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Pheno- and Genotypic Features of Epstein-Barr Virus Associated B-Cell Lymphoproliferations in Peripheral T-Cell Lymphomas

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Abstract Among the 300 peripheral T-cell lymphomas (PTCL) searched for EBV positive non-resting B-cells by EBER in situ hybridization 12 have been identified with various forms of EBV-driven B-cell proliferation. This could be categorized into three major forms. *i.* In the first form scattered immature, mononuclear B-cells of immuno-, centroblastic type with CD20+. CD30+ CD45+, LMP1+ phenotype, reactive appearance and polyclonal immunoglobulin heavy chains gene rearrangement (IgH-R) were admixed to the PTCL cells. *ii.* The second form mimicked diffuse large B-cell lymphoma as homogenous sheets, largely demarcated from the PTCL, of mononuclear, immature B-cell of CD20+, CD30+, CD45+, LMP1+, EBNA-2+ phenotype but with lack of monoclonal IgH-R were present. *iii.* In the third form scattered Hodgkin-Reed-Sternberg (HRS) type of cells were noticed which exhibited the CD15+/−, CD20−/+, CD30+, CD45−, LMP1+, EBNA-2- phenotype and in 50% showed clonal IgH gene rearrangement in whole tissue DNA extract. The IgH associated transcription factors' (OCT2, BOB.1/OBF.1, PU.1) expression patterns in these cells corresponded to those of HRS cells in cHL. Based on analysis of 65 PTCLs, we have identified in the positive cases a highly significant increase of EBV+ small, reactive, resting B-cell compartment (75.9 / 100 HPF in PTCL vs. 1.5 / 100 HPF in control lymph nodes) likely to be due to the decreased immune surveillance. This progressive accumulation of EBV+ bystander B-cell population in PTCLs might be the source of various B-cell proliferations, which in any form represent major diagnostic pitfalls and require a careful differential diagnostic procedure.

Keywords B-cell lymphoproliferation · Epstein-Barr virus · T-cell lymphoma

Abbreviations

CDR	complementary determining region
CHL	classical Hodgkin lymphoma
DLBCL	diffuse large B-cell lymphoma
EBER	Epstein Barr early response gene
EBNA	Epstein Barr nuclear antigen
EBV	Epstein Barr virus
FR	framework region
HPF	high power field
HRS	Hodgkin Reed Sternberg
IgH-R	immunoglobulin heavy chain gene rearrangement
LBC	large B-cell
LBCR-TCL	large B-cell rich T-cell lymphoma
LMP-1	latent membrane protein-1
NOS	not otherwise specified
PCR	polymerase chain reaction
PTCL	peripheral T-cell lymphoma
PTCL-LB	PTCL with proliferation of large B-cells

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TCR-BCL	T-cell rich B-cell lymphoma
TCR-γ-R	T-cell receptor gamma gene rearrangement
TF	transcription factor
TL	T-cell lymphoma

Introduction

Peripheral T-cell lymphoma (PTCL) can display not only a morphological, but also an immunophenotypical overlap with classical Hodgkin lymphoma (cHL) as PTCL may contain CD30 and CD15 as well as T-marker positive Hodgkin-Reed-Sternberg (HRS) cells of cHL type in a mixed inflammatory background of eosinophils, lymphocytes, histiocytes and plasma cells [1]. The distinction between PTCL and cHL is further complicated by the rare variant of PTCL resembling cHL because of the presence of HRS type of cells, which are constantly Epstein-Barr virus (EBV) and CD30 positive. Single cell molecular analysis of these cells indicated B-cell genotype, but controversy regarding clonality at the immunoglobulin heavy chain gene rearrangement (IgH-R) [2, 3]. PTCL morphology might be complicated by admixed EBV positive scattered immunoblastic type of cells or homogeneous sheets of EBV positive immature B-cells, making the differential diagnostics between PTCL and T-cell rich B-cell lymphoma (TCR-BCL) as well as PTCL composite with either cHL or diffuse large B-cell lymphoma (DLBCL) demanding [4–6].

Because of these special constellations and at least partially unclear pathogenesis, we have investigated a series of three hundred PTCLs for EBV+ non-T-cell proliferation with special emphasis on the frequency of this phenomenon, the growth pattern, phenotype as well as the immunoglobulin heavy chain gene rearrangement (IgH-R) of the the EBV+ B-cells. We have also analyzed the number of EBV positive non-proliferating, resting small B-cells in PTCLs as compared with that in reactive lymph nodes to see if expansion of the EBV+ by-stander memory B-cells accompanies development of PTCLs.

Materials and Methods

Histology and Immunohistology Tissue specimens of altogether 365 T-cell lymphomas and 20 reactive lymph nodes were fixed by 4% formaldehyde in phosphate buffered saline (pH 7.2) for 24 h and embedded into paraplast using standard techniques. Three cases have already been published from other aspects by us [3]. Four-micrometer-thick sections were stained for hematoxylin-eosin, methylated-Giemsa and periodic acid-Schiff. For immunohistology CD3, CD4, CD5, CD10, CD20, OCT2 primary monoclonal antibodies were obtained from Novocastra Laboratories Ltd. (Newcastle, UK), CD8, CD15, CD21, CD30, CD45, UCHL-1, CD79a, CD138, ALK-1, granzyme-B, BCL-6, EBV related latent membrane protein-1 (LMP-1), Epstein-Barr nuclear antigen-2 (EBNA-2), kappa and lambda light chains from DakoCytomation (Glostrup, Denmark), T-cell intracellular antigen-1 (TIA-1) from Immunotech (Marseille, France), PU.1 from BD Biosciences Pharmingen (Heidelberg, Germany) and OBF.1/BOB.1 from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Target retrieval was adjusted for the individual antibodies. The reactions were developed by either iView Detection Kit (Ventana Medical System Inc., Tucson, AZ, USA) or LSAB-2-System-HRP as well as EnVision using 3,3′-diaminobenzidine (all three from DakoCytomation) or occasionally 3-amino-9-ethylcarbazole (Vector Laboratories Inc., Burlingame, CA, USA) as chromogens in Ventana NEXES 5.1 (Ventana Medical Systems Inc.) or Dako Autostainer (DakoCytomation). Light microscopical images were acquired using a Nikon Eclipse E400 microscope and a Nikon Coolpix 4500 camera. Only adjustment of contrast, when needed, was made using the Adobe Photoshop 5.0 software.

EBER In Situ Hybridization (ISH) on Slides Epstein-Barr virus encoded RNA (EBER) were detected using Epstein-Barr virus probe (Novocastra Laboratories Ltd.) on paraffin sections according to the manufacturers' instructions except that microwave pre-treatment (3×5 min, 900 W in citrate buffer, pH 6.0) was used instead of proteinase K digestion [7]. The hybridization product was detected by the Amplification kit (Novocastra Laboratories Ltd.) for EBER using Fast Red TR Salt (Sigma-Aldrich Corp., St. Louis, Missouri, USA) as chromogenic substrate.

DNA Extraction Genomic DNA was extracted from one or two 10 μm thick formol-paraffin sections. The sections were deparaffinized, the remaining tissue pellet was digested in 100–200 μl digestion buffer (10 mM Tris-HCl / pH: 8.3/, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Triton X-100, 0.45% Tween-20) containing 200 μg/ml proteinase K (Sigma-Aldrich Corp.) at 56°C, overnight. After digestion, proteinase K was inactivated by incubating at 95°C for 10 min, the digest was pelleted and the supernatant as crude lysate was directly used for PCR amplification.

T-Cell Receptor Gamma (TCR-γ) Gene Rearrangement Analysis Monoclonal TCR-γ gene rearrangements were identified with multiplex PCR using V and J primers as described [8]. The polymerase chain reaction was accomplished in the presence of 200 μM dNTPs (Invitrogen, San Diego, CA, U.S.A), 50 mM KCl, 10 mM Tris-HCl (pH:

8,3), 1,5 mM $MgCl_2$, 20 pmol of each primers and 1,25 U Taq DNA polymerase (Sigma-Aldrich Corp.). After initial denaturation at 94°C for 5 min the mixture was subjected to 40 cycles of 40 s at 94°C, 60 s at 61°C, 60 s at 72°C followed by final elongation at 72°C for 5 min in a thermocycler (MiniCycler, MJ Research, Watertown, Massachusetts, USA). PCR product was size fractionated on 10 % polyacrylamide and / or 2% agarose gel by electrophoresis and visualized with 0,5 μg/ml ethidium-bromide.

Immunoglobulin Heavy Chain Gene Rearrangement (IgH-R) PCR amplification was done as described with slight modifications [9]. The CDR III region of the rearranged IgH gene was amplified using consensus V_H (5′- CTG TCG ACA CGG CCG TGT ATT ACT G –3′) and J_H (5′- AAC TGC AGA GGA GAC GGT GAC C –3′) primers. Genomic DNA was amplified in the presence of 200 μM dNTPs (Invitrogen), 50 mM KCl, 10 mM Tris-HCl (pH: 8,3), 1.5 mM $MgCl_2$, 20 pmol of each primers and 1.25 U Taq DNA polymerase (Sigma-Aldrich Corp.). The mixture was subjected to 40 cycles of 40 s at 94°C, 30 s at 55°C, 40 s at 72°C in a thermocycler (MiniCycler, MJ Research). PCR product was run in 10% polyacrylamide and / or 2% agarose gel by electrophoresis and visualized with 0.5 μg/ml ethidium-bromide. Using this method the amplicons range from 100 to 150 base pairs in molecular weight.

Results

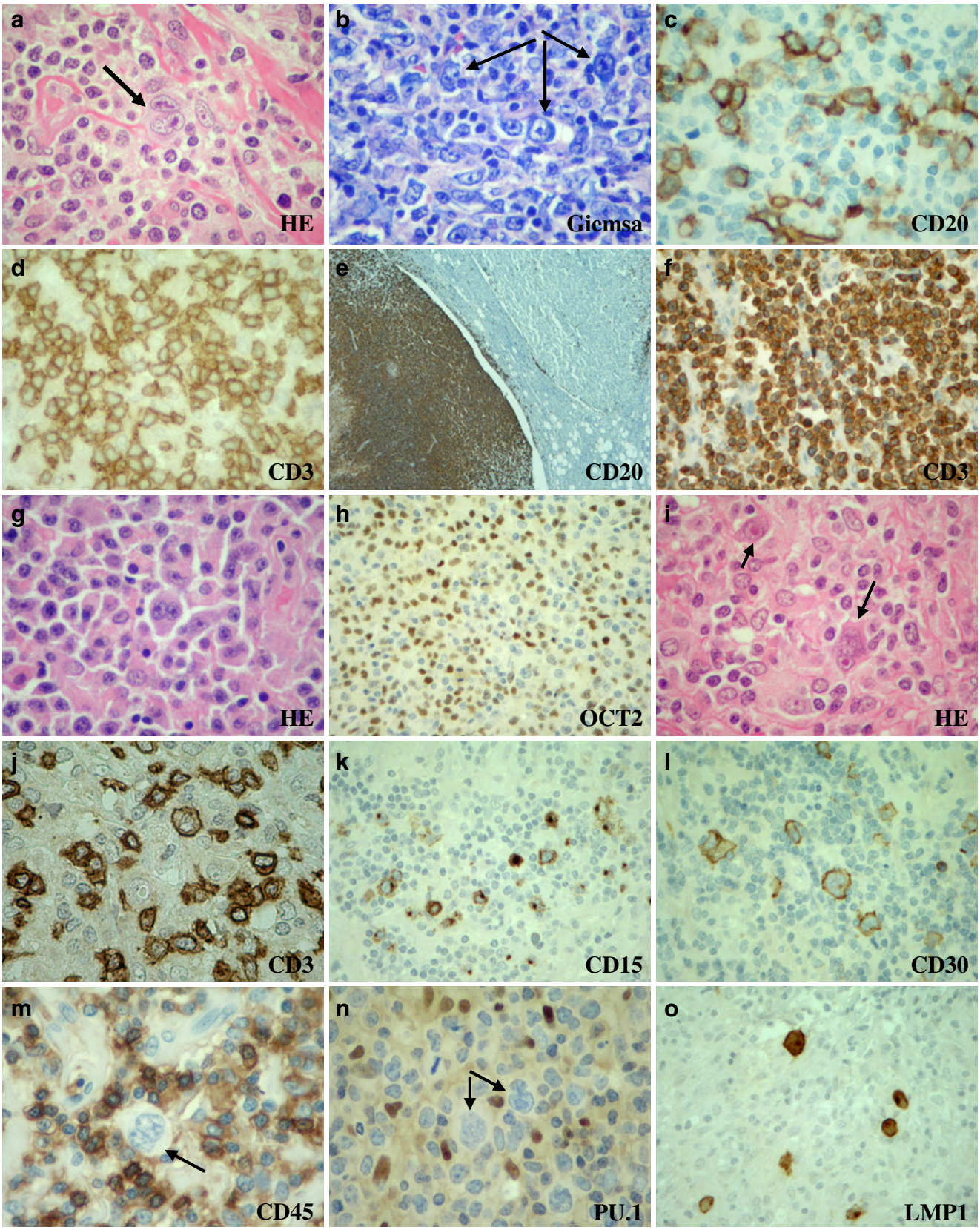
Among the three hundred T cell lymphomas twelve featured various type of large cell proliferation with nuclear EBER positivity. Out of these twelve cases one corresponded to angioimmunoblastic T-cell lymphoma (AIL) and eleven fell into the peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS). The cytological atypia ranged from moderate to pronounced, occasionally with granulomatous–inflammatory background reaction. Phenotypically the T-cell lymphoma cells expressed CD3 in all the 12 cases and, when investigated in 10 of the 12 cases, at least one or two additional T-cell marker positivity could be detected. In 9 out of 12 cases the TCR-γ gene rearrangement PCR test indicated monoclonality, in the remaining three cases the amplification was unsuccessful most likely to be due to the poor quality of the particulate region of genomic DNA extracted from formol-paraffin blocks.

The common features of the large cells, which could or could not be distinguished from the underlying T-cell lymphoma on morphological ground, were the EBER nuclear positivity, the combined cytoplasmic and membranous LMP-1 and CD30 positivity as well as lack of T-cell marker expression investigated. Without EBV directed ISH and immunohistochemistry, these large cells in the T-cell lymphoma with significant cytological atypia could be easily mistaken as part of the T-cell lymphoma proliferation. On the other hand, in those PTCL-NOS with moderate cytological atypia, the process was reminiscent in various extend to classical Hodgkin lymphoma (Fig. 1a-b). According to the growth pattern, cytology and immunophenotype of the EBER positive large cells the twelve cases could be classified into three groups (Table 1).

Three cases belonged into *group 1* hallmarked by scattered large and immature cells with rather immunoblastic / centroblastic appearance without overt cytological atypia, bi- or multinucleation and inclusion-like large nucleoli, respectively (Fig. 1b–d). In addition to the common phenotypic features as above, large cells in all these cases exhibited the CD15−, CD20+, CD45+ phenotype and—whenever it was available—expressed the IgH associated transcription factors (IgH-TF) analyzed. EBNA-2 nuclear positivity could be detected in a small fraction (20%) of cells in 1 out of the 3 cases. The IgH-R PCR assay performed on whole tissue DNA extract proved to be polyclonal in 2 out of the 3 cases, in 1 case the assay was unsuccessful.

Group 2 was represented by 1 case in which the large immature EBER+, LMP-1+, CD30+, T-cell marker negative cells did not differ cytologically from those in group 1, but instead of scattered distribution, they formed homogeneous sheets without being admixed to, but sharply demarcated from the PTCL cells (Fig. 1e–g). This arrangement imparted a tumor (DLBCL)-like growth pattern to the lesion. Other phenotypic features were also the same as those of large cells in group 1, i.e., they had the CD15−, CD20+, CD45+ phenotype, expressed the IgH-TFs, but, as a distinctive feature, they homogeneously expressed EBNA-2 EBV-related protein (Fig. 1h). As a molecular hallmark, IgH-R PCR assay in DNA extracted from 5 different blocks of the lymph node, all of them harboring the large cell, EBER+ lesion, could not reveal monoclonality at this level.

Group 3 included 8 cases and characterized by scattered large, T-cell marker negative cells, which, however, exhibited definitive cytological atypia, multinucleation and large, inclusion-like, eosinophilic nucleoli were common findings. Thus morphologically these cells were largely consistent with Hodgkin-Reed-Sternberg (HRS) cells of cHL (Fig. 1i–o). These cells were not specifically located in a granulomatous microenvironment with inflammatory background; instead they were studded as isolated cells among the T-cell lymphoma elements. In all but one cases the HRS-like cells were negative for CD45 and did not express at least one out of the IgH-TFs investigated. In fact, BOB.1, Oct-2 and PU.1 could not be detected in 7/8,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◀ **Fig. 1** **a** HRS-type of giant cell (arrow) among the homogeneous proliferation of small to medium sized T-cells with broad, clear cytoplasm representing atypia (400×, case N° 10); **b**–**d** the large immunoblastic and centroblastic type of cells (arrows) are not sharply deliniated from the background T-lymphoma cells with agressive cytology, but CD20 stain (**c**) clearly show that these scattered activated cells are B-cell in the sheets of medium sized, atypical CD3+ T-lymphoma cells (**d**) (all 400×, case N° 2); **e**–**h** a diffuse, large B-cell lymphoma type of CD20+ B-cell proliferation, sharply demarcated from the surrounding CD3+ T-lymphoma cells with mild atypia (**f**), with immunoblastic—plasmoblastic morphology (**g**) and nuclear OCT-2 expression (**h**) (20× – 200× – 400× – 200×, case N° 4); **i**–**o** classical Hodgkin lymphoma-like morphology with scattered HRS-type of cells (arows) admixed with medium sized, clearly atypical, CD3+ T-lymphoma cells, the large HRS-type cells exhibit the CD3−, CD15+, CD30+, CD45− (arrow), PU.1− (arrows), LMP-1+ phenotype (**k** and **l**: 200×, others: 400×, case N° 9)

3/8 and 4/8 cases, respectively. In 2 out of 8 cases two TFs, in another 2 all three TFs were absent. In 6 out of 7 cases the CD45− / at least 1 TF-, whereas in 4 out of 7 ones the CD15+ / CD45− / at least 1 TF- phenotype for the HRS-like cells could be determined. All four cases with monoclonal IgH-R fell into group 3 and all four corresponded to lymph node lesions having HRS-like cells with the last two phenotype.

A separate 65 TLs without CD30 and EBER co-expressing large cells have been reviewed for the presence and number of EBER+, CD30−, LMP-1-, EBNA-2- small lymphocytes with reactive appearance and data have been compared with those obtained from 20 reactive lymph nodes. The EBER+ small resting lymphocytes could be identified in 15 T-cell lymphoma (23 %) with and average number of 75,9 / 100 high power field (HPF) (range: 1–300) contrasting with the figure of 1.5 / HPF (range: 1–2) obtained in 11 positive, reactive lymph node out of 20 ones.

Discussion

Pathomorphology of PTCLs especially with minimal to moderate cytological atypia and with scattered large cells usually cause significant diagnostic difficulties and require a careful differential diagnostics. Because of the frequent mixed inflammatory background and / or granulomatous nature not only cHL, but also TCR-BCL or lymphomatoid granulomatosis need to be considered. In any suspected atypia of the overwhelming T-cells in the background of large, immature mono- or multinucleated cells, first, meticulous scrutiny for cytological derangement, search for an aberrant T-phenotype (absence of any physiological T-cell marker or homogeneous helper / cytotoxic phenotype), increased mitotic activity or high proliferation rate are required. By this approach the T-cell lymphoma can be reliably diagnosed and confirmed by the monoclonality of the TCR-γ rearrangement. Then the nature of the large cells need to be clarified which might be troublesome as PTCLs with scattered or diffuse large cells expressing CD15 and CD30 have also been described [1]. In those cases, however, the large cells always expressed at least one T-cell markers and atypical T-phenotype being concordant to that of the background T-lymphoma cells, furthermore, they proved to be negative for EBV. If the large cells in a TL are consistently negative for several T-cells markers, but positive for EBV, an accompanying or secondary B-cell proliferation might be suspected, which have been described with phenotypic heterogeneity and some controversy regarding the genotypic features [2, 3, 6, 10, 11]. Quintanilla-Martinez et al (1999) analyzed three such cases and found for the scattered large cells either an activated B-cell (CD15−, CD30+, CD20+) or cHL-HRS cell (CD15+, CD30+, CD20+) phenotype, polyclonal / oligoclonal IgH-R in microdissected cells and no progression to disseminated cHL. EBNA-2 and CD45 have not been investigated [2]. Higgins et al (2000) coined the name of large B-cell rich T-cell lymphoma (LBCR-TCL) for the PTCLs with large B-cells and could detect in 1 out 9 such cases monoclonal IgH-R [10]. Unless there are overt morphological features of a B-cell lymphoma, Reichard et al (2006) suggested reporting such cases as PTCL complicated with proliferation of large B-cells (PTCL-LB) with the clonality analysis included [11]. Zettl et al (2002) found 17 among 600 TLs with coexisting or subsequent EBV+ B-cells lymphoproliferation [6]. They observed subsequently developing EBV+ DLBCL (4 patients), 3 PTCL with coexisting EBV+ DLBCL and 10 T-cell lymphoma with scattered large immunoblastic-type cells, occasionally with HRS cell morphology and monoclonality, but in all cases a CD15−, CD45+ phenotype for the large, EBV+ B-cells

In the present study, we have identified 12 cases, among three hundred TLs, with EBV-driven B-lymphoproliferation, which were grouped into three according to the cytology and growth pattern. The lesional cells in group 1 and 2 represent those, which have already been described in the papers as above, and the phenotype was also consistent with that of an activated B-cell one. The only one case in group 2 differed from those in group 1 in growth pattern, but—despite the homogenous sheets and well demarcated nature of the large B-cells (LBC)- lack of overt cytological atypia and monoclonal IgH-R during extensive molecular sampling were against a DLBCL-type or proliferation. Although, an activated B-cell cytology and phenotype were noticed in all four cases in group 1 and 2, LBCs displayed only in one case, and fractionally in another one, the lymphoblastoid latency III profile by expressing EBNA-2, in addition to LMP-1. This phenomenon can be hardly explained on the basis of the currently available knowledge regarding EBV latency profiling in the course of virus—host interaction [12–14].

Table 1 Pheno- and genotypic features of the peripheral T-cell lymphomas with large B-cell (LBC) proliferation classified into three groups

	Patient N°	T-cell lymphoma type	Morphology of LBSs	Immunophenotype of LBCs							Molecular findings		EBV status		
				CD15	CD20	CD30	CD45	Bob-1	Oct-2	PU.1	TCR-y	IGH	EBER	LMP-1	EBNA-2
Group 1	1.	PTCL-NOS	activated B-cell	−	+	+	+	n.d.[a]	n.d.	n.d.	M[c]	P[d]	+	+	−/+
	2.	PTCL-NOS	activated B-cell	−	+	+	+	+	+	+	M	P	+	+	−
	3.	PTCL-NOS	activated B-cell	n.d.	+	−	+	n.d.	n.d.	n.d.	M	n.d.	+	+	n.d.
Group 2	4.	PTCL-NOS	activated B-cell	−	+	+	+	−/+	+	n.d.	M	P	+	+	+
Group 3	5.	PTCL-NOS	HRS	−	−	+	−	n.d.	n.d.	n.d.	M	P	+	+	−
	6.	PTCL-NOS	HRS	−	−	+	−	−	+	n.d.	M	M	+	+	−
	7.	PTCL-NOS	HRS	+	−	+	−	−	−	+	u.a[b]	M	+	+	−
	8.	PTCL-NOS	HRS	+	+	+	−	−	+	+	M	M	+	+	−
	9.	PTCL-NOS	HRS	+	+/−	+	−	−	−	−	M	P	+	+	−
	10.	PTCL-NOS	HRS	+	−	+	−	−	−	−	u.a.	M	+	+	−
	11.	AIL	HRS	−	+	+	+	−	+/−	−	u.a.	P	+	+	−
	12.	PTCL-NOS	HRS	−	+	+	−	+	+	−	M	P	+	+	−

[a] not done

[b] unsuccessful amplification

[c] monoclonal

[d] polyclonal

In group 3 we have identified HRS-like morphology and a unique phenotype for the EBV+ large cells. The CD15+/−, CD20−/+, CD30+, CD45−, TF- expression pattern and the clonal IgH-R+/− genotype in close to 90% of these cases fully fits to the pheno- and genotypic features of the HRS cells in cHL [15, 16]. This phenotype under these circumstances—CD45, TF and EBNA-2 negativity beside CD30, LMP-1 positivity being the most important—has not been described yet in the literature available for us, in fact CD45 expression data were not always available and the IgH-TFs have not been investigated at all in the referred papers. Our observations—together with previous result on the molecular characteristics of FRI–CDRIII region in the IgH gene of sorted LBCs in such a case—imply that EBV infected LBCs indistinguishable in morphology, pheno- and genotype from those of HRS cells in cHL may also arise in TLs [3]. These cases should not be called TL composite with cHL as the unique, reactive cellular background of cHL was missing. We believe, however, that these HRS-like cells are, in fact, HRS cells and serve as precursor elements of a subsequently developing cHL, whenever it may occur. One of our patient (N° 8) whose lymph node biopsy displayed the features as in group 3, but another lymph node in a different region of the still untreated patient exhibited PTCL composite with a spatially separated cHL might be a direct evidence for such pathogenetic pathway.

Finally, we can confirm other authors' data that the number of small EBV+ lymphocytes in PTCLs is significantly higher in comparison with that in reactive lymph nodes and this might indicate that the PTCL related immunosuppression is behind the accumulation of EBV+ cells [4, 17, 18]. Our data indicate that these cells may develop upon reactivation from latency [14] into various directions: polyclonal immunoblastic proliferation, DLBCL but also into cHL precursor tumor cells.

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References

1. Barry ST, Jaffe SE, Sorbara L et al (2003) Peripheral T-cell lymphomas expressing CD30 and CD15. Am J Surg Pathol 27:1513–1521
2. Quintanilla-Martinez L, Fend F, Rodriguez Moguel L et al (1999) Peripheral T-cell lymphoma with Reed-Sternberg-like cell of B-cell phenotype and genotype associated with Epstein-Barr virus infection. Am J Surg Pathol 23:1233–1240
3. Pajor L, Kajtár B, Jáksó P et al (2006) Epstein-Barr virus-induced B-cell proliferation of Hodgkin's and Reed-Sternberg cell pheno- and genotype may develop in peripheral T-cell lymphomas. Histopathology 49:553–557
4. Ho WYJ, Ho CSF, Chan CLA et al (1998) Frequent detection of Epstein-Barr virus-infected B cells in peripheral T-cell lymphomas. J Pathol 185:79–85
5. Niedobitek G, Baumann I, Brabletz T et al (2000) Hodgkin's disease and peripheral T-cell lymphoma: composite lymphoma with evidence of Epstein-Barr virus infection. J Pathol 191:394–399
6. Zettl A, Lee S-S, Rüdiger T et al (2002) Epstein-Barr virus-associated B-cell lymphoproliferative disorders in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified. Am J Clin Pathol 117:368–379
7. Tornoczky T, Kelényi G, Pajor L et al (1998) EBER oligonucleotide RNA in situ hybridization in EBV associated neoplasms. Patol Oncol Res 4:201–205
8. Trainor KJ, Brisco MJ, Wan JH et al (1991) Gene rearrangement in B- and T-lymphoproliferative disease detected by the polymerase chain reaction. Blood 78:192–196
9. Pajor L, Matolcsy A, Vass JA et al (1998) Phenotypic and genotypic analyses of blastic cell population suggest that pure B-lymphoblastic leukaemia may arise from myelodysplastic syndrome. Leuk Res 22:13–17
10. Higgins PTJ, Rijn M, Jones DC et al (2000) Peripheral T-cell lymphoma complicated by a proliferation of large B cells. Am J Clin Pathol 114:236–247
11. Reichard KK, Schwartz JE, Higgins PJ et al (2006) CD10 expression in peripheral T-cell lymphomas complicated by a proliferation of large B-cells. Modern Pathol 19:337–343
12. Thorley-Lawson AD, Gross A (2004) Persistence of the Epstein-Barr virus and the origins of associated lymphomas. N Engl J Med 350:1328–1337
13. Klein E (2004) Non-proliferative interactions of Epstein-Barr virus and human B lymphocytes. Fiola Biol-Prague 50:131–135
14. Amon W, Farrell JP (2005) Reactivation of Epstein-Barr virus from latency. Rev Med Virol 15:149–156
15. Atayar C, Poppema S, Blokzijl T et al (2005) Expression of the T-cell transciption factors, GATA-3 and T-bet, in the neoplastic cells of Hodgkin lymphomas. Am J Pathol 166:127–134
16. McCune CR, Syrbu IS, Vasef AM (2006) Expression profiling of transcription factors Pax-5, Oct-1, Oct-2, BOB.1, and PU.1 in Hodgkin's and non-Hodgkin's lymphomas: a comparative study using high throughput tissue microarrays. Modern Pathol 19:1010–1018
17. Korbjuhn P, Anagnostopoulos I, Hummel M et al (1993) Frequent latent Epstein-Barr virus infection of neoplastic T cells and Bystander B cells in human immunodeficiency virus-negative European peripheral pleomorphic T-cell lymphomas. Blood 82:217–223
18. Suwiwat S, Pradutkanchana J, Ishida T et al (2007) Quantitative analysis of cell-free Epstein-Barr virus DNA in the plasma of patients with peripheral T-cell and NK-cell lymphomas and peripheral T-cell proliferative diseases. J Clin Virol 40:277–283