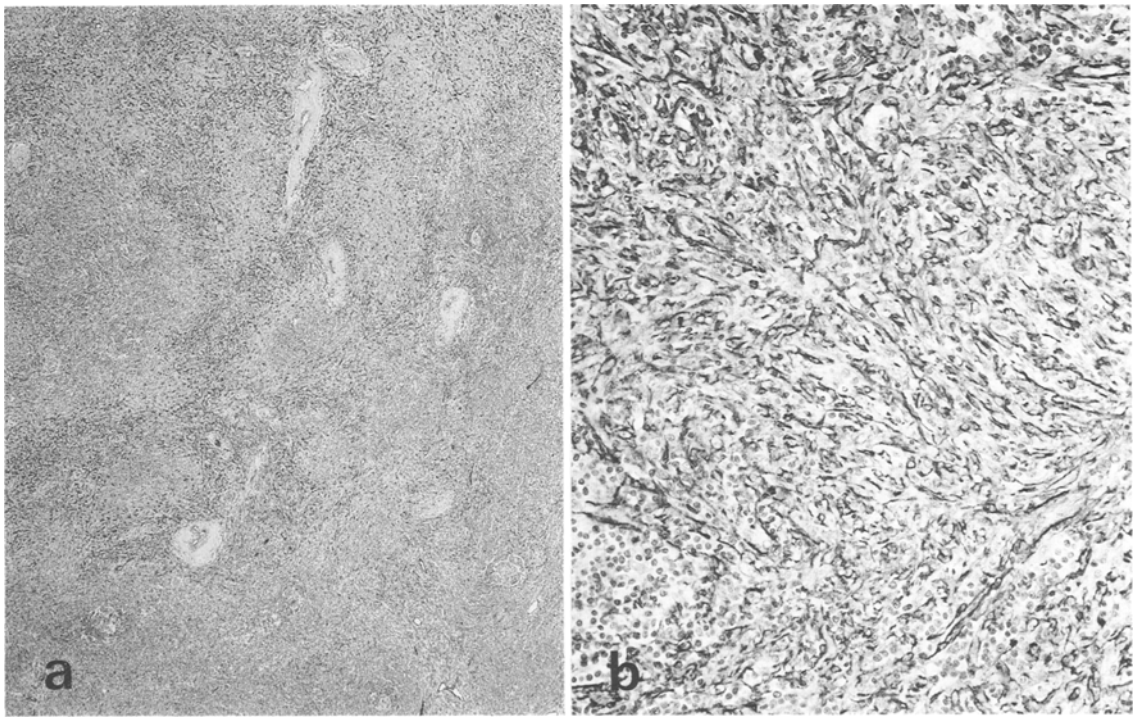

Fig. 10. The focus in Fig. 9a shows a richer vascular component than the surrounding interfollicular areas (**a**, immunoperoxidase with anti-factor 8 antibody, ×30) and a dense network of spindle and dendritic actin-positive cells (**b**, immunoperoxidase, ×200)

In 1 case containing an "angio-myoid" focus, a different proliferation was also seen, forming a larger, poorly defined nodule. This was also characterized by an increased, complex network of strongly F8- and CD34-positive and HECA/MECA-negative blood vessels, but contained abundant lymphocytes and showed no prominent cell spindling or storiform pattern (Fig. 11). The predominant cells had a dendritic morphology and strong reactivity with both KP1 (Fig. 12a) and anti-vimentin and are thus interpretable as HRCs. Other cell types, such as KP1-positive plasmacytoid monocytes, S100-protein-positive cells and, especially,

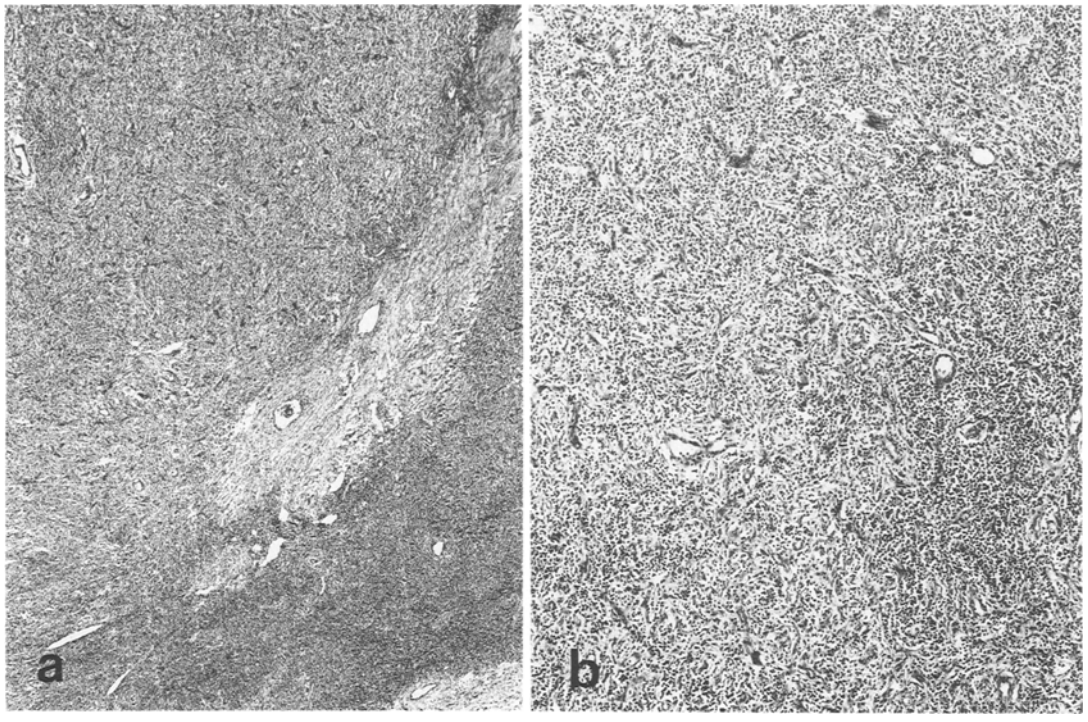

Fig. 11a, b. Angio-histiocytic reticulum cell focus. A hypervascular nodule, in the upper left half of the field (**a**, H&E, ×30) shows abundant lymphocytes, but no spindle cells (**b**, H&E, ×75)

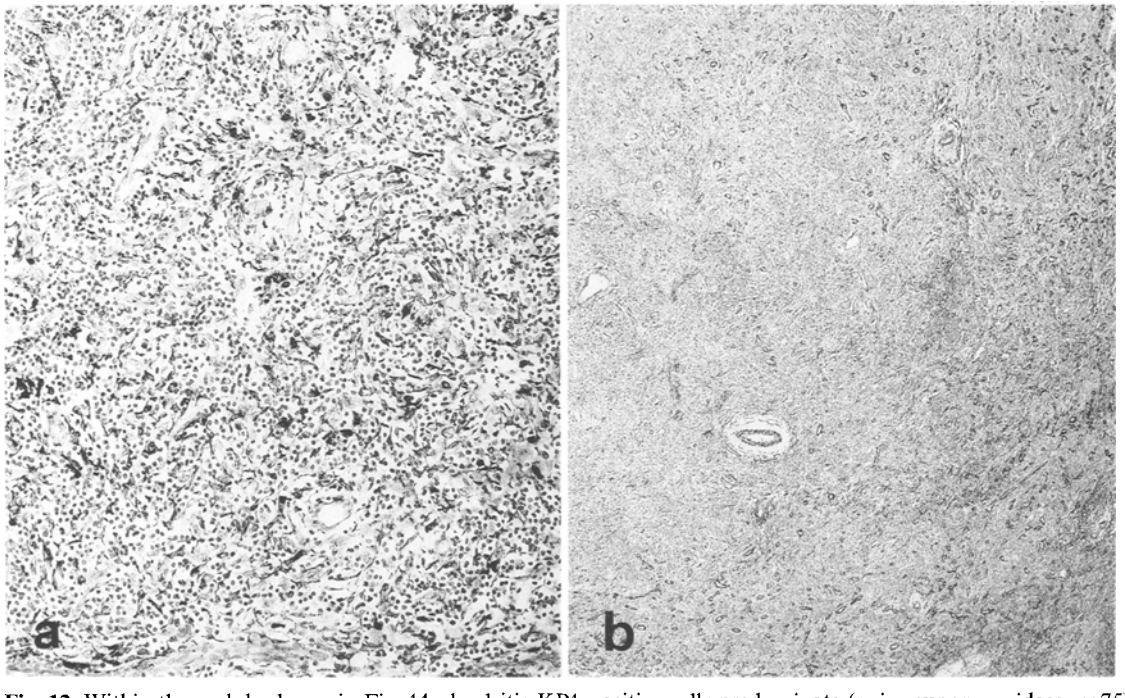

Fig. 12. Within the nodule shown in Fig. 11, dendritic KP1-positive cells predominate (**a**, immunoperoxidase, ×75), but the actin-positive stromal cell network is decreased, as compared to the adjacent tissue in the right and lower third of the field (**b**, immunoperoxidase, ×30)

stromal actin-positive cells (Fig. 12b) were very sparse within this area, as were tiny, contracted CD21-positive aggregates. This focus may be described as an "angio-HRC" proliferation.

Finally, one other case showed two large, poorly defined foci, where recognizable follicles were separated by a loose, storiform, vaguely nodular proliferation of thin spindle cells, intermixed with abundant small lymphocytes (Fig. 13) very reminiscent of FDRC sarcoma (Monda et al. 1986). The nodular areas were highlighted with CD21 (Fig. 14a), the anti-vimentin antibody and, less strongly, with KP1 (Fig. 14b), which all showed an

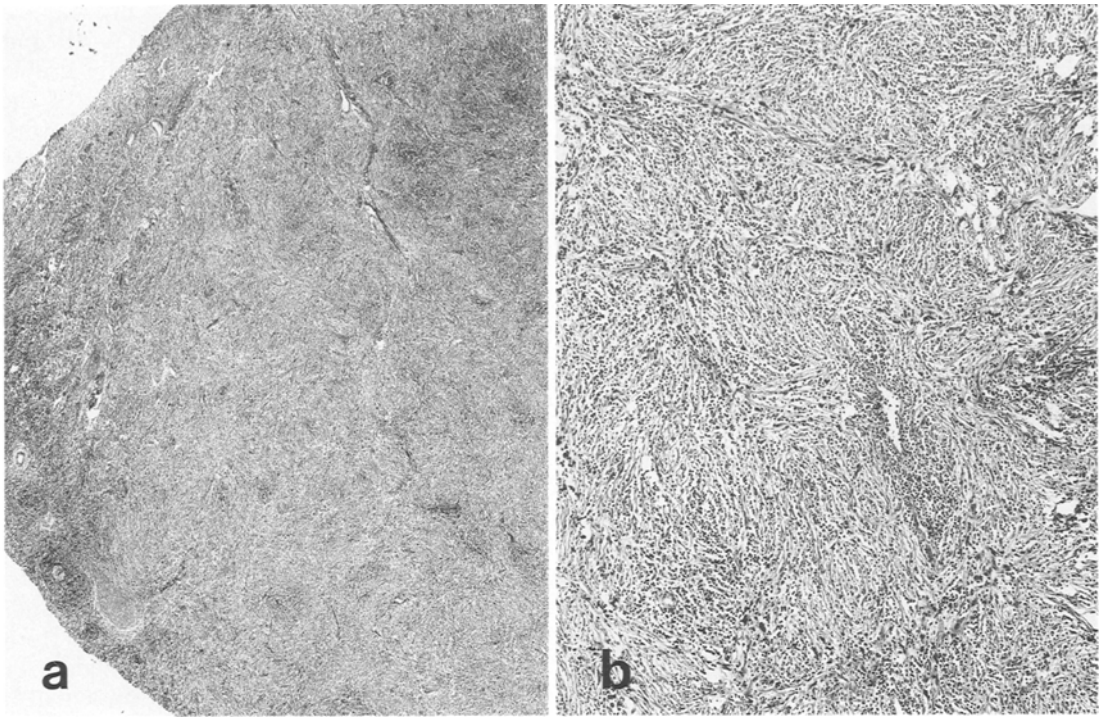

Fig. 13a, b. Follicular dendritic reticulum cell focus. A vaguely nodular proliferation, adjacent to a stroma-rich area (left fifth of the field; **a**, H&E, ×15) is composed of interlacing bundles of thin spindle cells, intermixed with lymphocytes (**b**, H&E, ×75)

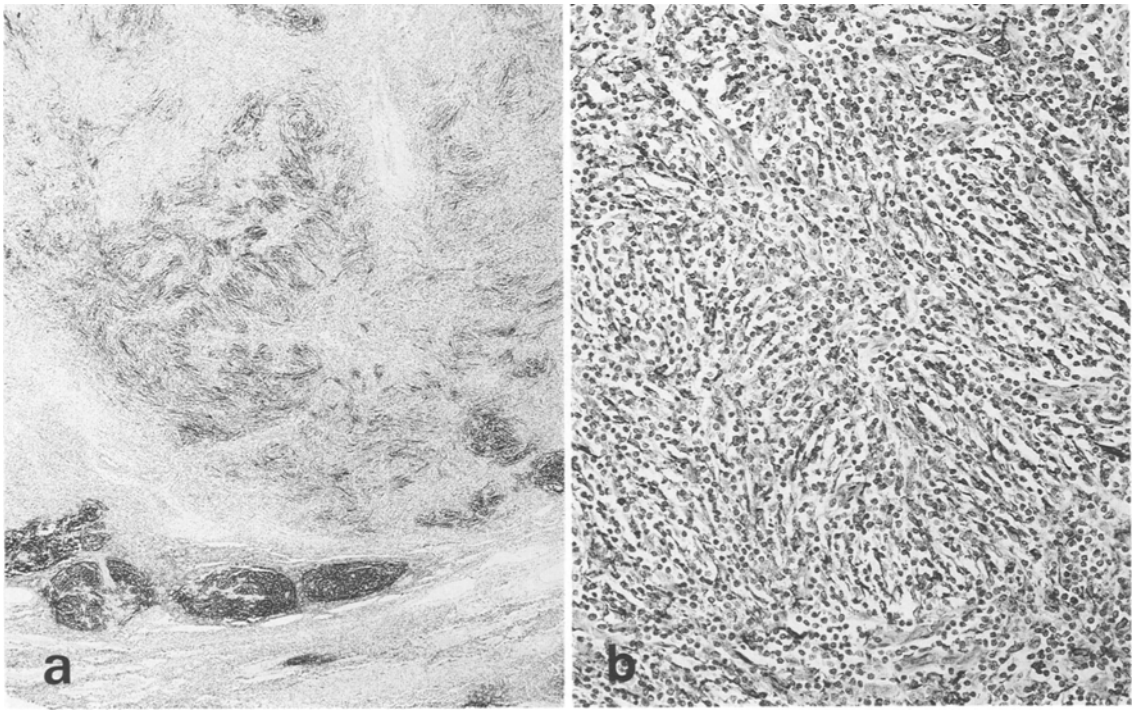

Fig. 14. The spindle cells in the nodule shown in Fig. 13 are CD21-positive (**a**, immunoperoxidase, ×30) and KP1-positive (**b**, immunoperoxidase, ×200)

identical pattern of loose, interlacing fascicles of reactive spindle cells. In contrast, staining for actin revealed, within the nodular areas, an interruption of the actin-positive reticular network seen in the adjacent areas. The blood vessels were spread out and thinned and their endothelia were strongly F8-positive, weakly CD34-positive, and all reacted with HECA-452 and, even more strongly, with MECA-79. We refer to this third focal proliferation as "FDRC" in type.

In 5 cases (2 follicular, 2 classic, 1 stroma-rich), the

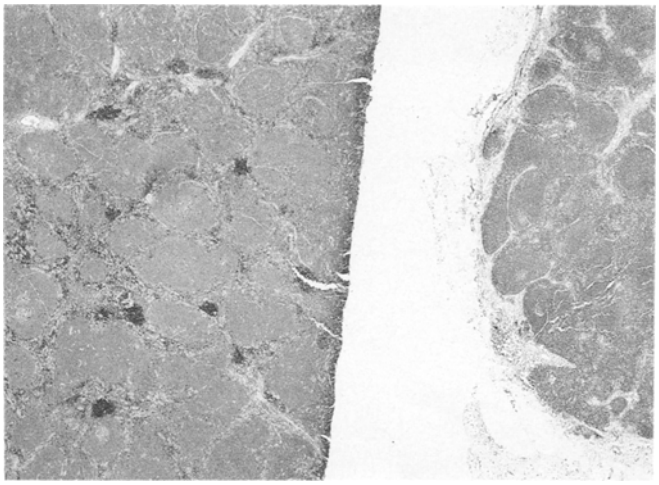

Fig. 15. In a section with two specimens from the same patient, the abundance of KP1-positive plasmacytoid monocytes in the tissue of HV CD contrasts with their absence in the uninvolved node (immunoperoxidase, ×15)

presence of a normal lymph node in the same section that contained tissue of HV CD allowed the comparison of structural and cytologic characteristics of normal versus abnormal tissue in the same patient. All these lymph nodes showed a distinct increase in follicles, with an accompanying decrease of the paracortical areas. The sinuses were frequently prominent due to histiocytosis; a focal loss of sinuses, also detected with anti-actin stain, was seen in one node. Nodular paracortical hyperplasia ("tertiary nodules") and microgranulomas were each present in 2 cases.

The follicles were very small, with minimal vascularity, and only few contained GCs. The IF areas showed a normal vascular pattern (the endothelia being reactive with anti-F8, CD34, HECA-452 and MECA-79) and actin- and vimentin-positive cell networks of variable intensity, not appreciably different from those of the adjacent tissue of CD. KP1 highlighted sparse cells with dendritic morphology, as well as plasmacytoid monocytes, either in clusters or scattered: these varied from uncommon (3 cases) to frequent (2 cases), but in all cases were distinctly less numerous than in the adjacent tissue of CD (Fig. 15).

The clinical findings are summarized in Table 3. Our patients ranged in age from 7 to 75 years (median: 30 years). The majority (66%) were females. A history of a mass growing slowly for many years (3–11) was elicited in many patients and, in one, the lesion was said to be a "recurrence". The sites involved were overwhelmingly nodal (95%) and, among these, predominantly the cervical (42%), mediastinal (31%) and abdominal (18%) regions. In the remaining 5% of patients the disease presented in extranodal sites: soft tissue of the arm in 2, and subcutis of the back, oropharynx and pericardium in 1 each. The size of the excised lesion varied from 2.5 to 10 cm in maximum diameter with a median of 7 cm. Follow-up was available in only 10 patients (5, 3, and 2 in the stroma-rich, classic and follicular categories, respectively) and varied from 3 to 16 years (median: 7.5); in none of these patients was there evidence of CD after excision of the original mass.

A comparison of the clinical findings in patients with the three variants (Table 3) showed no substantial differences between patients with the follicular and those with the classic variant. However, those with the stroma-rich morphology had a distinct presentation in two regards: first, all were adults (18 or older), whereas 5 of 29 (17%)

Table 3. Clinical findings

	All patients	Follicular variant	Classic variant	Stroma-rich variant
Age (years):				
range	7–75	9–75	7–63	18–74
median	30	27	29.5	32.5
Gender:				
male: female	32:61	11:18	17:27	4:16
% female	66	62	61	80
Sites –				
lymph nodes: cervical	38	15	23[a]	–
axillary	3	1	2	–
inguinal	5	1	3[a]	1
mediastinal	28	9	14	5
intra-abdominal	7	2	–	5
retroperitoneal	9	–	3	6
extranodal	5	2	–	3
Follow-up (years):				
range	3–16	3, 5	3, 10, 11	3, 5[b], 12, 15[c], 16[b]
median	7.5	–	10	12

[a] Both sites were involved in one patient
[b] Two of the patients with angio-myoid cell foci
[c] The patient with follicular dendritic reticulum cell proliferation

with the follicular form and 9 of 44 (20%) with the classic form were younger than 18 years; and, second, in contrast with the predominantly cervical and mediastinal nodal involvement observed in the other variants, their disease was most frequently abdominal. This difference in distribution is statistically significant ($P<0.001$ by the χ^2-test). In addition, their lesions tended to be larger ($P=0.058$), their median maximum diameter being 7.5 cm, versus 4.0 cm and 5.0 cm in the follicular and classic form, respectively.

Discussion

The histopathologic and immunophenotypic findings of this study, on one band, confirm the uniqueness of the cyto-architectural characteristics of the lymphoid tissue of HV CD. On the other hand, they expand the spectrum of changes to be recognized under this diagnosis, by bringing out a "stroma-rich" variant and a variety of cell proliferations occurring in it that may be misdiagnosed as stromal malignancies. Finally, our findings also offer clues to a new pathogenetic interpretation.

Common to all of our cases was a combination of: abnormal follicles; an IF tissue with increased vascularity and unusual cell composition; and loss of lymph node sinuses. The follicles were characterized by poorly formed GCs, composed of tight aggregates of CD21-positive, and often KP1-positive, FDRCs; and by a predominant MZ, with a looser, variable dendritic cell network and increased vascularity. We found no abnormality in the composition, or phenotypic characteristics, of the lymphoid component of the follicles. The composition of the IF areas was in some ways similar to that of the normal nodal paracortex, with HECA-452- and MECA-79-positive high endothelial venules (Duijvestijn et al. 1988; Streeter et al. 1988), a predominance of T cells, and a network of dendritic or spindle cells identified in both anti-vimentin and anti-actin stains and corresponding to the fibroblastic reticulum cells of Mueller-Hermelink et al. (1981) and the myoid cells described by Pinkus et al. (1986) and Toccanier-Pelte et al. (1987). However, in contrast to the paracortex of the normal lymph node, the IF tissue of CD showed: a distinct increase in the number of blood vessels; a large number of KP1-positive cells, including plasmacytoid monocytes and non-phagocytic stromal cells with dendritic processes, for which we use the term of HDCs; a marked increase in S100-protein-positive small lymphocytes (Takahashi et al. 1987), found in 35% of cases; and an extreme paucity of plasma cells, eosinophils, IDRCs or epithelioid cells in all cases. The third remarkable characteristic of our cases was the total lack of sinuses within the lesion (even though they could be often seen at its periphery): this is one of the essential differences that separate the HV from the PC type.

Within the framework of these three features, common to all cases, there were variations in the proportion of the F to the IF component, which allowed a basic categorization of HV CD into three forms: "classic," with an approximately equal proportion of F and IF tissue; predominantly "follicular"; and "stroma-rich." However, the distinction between the first two forms is blurred by both the subjectivity of this quantitative evaluation and the lack of any qualitative differences in immuno-/cyto-architectural characteristics. In fact, the recognition of a follicular variant might be useful only in reminding pathologists to also consider HV CD when confronted with a lymphoid process with follicular pattern, in order to avoid a misdiagnosis of MZ lymphoma, follicular lymphoma, or monocytoid B cell lymphoma.

The stroma-rich type, in contrast, stands out as a distinct histo-immunophenotypic variant, due not only to quantitative but substantial qualitative differences. The follicles, overrun by the IF tissue, were reduced in size and their periphery, still recognizable as a dense aggregation of lymphocytes, seemed to acquire IF characteristics (stronger F8 expression by blood vessels and often prominent KP1- and vimentin-positive dendritic network). The IF tissue was distinguished by loss of HECA and MECA reactivity (less specialized blood vessels), decreased numbers of plasmacytoid monocytes and, instead, increased numbers of myoid cells and HRCs. Both this rich, diffuse stromal cellularity and the occasional occurrence of discrete foci of hyperproliferation may lead, in this variant, to a misdiagnosis of stromal malignancy.

We found a variety of such focal proliferations which may be similar to others illustrated in the literature (case 9 of Nagai et al. 1986; cases 1 and 4 of Gerald et al. 1990). The most common among our cases featured a storiform pattern, due to crowded small vessels (with thick HECA-negative endothelium) and to plump spindle cells of myoid type; a pattern which we thus refer to as "angio-myoid." A second type also showed a florid aggregation of HECA-negative small blood vessels, but had no spindling at all and the predominant cells were KP1-positive elements of dendritic morphology; a combination which we summarized with the term "angio-HRC" proliferation. These two types of vascular proliferation may be confused with an angiosarcoma, a neoplasm which may indeed develop in CD (as proven by the metastatic dissemination of two such cases in the series by Gerald et al. 1990). However, they lack atypia and mitoses and, we believe, are not vascular neoplasms, but an exaggeration of the hypervascularity of CD in general and of the stromal cell outgrowth characteristic of the stroma-rich variant. A third type of focal growth in our cases featured no vascular prominence at all, but rather a spindle cell proliferation centered on, and disrupting, the follicles. In the absence of double staining, we cannot say with certainty whether one or more cell types of spindle shape are involved. However, the close similarity in the pattern of reactivity with three antibodies suggests a proliferation of CD21-positive FDRCs, which also express the KP1 antigen and vimentin (the latter being a feature of MZ reticulum cells; Gloghini et al. 1990). This interpretation is also supported by the close similarities (nodularity, spindling, abundance of intermixed lymphocytes and CD21 reactivity) between this third type of proliferation and the FDRC sarcoma (Monda et al. 1986). The distinction be-

tween the two, in fact, may be debatable and would need to rely on the absence, or presence, of atypia, mitoses and invasion of adjacent soft tissues.

In conclusion, while we agree with Ruco et al. (1991) that some "poorly differentiated tumors composed of spindle shaped cells" that arise in HV CD may be proliferations of FDRCs, rather than endothelia (our "FDRC" pattern) we consider that the stroma-rich variant of this disease is a fertile ground for other spindle cell proliferations, composed of endothelia and myoid cells ("angio-myoid"), as well as for non-spindle cell proliferations involving blood vessels and HRCs ("angio-HRC"). These diverse growths may develop simultaneously within the same lesion, as proven by one of our cases, and may in fact be related.

Whether the morpho-immunophenotypic variants we have described also have relevant clinical correlates is uncertain but our data are sufficient to support the distinctiveness of the stroma-rich variant. When compared with the other two, it affected only adults (18 years or over) and abdominal nodes significantly more than peripheral and mediastinal nodes, and tended to produce larger masses. Although we have follow-up in only 5 patients (including 3 with focal proliferations) data at 3–16 years provided no evidence of recurrence or dissemination of the stromal growths, supporting their hyperplastic, rather than neoplastic, nature.

The very distinct morpho-phenotypic findings of HV CD still require a coherent pathogenetic explanation. The contrasting combination of features which characterizes CD of PC type (preservation of the nodal architecture – including sinuses – hyperplasia of the GCs and abundance of PCs) has been seen, since its original description (Flendrig 1970; Keller et al. 1972), as the expression of an abnormal immune response. More recently, it has been suggested that the key cells in this response may be an abnormal mantle cell population of CD5-positive B cells (Hall et al. 1989; Isaacson 1989) and that the stimulus to their proliferation and differentiation to PCs could come from excessive interleukin-6 (IL-6) production by the hyperplastic GCs (Yoshizaki et al. 1989; Leger-Ravet et al. 1991). These explanations for the PC type, however, can hardly be valid for a lesion as histopathologically different from it as the HV form.

In the past this type has been considered a hyperplastic process (Castleman et al. 1956; Flendrig 1970; Keller et al. 1972) or a hamartoma of the lymphoid tissue (Abell 1957; Lattes and Pachter 1962; Neerhout et al. 1969; Symmers 1978). More recent studies, directing their attention especially to the abnormal follicles, have suggested a role for "an acquired T-cell immunodeficiency that results in ... abnormal follicle formation with diminished B-cell differentiation to PC" (Weisenburger et al. 1986) or have presented convincing evidence for a "dysplasia" of FDRCs, which would somehow lead to "a defect in GC organization" (Ruco et al. 1991). A complete pathogenetic theory for the HV type, however, needs to explain not only the abnormalities of the follicles (poor development of the GCs and preponderance of the MZ), but a variety of other, seemingly unrelated features (lack of sinuses, increase in plasmacytoid monocytes, and hypervascularity) as well as the continuum from a predominantly lymphoid, to a predominantly stromal, growth.

As to the first of these features, we agree with Ruco et al. (1991) that a primary developmental defect of the GC is a more likely explantation for its classic HV changes than regressive phenomena occurring after its formation, since it better explains the consistent presence of these changes in all the variants of HV CD. We also, like they and others (Harris and Bahn 1985), suspect that these changes reflect an abnormality of the FDRCs rather than of the lymphoid cells. We found, in fact, no abnormalities or differences among the three variants, in the distribution or phenotype of the T and B cell components except what we interpret as a secondary decrease in both, as a result of a progressive increase in blood vessels and stromal cells from the follicular, through the classic, to the stroma-rich variant.

Abnormalities of FDRCs, such as their extension from the GC into the MZ and the abnormal expression of the adhesion molecule V-CAM-1, which binds B lymphocytes (Ruco et al. 1991), might also play a role in another feature of the follicles in HV CD, namely, the expanded MZ. However, this may be better explained with the continuous accumulation of recirculating IgM+D+ small lymphocytes (Harris and Bahn 1985; Martin et al. 1985; Carbone et al. 1986; Weisenberger et al. 1986), which can enter the tissue via functional (HECA/MECA-positive) high endothelial venules and home in the MZs (as they do in normal lymph nodes), but are hindered in their egress from it by the paucity, or absence, of the sinuses.

This third essential feature of HV CD would also cause a decreased influx of antigens (de Wolf-Peeters and Delabie, in press) and so help explain the poor reactivity of this lymphoid tissue, as reflected in the lack of hyperplastic GCs (which are a result of continuous exposure to antigen; Kroese et al. 1990); the weak expression of adhesion molecules in the interfollicular areas (Ruco et al. 1991); and the paucity of reactive cells (plasma cells, eosinophils, epithelioid histiocytes). However, the lack of sinuses itself has no obvious explanation at this point.

Plasmacytoid monocytes are often seen focally in reactive nodes (Facchetti et al. 1988) and are a prominent cell component of Kikuchi's disease (Facchetti et al. 1989a). Harris and Bhan (1987) and Hunt and Anderson (1989) have reported their presence in three cases of CD. We found a marked increase of these cells in all of our cases, suggesting that they may have an essential role in the pathogenesis of CD, HV type. Although no definite function is yet recognized for them, plasmacytoid monocytes are thought to be antigen-presenting cells and to have secretory properties (Mueller-Hermelink et al. 1983; Facchetti et al. 1989a). In our cases, the question is how their abundance is related to other features of HV CD, which we have discussed.

A possible explanation, that would link plasmacytoid monocytes, lack of sinuses and poor GC formation, may lay, on one hand, in the very close immunophenotypic and ontogenetic relationship between sinus lining cells and FDRCs (Wacker et al. 1992) and, on the other, in

the hypothesis that plasmacytoid monocytes are the precursors of both of these cell types (Prof. Parwaresch: personal communication). If such a working hypothesis is true, one may visualize that the lymphoid tissue of HV CD would originate wherever an antigenic stimulus involves a lymph node containing abnormal plasmacytoid monocytes that are unable to develop into sinus lining cells and FDRCs. The ensuing response would be abnormal because stimulated, but functionally blocked, plasmacytoid monocytes would accumulate and no sinuses or functional GCs would form. As already suggested, recirculating lymphocytes would still enter the tissue through normal high endothelial venules and accumulate in the MZ, resulting in a slow but progressive mass formation.

Although this proposed pathogenesis appears to connect most features of HV CD we still have no suggestions to offer for the marked increase in vascularity of this tissue and the variety of stromo-vascular responses seen in our cases and in the literature (Nagai et al. 1986; Gerald et al. 1990), nor for the spectrum of morphologic variants within HV CD. We have no evidence to decide whether the stroma-rich form is a late phase in the evolution of the follicular and classic forms, or whether the three variants represent diverse unrelated tissue responses. We have already mentioned that the very different response observed in PC CD, which is one of B cell hyperreactivity, is most likely due to overproduction of a specific B cell growth factor, IL-6 (Yoshizaki et al. 1989; Leger-Ravet et al. 1991). We believe that, in HV CD, other factors need to be considered: some that affect the plasmacytoid monocytes, thus leading to an accumulation of lymphoid cells, and others that act mainly on the stromal component, stimulating angiogenesis and stromal cell outgrowth. To determine which factors these are, what their cell(s) of origin may be and what their interactions may be, is a challenge for future studies.

References

Abell MR (1957) Lymphnodal hamartoma versus thymic choristoma of pulmonary hilum. AMA Arch Pathol 64:584–588

Carbone A, Manconi R, Volpe R, Poletti A, De Paoli P, Tirelli U, Santini G (1986) Immunohistochemical, enzyme histochemical, and immunologic features of giant lymph node hyperplasia of the hyaline-vascular type. Cancer 58:908–916

Castleman B, Iverson L, Menendez VP (1956) Localized mediastinal lymph node hyperplasia resembling thymoma. Cancer 9:822–830

Diebold J, Tulliez M, Bernadou A, Audouin J, Tricot G, Reynes M, Bilski-Pasquier G (1980) Angiofollicular and plasmacytic polyadenopathy: a pseudotumorous syndrome with dysimmunity. J Clin Pathol 33:1068–1076

Duijvestijn AM, Horst E, Pals ST, Rouse BN, Steere AC, Picker LJ, Meijer CJLM, Butcher EC (1988) High endothelial differentiation in human lymphoid and inflammatory tissues defined by monoclonal antibody HECA-452. Am J Pathol 130:147–155

Facchetti F, Wolf-Peeters de C, Oord JJ van den, De Vos R, Desmet VJ (1988) Plasmacytoid T cells: a cell population normally present in the reactive lymph node. An immunohistochemical and electron microscopic study. Hum Pathol 19:1085–1092

Facchetti F, Wolf-Peeters C de, Oord JJ van den, De Vos R, Desmet VJ (1989a) Plasmacytoid monocytes (so-called plasmacytoid T cells) in Kikuchi's lymphadenitis: an immunohistological study. Am J Clin Pathol 92:42–50

Facchetti F, Wolf-Peeters de C, Oord JJ van den, Meijer CJLM, Pals ST, Desmet VJ (1989b) Anti-high endothelial venule monoclonal antibody HECA-452 recognizes plasmacytoid T cells and delineates an "extranodular" compartment in the reactive lymph node. Immunol Lett 20:277–281

Flendrig JA (1970) Benign giant lymphoma: clinicopathologic correlation study. In: Clark RL, Cumley RW (eds) The year book of cancer. Year Book Medical, Chicago, pp 296–299

Frizzera G, Banks PM, Massarelli G, Rosai J (1983) A systemic lymphoproliferative disorder with morphologic features of Castleman's disease. Pathological findings in 15 patients. Am J Surg Pathol 7:211–231

Frizzera G, Peterson BA, Bayrd ED, Goldman A (1985) A systemic lymphoproliferative disorder with morphologic features of Castleman's disease: clinical findings and clinicopathologic correlations in 15 patients. J Clin Oncol 3:1202–1216

Gerald W, Kostianovsky M, Rosai J (1990) Development of vascular neoplasia in Castleman's disease. Report of seven cases. Am J Surg Pathol 14:603–614

Gloghini A, Volpe R, Carbone A (1990) Vimentin immunostaining in fibroblastic reticulum cells within human reactive and neoplastic lymphoid follicles. Hum Pathol 21:792–798

Hall PA, Donaghy M, Cotter FE, Stansfeld AG, Levison DA (1989) An immunohistological and genotypic study of the plasma cell form of Castleman's disease. Histopathology 14:333–346

Harris NL, Bhan AK (1985) Immunohistology of Castleman's disease: a monoclonal antibody study (abstract). Lab Invest 52:28A

Harris NL, Bhan AK (1987) "Plasmacytoid T cells" in Castleman's disease: immunohistologic phenotype. Am J Surg Pathol 11:109–113

Harrison EG, Bernatz PE (1953) Angiofollicular mediastinal lymph node hyperplasia resembling thymoma. AMA Arch Pathol 75:284–292

Hsu SM, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Hunt SJ, Anderson WD (1989) Giant lymph node hyperplasia of the hyaline vascular type with plasmacytoid T-cells and presentation in infancy. Am J Clin Pathol 91:344–347

Isaacson PG (1989) Commentary: Castleman's disease. Histopathology 14:429–432

Keller AR, Hochholzer L, Castleman B (1972) Hyaline-vascular and plasma-cell types of giant lymph node hyperplasia of the mediastinum and other locations. Cancer 29:670–683

Kroese FGM, Timens W, Nieuwenhuis P (1990) Germinal center reaction and B lymphocytes: morphology and function. In: Grundmann E, Vollmer E (eds) Reaction patterns of the lymph node, part 1. Cell types and functions. Springer, Berlin Heidelberg New York, pp 103–148

Lattes R, Pachter MR (1962) Benign lymphoid masses of probable hamartomatous nature. Analysis of 12 cases. Cancer 15:197–214

Leger-Ravet MB, Peuchmaur M, Devergne O, Audouin J, Raphael M, Van Damme J, Galanaud P, Diebold J, Emilie D (1991) Interleukin-6 gene expression in Castleman's disease. Blood 78:2923–2930

Martin JME, Bell B, Ruether BA (1985) Giant lymph node hyperplasia (Castleman's disease) of hyaline vascular type. Clinical heterogeneity with immunohistologic uniformity. Am J Clin Pathol 84:439–446

Monda L, Warnke R, Rosai J (1986) A primary lymph node malignancy with features suggestive of dendritic reticulum cell differentiation. A report of 4 cases. Am J Pathol 122:562–572

Mueller-Hermelink HK, Lennert K (1978) Stationary elements of the lymph node. In: Lennert K (ed) Malignant lymphomas other than Hodgkin's disease. Histology-cytology-ultrastruc-

ture-immunology. Springer, Berlin Heidelberg New York, pp 51–71

Mueller-Hermelink HK, Gaudecker B von, Drenckhahn D, Jaworsky K, Feldmann C (1981) Fibroblastic and dendritic reticulum cells of lymphoid tissue. Ultrastructural, histochemical, and 3H-thymidine labeling studies. J Cancer Res Clin Oncol 101:149–164

Mueller-Hermelink HK, Steinman G, Stein H, Lennert K (1983) Malignant lymphoma of plasmacytoid T-cells. Morphologic and immunologic studies characterizing a special type of T-cell. Am J Surg Pathol 7:849–862

Nagai K, Sato I, Shimoyama N (1986) Pathohistological and immunohistochemical studies on Castleman's disease of the lymph node. Virchows Arch [A] 409:287–297

Neerhout RC, Larson W, Mansur P (1969) Mesenteric lymphoid hamartoma associated with chronic hypoferremia, anemia, growth failure and hyperglobulinemia. N Engl J Med 280:922–925

Pinkus GS, Warhol MJ, O'Connor EM, Etheridge CL, Fujiwara K (1986) Immunohistochemical localization of smooth muscle myosin in human spleen, lymph node, and other lymphoid tissues. Unique staining patterns in splenic white pulp and sinuses, lymphoid follicles, and certain vasculature, with ultra-structural correlations. Am J Pathol 123:440–453

Ruco LP, Gearing AJH, Pigott R, Pomponi D, Burgio VL, Cafolla A, Baiocchini A, Baroni CD (1991) Expression of ICAM-1, VCAM-1 and ELAM-1 in angiofollicular lymph node hyperplasia (Castleman's disease): evidence for dysplasia of follicular dendritic reticulum cells. Histopathology 19:523–528

Sethi G, Kepes JJ (1971) Intrathoracic angiomatous lymphoid hamartomas. A report of three cases, one of iron refractory anemia and retarded growth. J Thorac Cardiovasc Surg 61:657–664

Streeter PR, Rouse BTN, Butcher EC (1988) Immunohistologic and functional characteristics of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. J Cell Biol 107:1853–1862

Symmers WStC (1978) The lymphoreticular system. In: Symmers WStC (ed) Systemic pathology. Churchill Livingstone, Edinburgh, pp 544–548

Takahashi K, Yoshino T, Hayashi K, Sonobe H, Ohtsuki Y (1987) S-100 beta positive human T lymphocytes: their characteristics and behavior under normal and pathologic conditions. Blood 70:214–220

Toccanier-Pelte M-F, Skalli O, Kapanci Y, Gabbiani G (1987) Characterization of stromal cells with myoid features in lymph nodes and spleen in normal and pathologic conditions. Am J Pathol 129:109–118

Tung KSK, McCormack LJ (1967) Angiomatous lymphoid hamartoma. Report of five cases with a review of the literature. Cancer 20:525–536

Wacker H-H, Heidebrecht HJ, Radzun HJ, Parwaresch MR (1992) Sinuswandzellen: Morphologie, Funktion und Neoplasie. Verh Dtsch Ges Pathol 76:219–225

Weisenburger DD, Nathwani BN, Winberg CD, Rappaport H (1985) Multicentric angiofollicular lymph node hyperplasia: a clinicopathologic study of 16 cases. Hum Pathol 16:162–172

Weisenburger DD, Lipscomb Grierson H, Purtilo D (1986) Immunologic studies of multicentric (M) and unicentric (U) angiofollicular lymphoid hyperplasia (AFH; abstract). Lab Invest 54:68A

Wolf-Peeters C de, Delabie J (1993) Anatomy and histophysiology of lymphoid tissue. Semin Oncol (in press)

Yoshizaki K, Matsuda T, Nishimoto N, Kuritani T, Taeho L, Aozasa K, Nakahata T, Kawai H, Tagoh H, Komori T, Kishimoto S, Hirano T, Kishimoto T (1989) Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease. Blood 74:1360–1367

Zettergren L (1961) Probable neoplastic proliferation of lymphoid tissue (follicular lymphoreticuloma). Report of four cases with a survery of the literature. Acta Pathol Microbiol Scand 51:113–126