Epstein-Barr Virus–Associated B-Cell Lymphoproliferative Disorders in Angioimmunoblastic T-Cell Lymphoma and Peripheral T-Cell Lymphoma, Unspecified

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Abstract

Various patterns of Epstein-Barr virus (EBV)-associated B-cell lymphoproliferation occur in patients with immunodeficiency. We studied 17 cases of T-cell lymphoma displaying extensive EBV-driven B-cell lymphoproliferation or simultaneous/subsequent EBV-associated B-cell lymphoma. In 10 cases of angioimmunoblastic T-cell lymphoma, an uncommonly prominent population of EBV+ atypical, activated, focally confluent large transformed B cells was found in the background of T-cell lymphoma. In 4 cases, an EBV-associated B-cell neoplasm (3 diffuse large B-cell lymphomas, 1 plasmacytoma) occurred in patients with T-cell lymphoma. Three cases were composite lymphomas of a peripheral T-cell lymphoma, unspecified, combined with EBV-associated diffuse large B-cell lymphoma. The transformed B-cell population displayed EBV latency types 2 and 3. Monoclonal and oligoclonal B-cell populations were detected in 5 and 6 cases, respectively. Similar to other states of immunodeficiency, disease-related and therapy-induced immunosuppression in T-cell lymphoma may lead to a prominent EBV-associated B-cell lymphoproliferation and to EBV+ B-cell neoplasms.

Among peripheral T-cell non-Hodgkin lymphomas (NHLs), angioimmunoblastic T-cell lymphoma (AILT, previously referred to as AILD) displays particular morphologic, clinical, and immunologic features. Morphologically, AILT is characterized by the destruction of the lymph node architecture with a polymorphous cellular infiltrate, a proliferation of follicular dendritic cells, and arborizing high endothelial venules.[1-3] Clinically, in addition to generalized lymphadenopathy and hepatosplenomegaly, AILT frequently is accompanied by immunologic dysfunctions, as reflected by systemic inflammatory symptoms, hypergammaglobulinemia, and autoimmune phenomena,[1,3] which are not encountered as commonly in other peripheral T-cell lymphomas.[4-6] In addition, patients with AILT share a particular propensity to develop opportunistic infections, reflecting disease-associated immunosuppression as a key feature of this disease.[4,7]

Scattered Epstein-Barr virus (EBV)-infected lymphocytes have been documented in up to 97% of cases of AILT.[8,9] Some authors argue that neoplastic T cells in AILT may be EBV-infected, with the virus possibly having a direct role in lymphomagenesis.[8] Other studies, however, showed EBV to be mainly[9-12] or exclusively[13] present in nonneoplastic bystander B cells. In contrast with most other T-cell lymphomas, the development of B-cell lymphomas has been reported in cases of AILT,[14] which in very rare cases have been shown to be EBV-associated.[15-18]

We studied 17 cases of T-cell lymphoma with an unusual spectrum of EBV-driven B-cell lymphoproliferation, including 10 cases with a prominent population of large, atypical, activated, focally confluent transformed EBV+ B cells in the background of AILT; 4 cases with a history of T-cell lymphoma

developing EBV-associated B-cell lymphoma; and 3 cases of peripheral T-cell lymphoma, not otherwise specified (PTCL NOS) with coexistent EBV-associated diffuse large B-cell lymphoma (DLBCL). The morphologic features of 10 cases of AILT with EBV-associated B-cell lymphoproliferation were compared with 98 cases of AILT without these features. Similar to posttransplantation status or HIV infection, decreased antiviral immunosurveillance in peripheral T-cell lymphoma, in particular in AILT, may favor the proliferation of latently EBV-infected B lymphocytes potentially developing to secondary EBV+ B-cell malignant neoplasms.

Materials and Methods

Between January 1989 and December 2000, more than 600 cases of nodal T-cell lymphoma, among them more than 170 cases of AILT, were seen at the Würzburg Lymph Node Reference Center, Würzburg, Germany. Among those cases, 10 cases of AILT, in addition to characteristic diagnostic features, showed an uncommonly prominent population of atypical, large, transformed B cells focally forming confluent sheets on H&E-stained slides and by immunohistochemical analysis. These cases were compared with 98 cases of AILT, all of which showed common histopathologic features of AILT without a comparably prominent B-cell lymphoproliferation. In addition, in 7 patients with T-cell lymphoma, the simultaneous presence or development of a secondary B-cell lymphoma was observed. All these cases were studied further. Two of the cases have been reported in short form.[18]

Information on clinical manifestation of the disease, stage, therapy, and follow-up was obtained from referring pathologists or clinicians.

Sections of formalin-fixed, paraffin-embedded lymph nodes, decalcified trephine bone marrow biopsy specimens, skin, and epidural tissue removed for diagnostic purposes were stained with H&E, periodic acid–Schiff, Giemsa, and Gomori silver impregnation.

Immunohistochemical Analysis

Immunophenotypic analyses were performed on paraffin-embedded tissue using a 3-stage indirect immunoperoxidase (Zymed, San Francisco, CA) or alkaline phosphatase technique (BioGenex, San Ramon, CA) after antigen retrieval by pressure cooking the slides in citrate buffer for 5 minutes. The cases were analyzed (clone and dilution in parentheses) for CD3 (PS1; 1:20; DAKO, Hamburg, Germany), CD4 (1F6; 1:5; Novocastra, Newcastle upon Tyne, England), CD8 (C8/144B; 1:10; DAKO), CD45RB/LCA (PD7/26, 2B11; 1:50; DAKO), CD20 (L26; 1:1,000; DAKO), CD23 (1B12; 1:20; Novocastra), immunoglobulin kappa chain (1:40,000; DAKO), immunoglobulin lambda chain (1:20,000; DAKO), CD79a (HM57; 1:10; DAKO), syndecan (CBL534; 1:500; Cymbus Biotechnology, Chandlers Ford, England), CD21 (1F8; 1:20; DAKO), CD35 (RLB25; 1:40; Novocastra), CD30 (BerH2; 1:40; DAKO), and CD15 (LeuM1; 1:30; DAKO) as previously described.[19]

In 4 cases (5, 7, 15, and 16), immunohistochemical double-staining experiments were performed. The first primary antibody was detected by the aforementioned peroxidase technique. After the diaminobenzidine reaction and washing in phosphate-buffered saline (PBS), a second primary antibody was applied. Slides then were incubated with a secondary link antibody (BioGenex) for 20 minutes and washed again in PBS. Subsequently, the alkaline phosphatase-streptavidin complex was applied. The color was developed using a solution of naphthol phosphate in tris(hydroxymethyl)aminomethane hydrochloride buffer after adding 1 tablet of fast red chromogen to 5 mL of substrate. After washing in PBS, the slides were counterstained in hematoxylin. The brownish staining result of the first reaction was easily distinguishable from the red chromogen applied in the second reaction.

Detection of EBV and Quantitative Analyses

In situ hybridization for EBV-encoded RNAs (EBERs) was performed according to previously published protocols.[20] Briefly, tissue sections were deparaffinized, dehydrated, and pretreated with Proteinase K. Hybridization was performed using commercially available digoxigenin-labeled EBER1 and EBER2 antisense RNA probes (DAKO) by overnight incubation at 55°C. Detection was accomplished using bromochlorindoxylphosphate/nitroblue tetrazolium development and fast red counterstaining. As a positive control, tissue sections from tonsils of patients with infectious mononucleosis were hybridized. Sections from lymph nodes removed during mastectomy served as negative controls.

For further characterization of EBV infection status, immunohistochemical stains were performed for EBV latency proteins LMP1 (CS1-4; 1:200; DAKO) and EBV nuclear antigen 2 (EBNA2) (PE2; 1:10; DAKO). Four cases (5, 7, 15, and 16) were analyzed further by immunohistochemical double staining for CD20 and LMP1, CD30 and LMP1, CD20 and CD30, and CD3 and LMP1, as described in the preceding section.[19] For detection of early lytic EBV infection, sections were immunostained for EBV immediate early protein BZLF1 (BZ1; 1:10; DAKO).

For quantitative analysis, the number of LMP1+ large B cells was determined per high-power field (HPF, ×400) using a 10× ocular lens and a 40× objective (Standard 25 microscope, Zeiss, Jena, Germany) in the 10 AILT cases with prominent EBV+ B-cell lymphoproliferation and compared with a reference series of 98 usual AILT cases. Both the total number of LMP1+ large transformed B cells in 10 randomly

selected HPFs and their number at hot spots, corresponding to the HPF with the highest number of LMP1+ transformed large B cells, were counted separately, followed by statistical analysis (Mann-Whitney test and Kolmogorov-Smirnov test; $P = .05$ or less).

Clonality Analysis

For molecular clonality studies, paraffin blocks were dewaxed and DNA was extracted according to routine protocols. Polymerase chain reaction (PCR) for detection of clonal B-cell populations was performed with consensus FR3A and LJH primers according to previously published protocols.[21] Clonal T-cell populations were detected using a mixture of specific and consensus primers for the T-cell receptor (TCR) gamma chain. Aliquots of PCR reaction products were mixed with size standard and formamide, denatured, and subjected to electrophoresis on a 373 DNA Sequencer (Perkin-Elmer, Weiterstadt, Germany). Data were obtained and analyzed automatically using Genescan software (Perkin-Elmer) as described in the manufacturer's manual.

Results

Histologic Features

According to the histopathologic features, 3 different groups of B-cell lymphoproliferations in T-cell lymphoma could be distinguished.

Group 1 consisted of 10 cases of AILT that, in addition to the diagnostic histologic features of AILT, showed an unusual and prominent population of atypical, large transformed lymphoid cells. These cells frequently resembled immunoblasts; however, occasionally they showed features of Hodgkin and/or Reed-Sternberg cells **Image 1A**. As a unifying feature, these cells formed large, partially confluent sheets of blasts, focally obscuring the coexisting T-cell lymphoma.

Group 2 comprised 4 cases with a history of T-cell lymphoma (3 cases of AILT, 1 case of PTCL NOS) and subsequent development of a secondary B-cell lymphoma. Three cases showed typical histologic features of AILT; a prominent population of large transformed lymphoid cells was missing at initial diagnosis. One case (13) did not show typical features of AILT. In this tumor, a diagnosis of PTCL NOS was favored in view of only scant angioproliferation, a polymorphous infiltrate of atypical T cells, and only a few reactive inflammatory cells. In 3 cases, follow-up biopsies revealed a diffuse infiltration of lymph nodes (cases 12 and 13) or of the subcutaneous tissue (case 11) by large centroblast- or immunoblast-like cells, sometimes with anaplastic features, consistent with DLBCL **Image 2A**. Although no clear-cut morphologic evidence for residual areas of T-cell lymphoma was seen in any of these cases, focal atypical T-cell infiltrates were noted (cases 13 and 14). In 1 case (14), a diffuse infiltrate of the lymph node by atypical, partially anaplastic plasma cells and plasmablasts, consistent with nodal plasmacytoma, was demonstrated in a subsequent biopsy specimen **Image 2E**. In this patient, no nodal or bone marrow involvement of T-cell lymphoma was evident; however, cerebrospinal fluid analysis revealed persistent involvement by T-cell lymphoma.

Group 3 (3 cases) showed a composite lymphoma consisting of a PTCL NOS and DLBCL. Two cases showed a pleomorphic infiltrate of medium-sized to large lymphocytes with atypical nuclei admixed with reactive inflammatory cells and with large immunoblast-like cells in a lymph node (case 16) and epidural soft tissue (case 15). One case (17) was characterized by a pleomorphic infiltrate of medium-sized lymphocytes in the bone marrow and a mixed infiltrate of pleomorphic atypical medium- to large-sized lymphocytes and immunoblasts in the skin. A discordant lymphoma with pleomorphic T-cell lymphoma of the bone marrow and a composite B- and T-cell lymphoma in the skin was diagnosed based on immunohistochemical results.

Immunophenotype

On immunohistochemical analysis, all cases of group 1 (AILT with EBV+ B-cell lymphoproliferation) showed a diffuse infiltrate of small to medium-sized CD3+ T cells admixed with small, diffusely scattered CD20+ B cells throughout the lymph node. Aggregates of small to medium-sized B lymphocytes were found, which in CD21 and CD35 staining corresponded to partially destroyed follicle centers. In addition, in all cases, except the initial biopsy specimen in case 10, an eminent population of large, atypical, transformed B cells, focally forming confluent sheets, was noted. Immunophenotypically, these cells were consistently CD20+, with staining intensity slightly reduced compared with small CD20+ B lymphocytes **Image 1B**. In addition, these cells were CD3– and CD30+ **Image 1C**. Occasionally, the large transformed B cells resembled Hodgkin and/or Reed-Sternberg cells, which were CD20+, CD30+/–, and, of note, CD15–. In 4 of 6 group 1 cases stained for CD45RB/LCA, the large, atypical, transformed cells were positive. In group 2, the 3 AILT cases showed analogous immunohistochemical distribution of T and B cells; however, confluent sheets of CD20+, CD30+ large transformed B cells were completely missing. In one case (13), a diagnosis of PTCL NOS was confirmed immunohistochemically by an extensive infiltrate of atypical CD3+, CD4+ T cells. In 3 cases (11-13), immunohistochemical analysis of follow-up biopsy specimens showed a diffuse infiltrate of CD20+ large transformed B lymphocytes, consistent with DLBCL. In 2 cases (11 and 12), strong CD30 coexpression was observed, whereas in case 13,

Table 1
T-Cell Lymphoma With Transformed Large EBV+ B Cells: Clonality Analysis and EBV Phenotype

Case No./Sex/ Age (y)	Diagnosis	Clonality		Phenotype of Transformed Large B Cells			
		TCR	IgH	EBER	CD30	LMP1	EBNA2
1/M/69	AILT with EBV + LP	Polyclonal	Monoclonal	+	+	+	(+)
2/M/69	AILT with EBV + LP	Oligoclonal	Oligoclonal	+	+	+	–
3/M/69	AILT with EBV + LP	Biclonal	Polyclonal	ND	+	+	(+)
4/F/80	AILT with EBV + LP	Polyclonal	Polyclonal	+	+	(+)	–
5/M/57	AILT with EBV + LP	Monoclonal	Oligoclonal	+	+	(+)	(+)
6/M/58	AILT with EBV + LP	Monoclonal	Polyclonal	+	+	(+)	(+)
7/M/73	AILT with EBV + LP	Oligoclonal	Oligoclonal	+	+	+	(+)
8/F/74	AILT with EBV + LP	Monoclonal	Polyclonal	+	+	(+)	–
9/F/75	AILT with EBV + LP	Monoclonal	Monoclonal	+	+	(+)	(+)
10/F/73	AILT with EBV + LP	Polyclonal	Monoclonal	+	+	+	(+)
11/M/68	AILT	ND	ND	ND	ND	ND	ND
	EBV-associated DLBCL	Polyclonal	Monoclonal	+	+	+	(+)
12/M/47	AILT	Monoclonal	Polyclonal	+	–	+	(+)
	EBV-associated DLBCL	Polyclonal	Oligoclonal	+	+	+	(+)
13/F/56	PTCL NOS	Monoclonal	Polyclonal	+	+	+	–
	EBV-associated DLBCL	Polyclonal	Polyclonal	+	(+)	(+)	–
14/M/61	AILT	Biclonal	Polyclonal	+	–	+	–
	EBV-associated plasmacytoma	Monoclonal	Oligoclonal	+	+	–	–
15/M/25	Composite lymphoma (PTCL NOS and EBV-associated DLBCL)	Monoclonal	Monoclonal	+	+	+	–
16/F/89	Composite lymphoma (PTCL NOS and EBV-associated DLBCL)	Polyclonal	Polyclonal	+	(+)	(+)	(+)
17/F/91	Composite lymphoma (PTCL NOS and EBV-associated DLBCL)	Monoclonal	Oligoclonal	+	+	+	+

AILT, angioimmunoblastic T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; EBER, EBV-encoded RNA; EBNA2, EBV nuclear antigen 2; EBV + LP, Epstein-Barr virus–associated B-cell lymphoproliferation; IgH, immunoglobulin heavy chain gene rearrangement; LMP1, latent membrane protein 1; ND, not done; PTCL NOS, peripheral T-cell lymphoma, not otherwise specified; TCR, T-cell receptor gamma-chain rearrangement; +, positive; (+), focally positive; –, negative.

only a minority of large transformed cells were CD30+. In 2 cases (13 and 14), small focal atypical infiltrates of small to medium-sized CD3+ T cells were noted. The nodal plasmacytoma (case 14) was CD20– and CD30– but CD79a+ and syndecan+.

In group 3, there was a diffuse admixture of 2 morphologically and immunohistochemically distinct cell populations. The pleomorphic infiltrate of medium-sized to large lymphocytes with atypical nuclei was CD3+ and CD4+ but CD20–, whereas the immunoblast-like transformed large cell population was CD20+ and CD30+. In case 16, immunophenotypic characterization of atypical T cells showed abnormal marker expression with loss of CD2 and CD7 expression. In 3 of 5 cases stained for CD45RB/LCA in groups 2 and 3, the large, atypical, transformed cells were positive.

EBV Status and Quantitation of Transformed Large B Cells

EBER, LMP1, and EBNA2

In all cases, the transformed large B cells showed positive hybridization signals in EBER in situ hybridization and in LMP1 immunostains **Table 1** (case 3, no EBER hybridization performed), **Image 1D**, **Image 1E**, and **Image 2B**. Occasional EBER+ small lymphocytes were seen as a minor population in all cases. EBER in situ hybridization also was positive for the plasma cells in the plasmacytoma **Image 2F**, which, however, was LMP1–. In 5 (56%) of 9 cases in group 1, 2 (50%) of 4 cases in group 2, and 2 (67%) of 3 cases in group 3, LMP1 expression paralleled EBER staining with almost equal numbers of positive transformed cells. In the remaining cases, a proportion of EBER+ cells expressed LMP1, these cells corresponding to the population of large transformed B cells. EBNA2 expression by large transformed B cells was noted in 7 (70%) of 10 cases in group 1, 2 (50%) of 4 cases in group 2 **Image 2C**, and 2 (67%) of 3 cases in group 3. However, in all but 1 case (17), these cells made up only a small percentage of the EBER+ and LMP1+ cell population. In 3 (30%) of 10 cases in group 1 and 3 (75%) of 4 cases in group 2, scattered small to medium-sized cells (1-5 per section) expressed EBV immediate early lytic protein BZLF1 **Image 2D**. In group 3, no BZLF1+ cells were found. In 3 cases with secondary EBV-associated B-cell lymphoma, EBER hybridization of the initial biopsy specimens diagnostic of AILT and PTCL NOS, respectively, showed only occasional EBV+ cells. In 1 case of AILT with EBV+ B-cell lymphoproliferation (case 10), a previous biopsy specimen diagnostic of AILT was EBV–.

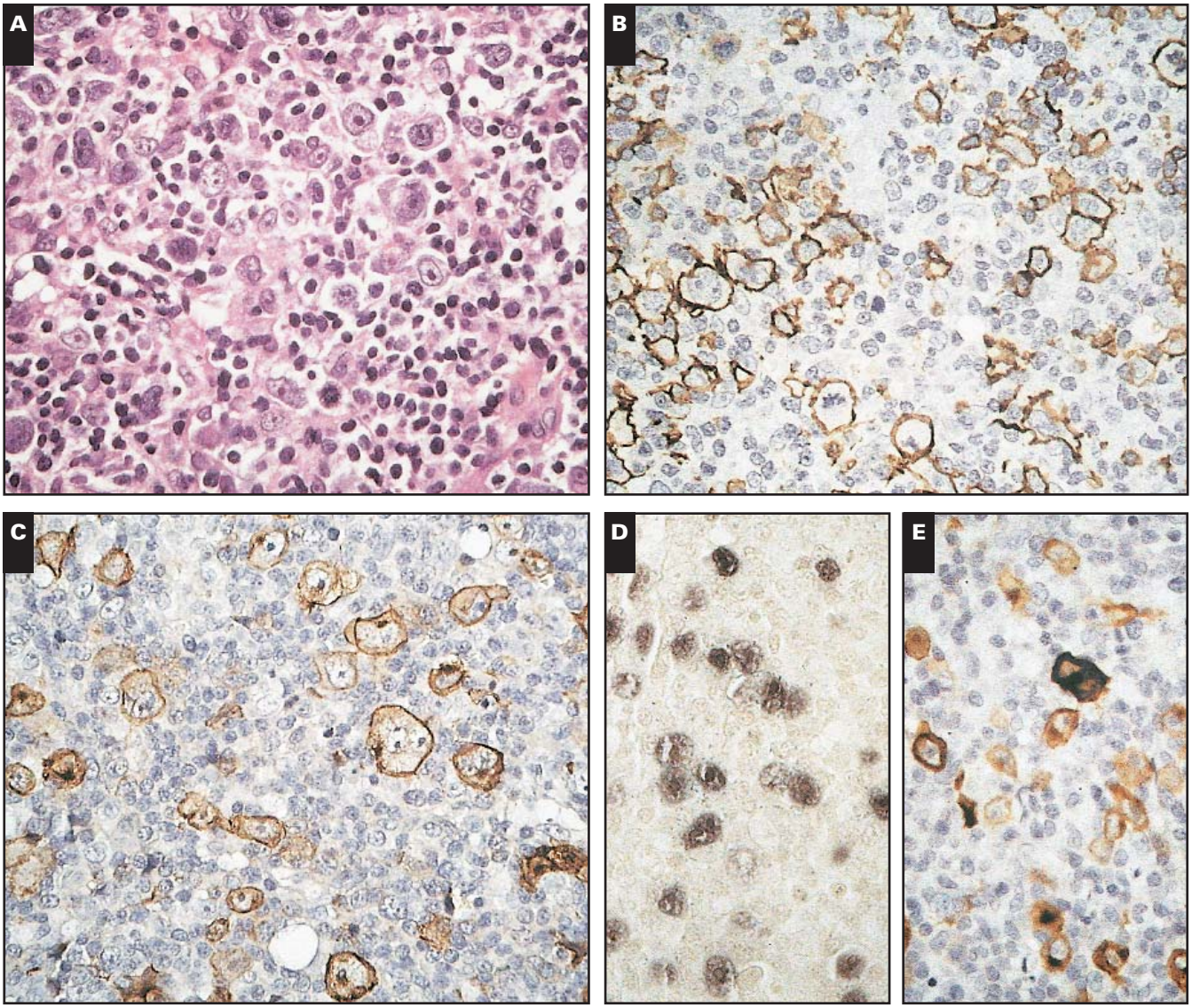

Image 1 Angioimmunoblastic T-cell lymphoma with Epstein-Barr virus (EBV)+ B-cell lymphoproliferation. (Case 7) **A**, A prominent large transformed cell population, partially with Hodgkin and Reed-Sternberg cell–like features, is seen in the polymorphous background of angioimmunoblastic T-cell lymphoma (H&E stain, ×250). **B** and **C**, Immunophenotyping by staining for CD20 (**B**) and CD30 (**C**) (immunoperoxidase with hematoxylin counterstain, ×250) shows positive (brownish) membrane staining of large transformed cells, with only a few additional CD20+ small to medium-sized cells. **D** and **E**, The large transformed cells display latent EBV infection, as shown by strong nuclear signals in EBV-encoded RNA in situ hybridization (**D**, bromochlorindoxylphosphate/nitroblue tetrazolium, fast red counterstaining, ×250) and positive immunohistochemical membrane staining for EBV latency protein LMP1 (**E**, immunoperoxidase with hematoxylin counterstain, ×250).

Double-Staining Experiments

Double-staining experiments were performed in 4 cases (5, 7, 15, and 16). By combining CD20 and LMP1 staining, the LMP1-expressing large transformed lymphoid cells were shown to be of B-cell lineage, with LMP1-expressing cells coexpressing CD20 **Image 1F**. No LMP1-expressing CD3+ cell population was noted when combining staining for CD3 and LMP1 **Image 1G**. Double staining for CD20 and CD30 showed the large transformed B-cell population to be CD30+ as well.

Comparative Quantitation of EBV+ Transformed Large B Blasts in AILT

The 10 cases of AILT with EBV+ B-cell lymphoproliferation were subjected to quantitative analysis of the LMP1+ large transformed B-cell population. The total number of LMP1+ large transformed B cells ranged between 8 and 95 in 10 unselected HPFs (average, 40/10 HPFs) and 8 to 80 in a single HPF at hot spots (average, 22/HPF at hot spots).

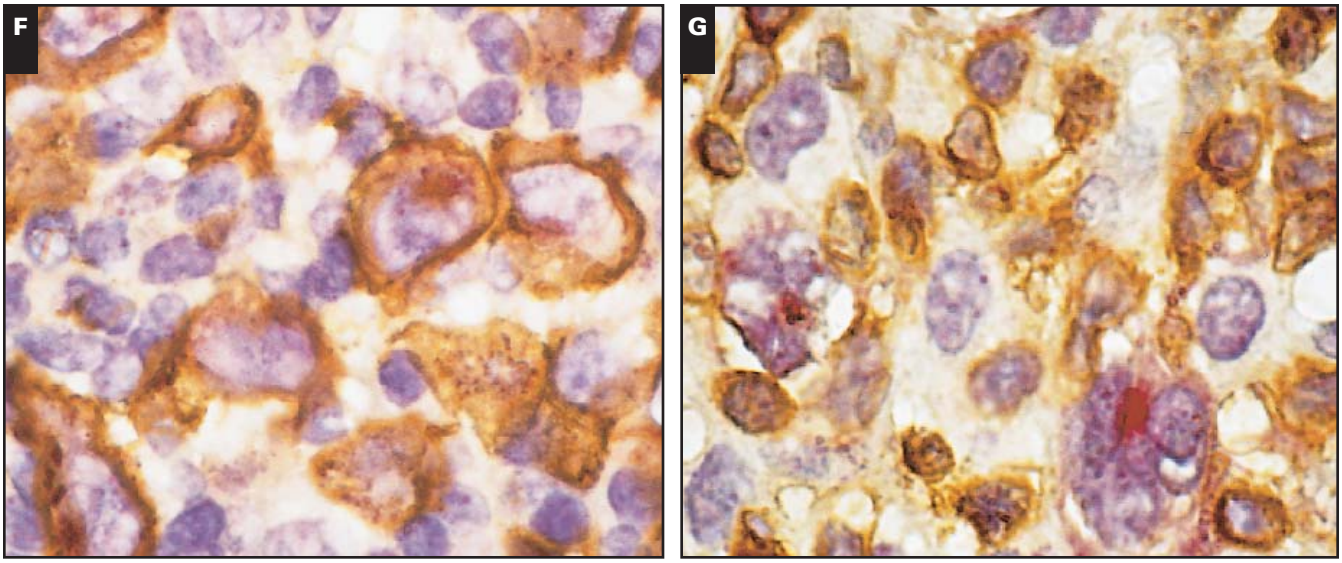

Image 1 (cont) **F**, Double staining for CD20 and LMP1 (CD20, brown staining, immunoperoxidase, visualized by application of diaminobenzidine; LMP1, red staining, alkaline phosphatase, visualized by application of fast red with hematoxylin counterstain, ×1,000) shows the large transformed B lymphocytes to be latently EBV infected, as highlighted by intense staining for both CD20 and LMP1 (with accentuation of paranuclear Golgi zone). (Case 15) **G**, Double staining for CD3 and LMP1 shows CD3 positivity but LMP1 negativity of small T cells and CD3 negativity but LMP1 positivity of large transformed cells (CD3, brown staining by immunoperoxidase, visualized by application of diaminobenzidine; LMP1, red staining, alkaline phosphatase, visualized by application of fast red with hematoxylin counterstain, ×1,000). Coexpression of CD3 and LMP1 was not observed.

For comparison, we analyzed 98 cases of AILT without the morphologic features of EBV-associated B-cell lymphoproliferation. In 50 (51%) of 98 cases, LMP1+ B cells were identified. In most instances, only single LMP1+ transformed large B cells were noted on the whole slide. In 20 of the cases of usual AILT showing the highest number of LMP1+ B cells, quantitative analysis was performed. In these cases, the number of LMP1+ B blasts ranged between 0 and 10 per 10 HPFs (average, 3.2/10 HPFs) and 1 to 11 per HPF at hot spots (average, 3.6/HPF).

Clonality Analysis

In the AILT cases with a prominent EBV+ large transformed B-cell population, TCR gamma rearrangement studies showed monoclonal, biclonal, and oligoclonal T-cell populations in 4, 1, and 2 cases, respectively, whereas in 3 cases, TCR gamma rearrangement was polyclonal (Table 1). Immunoglobulin heavy chain gene (IgH) rearrangement studies demonstrated monoclonal and oligoclonal B-cell populations in 3 cases each. In the secondary EBV-associated B-cell lymphomas, TCR gamma rearrangement was polyclonal in 3 cases and monoclonal in 1 case, whereas monoclonal or oligoclonal rearrangements of IgH were detected in 1 and 2 cases, respectively. In case 14, clonality study of the plasmacytoma displayed monoclonal TCR gamma rearrangement, the PCR product showing a length identical to 1 of the 2 bands of the biclonal AILT diagnosed 7 years earlier. Two cases of composite B- and T-cell lymphoma showed monoclonal TCR gamma rearrangement; the third case was polyclonal for TCR gamma. IgH rearrangement was monoclonal, oligoclonal, and polyclonal in 1 case each.

Clinical Features

There were 7 women and 10 men **Table 2**, **Table 3**, and **Table 4**. The age at disease onset of 14 patients diagnosed with AILT ranged from 47 to 80 years (mean, 67 years). All patients except one (case 10) were untreated at the time of biopsy; biopsies provided the initial diagnostic specimens. The 4 patients diagnosed with PTCL NOS were 25, 56, 89, and 91 years old. Sixteen of 17 patients had generalized lymphadenopathy at diagnosis, and most patients showed systemic symptoms. At diagnosis, case 15 had an epidural tumor of the lumbar spine causing paresthesia of the legs. At diagnosis, most patients had disease with an advanced Ann Arbor stage (9 cases with stage IIIA/B and 4 cases with stage IVA/B vs 1 case with stage II disease; data were not given for 3 cases).

In patients with a history of T-cell lymphoma developing EBV-associated DLBCL, the interval between initial diagnosis of T-cell lymphoma and secondary B-cell lymphoma ranged between 29 and 40 months (Table 3). In case 14, EBV+ nodal plasmacytoma developed 8 years after the initial diagnosis of AILT. Of note, all but 1 patient had nodal manifestations of the secondary B-cell lymphoma; in

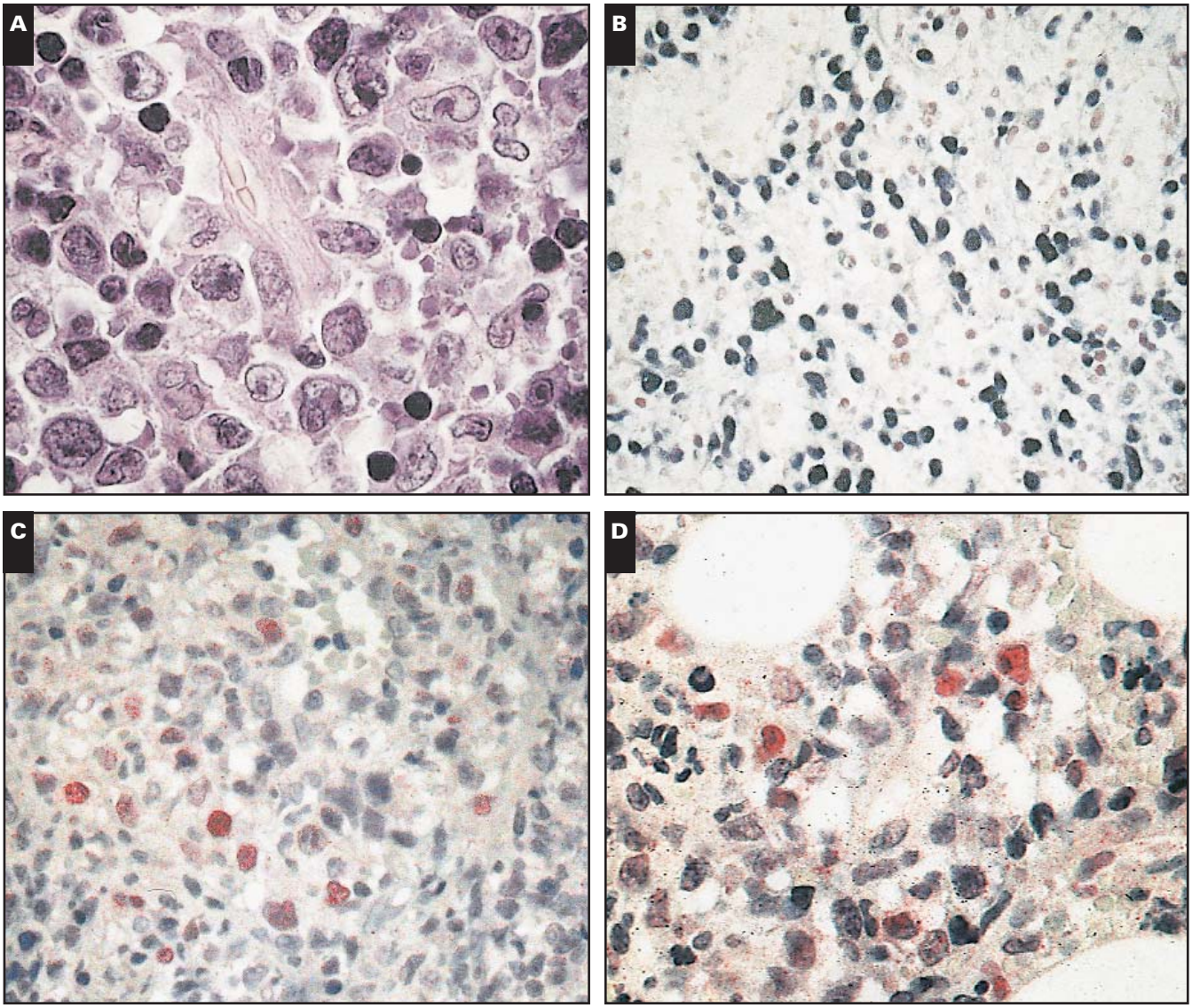

Image 2 (Case 11) Epstein-Barr virus+ B-cell lymphomas arising in angioimmunoblastic T-cell lymphoma (AILT). **A**, A diffuse infiltrate of atypical large transformed lymphoid cells arising as a subcutaneous mass (H&E, ×1,000). Their B-cell origin was demonstrated by CD20 staining (data not shown). **B**, EBV association of the lymphoma was demonstrated by EBV-encoded RNA (EBER) in situ hybridization, showing intense nuclear hybridization signals (bromochlorindoxylphosphate/nitroblue tetrazolium, fast red counterstain, ×250). **C** and **D**, Occasional lymphoma cells, here showing focal angioinvasion, express EBV latency protein EBV nuclear antigen 2 (**C**) and EBV immediate early lytic protein BZLF1, as shown by intense red nuclear staining (**D**) (alkaline phosphatase visualized by application of fast red with hematoxylin counterstain, ×250).

case 11, however, the B-cell lymphoma initially manifested as a localized subcutaneous mass.

Patients diagnosed with composite T- and EBV-associated B-cell lymphoma were case 15 with epidural, case 16 with nodal manifestations, and case 17 with PTCL NOS in the bone marrow and simultaneous PTCL NOS and DLBCL in the skin (Table 4).

The follow-up periods varied substantially, between 1 and 101 months after initial diagnosis. Four patients were lost to follow-up. Of the remaining 13 patients, 8 had died at the time of manuscript submission, 3 of infectious complications (case 3, fulminant septicemia; case 11, *Mycobacterium tuberculosis* infection; case 12, *Aspergillus* pneumonia). Among the remaining 5 patients, 2 were in remission and 3 were alive with disease at last follow-up. In none of the patients was there any evidence for primary immunodeficiency or HIV-infection. Serologic studies for EBV were not available for the cases reported.

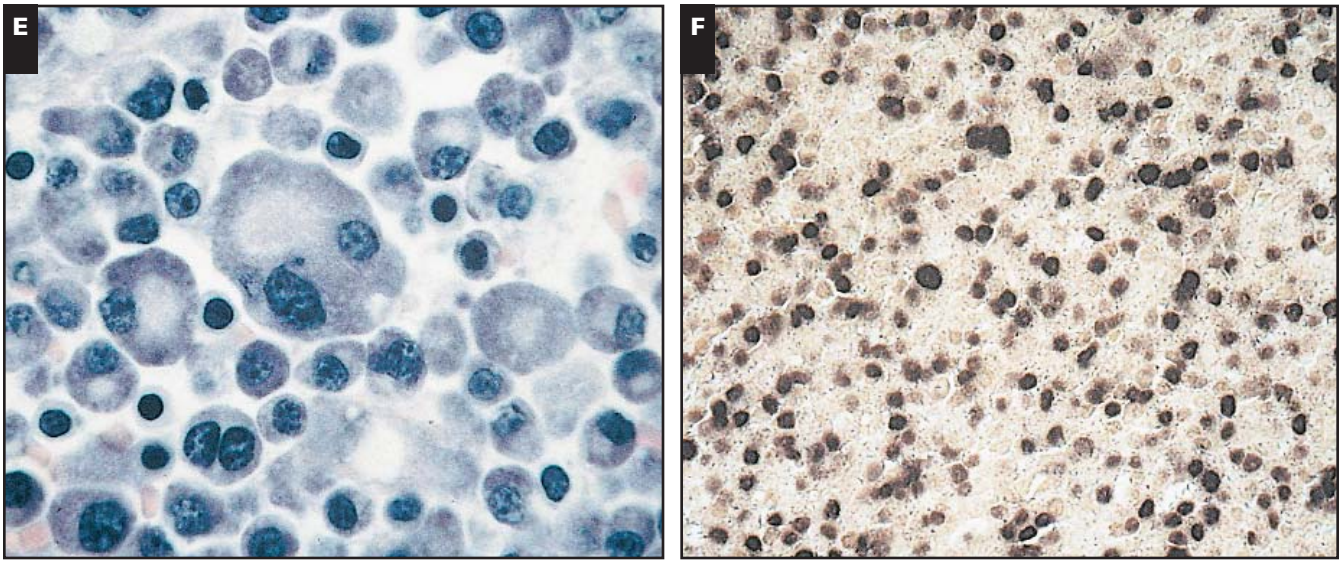

Image 2 (cont) (Case 14) **E**, Nodal plasmacytoma in an inguinal lymph node 8 years after initial diagnosis of AILT (periodic acid–Schiff, ×1,000). **F**, Latent EBV infection of atypical plasma cells demonstrated by EBER in situ hybridization (×250).

Table 2
Clinical Data for Patients With AILT With EBV-Associated B-Cell Lymphoproliferation*

Case No./ Sex/Age (y)	Clinical Findings	Ann Arbor Stage	Therapy	Follow-up (Time After Initial Diagnosis)
1/M/69	Generalized lymphadenopathy; hypergammaglobulinemia	IIIB	CC	Alive, in remission (24 mo)
2/M/69	Generalized lymphadenopathy; hepatosplenomegaly	IVA	CC	Alive with disease (25 mo)
3/M/69	Ascites; generalized lymphadenopathy; splenomegaly	IIIA	CC	Died (68 mo, of septicemia)
4/F/80	Generalized lymphadenopathy; splenomegaly	IIIB	NA	Lost to follow-up
5/M/57	Upper gastrointestinal bleeding; cervical and axillary lymphadenopathy; splenomegaly	NA	CC	Died (3 wk, of tumor lysis syndrome)
6/M/58	Bronchopneumonia; aphthous stomatitis; generalized lymphadenopathy; hepatosplenomegaly; Coombs+ autoimmune hemolytic anemia	IIIB	CC	Alive with disease (24 mo)
7/M/73	Inguinal and retroperitoneal lymphadenopathy; splenomegaly; hypergammaglobulinemia	IIB	CC	Died (4 mo, of tumor cachexia)
8/F/74	Generalized lymphadenopathy; pulmonary and hepatic infiltrates; Coombs+ autoimmune hemolytic anemia	IVA	CC	Lost to follow-up
9/F/75	Generalized lymphadenopathy; splenomegaly; Coombs+ autoimmune hemolytic anemia	IVB	CC	Died (15 mo, of tumor cachexia)
10/F/73	Recurrent generalized lymphadenopathy 24 mo after diagnosis of AILT	NA	NA	Lost to follow-up

AILT, angioimmunoblastic T-cell lymphoma; CC, combination chemotherapy; EBV, Epstein-Barr virus; NA, not available.
* The biopsy site for all patients was lymph node.

Discussion

In this study, 17 unusual cases of T-cell lymphoma with a prominent B-cell lymphoproliferative disorder present at initial diagnosis or revealed in follow-up biopsies were investigated. Group 1, consisting of 10 cases, was characterized by a notable population of large atypical, activated, focally confluent transformed B cells in a background of AILT. Group 2 comprised 4 patients with a history of T-cell lymphoma developing secondary DLBCL or plasmacytoma. Group 3 consisted of 3 patients with composite lymphoma of PTCL NOS and DLBCL. The unifying feature in all cases was the association of the transformed large B-cell lymphoproliferation with EBV and, in particular, with LMP1 expression, the major EBV-encoded B-cell transforming latency protein.[22]

Group 1 was notable because in all cases, the EBV-associated B-cell lymphoproliferation occurred in the setting of AILT. Depending on the method applied, EBV-infected lymphocytes can be detected in up to 97% of typical AILT cases.[8,9] Most studies showed that latent EBV infection is a frequent feature of scattered nonneoplastic bystander B cells,[8,9,11-13] whereas EBV infection of neoplastic T cells in AILT remains controversial. The AILT cases reported herein add a further pattern to the spectrum

Table 3
Clinical Data for Patients With T-Cell Lymphoma With Secondary EBV-Associated B-Cell Neoplasm

Case No./ Sex/Age (y)	Clinical Findings	Biopsy Site	Diagnosis	Ann Arbor Stage	Treatment	Clinical Course
11/M/68						
At diagnosis	Generalized lymphadenopathy; skin rash; hypergammaglobulinemia	LN	AILT	IIIB	Observation	Spontaneous remission
At 34 mo	Generalized lymphadenopathy; pulmonary infiltrates; subcutaneous nodules, right thigh	Soft tissue	EBV with DLBCL	NA	Glucocorticoids	Died 4 mo later of tuberculosis
12/M/47						
At diagnosis	Generalized lymphadenopathy	LN	AILT	IIIA	CC; BMT	Complete remission
At 29 mo	Cervical lymphadenopathy	LN	EBV with DLBCL	NA	CC; involved field radiation	Complete remission
At 39 mo	Cervical lymphadenopathy; tonsillar swelling	LN	EBV with DLBCL	NA	CC	Died 3 mo later of *Aspergillus* pneumonia
13/F/56						
At diagnosis	Cervical lymphadenopathy	LN	PTCL NOS (initially misdiagnosed as lymphadenitis)	NA	Observation	Spontaneous remission
At 38 mo	Generalized lymphadenopathy; pleural effusion	LN	PTCL NOS (misdiagnosed as viral lymphadenitis)	NA	Interferon alfa-2a	Progressive disease
At 40 mo	Progressive generalized lymphadenopathy	LN	EBV with DLBCL	IIIB	CC	Died 34 mo later of tumor cachexia
14/M/61						
At diagnosis	NA	LN	AILT	NA	NA	NA
At 96 mo	Axillary lymphadenopathy	LN	EBV with plasmacytoma	NA	NA	Alive with disease 24 mo later

AILT, angioimmunoblastic T-cell lymphoma; BMT, bone marrow transplantation; CC, combination chemotherapy; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; LN, lymph node; NA, not available; PTCL NOS, peripheral T-cell lymphoma, not otherwise specified.

Table 4
Clinical Data for Patients With Composite T-Cell Lymphoma and EBV+ B-Cell Lymphoma

Case No./ Sex/Age (y)	Clinical Findings	Biopsy Site	Diagnosis	Ann Arbor Stage	Treatment	Clinical Course
15/M/25	Epidural tumor; generalized lymphadenopathy; splenomegaly	Tumor	PTCL and EBV with DLBCL	IVB	CC	Alive, in remission 101 mo after initial diagnosis
16/F/89	Generalized lymphadenopathy	LN	PTCL and EBV with DLBCL	IIIB	None	Lost to follow-up
17/F/91	Nodular and diffuse skin infiltrates; generalized lymphadenopathy	Skin; bone marrow	PTCL NOS and EBV with DLBCL (skin); PTCL NOS (bone marrow)	IIIA	Topical therapy, methotrexate	Died 6 mo after initial diagnosis of tumor cachexia

CC, combination chemotherapy; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; LN, lymph node; NA, not available; PTCL NOS, peripheral T-cell lymphoma, not otherwise specified.

of the EBV association of AILT.[8,9,11-13] On H&E-stained sections, a population of numerous atypical, transformed large cells, focally forming confluent clusters and partially exhibiting Reed-Sternberg or Hodgkin cell–like features, not commonly met in AILT, has been noted. Second, on immunohistochemical stains and in EBER hybridization, these large transformed lymphoid cells were CD20+, CD30+, and EBV+; in particular, they showed positivity for LMP1. Comparison of these cases with common AILT without similar histomorphologic characteristics revealed that the average total number of LMP1+ large transformed B cells was significantly higher (40/10 HPFs vs 3.2/10 HPFs; $P = .05$ or less) in cases with EBV+ B-cell lymphoproliferation. In particular, the number of large transformed LMP1+ B cells at hot spots was significantly accentuated in AILT with EBV+ B-cell lymphoproliferation (22.1 vs 3.6/HPFs; $P = .05$ or less). Therefore, in addition to latent EBV infection of bystander B cells,[8,9,11-13] a prominent EBV-driven activation and proliferation of LMP1+ B cells may occur in AILT, potentially representing precursor lesions of EBV-associated secondary B-cell lymphoma. In contrast with the frequently

detectable latently infected bystander B cells in AILT, cases with a prominent EBV+ (LMP1+) B-cell lymphoproliferation seem to be rare. The cases were observed among more than 170 cases of AILT in the files of our department, suggesting that only about 5% of AILT cases exhibit the massive EBV-triggered B-cell lymphoproliferation described herein.

Group 2 was characterized by the development of EBV-associated B-cell lymphoma in patients with preexisting T-cell lymphoma. Only few cases of secondary EBV-associated DLBCLs in AILT have been reported in the literature, occurring between 8 months and 10 years after the initial diagnosis of AILT.[15-18] In our series, secondary EBV-associated B-cell lymphomas developed between 29 months and 8 years after initial diagnosis of T-cell lymphoma. Given the rarity of follow-up biopsies in AILT, the actual frequency of EBV-associated B-cell lymphoproliferations and B-cell lymphoma in AILT may be underestimated. One case showed the development of a nodal EBV+ plasmacytoma. EBV+ plasmacytoma has not been reported so far in T-cell lymphoma, yet it has been described in the setting of post-transplantation lymphoproliferative disorder.[23] The occurrence of EBV-associated B-cell lymphoma in T-cell lymphoma is most common in, but not restricted to, AILT. This series includes 3 patients diagnosed with composite PTCL NOS and EBV-associated DLBCL and 1 patient in whom PTCL NOS was followed by EBV-associated B-cell lymphoma. The development of secondary or simultaneous EBV+ B-cell lymphoma in nodal T-cell lymphoma other than AILT has been reported only very rarely.[24,25]

With regard to the pathogenesis of EBV-associated lymphoproliferation in T-cell lymphoma, small numbers of latently EBV-infected B lymphocytes can be detected in the peripheral blood and lymph nodes of healthy individuals. Although immortalization of infected B lymphocytes has been shown in vitro to be induced by the transforming properties of EBV latency proteins LMP1 and EBNA2, their proliferation is tightly controlled in vivo by the cellular immune system.[22] Among T-cell lymphomas, AILT is characterized by a particular propensity of patients to develop fatal infections,[4,7] and it shares immunologic dysfunctions with later stages of HIV infection.[6] Potentiating lymphoma-associated impairment of the immune system, treatment protocols of AILT include systemic chemotherapy or administration of immunosuppressive agents, which further inhibit cellular immunity and may favor the outgrowth of latently EBV-infected B cells.[4,7] All of the transformed large B cells showed LMP1 positivity, suggesting EBV as the causative factor for B-cell lymphoproliferation. Since all but 1 patient with AILT with EBV+ lymphoproliferation showed B-cell proliferation in the initial diagnostic specimen before institution of treatment, therapy-associated immunosuppression may have a more important role in the development of overt secondary EBV+ B-cell neoplasm. In addition to immunosuppression in AILT, proliferation of EBV-infected B lymphocytes may be further promoted by activated lymphoma or bystander T cells. In vitro, the proliferation of EBV-immortalized lymphoblastoid cell lines is dependent on the secretion of autocrine growth factors, such as lymphotoxin and interleukin-6.[26,27] Both cytokines are produced by atypical T cells and by EBV-infected B-immunoblasts in AILT in a higher degree than in other peripheral T-cell lymphomas.[5] Thus, lymphoma- and therapy-associated immunosuppression, together with abnormal T-cell activation and cytokine patterns in AILT, may explain the increased detection of EBV in AILT compared with other T-cell lymphoma types.[8,9] In selected cases, as shown herein, a prominent EBV-associated B-cell lymphoproliferation comparable to that found in other settings of immunosuppression, eg, AIDS or posttransplantation, may occur. However, since the extent of EBV-associated B-cell lymphoproliferation we describe is found only in a minority of cases of AILT, further patient- or virus-specific factors are likely to contribute.[16]

Depending on the profile of EBV latency proteins expressed, 3 types of viral latency have been defined in vitro.[22] Based on the results of immunohistochemical detection of LMP1 and EBNA2, both AILT with a prominent proliferation of transformed B cells and the secondary B-cell lymphomas (with the exception of the plasmacytoma) displayed latency types 2 or 3, similar to EBV+ high-grade B-cell lymphomas in AIDS and in posttransplantation lymphoproliferative disorders.[28,29] In addition to EBV latency proteins, lytic EBV product BZLF1 was detected in scattered lymphocytes in 6 cases. The detection of lytic EBV infection in T-cell lymphoma has been reported only very rarely.[10,11] However, lytic EBV infection is a more frequent feature in posttransplantation lymphoproliferative disorders and AIDS-related B-cell lymphomas,[28-30] again showing parallels between EBV-associated B-lymphoproliferation in T-cell NHL and in the setting of immunosuppression.

With regard to clonality, 7 of 10 cases of AILT with EBV-associated lymphoproliferation showed monoclonal or oligoclonal B-cell populations. Monoclonal IgH rearrangement has been reported in 19% to 30% of typical cases of AILT.[31,32] Although 3 of 4 cases with a particularly prominent EBV+ transformed B-cell population but still discernible AILT showed monoclonal or oligoclonal IgH rearrangement, the monoclonal B-cell populations need not necessarily represent the EBV+ large transformed cell population. Microdissection studies were not performed. Similar to EBV-associated B-cell neoplasms in immunosuppressed patients, most but not all of our cases of secondary EBV+ B-cell lymphomas were clonal, with clonality studies demonstrating monoclonality or oligoclonality in 5 of 7 cases.

Clinically, EBV-associated B-cell lymphoproliferation occurring in AILT does not seem to be associated with a more aggressive clinical course than typical AILT. Since no follow-up biopsy specimens were available, we could not document progression of EBV-associated B-cell lymphoproliferation to DLBCL. Remarkably, in 5 of 7 cases of EBV+ B-cell NHL in T-cell NHL, secondary EBV+ B-cell lymphomas showed nodal manifestations. In contrast to EBV-associated posttransplantation lymphoproliferation and AIDS-associated lymphoma, which frequently manifest as extranodal disease, an intimate interaction between T-cell lymphoma, latently EBV-infected B lymphocytes, and other components of the lymph node parenchyma may be pivotal in EBV-associated B-cell lymphomagenesis in T-cell NHL.

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