Heterogeneous Epstein-Barr Virus Infection Patterns in Peripheral T-Cell Lymphoma of Angioimmunoblastic Lymphadenopathy Type

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In this study, 32 cases of T-cell lymphoma of angioimmunoblastic lymphadenopathy type (AILD-TCL) were investigated for their association with Epstein-Barr virus (EBV). For this purpose, three different approaches were applied: polymerase chain reaction (PCR) for the presence of EBV-DNA, in situ hybridization (ISH) for EBV-encoded small nuclear RNAs (EBER), and immunohistology for EBV-encoded latent membrane protein (LMP). PCR and EBER-ISH produced almost identical results, showing that all but one case of AILD-TCL contained EBV genomes. Three distinctive patterns of EBV infection were observed after immunophenotypical characterization of EBER-positive cells: (1) in 26% of the cases, B and T cells were infected, the majority of which were B cells of immunoblastic morphology located in the remnants of lymphoid follicles; (2) in 42% of the cases, the vast majority of infected cells were neoplastic T cells diffusely distributed in the lymph nodes, but infected B cells were also present; and (3) in 32% of the cases, there were only a few infected small lymphoid cells. Detectable LMP was frequent in cases exhibiting patterns 1 and 2. These findings suggest that in AILD-TCL patients, B cells and especially T cells are highly susceptible to a persistent EBV infection, which often leads to a growth advantage of the infected cells. Thus EBV, in conjunction with genetic abnormalities and selective defects of the immune system, might be involved in the pathogenesis of AILD-TCL.

IN THE EARLY 1970s, several investigators described a disease entity in patients presenting with generalized lymphadenopathy, hepatosplenomegaly, hypergammaglobulinemia, hemolytic anemia, and other autoimmune phenomena. Histologically, the lymph nodes were characterized by a proliferation of medium-sized to large immunoblast-like lymphoid cells and arborizing venules. Terms used to define this disease included immunoblastic lymphadenopathy (IBL),[1] angioimmunoblastic lymphadenopathy with dysproteinemia (AILD),[2] and lymphogranulomatosis X (LGRX).[3,4] The disease was generally considered to be an abnormal hyperimmune reaction, even though most cases had a rapidly fatal outcome. Immunophenotypical studies have identified the vast majority of proliferating cells as T cells of either CD4 (most commonly) or CD8 type.[5-7] More importantly, cytogenetical studies have disclosed chromosomal abnormalities (most frequently trisomy 3 or 5, or both),[8-10] and molecular studies have shown clonal rearrangements of the T-cell receptor genes and, in some instances, immunoglobulin genes or both.[11-16] On the basis of these data, most cases previously diagnosed as AILD are now regarded as belonging to the distinct entity of peripheral T-cell lymphoma of AILD type (AILD-TCL).

In 1976, the screening of 27 cases of lymphoid tissue lesions for Epstein-Barr virus (EBV)-DNA using nucleic acid reassociation kinetics led to the detection of one virus-positive case of AILD.[17] Subsequently, there were sporadic publications based on serologic[18] and Southern blot analysis[19,20] that reported on the presence of EBV in single cases of AILD. In a recent study using the more sensitive polymerase chain reaction (PCR) technique, EBV-DNA was demonstrated in five of eight cases.[21] These observations prompted the following questions: How frequently is AILD-TCL associated with EBV when investigated in a larger series? Which cells harbor the EBV genome? Do the EBV-positive cells belong to the neoplastic clone? Is the EBV only a "silent passenger" or is it involved in the pathogenesis of the disease? To clarify these points, a collection of 32 cases of AILD-TCL from three lymphoma research centers (Berlin, Kiel, Oxford) were investigated for the presence of EBV by the demonstration of EBV-DNA in extracted DNA, as well as EBV-encoded small nuclear RNAs (EBER)[22,23] and EBV-encoded latent membrane protein (LMP)[24-26] in routinely processed tissue sections. This report describes the results obtained with these approaches.

MATERIALS AND METHODS

Specimens and Diagnostic Criteria

Paraffin-embedded lymph node and skin biopsy specimens from 32 patients with the diagnosis of AILD-TCL were selected from the files of the Institute of Pathology, Klinikum Steglitz, Free University, Berlin, Germany, from the Lymph Node Registry, University of Kiel, Germany, and from the Department of Pathology, University of Oxford, John Radcliffe Hospital, Oxford, UK. Serological data as to previous or current EBV infection were only available for cases 3, 9, 10, 11, 23, and 30. These sera contained antibodies against the nuclear antigen of EBV (EBNA), as well as antibodies of IgG isotype against the viral capsid antigen (VCA). Antibodies of IgM isotype against VCA were not observed. The Paul-Bunnell test was negative in all of these cases. Multiple, small axillary lymph nodes removed from 12 patients during the course of mastectomy were used as controls. Histologically, they displayed no signs of activation and are therefore referred to as "normal lymph nodes."

Four-micrometer sections were cut and stained with hematoxylin

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Table 1. Monoclonal Antibodies Used

Antibody Designation	Cluster of Differentiation	Specificity	Source
βF1		T-cell receptor β chain	T-Cell Sciences
UCHL1	CD45RO	Pan-T cell	Dako
DF-T1	CD43	Pan-T cell	Dako
UCHT1	CD3	Pan-T cell	Dako
MT310	CD4	T-helper cell	Dako
DK25	CD8	T-suppressor cell	Dako
L26	CD20	Pan-B cell	Dako
Ki-B3	CD45RB	B-cell subset	Lab. Parwaresch
4KB5	CD45RA	Most B cells	Dako
1F8	CD21	Follicular dendritic cells (FDC), B-cell subset*	Dako
Ki-M4p		FDC	Lab. Parwaresch
Ber-H2	CD30	Activated T and B blasts, plasma cell subset	Dako
PG-M1	CD68	Macrophages	Dako
KP1	CD68	Granulopoetic cells, macrophages	Dako
Ki-M1p	CD68	Macrophages	Lab. Parwaresch
CS 1-4		EBV-encoded latent membrane protein (LMP)	Lab. Rowe; Dako

Manufacturer locations: T-Cell Sciences: Cambridge, MA; Dako: Glostrup, Denmark; Lab. Research: Kiel, Germany; Lab Rowe: Birmingham, UK.

*Predominant reactivity with FDC in paraffin sections.

and eosin (H&E), Giemsa, periodic acid–Schiff (PAS), and Gomori silver impregnation. Immunohistological analysis involved the use of monoclonal antibodies (MoAbs) for the detection of T- and B-cell antigens, immunoglobulin light chains, CD21 antigen, etc (see Table 1 for clone designation of most frequently used MoAbs). The diagnosis of AILD-TCL was established on the basis of the following criteria: (1) diffuse effacement of nodal architecture replaced by a mixture of small, medium, and large T cells, B lymphocytes, B immunoblasts, plasma cells, macrophages, epithelioid cells, and occasional neutrophils and eosinophils; (2) marked proliferation of high endothelial venules (HEV) with arborizing pattern and PAS-positive hyalinized distension of basement membranes; and (3) "burned out" germinal centers with ill-defined, stretched meshworks of follicular dendritic cells (FDC), usually in direct association with the proliferated HEV.

Immunohistology

The bound antibodies were made visible by using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method according to Cordell et al[27] and/or a modified streptavidin-biotin-complex (ABC) method according to Hsu et al.[28]

PCR

Ten-micrometer thick paraffin sections were cut from each specimen under conditions preventing DNA cross-contamination. Sections were dewaxed in xylol, precipitated with ethanol, and digested overnight with proteinase K (1 μg/μL) at 37°C. One tenth of the digested material was used for amplification after boiling for 15 minutes. PCR was performed in a thermocycler (BioMed 60, BioMed, Theres, Germany) using AmpliTaq (Perkin Elmer-Cetus, Norwalk, CT) for 40 cycles as previously described.[29] For reamplification, 1% of the volume of the first round of amplification was submitted to another 40 rounds of PCR using a set of nested primers (see Table 2 for list of primers used). The optimal concentration of $MgCl_2$ for each pair of primers was empirically determined. Ten to twenty microliters of the PCR product was examined with a 2% ethidium-bromide–stained agarose gel for the presence of appropriate bands, blotted on nylon membranes[30] (Hybond N, Amersham, Germany), and hybridized with a ^{32}P terminal-transferase–labeled internal oligonucleotide. The positive controls used for each stage of experimentation were the Namalwa cell line harboring one to two copies of EBV,[31] as well as an EBV-positive case of Hodgkin's disease (HD). The negative controls were the EBV-negative cell line HUT102[32] and DNA-negative samples (data not shown). The amplification of a section of the β-globin gene served to determine the amplifiability of extracted DNA (Table 2). The sensitivity of the PCR approach was determined by an experiment using serial dilutions of Namalwa cell-line DNA, in which only a single copy viral genome could be detected (not shown).

In Situ Hybridization

Plasmids and probes. Plasmids pJJJ1 and pJJJ2 containing EBER1 and EBER2, kindly donated by Dr J. Arrand, Manchester, UK,[33] were linearized before in vitro transcription with a combination of T3 or T7 RNA polymerases[34] (Bethesda Research Laboratories, Gaithersburg, MD) and ^{35}S-UTP or digoxigenin (DIG)-UTP.

Hybridization protocol. Hybridization was performed according to established protocols[35] on paraffin-embedded tissue sections with either radioactive (^{35}S) or nonisotopic (DIG) labeled in vitro transcribed sense and antisense EBER probes. To increase the sensitivity of in situ hybridization (ISH), a mixture of EBER1 and EBER2 antisense probes was applied. A cocktail of the sense

Table 2. Primers for Nested Amplification and Detection of EBV-DNA and β-Globin Sequences

Primer	Sequence	Use	Position
EBV up	5′-GCAGTAACAGGTAATCTCTGG-3′	1st PCR	20124-20145
EBV low (R)	5′-ACTTTAGAGGCGAATGGGCG-3′	2nd PCR	20210-20230
EBV low	5′-ACCAGAAATAGCTGCAGGACC-3′	1st PCR	20524-20504
EBV low (R)	5′-TGGCTGCTGTCTGGCTTACG-3′	2nd PCR	20459-20439
β-globin up	5′-ATGGTGCACCTGACTCCTGAGG-3′	1st PCR	2205-2226
β-globin up (R)	5′-ATAACAGCATCAGGAGTGG-3′	2nd PCR	2229-2247
β-globin low	5′-GCCATCACTAAAGGCACCGAGC-3′	1st PCR	2259-2538
β-globin low (R)	5′-AAGTCTGCCGTTACTGCCC-3′	2nd PCR	2501-2483
EBV probe	5′-TATCTTTAGAGGGGAAAAGAGGAATAAG-3′	Detection	20314-20341

Abbreviation: (R), reamplification.

Table 3. EBV-DNA, Distribution, Number, and Morphology of EBER- and LMP-Positive Cells in 32 Cases of AILD-TLC

Case No.	EBV-DNA by PCR	Distribution and No. of EBV+ Cells	Morphology of EBER+ Cells (ISH)	Morphology of LMP+ Cells (immunostaining)
First Group				
1	Positive	Nodular, many cells	B-IB, RS-cell-like & B-lymphocytes	B-IB, RS cell-like
2	No β-globin amplification	Nodular, many & diffuse, single cells	Small B and T lymphocytes	No positive cells
3	Positive	Nodular, many & diffuse, single cells	Small B lymphocytes & medium sized pleom T cells (tumor cells)	No positive cells
4	Positive	Nodular, many & diffuse, single cells	B-IB, RS cell-like & small T lymphocytes	B-IB, RS cell-like
5	No β-globin amplification	Nodular, many & diffuse, single cells	B-IB & small T lymphocytes	B-IB
6	No β-globin amplification	Nodular, many & diffuse, single cells	B-IB & medium sized pleom T cells (tumor cells)	No positive cells
7	Positive	Nodular, many & diffuse, single cells	Small B & T lymphocytes	Small B & T lymphocytes & medium sized pleom T cells (tumor cells)
8	Positive	Nodular, many cells	B-IB, HD-cell-like	B-IB, HD-cell like
Second Group				
9	Positive	Diffuse, many cells	20% of tumor cells & B-IB & few small B lymphocytes	B-IB & tumor cells
10	Positive	Diffuse, many cells	15%-20% of tumor cells	Single tumor cells
11	Positive	Diffuse, many cells	5%-10% of tumor cells & B-IB	B-IB & small T lymphocytes
12	Positive	Diffuse, single cells	5% of tumor cells	No positive cells
13	No β-globin amplification	Diffuse, many cells	5% of tumor cells & B-IB	Single tumor cells & B-IB
14	Positive	Diffuse, many cells	20% of tumor cells & B-IB	B-IB & small T lymphocytes
15	No β-globin amplification	Diffuse, few cells	5% of tumor cells & single B-IB	B-IB, RS-cell-like & small T lymphocytes
16	Positive	Diffuse, few cells	1%-5% of tumor cells & small B lymphocytes	No positive cells
17	No β-globin amplification	Diffuse, few cells	2% of tumor cells & small B lymphocytes	Small T- & B-lymphocytes
18	No β-globin amplification	Diffuse, few cells	2% of tumor cells	No positive cells
19	No β-globin amplification	Diffuse, many cells	15% of tumor cells	Single tumor cells
20	Positive	Follicular, many cells	20% of tumor cells & B-IB	B-IB, HD cell-like
21	No β-globin amplification	Diffuse, few cells	2% of tumor cells in cutaneous infiltrate	No positive cells
Third group				
22	No β-globin amplification	Diffuse, few cells	Small T & B lymphocytes	No positive cells
23	Positive	Single cells	Small T lymphocytes	No positive cells
24	Positive	Single cells	Small T & B lymphocytes	Small T & B lymphocytes
25	No β-globin amplification	Single cells	Small T lymphocytes	No positive cells
26	Positive	Single cells	Medium sized pleom T cells	Medium sized pleom T cells
27	Positive	Single cells	Small T lymphocytes	No positive cells
28	Positive	Diffuse, few cells	Small B lymphocytes	No positive cells
29	Positive	Single cells	Small T lymphocytes	No positive cells
30	Negative	Single cells	Small T lymphocytes	No positive cells
31	Positive	Single cells	Small B & T lymphocytes	Small B lymphocytes
32	No β-globin amplification	No labeled cells	—	—

Abbreviations: B-IB, B immunoblasts; RS cell-like, similar to Reed-Sternberg cells; pleom, pleomorphic; HD cell-like, similar to Hodgkin cells.

control probes was also used. After dewaxing with xylene and digestion with proteinase K (500 μg/mL), the slides were incubated for 12 hours at 37°C with a hybridization solution containing either 1.2×10^7 cpm/mL of ^{35}S-labeled or 1 ng of DIG-labeled EBER cocktail per section. After washing in 50% formamid and RNAse A treatment, immobilized radioactive probes were detected by dipping the slides into a 1:2 solution of Ilford G5 (Ilford, Mobberly, UK) emulsion, followed by exposure for 3 to 10 days at 4°C. The slides were then developed and counterstained. An immunologic detection was performed in the case of DIG-labeling. Briefly, the washed slides were incubated with a DIG-specific phosphatase-conjugated Fab fragment and developed with Naphtol-as-biphosphate and New Fuchsin (Merck, Darmstadt, Germany).[36] All steps comprising pretreatment of the slides and hybridization were performed under conditions avoiding RNAse contamination. To demonstrate the RNA origin of our targets, an RNAse predigestion step was introduced for several cases, resulting in complete disappearance of the signal. Additional ISH with a cocktail of labeled unrelated oligonucleotides on several cases did not lead to any cross-reactivity. Furthermore, no hybridization signal was detected using the EBER sense probes. Sense and antisense probes were checked for their suitability on several EBER-positive and -negative cell lines. The case that was negative for EBER-ISH (case 32) was hybridized with an oligo(dT) primer to ascertain the presence of tissue RNA.

Double labeling. For double labeling, the immunohistologic demonstration of T-cell–specific or characteristic (β-F1 and CD45RO), B-cell–specific (CD20) and FDC-characteristic (CD21) antigens was performed before ISH under RNAse-free conditions. In specific, the APAAP technique was used to visualize the detection of the antigens before the application of ^{35}S-labeled EBER probes, whereas the ABC system was applied in the case of DIG-labeled EBER probes.

RESULTS

EBV-DNA Detection by PCR

In 12 of the 32 cases of AILD-TCL and 2 of the 12 normal lymphoid tissue samples, the extracted DNA was not amplifiable as shown by the nonappearance of a β-globin–specific band after amplification and reamplification with appropriate primers. In 19 of the remaining 20 AILD-TCL cases, PCR analysis showed EBV-specific sequences (Table 3; Fig 1). In contrast, EBV-specific DNA was demonstrable with the same technique in only 2 of the remaining 10 normal lymphoid tissue samples.

Detection of EBER by ISH

Both radioactive and nonradioactive antisense probes specific for EBER were applied and gave identical results: EBV-infected cells were demonstrable in all but 1 of the 32 AILD-TCL cases (see Table 3), as well as in 3 of the 12 normal lymphoid tissue samples.

The comparison of these data with the PCR results showed that the latter data were congruent with the findings of EBER-ISH in all but one instance. In this one exception (case no. 30), EBER-ISH detected only 16 labeled cells in the whole section.

Both the radioactive and nonradioactive EBER-specific probes were suitable for the determination of the overall frequency and distribution of EBV-infected cells. The nonradioactive probes proved to be superior for the morphologic evaluation of the EBV-infected cells. With the latter reagents, it could be clearly shown that only lymphoid cells were EBV-positive. All other cell types, including granulocytes, macrophages, endothelial cells, fibrocytes, and plasma cells, were EBV-negative.

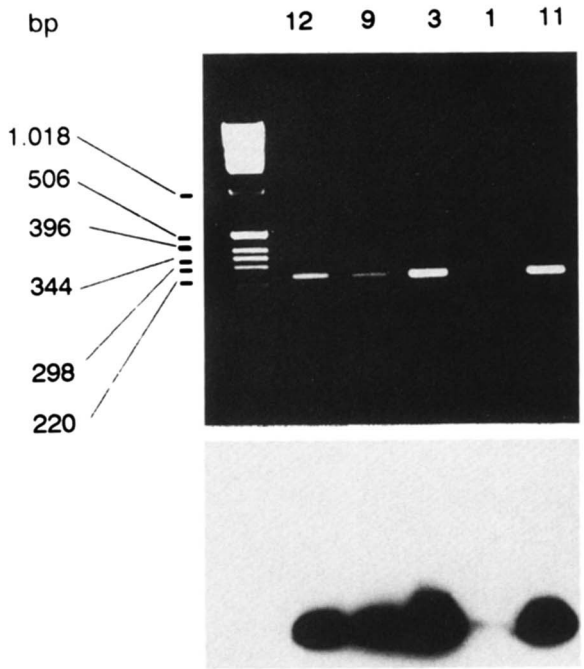

Fig 1. PCR amplification products of EBV-specific DNA sequences in extracted tissue DNA from lymph nodes of five different AILD-TCL cases. Products are visible in ethidium-bromide-stained agarose gel as bands of 249 base pairs (bp) in various quantities due to different amounts of amplifiable DNA (upper part). Hybridization of corresponding Southern blot with ^{32}P-labeled internal oligonucleotide disclosed bands of appropriate size in all cases after 12 hours exposure (lower part).

To identify the lineage allocation of the EBV-infected lymphoid cells, the EBER-ISH labeling was compared with the immunohistological staining reactions obtained on serial sections using the MoAbs listed in Table 1. Three patterns of EBV-infection were distinguished: (1) A nodular distribution of EBER-positive lymphoid cells predominantly located in the remnants of lymphoid follicles in 8 of 31 cases (Fig 2A). The majority of these cells exhibited the morphology of immunoblasts with large oval, round, or slightly lobated nuclei and usually a centrally located, prominent nucleolus (Fig 2C). In several specimens, some EBER-positive cells displayed one or two large nuclei, similar to small-sized variants of HD and Reed-Sternberg (RS) cells (Fig 2C, insert). In addition, EBER signals were observed in medium-sized cellular elements showing the morphology of proliferating (neoplastic) T cells. (2) A diffuse distribution of many small, medium, and large EBER-positive lymphoid cells in 13 of 31 cases (Fig 3A). They were identical in size and cytology to (neoplastic) cells expressing T-cell antigens. In addition, there were EBER-positive cells with the morphology of immunoblasts. In several instances, EBER-positive cells were in mitosis. (3) An occasional occurrence of EBER-positive cells in 10 of 31

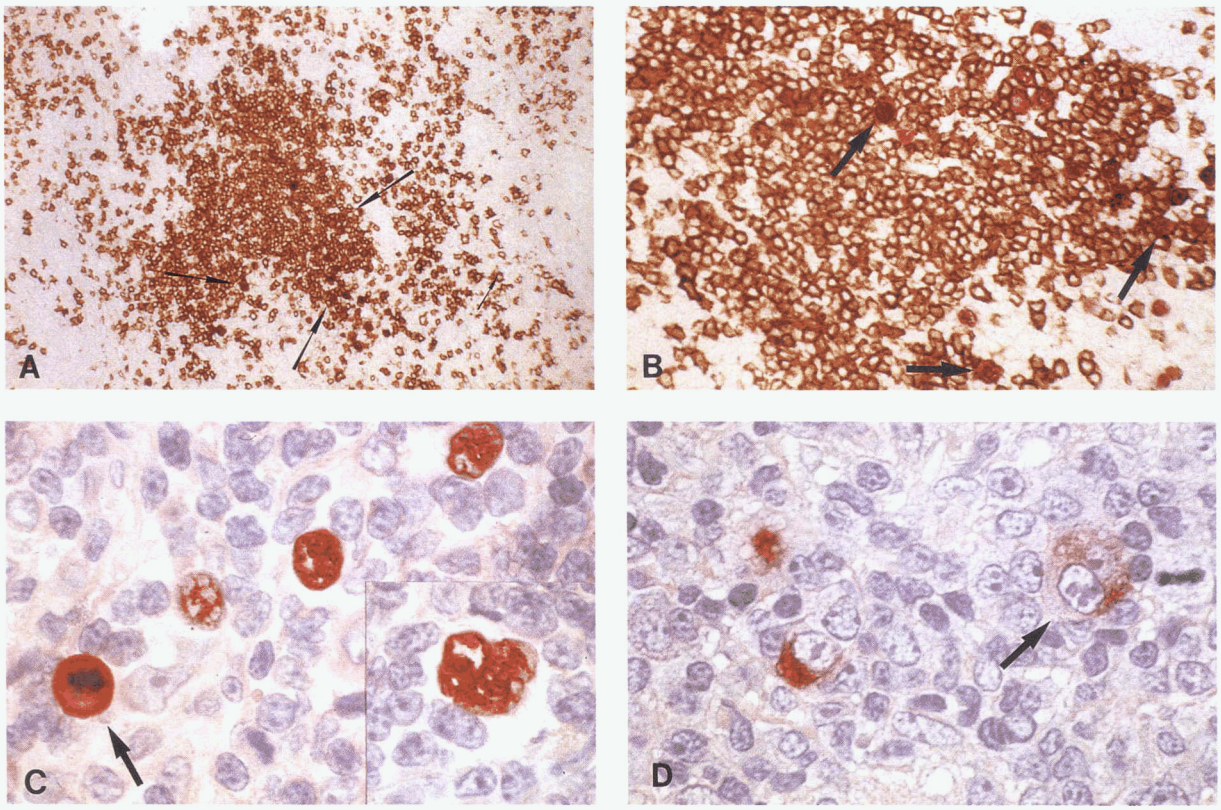

Fig 2. Nodular EBV-infection pattern in AILD-TCL. (A) Double labeling by using DIG-labeled EBER1- and EBER2-specific probes and MoAb L26 (CD20). Immunoenzymatic EBER-ISH (red label) was performed after detection of the L26 binding visualized by the ABC method (brown label). Note the nodular distribution of EBER-positive cells (arrows) in the remnant of a B zone, which is referred to as EBV-infection pattern 1. (Original magnification × 160.) (B) Detail of (A). Note that EBER-positive cells are also labeled with the MoAb L26 (arrows). (Original magnification × 400.) (C) ISH by using DIG-labeled EBER1- and EBER2-specific probes. Labeled cells correspond morphologically to immunoblasts. (Original magnification × 1,000.) (Insert) One labeled cell is in mitosis (arrow); there are occasional binucleated RS-like cells (Original magnification × 1,000). (D) LMP immunostaining in a case of AILD-TCL with the MoAb CS1-4. The labeling is confined to immunoblasts and RS-like cells (arrow). (APAAP; original magnification × 1,000.)

cases (Fig 4) These cells were small and possessed a round or slightly irregularly shaped nucleus, resembling the occasional EBER-positive lymphoid cells occurring in 3 of 12 normal lymphoid tissue samples (Fig 5).

Double Labeling for EBER and Lineage Markers

All AILD-TCL cases were analyzed using a combination of labeling with nonisotopic EBER probes and immunohistology. Radioactively labeled EBER probes were applied in six cases, two of which were of EBV infection pattern 1 and four of EBV infection pattern 2. Both approaches produced congruent results. The majority of EBER-positive, immunoblast-like cells of the cases showing infection pattern 1 reacted with L26, confirming the B-cell nature of most of these cells (Fig 2B). The majority of the EBER-positive small, medium, and large cells, as well as many of those in mitosis present in the cases showing infection pattern 2, were reactive with the MoAbs βF1 and/or UCHL1, hence verifying their T-cell nature (Fig 3B). In addition, several of the immunoblast-like EBER-positive cells observed in this group of cases reacted with L26. The single EBER-positive lymphocytes present in the cases showing infection pattern 3 were reactive with either MoAbs βF1 and/or UCHL1 or with L26, indicating their T- or B-cell nature (Fig 4, insert). Double labeling for EBER and CD21 did not lead to a colocalization of the labeling in any of the cases studied, which means that all of the CD21-positive cells, including all FDC, were not infected by EBV.

Detection of EBV-Encoded LMP

The cocktail of four MoAbs directed against different epitopes on the LMP led to the staining of lymphoid cells in 17 of the 32 AILD-TCL. The number of LMP-positive cells was, in all instances, less than the number of EBER-positive cells. LMP was demonstrated on the cell membrane, usually in a patchy distribution, and, in addition, in the cytoplasm. The intensity of the LMP staining varied considerably both within the same case and between cases. The comparison of the LMP staining with the three EBV infection patterns seen with the EBER-ISH led to the following findings: Of the eight AILD-TCL cases displaying infection pattern 1, five contained LMP-positive cells. In four cases, the nodular distribution of the LMP-positive cells was similar to that of the EBER-positive cells, which had the morphology of B immunoblasts and, occasionally, that of binucleated RS cells (Fig 2D). In the fifth case, the

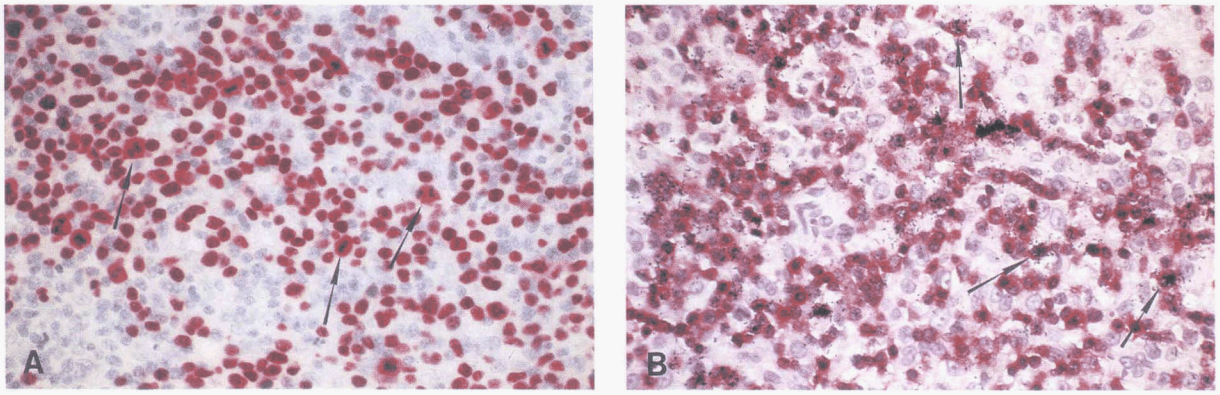

Fig 3. Diffuse EBV infection pattern in AILD-TCL. (A) ISH by using DIG-labeled EBER1- and EBER2-specific probes. Detection of many positive cells diffusely distributed in the lymph node, which is referred to as "EBV infection pattern 2." Positive nuclei vary in size from small, to medium, to large. Note the labeled mitotic figures (arrow). (Original magnification × 400.) (B) Double labeling for CD45RO (MoAb UCHL-1; APAAP technique) and EBER (ISH with EBER-specific ^{35}S-labeled probes; exposure time, 5 days). Many EBER-positive cells are labeled with MoAb UCHL-1 (arrows). (Original magnification × 400.)

LMP positivity was associated with medium-sized lymphoid cells, which—as shown in adjacent sections—were reactive with anti–T-cell antibodies. In the second group of 13 cases showing infection pattern 2, nine were LMP-positive. The LMP-positive cells were diffusely scattered throughout the diseased tissue. Most of the LMP-positive cells resembled small, medium, and large pleomorphic T cells. In several cases, some of the LMP-positive cells were similar to immunoblasts, HD cells, or RS cells. In the group of 10 cases with infection pattern 3, only three cases contained occasional LMP-positive cells, most of which were the size of small lymphoid cells.

There were no LMP-positive cells detectable in the 12 normal lymphoid tissue samples, including those that contained EBER-positive cells.

DISCUSSION

The data presented here indicate a strong association of AILD-TCL with EBV (97% of cases studied). This evaluation was accomplished by the application of two different and independent nucleic acid detection methods: the PCR technique for the demonstration of EBV-specific DNA sequences and ISH for the detection of EBER. Both methods produced almost identical results, showing that EBER-ISH was no less sensitive than PCR. The high sensitivity of the EBER-ISH is not surprising when the high copy number of EBER (up to 10^6 per infected cell) in the nuclei of the infected cells is considered. EBER-ISH proved to be superior to PCR as demonstrated by the fact that the DNA of 11 cases was not amplifiable (as shown by nonappearance of a β-globin band), whereas the application of EBER-ISH produced clearly specific signals in these cases.

When compared with normal lymphoid tissue, it became evident that EBV is more frequently present in AILD-TCL (97%) than in nondiseased lymphoid tissue (25%), which suggests a strong involvement of EBV in the pathogenesis of AILD-TCL. To assess the possible role of EBV in AILD-TCL, the number and identity of EBV-infected cells

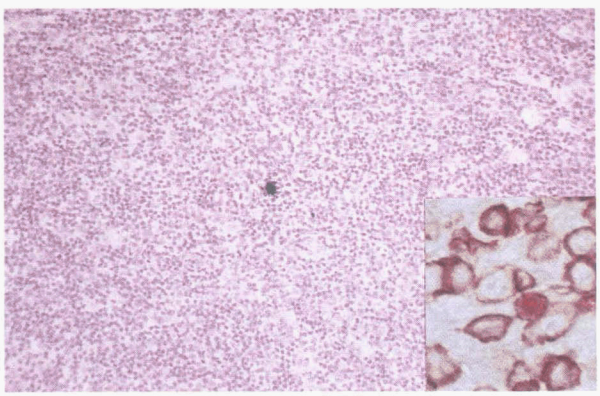

Fig 4. Occasional EBV infection in AILD-TCL. ISH by using ^{35}S-labeled EBER1- and EBER2-specific probes (exposure time, 3 days). Only a single cell is labeled and is therefore referred to as "EBV-infection pattern 3." (Original magnification × 160.) Additional double labeling for EBER using DIG-labeled specific probes and MoAb βF1 for the β-chain of the T-cell receptor (insert). Note that the EBER-positive cell (with red nucleus) is colabeled with the MoAb βF1 demonstrated by ABC reaction (brown label). (Original magnification × 1,000.)

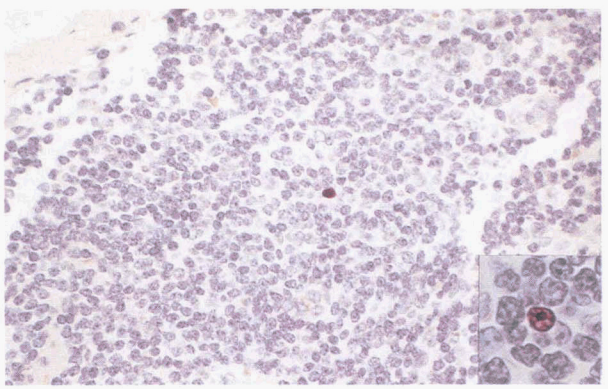

Fig 5. EBV infection pattern in normal lymph nodes. ISH for EBER in normal lymphoid tissue by using DIG-labeled EBER1- and EBER2-specific probes. Note that the single EBER-positive cell (insert) resembles the other small B lymphocytes in morphology and size. (Original magnification × 160; insert × 1,000.)

were investigated. In these experiments, the nonradioactive EBER-specific probes proved to be ideal for the morphologic evaluation of the infected cells, whereas ISH with radioactive EBER-specific probes could be well combined with immunohistologic labeling for the determination of the antigenic profile of the infected cells. Through the correlation of the ISH results with morphology and immunohistology, three patterns of EBV-infection were observed: (1) predominant EBV infection of large immunoblast-like B cells nodularly distributed in the area of residual lymphoid follicle and minor infection of neoplastic T cells (8/31 cases); (2) predominant EBV infection of many small, medium, and large neoplastic T cells, including those in division, and minor infection of B cells (13/31 cases); and (3) EBV infection of occasional small lymphoid cells (10/31 cases).

LMP was detectable in all three infection pattern groups, with a predominance in the cases of groups 1 and 2. This indicates the transcriptional and translational activity of EBV genes in AILD-TCL in contrast to normal lymphoid tissue, in which the EBV-positive cells were consistently LMP-negative. LMP expression of the EBV-infected cells in AILD-TCL appears to be relevant, as it has already been demonstrated that, in vitro, LMP has transforming potential[37-39] and can prolong cell survival (probably by blockage of programmed cell death through induction of *bcl-2* expression).[40]

The predominant infection of B cells in the nodularly EBV-positive cases and the predominant infection of T cells in the diffusely EBV-positive cases was confirmed by double labeling for EBER and lineage markers performed on all AILD-TCL cases. The pathway of B- and T-cell infection by EBV in AILD-TCL is unclear, as cells harboring EBV expressed no detectable levels of the EBV-receptor antigen molecule CD21.[41-44] Interestingly, the proliferated FDC also did not contain any detectable EBV, although the EBV-receptor molecule CD21 was expressed—as in normal and other lesions[45]—in all instances at a high density.

The EBV infection of two cell lineages would appear to be unique to AILD-TCL, as this has not yet been reported for other malignancies. This may reflect the differences in the chromosomal aberrations and the configuration of antigen receptor genes between AILD-TCL and other malignant lymphomas. In AILD-TCL, simultaneous rearrangements of T-cell receptor and immunoglobulin genes can be found in a number of instances.[16] Over the course of time, rearrangements disappeared and were occasionally replaced by new ones.[15] The frequent presence of karyotypically unrelated abnormal clones and/or cells with nonclonal chromosomal abnormalities and a large proportion of normal mitotic cells, as well as a high incidence of trisomy 3 and 5, have been reported.[8,9] Sequential studies have found different patterns of cytogenetic abnormalities, with some clones emerging and predominating over time, and others disappearing completely.[8,10] With regard to these findings, it is tempting to speculate that AILD-TCL develops from a persistent EBV-infection during which EBV infects different cell lineages and possibly different cell clones within a cell lineage. Dependent on growth advantage due to genetic alterations and the T-cell reaction against the EBV-infected cells, certain EBV-positive or EBV-negative clones expand or diminish, the result of which is the heterogeneity in the EBV infection patterns described above. Hence, it would appear possible that the disease begins with a deregulated response to antigenic stimulation as a consequence of damage or loss of regulatory cell populations that might otherwise confine EBV infection and limit lymphocyte expansion during immunologic challenge.

A challenge for future research is to elucidate the precise role of EBV in the pathogenesis of AILD-TCL. One possible step in this direction would be the correlation of the EBV detection at the single-cell level with the clinical presentation, appearance, and disappearance of antigen receptor rearrangements and chromosomal abnormalities, mutated oncogenes, and suppressor genes, as well as the course of the disease. Given the rarity of this lymphoma entity, such investigations could only be achieved by a multiinstitutional study.

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