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Follicular dendritic cells in extranodal non-Hodgkin lymphomas of MALT and non-MALT type

C. Fellbaum [1], J. Sträter [2], M.-L. Hansmann [2]

[1] Institute of Pathology, School of Medicine, Technical University of Munich, Munich, Germany
[2] Institute of Pathology, School of Medicine, University of Cologne, Cologne, Germany

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Abstract. Extranodal lymphomas of the thyroid ($n=19$), kidney ($n=15$) and testis ($n=30$) were investigated histologically and immunohistochemically for follicular dendritic cell pattern using the monoclonal antibody Ki-FDC1P. This recognizes follicular dendritic cells in paraffin sections. Follicular dendritic cells were most predominant in lymphomas of the thyroid. These thyroid lymphomas showed the morphological features of mucosa-associated lymphoid tissue (MALT) type lymphomas in 18 of 19 cases and were classified as high-grade malignant lymphoma of MALT type with evidence of a low-grade malignant component ($n=18$). Ten of these cases contained destroyed reactive follicles of follicular dendritic cells. In 6 of these 10 cases follicular dendritic cells occurred in a pattern of tumour-associated abortive follicle type. The remaining lymphoma of the thyroid was an immunoblastic lymphoma of B-cell type showing no detectable follicular dendritic cells. In extranodal lymphomas of non-MALT type follicular dendritic cells occurred in only two cases where immunocytoma involved the kidney. Malignant lymphomas of the kidney (chronic lymphocytic leukaemia, $n=2$; immunocytoma, $n=4$; centroblastic lymphoma, $n=9$) and of the testis (immunocytoma, $n=2$; centroblastic lymphoma, $n=27$; immunoblastic lymphoma of B-cell type, $n=1$) revealed no characteristics of MALT type lymphoma, cytologically or with respect to follicular dendritic cells. Classical lymphoepithelial lesions formed by centrocyte-like cells, a hallmark of MALT, occurred exclusively in thyroid lymphomas of MALT type. Although occurrence of classical lymphoepithelial lesions formed by centrocyte-like cells was limited to thyroid lymphomas of MALT type, a growth pattern of lymphoid blasts, with formation of lesions mimicking lymphoepithelial lesions superficially, was found in 6 of 27 testicular centroblastic lymphomas. Follicular dendritic cells in non-Hodgkin's lymphomas of MALT type show distinct follicular patterns not found in other extranodal lymphomas such as those found in the kidney and testis.

Key words: Follicular dendritic cells – Lymphoma of MALT type – Extranodal lymphoma

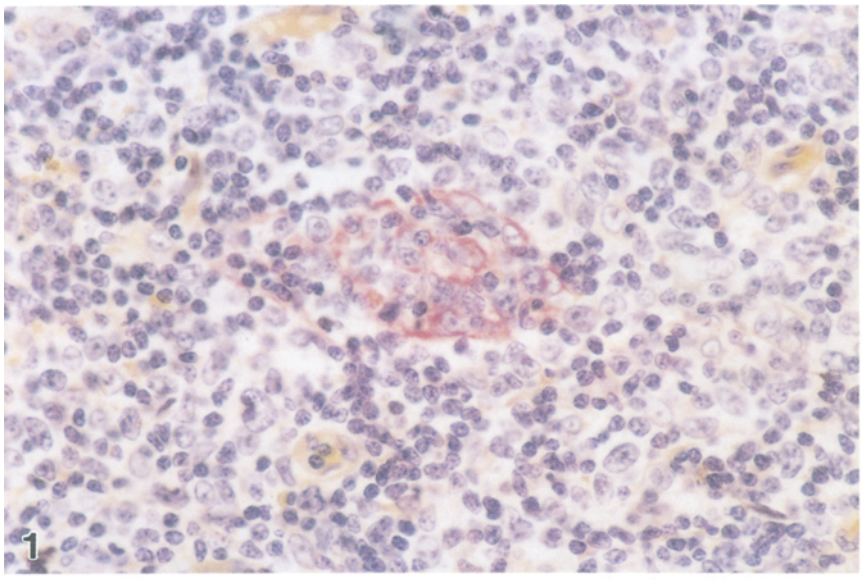
Correspondence to: C. Fellbaum, Institute of Pathology, School of Medicine, Technical University of Munich, Ismaninger Strasse 22, D-81675 Munich, Germany

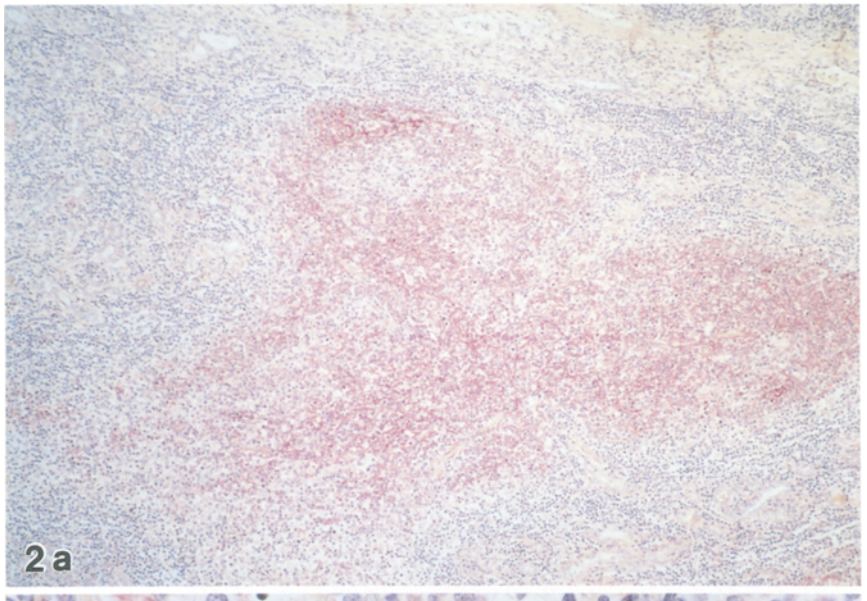
Introduction

Follicular dendritic cells (FDC) play an important role as accessory cells in the immune response. They induce proliferation of B cells by presenting antigen or immune complexes processed on their dendritic network to immunocompetent B cells, resulting in formation of germinal centres (Klaus et al. 1980). FDC patterns and functions in reactive lymphoid tissue have been described in detail in the literature (Van den Oord et al. 1985; Guettier et al. 1986; Hansmann and Wacker 1990). Distinctive FDC patterns have been reported in nodal non-Hodgkin's lymphomas (NHL; Scoazec et al. 1989) as well as in Hodgkin's disease (Alavaikko et al. 1991). In addition, recent cell culture experiments of isolated FDC suggest a nurse function of FDC for lymphoid cells (Tsunoda et al. 1992).

NHL of mucosa-associated lymphoid tissue (MALT) type are defined as morphologically and prognostically distinct entities (Isaacson and Spencer 1987; for review see Isaacson 1990), including NHL of the thyroid gland (Hyjek and Isaacson 1988). NHL of MALT type are frequently associated with chronic inflammation and often exhibit reactive, non-neoplastic lymphoid follicles with germinal centres. In gastrointestinal NHL of MALT type several different patterns of FDC have been described (Tabrizchi et al. 1990). A functional association of autoimmune diseases like Hashimoto's thyroiditis with the presentation of autoantigens by FDC has also been discussed (Imai et al. 1991).

The aim of this study was to examine FDC in thyroid lymphomas of MALT type and compare them with FDC in other extranodal lymphomas (kidney and testis) assumed not to belong to the MALT type.





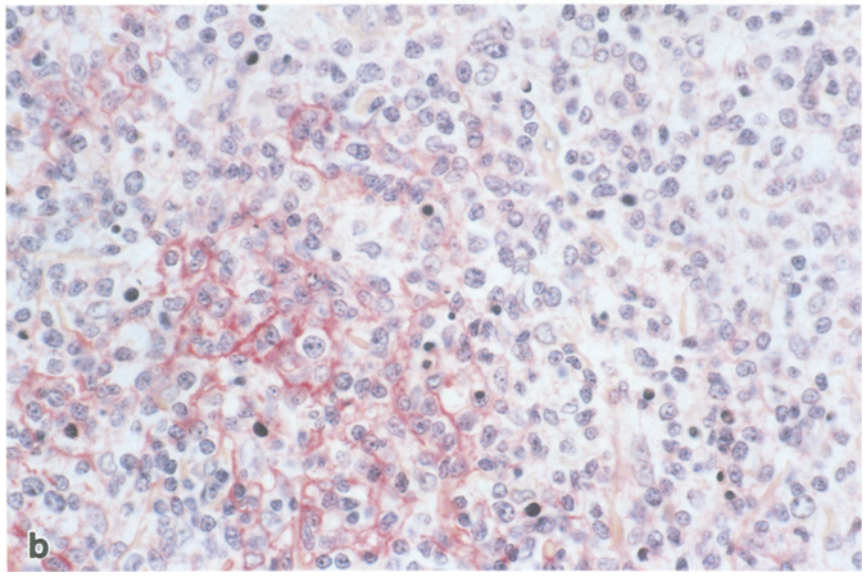


Fig. 1. Low-grade component of malignant thyroid lymphoma of mucosa-associated lymphoid tissue type: abortive follicle type composed of a loose cluster of few follicular dendritic cells intermingled with lymphoid tumour cells having "centrocyte-like" morphology and with a few lymphoid blasts. Ki-FDClP, APAAP method, ×320

Fig. 2a, b. Low-grade component of malignant thyroid lymphoma of mucosa-associated lymphoid tissue type: "destroyed reactive follicle" of follicular dendritic cells, enlarged and with ill-defined borders, invaded by lymphoid tumour cells with "centrocyte-like" morphology. Some small B-cells show a slight co-reaction with the antibody. Ki-FDClP, APAAP method,
a ×63, **b** ×315

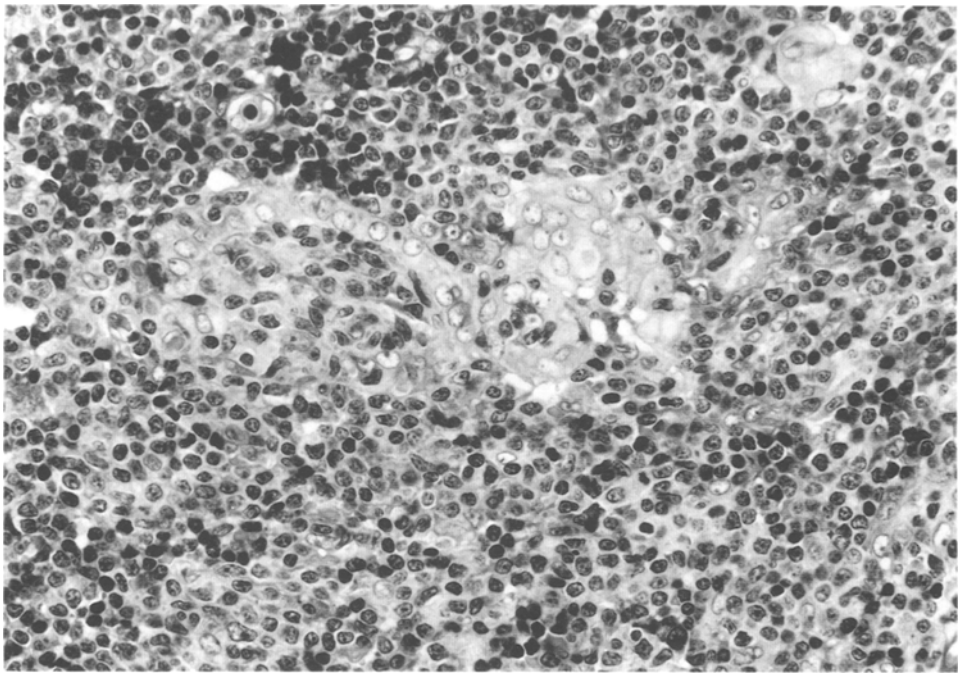


Fig. 3. Lymphoepithelial lesion: thyroid follicles with invasion and destruction of follicular epithelium by centrocyte-like lymphoid tumour cells. Giemsa, ×320

Table 1. Follicular dendritic cells in lymphomas of mucosa-associated lymphoid tissue (MALT) type and non-MALT type

Tumour site/type	*n*	Ki-FDC1P-positive "abortive follicle type"	Ki-FDC1P-positive "reactive destroyed follicle type"	LEL *n*
Thyroid ($n=19$)				
High-grade malignant NHL of MALT-type with evidence of low-grade malignant component	18	6	10	17
IB	1	–	–	–
Kidney ($n=15$)		–	–	–
CLL	2	–	–	–
IC	4	–	–	–
CB	9	–	–	–
				LEL-mimicking lesion
Testis ($n=30$)				
IC	2	–	–	–
CB	27	–	–	6
IB	1	–	–	–

FDC, Follicular dendritic cell; LEL, lymphoepithelial lesion; NHL, non-Hodgkin lymphoma; MALT, mucosa-associated lymphoid tissue; CLL, chronic lymphocytic leukaemia of B-cell type; IC, immunocytoma; CB, centroblastic lymphoma; IB, immunoblastic lymphoma of B-cell type

Materials and methods

Sixty-four cases of extranodal NHL, taken from the files of the lymph node registry at the Institute of Pathology, University of Kiel, Germany, were investigated. These included primary thyroid NHL ($n=19$), NHL of the kidney ($n=15$) and NHL of the testis ($n=30$). No definitive data regarding primary or secondary involvement of the kidney or testis were available.

Tumours were classified according to the updated Kiel classification and the classification of NHL of MALT type (Isaacson et al. 1988; Stansfeld et al. 1988; Lennert and Feller 1992). Paraffin sections were stained with haematoxylin and eosin, Giemsa, periodic acid-Schiff and by silver impregnation (Gomori). Using the alkaline phosphatase-antialkaline phosphatase (APAAP) method (Cordell et al. 1984), FDC were stained with the monoclonal antibody Ki-FDC1P (gift of Prof. Parwaresch, Institute of Pathology, University of Kiel, Kiel, Germany) which recognizes an antigen closely related to CD21 (Tabrizchi et al. 1990). For immunohistochemical staining with Ki-FDC1P, deparaffinized and rehydrated sections were treated with 0.1% pronase (Sigma, Deisenhofen, Germany; pH 7.4; 37° C) for 10 min. The primary antibody Ki-FDC1P was diluted 1:800 in a solution of RPMI (Seromed Biochrom, Ger-

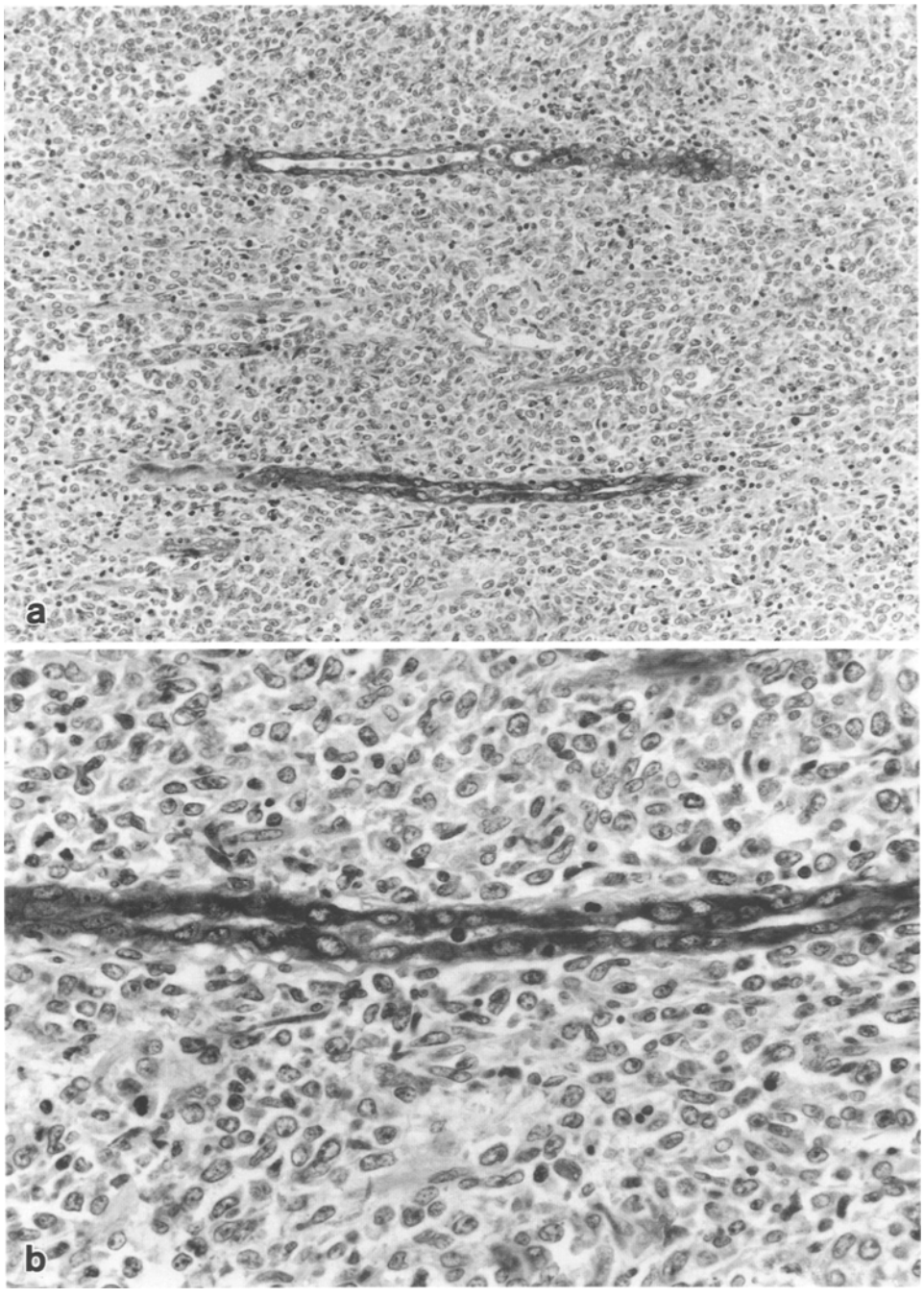


Fig. 4a, b. Centroblastic lymphoma of the kidney: tumour cells surrounding and compressing cytokeratin-positive tubule without invasion or destruction of epithelial layer. Kl-1, APAAP method, **a** ×150, **b** ×320

many), aqua bidest. and bovine serum (Seromed Biochrom) 1:8:1. As secondary antibody we used the rabbit anti-mouse-antibody Z259 (Dakopatts, Hamburg, Germany) 1:20 in RPMI-human serum (RPMI with 10 ml/dl human serum). APAAP complex was produced by adding 10 mg alkaline phosphatase (Sigma) to 1 ml of a mouse anti-alkaline phosphatase antibody (Prof. Parwaresch) and was diluted 1:20 in RPMI-human serum. Incubation steps with secondary antibody and APAAP complex were repeated twice. The enzyme label was developed with hexazotized new fuchsin. Negative controls were performed by omitting the primary antibody.

In addition, the CD20 antibody (L26; Dakopatts), the polyclonal CD3 antibody (Dakopatts) and a marker for keratin (Kl-1; Dianova, Hamburg, Germany) were applied.

Results

The results of the study are summarized in Table 1.

Eighteen cases of **NHL of the thyroid** were classified as high-grade malignant NHL of MALT type with evidence of a low-grade malignant NHL component. The majority of nodular and diffusely arranged tumour cells showed centrocyte-like morphology with small to medium-sized lymphoid cells with an indented or grooved nucleus and sparse to moderate clear cytoplasm. In all of these cases, sheets of lymphoid blasts occurred focally which microscopically filled at least one high power field. In 12 of these cases the sheet-forming blasts resembled centroblasts possessing a round nucleus with sparse

chromatin and multiple small to medium-sized nucleoli located near the nuclear membrane. The blasts in the remaining 6 cases were not classifiable.

Immunohistochemically, FDC were detectable with the monoclonal antibody Ki-FDClP in 10 of the thyroid NHL cases. In general, FDC occurred in areas of low-grade malignancy NHL. Two different patterns of FDC were distinguishable. The first pattern, found in 6 of 10 cases positive for Ki-FDClP, was characterized by small ill-defined loose clusters of up to approximately 10 FDC (so-called abortive follicle type; Fig. 1). In the second pattern, found in all Ki-FDClP-positive cases, FDC were seen in larger groups and resembled reactive follicles, but possessed ill-defined borders where intermingling with the lymphoid centrocyte-like tumour mass occurred (so-called destroyed reactive follicle type; Fig. 2a, b). FDC follicle number varied considerably from case to case. Additionally, a few dense concentrically arranged concentrations of FDC (corresponding to regressively transformed follicle centres) occurred in those cases positive for Ki-FDClP. Some mantle zone cells and a few plasma cells showed a slight positivity with Ki-FDClP. Lymphoid tumour cells showed no pronounced reaction with Ki-FDClP.

In all but 1 case of thyroid NHL of MALT type, centrocyte-like tumour cells showed invasion and destruction of follicular epithelium forming lymphoepithelial lesions (LEL; Fig. 3). In 3 cases, these LEL were detectable only by staining epithelial remnants with the anti-cytokeratin antibody Kl-1.

In the remaining case the tumour consisted of sheets of blasts containing basophilic cytoplasm and large round nuclei with prominent solitary nucleoli. This case was classified as immunoblastic NHL (of B-cell type). FDC were not detectable and no LEL could be demonstrated.

Evidence of Hashimoto's thyroiditis (including focal interstitial lymphocytic infiltrates with reactive germinal centres and oncocytic metaplasia of follicular epithelium) was found in all those cases having preserved, non-neoplastic, tumour-free thyroid tissue areas ($n=9$). Representative areas revealed sharply demarcated follicular networks of FDC. All 9 cases showed NHL of MALT type elsewhere in the thyroid gland. The remaining 10 cases contained no representative preserved thyroid tissue for evaluation.

NHL of the kidney were classified as chronic lymphocytic leukaemia of B-type ($n=2$), immunocytoma ($n=4$) and centroblastic lymphoma ($n=9$). Chronic lymphocytic leukaemia was composed of small lymphocytes with round nuclei and scanty cytoplasm with a few large immunoblast-like cells. Immunocytoma exhibited small lymphocytes and lymphoplasmacytoid cells as well as a few "Dutcher bodies". Centroblastic lymphoma consisted of lymphoid blasts with round or multilobated pale nuclei with several nucleoli. Immunohistochemically, FDC positive for Ki-FDClP were detectable in 2 cases of immunocytoma, forming large networks lacking a distinct follicular pattern.

Tumour cells of all NHL entities displaced parenchyma by separating tubuloglomerular structures without forming LEL (Fig. 4a, b).

NHL of the testis were classified as immunocytoma ($n=2$), centroblastic lymphoma ($n=27$) and immunoblastic lymphoma of B-cell type ($n=1$). In general, NHL showed a diffuse growth pattern with interstitial infiltration dissecting the tubular testicular architecture. In 6 cases of centroblastic NHL, lymphoid blasts invaded ductuli seminiferi without complete destruction of the basal membrane (Fig. 5).

Immunohistochemically, none of the tumours contained FDC positive for Ki-FDClP.

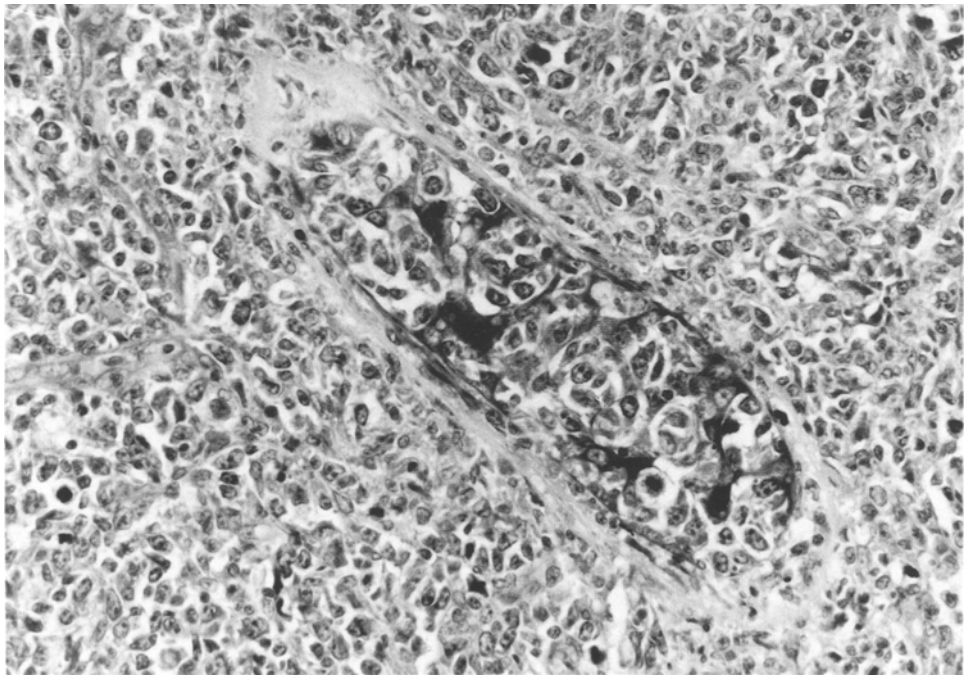


Fig. 5. Centroblastic lymphoma of the testis: tumour cells invading testicular tubule with destruction of seminiferous cell layer by tumour cells, leaving the basal membrane mostly intact. Kl-1, APAAP method, ×360

Discussion

The vast majority of thyroid NHL investigated in this study were of MALT type as defined by Hyjek and Isaacson (1988), including the occurrence of centrocyte-like cells and LEL. In addition, all the cases with sufficient residual lymphoid tissue revealed histological indications of Hashimoto's thyroiditis, in accordance with the studies of Woolner et al. (1966), Burke et al. (1977) and Aozasa (1990). Hyjek and Isaacson (1988) describe a continuous morphological development to high-grade malignant NHL of MALT type. All our cases showed frank malignant lymphoma. Surprisingly, in all cases, the morphological criteria of high-grade malignancy were fulfilled focally.

FDC occurring in low-grade malignant areas showed two patterns, corresponding to the findings in gastrointestinal lymphomas of MALT type (Tabrizchi et al. 1990): small "abortive FDC follicles" and large disrupted "reactive" FDC follicles. The latter corresponds to the second type of follicle reported by Isaacson et al. (1992). This "reactive" follicle type represents a preexisting reactive lymphoid follicle colonized by lymphoid tumour cells, as recently confirmed by Isaacson et al. (1992), who found no hint of chromosomal translocation t(14;18) in these areas, supporting the inclusion of primary thyroid lymphomas into the MALT category and arguing against a follicular centre cell lineage. The similarities of FDC patterns seen in NHL of MALT type in the thyroid and in the gastrointestinal tract (Tabrizchi et al. 1990) and the observation of thyroidal MALT type lymphomas spreading to other sites of MALT (such as the gastrointestinal tract; Anscombe and Wright 1985) may lead to speculation of a possible functional role of FDC in B-cell homing of MALT (Moore and Wright 1984). However, no conclusions on function or kinetics can be drawn from our study.

In 8 of our cases of thyroid lymphomas of MALT type, no FDC were detectable. It is likely that follicular structures were completely destroyed by tumour cells in these cases. This finding may argue against a possible neogenesis of FDC follicles within the tumour. FDC-negative cases may also represent case heterogeneity; for example this may be due to different pathogenetic mechanisms in FDC-positive and FDC-negative cases. However, one should also consider that lack of detectable FDC networks may be due to technical problems of antigen preservation in formalin-fixed tissue.

LEL were detectable in all except one of our cases of thyroid lymphomas of MALT type. However, their detection should not lead to an overdiagnosis of malignant lymphoma of the thyroid, since LEL of centrocyte-like cells are also found in reactive states like Hashimoto's thyroiditis (Hyjek and Isaacson 1988; Isaacson 1990; Matias-Guiu and Esquius 1991). In the cases we examined, malignancy of the lymphoid tumour was associated with focal detection of sheets of monomorphic lymphoid blasts. Within these blastic areas no FDC were detectable, thus further excluding a misinterpretation of these high-grade malignant foci as, for example, reactive germinal centre areas.

Primary lymphomas of the kidney are extremely rare and are the subject of few studies (Falconieri and Melato 1987; Osborne et al. 1987; Leoncini et al. 1988). Most cases of renal NHL are reported as secondary involvement of primary nodal NHL (Richards et al. 1990), occurring in up to 42% of patients with malignant nodal lymphoma (Martinez-Maldonado and De Arellano 1966). Lymphomatous involvement represents the third most common cause of renal involvement by metastatic disease after lung and breast cancer (Deuskar et al. 1987). No association of NHL of the kidney and the category of NHL of MALT type is apparent, as primary NHL or as secondary involvement. Two cases of renal involvement by immunocytoma revealed diffusely arranged FDC, but no distinct follicular FDC pattern was found.

In testicular NHL no FDC could be demonstrated. Of the 30 cases of testicular NHL included in this study, 28 were of high-grade malignancy, comprising 27 centroblastic lymphomas and 1 immunoblastic lymphoma of B-cell type. This result is in agreement with previous studies, which report the majority of testicular lymphomas to be centroblastic NHL (Wilkins et al. 1989) or "histiocytic type" according to the Rappaport classification, corresponding to centroblastic and immunoblastic NHL in the Kiel classification (Sussman et al. 1977; Paladugu et al. 1980; Turner et al. 1981; Doll and Weiss 1986; Nonomura et al. 1989). In 6 of our cases of centroblastic NHL, invasion of seminiferous tubules by blastic lymphoid tumour cells resulted in a lesion mimicking the tumour growth pattern in LEL of MALT type lymphoma. A tubulo-invasive tumour growth pattern has been observed by Paladugu et al. (1980), Turner et al. (1981) and Doll and Weiss (1986). Wilkins et al. (1989) found this pattern to be associated with primary testicular lymphomas (in contrast to a predominantly interstitial pattern in cases of secondary involvement). However, in our cases the basal membrane seems to be mostly intact – a finding which suggests invasion of ductuli seminiferi from the site of the lumen and is in contrast to true LEL as found in the thyroid cases. Moreover, the cells invading the testicular tubule wall are blasts: This is in contrast to predominantly small "cytic" lymphoid cells in the LEL of lymphomas of MALT type (Moore and Wright 1984), which were not found in our testicular cases. The lack of a low-grade malignant component of testicular NHL with morphological features of NHL of MALT type, such as centrocyte-like cells and the lack of FDC pattern as described for lymphomas of MALT type, suggest that testicular lymphomas are not lymphomas of MALT type.

In summary, distinct patterns of FDC, namely the "abortive follicle type" and the "destroyed reactive follicle type", occurred only in NHL of MALT type and not in NHL of non-MALT type in our series.

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