

Burkitt lymphoma with unusual granulomatous reaction. A case report

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SUMMARY

Formation of epithelioid histiocytic cell granulomas has been described in the past in various neoplasms, hematologic malignancies included. Among lymphoproliferative disorders such changes are commonly found in Hodgkin lymphoma and T-cell non-Hodgkin lymphomas (NHL), but are rarely described in B-NHL, like Burkitt lymphoma. This report presents a case of sporadic Burkitt lymphoma accompanied by a sarcoid-like reaction without any clinical, laboratory or histological evidence of microorganisms nor sarcoidosis. Using *in situ* hybridization and polymerase chain reaction the presence of the Epstein-Barr virus (EBV) was detected in the analyzed lymphoma cells. EBV demonstrated latency I phenotype as defined by the lack of immunohistochemical positivity of latent membrane protein 1 (LMP1). Cytogenetic investigation using fluorescence *in situ* hybridization uncovered c-MYC mutation and provided indirect indication for the MYC/IgI fusion gene. The lack of EBV positivity in histiocytes indicated the reactive character of the granulomatous reaction in relation to the neoplasm. The role of the granulomatous reaction in the biology and prognosis of Burkitt lymphoma and the function of EBV infection in its development remain to be established.

Key words: Burkitt lymphoma - granulomatous reaction - Epstein-Barr virus - EBV

Burkittov lymfóm s nezvyčajnou granulomatóznou reakciou. Kazuistika

SÚHRN

Výskyt granulomatózne reakcie sprevádzajúcej rôzne druhy nádorov bol už v minulosti opísaný. Spomedzi lymfoproliferatívnych chorôb, vykazuje tvorbu granulómov predovšetkým Hodgkinov lymfóm a T-bunkové non-Hodkinove lymfómy (NHL). V B-bunkových NHL, ako je Burkittov lymfóm, sú granulomatózne reakcie zriedkavé. V tejto práci predstavujeme prípad sporadického Burkittovho lymfómu sprevádzaného tvorbou granulómov pripomínajúcich sarkoidózu. Klinické, laboratórne, ani histologické vyšetrenie nepreukázalo prítomnosť infekčných agens, resp. nesvedčilo pre diagnózu sarkoidózy. *In situ* hybridizáciou a polymerázovou reťazovou reakciou sa v tkanive dokázala prítomnosť Epstein-Barrovej vírusu (EBV). Negativita imunohistochemicky stanovovaného vírusového latentného membránového proteínu 1 (LMP1) spolu s predchádzajúcimi vyšetreniami svedčí v tomto prípade pre typ I latentnej EBV infekcie. Cytogenetická analýza s použitím fluorescenčnej *in situ* hybridizácie odhalila mutáciu génu c-MYC spolu s naznačenou fúziou MYC/IgI. Absencia EBV v histiocytoch podporuje skôr reaktívny charakter granulomatózne reakcie na nádorový proces. Biologický a prognostický význam vzniku týchto granulómov sprevádzajúcich Burkittov lymfóm a úloha EBV infekcie v tomto vzťahu zostáva však nejasná.

Kľúčové slová: Burkittov lymfóm – granulomatózna reakcia - Epstein-Barrovej vírus - EBV

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Tumor-related tissue reactions as the formation of epithelioid cell granulomas have been reported in association with several neoplasms (1). Such a sarcoid-like reaction may occur in solid tumors, particularly keratinizing squamous cell carcinoma and seminoma (2). In sarcomas the presence of such histological changes is extremely rare (3). Formation of granulomas has been also described in hematologic malignancies (1), mainly in Hodgkin lymphomas. The incidence of granulomatous reaction in patients with Hodgkin disease is reported to be 13.8% (4), whereas the frequency in non-Hodgkin lymphomas (NHL) ranges from 3.6% (5) to

7.3% (3). From the latest group, sarcoid-like granulomas most commonly occur in T-cell-derived non-Hodgkin lymphomas (6), including a marked number of cutaneous lymphomas like mycosis fungoides and subcutaneous panniculitis-like T-cell lymphoma. Epithelioid cell granulomas are less frequent in B-cell NHL. They have been reported in follicular center cells lymphomas, small lymphocytic lymphomas, large cell lymphomas (5) and in a few cases of sporadic Burkitt lymphoma (6-8). The appearance of these coincidental granulomas has been described in regional lymph nodes either involved or uninvolved by the tumour, in sites of distant metastases and in non-involved organs, respectively (2).

The presented paper reports a case of a patient with sporadic Burkitt lymphoma accompanied by a sarcoid-like reaction, which emerged in lymph nodes nearby the ligamentum hepatoduodenale.

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MATERIALS AND METHODS

The patient, a 58-year old woman, presented with an accidental finding of a retroperitoneal mass on abdominal CT. The clinical preliminary diagnosis of a benign soft tissue tumour was established. To specify the process an open biopsy of the lymph nodes from the area of the ligamentum hepatoduodenale was performed.

Biopsy samples were formalin-fixed and routinely processed for histological evaluation. Beside routine hematoxylin and eosin staining, Ziehl-Neelsen, Gram and Giemsa staining and the periodic acid-Schiff (PAS) reaction, as well as immunohistochemical staining for CD20 (DakoCytomation, USA), CD45RO (DakoCytomation, USA), CD3 (DakoCytomation, USA), CD10 (DakoCytomation, USA), Bcl-6 (DakoCytomation, USA), Bcl-2 (DakoCytomation, USA), CD68 (DakoCytomation, USA) and Ki-67 (DakoCytomation, USA) were performed. For EBV detection immunostaining with antibodies against viral latent membrane protein (LMP) from two different manufacturers (DakoCytomation, USA; Labvision, USA), as well as *in situ* hybridization with the fluorescein-conjugated EBV Probe ISH Kit (Vector Laboratories, USA) and polymerase chain reaction were used. DNA iso-

lation for PCR was achieved from a FFPE sample with a QIAamp DNA FFPE Tissue Kit (Qiagen, USA). Isolated DNA and primers for the EBNA1 EBV gene providing a 110bp product were used for PCR, followed by gel electrophoresis in a 2% agarose gel. Fluorescence *in situ* hybridization with the use of MYC (8q24) and IgL (22q11) DNA split signal probes were used for rearrangement detection.

RESULTS

The hematoxylin and eosin stained sections uncovered in the fat tissue embedded lymph nodes with architecture almost completely effaced by a monotonous infiltrate of medium-sized lymphoid cells spreading beyond the borders of the lymph nodes. The infiltrating lymphocytes had scant basophilic cytoplasm, round nuclei with clumped chromatin and conspicuous nucleoli. The mitotic activity of these cells was high (6-13/HPF). Scattered macrophages between the neoplastic cells formed a typical starry sky pattern. The lymphoma infiltrate was surrounded or interspersed by an extensive histiocytic infiltrate partly forming epithelioid granulomas containing large multinucleated Touton cells with foamy cytoplasm and areas of non-caseous necrosis present mainly in the lymphocytic infiltrates (Fig.1). No microorganisms were detected by Ziehl-Neelsen, Gram and Giemsa staining and the PAS reaction.

Immunophenotype of the neoplastic lymphocytes was positive for CD20, CD10 and Bcl-6, negative for Bcl-2 and 95% of cells were positive for Ki-67. Small lymphocytes scattered at the edge of the CD20 positive cell groups, showed CD45RO and CD3 positivity. Epithelioid and giant cells in the lesion were stained positively with anti-CD68 antibody.

The neoplastic cells showed no immunoreactivity for viral LMP. EBV infected cells were identified by *in situ* hybridization, demonstrating positivity of virus-encoded small nuclear non-polyadenylated RNAs (EBER). Burkitt lymphoma cells showed a nuclear positivity pattern in neoplastic lymphocytes, the other cells, including histiocytes, were negative (Fig.2). PCR analysis detected EBNA 1 gene products in the samples of the tumor.

Fluorescence *in situ* hybridization with the use of MYC DNA split signal probe identified c-myc rearrangement in the locus (8q24). IgH/myc fusion corresponding to the translocation t(8;14) was negative. The IgL (22q11) split signal probe detected rearrangement of the gene and provided indirect proof for IgL/MYC t(8;12) translocation associated with Burkitt lymphoma.

DISCUSSION

Burkitt lymphoma is an aggressive mature B-cell non-Hodgkin lymphoma, which follows a rapid clinical course and can be fatal within months if left untreated (9). Early assessment of the correct diagnosis is crucial for disease management. The presence of a histiocytic granulomatous reaction, particularly if it is extensive, could cause a diagnostic dilemma by obscuring the underlying malignant process (10,11). Therefore, it is important to be aware of this phe-

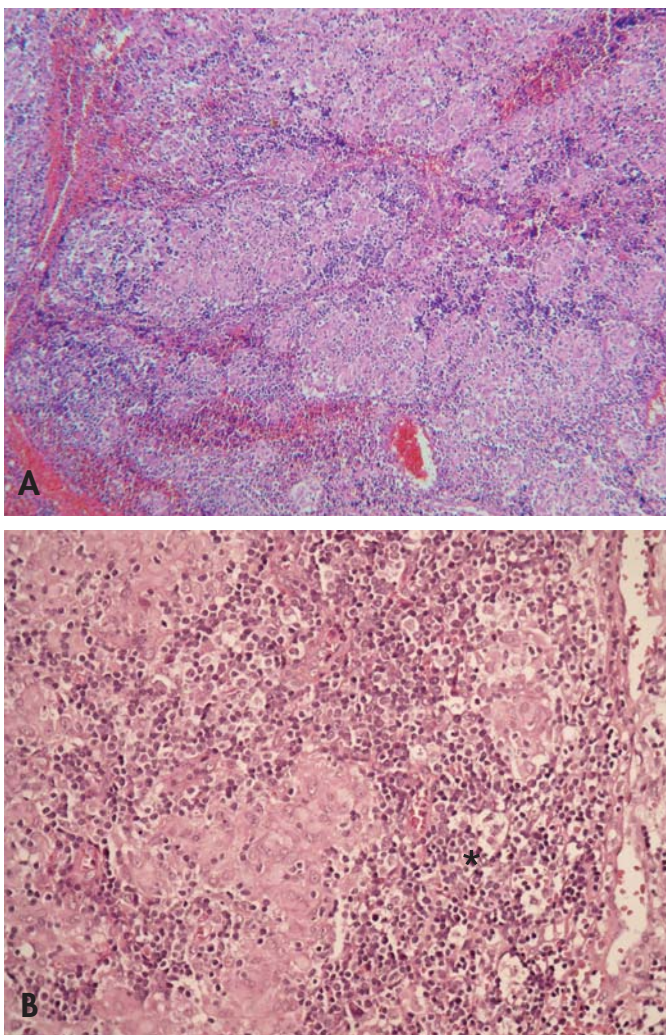


Figure 1. Histological picture of the tumour composed prevalently of granulomatous histiocytic reaction (A) with capture of the borderline between Burkitt lymphoma (*) and the histiocytic infiltrate (B). HE, 100x (a), 200x (b).

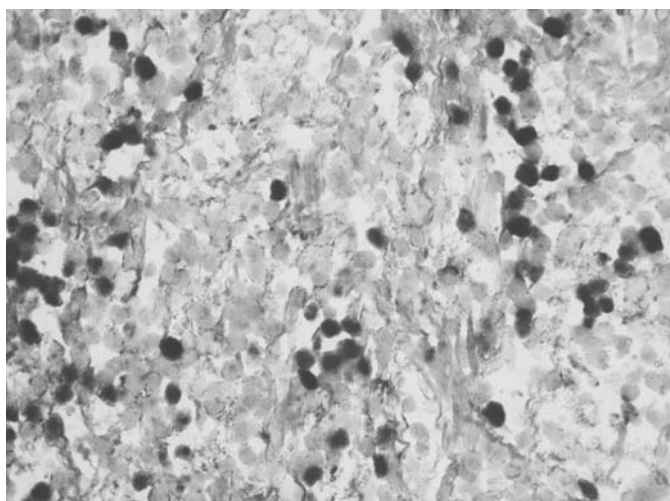


Figure 2. In situ hybridization reaction with EBER showing positivity for EBV presence in the nuclei of neoplastic lymphocytes (dark) and negative nuclei of histiocytes and other cell types. 400x.

nomenon, to prevent false diagnostic conclusions and delayed treatment (12). Correlation of the clinical findings with the results of histological examination and the exclusion of an infectious process are essential for establishing the correct diagnosis (13).

Problems may also arise in distinguishing between tumor-related sarcoid reactions and true systemic sarcoidosis (3). However, sarcoid-like granulomas are usually accompanied by no clinical symptoms of sarcoidosis; to rule out this diagnosis it is necessary to confront the results of appropriate clinical, biochemical and radiological examination with the histological findings (5).

An interesting aspect for discussion represents the possible prognostic significance of such granulomatous reactions. Chopra et al. (14) studied the prognostic value of epithelioid granulomas in association with Hodgkin disease. Comparing cases of Hodgkin lymphoma with and without a granulomatous reaction indicated that the group with granulomas showed a tendency towards better survival with a lesser number of relapses and longer remissions. A favorable prognosis in Hodgkin patients with epithelioid granulomas was also reported by other authors (3,15,16). In patients with Burkitt lymphoma this relationship is not clear at present. However, recent studies suggest an association between the granulomatous response and the favorable outcome in cases of sporadic Burkitt lymphoma (6,8). Burkitt lymphoma is known by a sudden onset and a rapid progression (9), which is in contrast with the clinical course in the presented patient suggesting at first a benign process. This indulgent behavior of the tumor could result from the suppressive effect of the granulomatous inflammatory reaction.

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The relationship between epithelioid granulomas and the underlying neoplastic process is at present not fully understood. Considering the localisation of the affected lymph nodes in the drainage area of the biliary tract, this phenomenon could represent a collision between reactive and neoplastic changes or the occurrence of malignancy in a terrain of granulomatous lymphadenopathy, respectively. Some histological features like a close spatial relation and the positivity of lymphoid antigens in the necrotic areas let us suggest, that the granulomatous reaction is related to the tumor. Some authors propound a defensive host response to the malignancy (4,17) or a reaction to the presence of necrotic and poorly viable parts of the tumor (7) as the most probable cause for this tissue reaction. Antigenic factors of the neoplastic cells could initiate a hypersensitivity reaction leading to the formation of epithelioid granulomas (3). This possible immunologically evoked anti-tumor effect would correspond with an improved prognosis of the patient in these cases (4). In Hodgkin disease and T-cell-derived non-Hodgkin lymphomas it is believed that the granulomatous reaction could be induced by aberrant cytokine production in the tumour cells or other cells forming the tumour background (6,18). Other authors consider the possible role of Epstein-Barr virus products in the development of epithelioid granulomas in Burkitt lymphoma (6). Several reports describe the development of EBV-associated granulomatous reactions in patients with infectious mononucleosis (19), systemic granulomatous arteritis (20), granulomatous pneumonitis (21) or bone marrow fibrin-ring granulomas (22). EBV was also causally associated with lethal midline granuloma, a subtype of nasal NK/T-NHL (23).

In the presented case of Burkitt lymphoma, EBV infection was detected by *in situ* hybridization and a PCR reaction. EBV-infected cells showed the gene expression pattern of latency type I infection, or resting state, defined by the absence of immunohistochemical LMP positivity and the presence of EBNA-1 (EBV nuclear antigen 1) gene fragment and small non-polyadenylated nuclear RNAs (EBER)(9). A similar expression pattern of EBV products was observed by Schrager et al. (8). In our case EBER positivity was detected only in tumor cells without any expression in histiocytes forming the epithelioid granulomas. This implies that the primary cause of this tissue reaction is not the infection itself and that the granulomatous reaction is related to the tumor.

As already discussed, the presence of epithelioid granulomas has been described in several neoplasms (1), including hematologic malignancies (24). There are only a few reports of granulomatous reactions in cases of Burkitt lymphoma. The exact cause and the role of these granulomas in the biology and prognosis of Burkitt lymphoma remain to be established.

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