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CASE REPORT

Christine Beham-Schmid · A. Beham · R. Jakse
L.Auböck · G. Höfler

Extranodal follicular dendritic cell tumour of the nasopharynx

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Abstract We report the first case of an extranodal follicular dendritic cell (FDC) tumour localized in the nasopharynx of a 44-year-old male patient. The tumour cells were characterized immunohistochemically by strong expression of CD21, HLA-DR and vimentin and focal expression of CD68 and cytokeratin. Electron microscopic examination revealed desmosomal cell junctions between adjacent cell processes. Molecular genetic analysis using polymerase chain reaction (PCR) showed germline configuration of immunoglobulin and T-cell receptor genes. Epstein-Barr virus (EBV) genomes were detectable by PCR. After complete surgical tumour removal and radiotherapy the patient is disease-free 20 months after the initial diagnosis.

Key words Follicular dendritic cell tumour · Nasopharynx · Immunohistochemistry · Electron microscopy · PCR

Introduction

Follicular dendritic cell (FDC) tumours are extremely rare neoplasms of the antigen-presenting cells of the B-cell follicles of lymphoid organs [21]. Although FDCs were identified as early as in the 1960s as the cells responsible for follicular antigen capture [17], their more detailed characterization has followed only in the past 15 years [3]. However, some controversies remain concerning their ontogeny and immunophenotype [5, 20, 22, 24, 26, 27]. It has recently been shown that FDCs, classically thought to be cells with long, branching cell processes that form desmosomal attachments with one another [6], reveal heterogeneous morphology and immunophenotype dependent both on their localization within the follicle and on the stage of germinal centre reaction [12, 19, 20]. So far only 36 well-documented FDC tumours have been described in the literature. Most of these neoplasms have occurred in lymph nodes [1, 7, 16, 21, 31], and only 14 cases have been reported in extranodal sites. Since extranodally located pre-existent or acquired B-cell follicles contain FDCs, the occurrence of extranodal FDC tumours is hardly astonishing. Of these extranodal cases, 7 were located in the oral cavity [1, 4, 15, 21], 3 in the neck [4], 2 in the gastrointestinal tract [8], 1 in the spleen [21] and 1 in the liver [28].

This report, to our knowledge, documents the first case of FDC tumour localized in the nasopharynx, the diagnosis of which was confirmed by both immunohistochemistry and electron microscopy. The aim of reporting this case was – in addition to further characterization of FDC tumours – to broaden the clinicopathological spectrum and to show up its morphologic and immunophenotypic heterogeneity compared with other FDC tumours.

C. Beham-Schmid (✉) · A. Beham · L.Auböck · G.Höfler
Institute of Pathology, University of Graz Medical School, Auenbruggerplatz 25, A-8036 Graz, Austria,
Tel.: (+43) 316-380-4406; Fax: (+43) 316-384329

R. Jakse
Department of ENT, Krankenhaus der Elisabethinen, Graz, Austria

Case report

In December 1995 a 44-year-old male patient consulted an ENT specialist because of recurrent nasal stuffiness, bloody nasal secretion and occlusion of the right ear for 2 years. A biopsy of a clinically evident tumour in the nasopharynx, measuring 2 cm in its greatest diameter, revealed the histopathological diagnosis of a tumour of FDCs. The patient was admitted to hospital in a good general condition. Diagnostic endoscopy of the nose and computer tomographic examination of the paranasal sinuses showed a tumourous expansion measuring 2 cm in its greatest diameter, extending from the cavum nasi to the right side of the nasopharynx into the fossa of Rosenmüller (Fig. 1a). The base of the sphenoid bone was intact and not infiltrated. The tumour was removed surgically. Postoperative local radiation therapy was given. The patient has been carefully monitored and is now – 20 months after tumour removal – free of recurrence and of systemic tumour spread.

Materials and methods

The majority of the material obtained by surgery was fixed in 10% formaldehyde solution, paraffin embedded and processed conven-

Table 1 List of antibodies used for immunohistochemical cell characterization

Antibody	Source	Type	Comment
CD20 (L26)	Dakopatts	Monoclonal	B cells
CD21	Dakopatts	Monoclonal	FDC,some B cells
CD79a (HM57)	Dakopatts	Monoclonal	B cells
CD3	Dakopatts	polyclonal	Peripheral T cells
CD45RO(UCHL1)	Dakopatts	Monoclonal	T cells,some B cells
CD45RB (PD7/26)	Dakopatts	Monoclonal	Panleukocytes
CD30(BerH2)	Dakopatts	Monoclonal	Activated lymphocytes
CD15(DakoM1)	Dakopatts	Monoclonal	Hapten-X
CD31	Dakopatts	Monoclonal	Endothelium, monocytes
CD34	Serotec	Monoclonal	Endothelium, stem cells
CD68	Dakopatts	Monoclonal	Macrophages
HLA-DR	Dakopatts	Monoclonal	MHC class II
SMA	Sigma	Monoclonal	Smooth muscle actin
Desmin	Dakopatts	Monoclonal	Muscle differentiation
HHF-35	Dakopatts	Monoclonal	Muscle specific actin
S-100 protein	Dakopatts	Polyclonal	IDC
Pankeratin	Boehringer Mannheim	Monoclonal	Keratin
EMA	Dakopatts	Monoclonal	Epithelial membrane antigen
Vimentin	Euro Diagnostic	Polyclonal	Mesenchymal cells
EBV (LMP CS 1–4)	Dakopatts	Monoclonal	Epstein Barr virus protein
MIB-1	Dianova	Monoclonal	Proliferating cells

(*IDC* interdigitating reticulum cells, *FDC* follicular dendritic cells)

tionally. Consecutive 4-µm sections were stained with haematoxylin and eosin (H&E).

Sections were immunostained with the antibodies listed in Table 1 using the streptavidin–biotin complex method, with prior trypsinization or microwave treatment as required. For control purposes, tissues known to contain the respective antigens were included (positive controls). Replacement of the primary antibody by normal serum always led to negative results (negative controls).

For electron microscopy, tissue was also fixed in 2.5% glutaraldehyde and processed according to standard procedures. Semithin and ultra-thin sections were cut with a Reichert OM U4 ultracut microtome. The ultra-thin sections were stained with uranylacetate and lead citrate and examined at 80 kV on a Philips EM 400 electron microscope.

Genomic DNA was prepared from formalin-fixed paraffin-embedded tissue specimens essentially as described elsewhere [23]. The immunoglobulin heavy chain gene was analysed according to Wan et al [30] with minor modifications. For the first PCR 1 µg of template DNA was used in a 50-µl PCR reaction containing dNTPs (0.8 mM), primers Fr3A and LJH (1 µM each), $MgCl_2$ (7.5 mM), KCl (50 mM), Tris-HCl pH 8.3 (10 mM) and 0.5 U Taq polymerase (Perkin Elmer Cetus). Thirty cycles of the reaction were carried out (denaturation, 94°C, 60 s; annealing, 50°C, 60 s; extension, 72°C, 110 s) followed by a 10-min final extension step at 72°C. A second PCR was performed out using 1 µl from the first PCR as template in 50 µl of the same buffer, except that primer VLJH was used instead of LJH and the $MgCl_2$ concentration was 1.5 mM. Twenty cycles were carried out, using the same temperature profile as in the first PCR. The T-cell receptor gamma gene was analysed according to McCarty et al. [14] with minor modifications. DNA amplification was carried out in the same buffer as above, except that the concentration of $MgCl_2$ was 1.5 mM and 1 U of Taq polymerase was used. Primer concentration was 0.5 µM for primers A and B and 1 mM for primer C (denaturation, 94°C, 60 s; annealing, 55°C, 90 s; extension, 72°C, 110 s). Forty cycles were carried out with an annealing temperature of 55°C. PCR products were analysed on 6% polyacrylamide vertical gel electrophoresis.

Detection of the EBNA-1 region of the EB virus was carried out essentially as already described [13]. A 200-bp stretch was amplified in a 50 µl reaction using primers (5' ACG AGG GGC CAG GTA CAG GA 3',5' CAC CAT CTC TAT GTC TTG GC 3') 1 µM (each), 10 mM Tris-HCL pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1.6 mM dNTP. Then 2.5 U Taq polymerase (Perkin Elmer Cetus) was added after an initial denaturation for 5 min at 94°C. Thirty-five cycles of denaturation, 94°C, 60 s; annealing, 55°C, 90 s; extension, 72°C, 110 s were carried out followed by a 10-min terminal extension at 72°C. Products were analysed on 3% agarose gels (NuSieve, FMC/SeaKem, FMC 2:1).

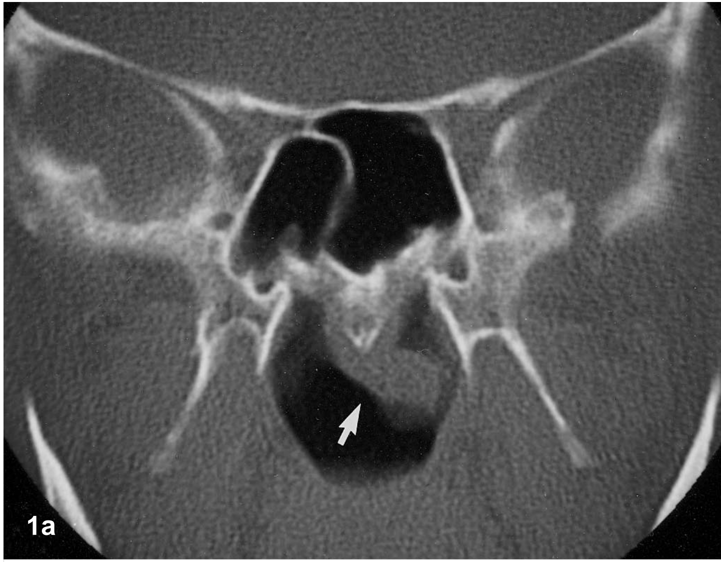
Results

Low-power examination revealed a cellular, fairly well-circumscribed tumour with occasional necrotic areas, partly covered by respiratory or squamous epithelium (Fig. 1b). Two main morphological patterns were formed by the tumour. The minor component had tumour cells with spindle cell differentiation; in this part the tumour cells were arranged haphazardly or in a focal storiform pattern. They were elongated, with rather pale eosinophilic cytoplasm and plump ovoid or fusiform nuclei with one or more inconspicuous nucleoli (Fig. 2a). The major part consisted of sheets of large cells with round nuclei and indistinct cell borders forming a syncytial growth pattern (Fig. 2b). In both areas a variable fibrous stroma was present with the focal occurrence of multinucleated giant cells. Especially in the neighbourhood of such giant cells, mitotic figures were frequent (up to 5 mitoses per high-power field; Fig. 2c). Between the tumour cells numerous small lymphocytes, occasionally forming variably sized lymphoid aggregates, plasma cells and macrophages could be seen.

Fig. 1 **a** Computer tomographic examination showing a tumour (*arrow*) extending from the cavum nasi to the right side of the nasopharynx. **b** The tumour is covered by respiratory or squamous epithelium. H&E, ×25 ▶

Fig. 2 Area **a** with mainly spindle cell differentiation and **b** with syncytial growth pattern. H&E, ×400. **c** Tumour giant cell and neighbouring mitosis. H&E, ×1,000

Fig. 3 **a** Strong immunoreactivity of tumour cells with an antibody to CD21. **b** Cytokeratin-positive tumour cells showing fine granular reactivity. Streptavidin–biotin complex method, ×1,000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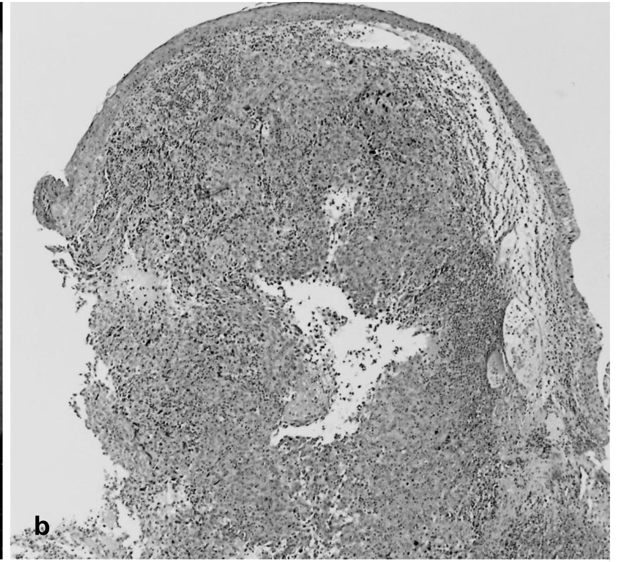
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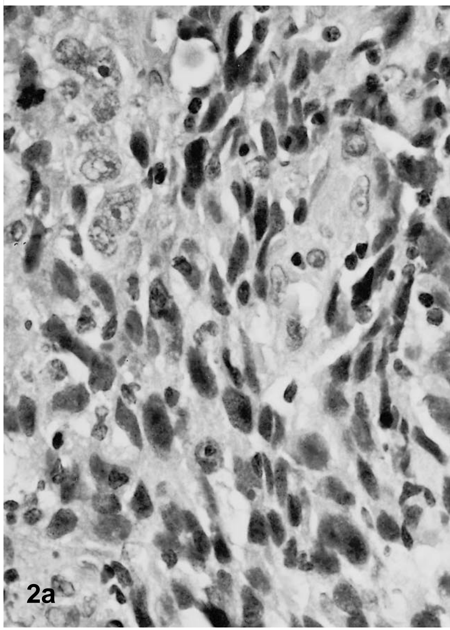
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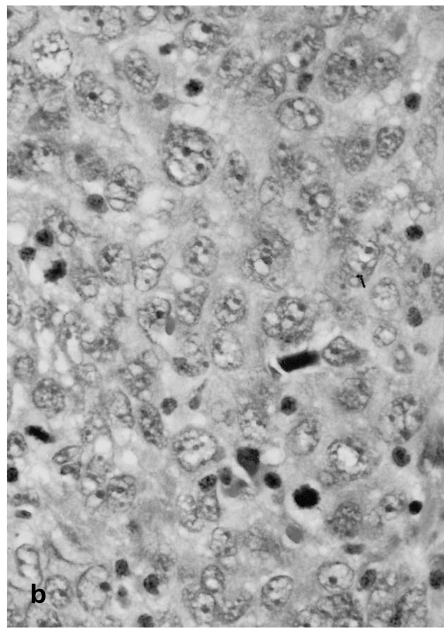
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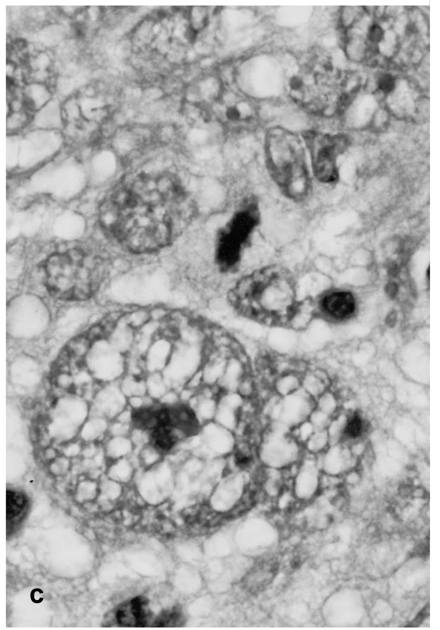
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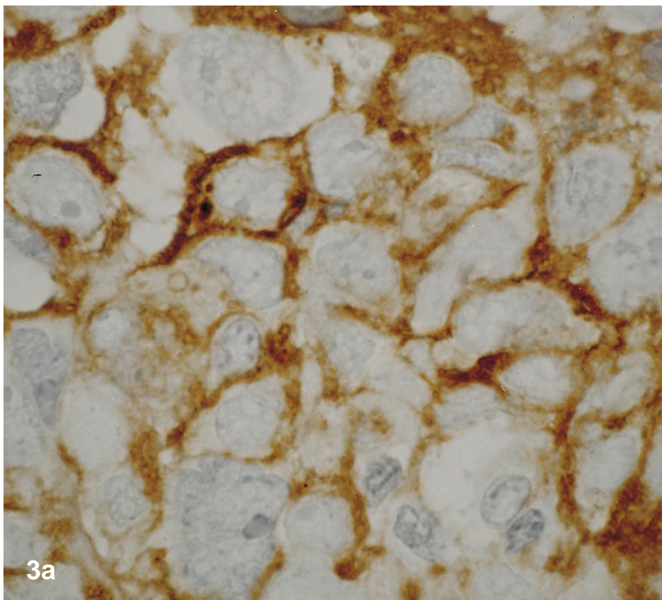
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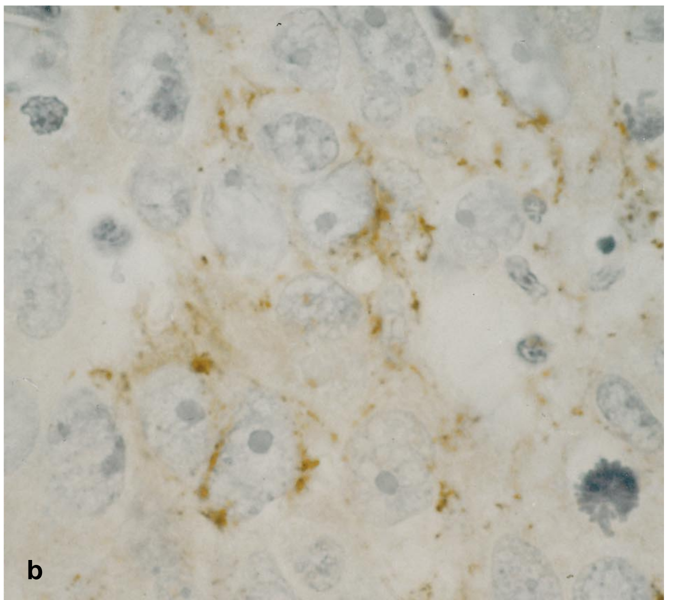
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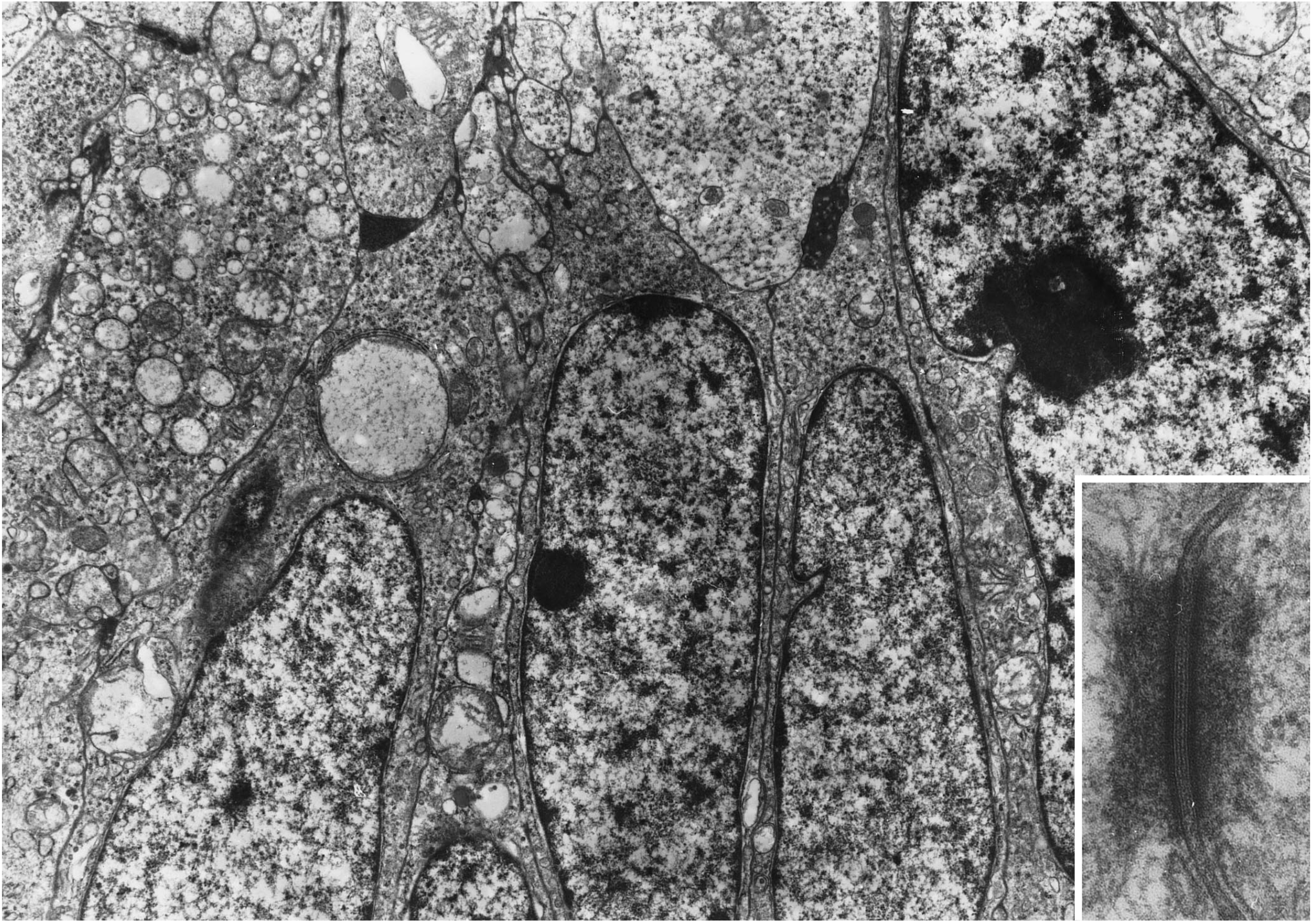

Fig. 4 Electron microscopy showing tumour cells with elongated nuclei and prominent nucleoli. ×7200. *Inset* reveals desmosomal cell junctions. ×98,000

The majority of tumour cells in all areas revealed strong membrane staining for CD21 and, to a lesser extent, cytoplasmic staining (Fig. 3a). A strong cytoplasmic reactivity was seen using vimentin antibodies. Most tumour cells showed predominantly membrane staining for HLA-DR. Occasional cells were cytoplasmically CD68 reactive and a few (less than 10%) were cytokeratin positive, and could be seen in a fine granular cytoplasmic and/or membranous distribution pattern (Fig. 3b). The proliferation fraction as assessed by MIB-1 was 40%. There was no tumour cell expression of any other antigens. The vast majority of the infiltrating small lymphocytes were T cells (CD3 and CD45RO positive).

Ultrastructural studies showed tumour cells containing round to oval euchromatic nuclei (Fig. 4). Cytoplasmic organelles included scattered polyribosomes and ribosomes, poorly developed rough endoplasmic reticulum, scattered mitochondria and an inconspicuous Golgi apparatus. Lysosomes were scanty. There were no tonofilaments, basal lamina, melanosomes, pinocytotic vesicles or filaments with focal densities. There were numerous thin long interwoven cell processes arising from the cell body. Cell junctions of desmosomal type were found between adjacent cell processes (Fig. 4, inset).

Gene rearrangement studies using PCR to determine the status of the immunoglobulin heavy chain gene and the gamma-T-cell receptor gene showed a germline configuration, providing no evidence for specific monoclonal B- or T-lymphocyte differentiation. DNA fragments with a molecular weight typical for EBV were detected after gel electrophoresis of PCR products obtained as described in ”Materials and methods”.

Discussion

Tumours of FDC appear to be very rare, although the occurrence of such tumours was predicted by Lennert [11] nearly 20 years ago. It is our impression, apparently shared by others [1, 7, 8], that this perception of rarity is exaggerated by underrecognition of the entity. However, since the characterization of normal FDC began only relatively recently and there are still many aspects of their immunohistochemical profile and ontogeny unresolved, the non-recognition of FDC tumours is not surprising. They may also be difficult to diagnose, since recent studies revealed morphological [24] and immunophenotypic [12] heterogeneity in normal FDC, which might cause diagnostic problems in recognizing characteristics of

FDC differentiation. FDC are an absolute requirement for germinal centres of B cell follicles, which are present in lymph nodes but also extranodally, either as acquired lymphoid tissue or as part of the organized constitutive lymphoid tissue. Thus, it is reasonable that tumours with FDC differentiation arise at extranodal locations. Consequently, 14 extranodal cases of FDC tumours have been reported so far [1, 4, 8, 15, 21, 28]. This report documents an additional extranodal FDC tumour, the first case localized in the nasopharynx.

Recognition of these tumours is of great clinical importance, as they behave in an indolent manner. Only 1 of 14 previously reported extranodal cases has pursued an aggressive course with intraperitoneal dissemination [8], and of the cases localized in lymph nodes only a low percentage developed local recurrence; only 1 has developed a distant metastasis [7, 16, 21, 31]. The histological differential diagnosis includes malignant tumours with much more aggressive behaviour, such as synovial sarcoma, malignant melanoma, undifferentiated carcinoma and, occasionally, malignant lymphoma with spindled appearance, which is mostly secondary to distortion of the tumour cells by sclerosis, rather than the intrinsic growth pattern of lymphoma cells. For reliable diagnosis of FDC tumours corroborative ultrastructural and/or immunophenotypic evidence of FDC differentiation is required. To date, FDC tumours have been treated with local excision, in some cases followed by radio- or chemotherapy. Recurrences of these tumours have been independent of the type of therapy received.

There is general consensus on the expression of some antigens by normal and malignant FDC tumours. Thus, the expression of complement receptors CR1 (CD35) and CR2 (CD21), applicable in paraffin sections, is good evidence of FDC differentiation. However, it must be taken into consideration that CD21 is not lineage specific for FDC but also detects some B-cells. Therefore negativity for B-cell markers (e.g. CD20) is indicative for FDC. Despite the fact that the expression of CD45RB (LCA) and HLA-DR by normal FDC remains controversial and tumours of FDC have shown variable positivity, these two antigens are described as useful in supporting the diagnosis of FDC sarcoma [8]. HLA-DR expression has been used to underline a bone marrow origin of FDC, since expression of MHC class II molecules is usually restricted to bone marrow-derived cells. Most studies, including ours, have shown this HLA-DR expression [20, 22, 26, 27] and few have refuted it [10, 25]. CD45RB (leucocyte common antigen; LCA) expression by FDC tumours is discordant with some cases showing LCA positivity [28, 29] and others [19], including ours, revealing negative results.

In accordance with previous reports on FDC tumours, the tumour cells in our case were uniformly negative for both B- and T-cell antigens. As shown previously [7, 28], most reactive intermingled lymphocytes were T cells.

Inconsistent staining of FDC tumours has been reported with histiocytic markers such as CD68 [1, 15, 19, 28, 31], which was shown to stain some tumour cells in our case. In contrast to other FDC tumours, the tumour cells in our case were not reactive with muscle-specific actin [1, 2] and CD30 [18] or S-100 protein, which has been reported to be positive in a few cases [21]. In contrast to our case, aberrant EMA – but not cytokeratin expression – has been demonstrated in some extranodal FDC tumours [8]. We found granular cytokeratin staining in a few tumour cells, most probably reflecting the ultrastructural findings concerning desmosomal structures. In concordance with genotypic studies performed previously on three cases [28, 31], immunoglobulin and T-cell receptor genes in our case were in germline configuration.

Although we were not able to show EBV-positive cells by immunohistochemistry, molecular genetic analysis, a much more sensitive technique, confirmed EBV positivity, a gene expression that was also detected in an FDC tumour of the liver [28]. EBV positivity in our case might suggest involvement of this virus in the pathogenesis of the tumour. However, there is the possibility that the presence of EBV is merely a ”bystander” effect, since PCR does not determine the cells that contain the EBV genome. Therefore, it is conceivable that the EBV-containing cells in this case are intermingled lymphocytes, especially as the anatomical region of the nasopharynx is a major site of EBV infection. This is evident from the frequency of EBV in the presence of malignant lymphomas of the upper respiratory tract [9].

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