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Evidence for Early B-Cell Activation Preceding the Development of Epstein-Barr Virus–Negative Acquired Immunodeficiency Syndrome–Related Lymphoma

By Grzegorz K. Przybylski, June Goldman, Valerie L. Ng, Michael S. McGrath, Brian G. Herndier, David P. Schenkein, John G. Monroe, and Leslie E. Silberstein

To investigate the origin and pathogenesis of acquired immunodeficiency syndrome (AIDS)-related lymphoma (ARL), we studied 14 cases in which Epstein-Barr virus (EBV) infection was not an etiologic factor. By histology, 8 of the specimens were of the small noncleaved cell type and 6 consisted of the large diffuse cell type. Southern analysis using a J_H probe was consistent with a monoclonal B-cell tumor in 13 cases. To characterize the expressed Ig genes, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) and direct sequencing of PCR products. Eight cases expressed IgM and 1 case expressed IgG. V_H3 genes were found in 5 cases, V_H4 genes in 3 cases, V_H1 genes in 2 cases, and a V_H7 gene in 1 case. The nucleotide homology to known germline V_H genes ranged from 80% to 97%, suggesting significant somatic diversification of expressed V_H genes. The large proportion of V_H3-expressing lymphomas in this series corresponds to the frequency of V_H3-expressing B cells in the peripheral blood from healthy and (recent) human immunodeficiency virus (HIV)-seropositve individuals and contrasts with the V_H3 clonal deficit observed in late stages of HIV infection. Similar to the Ig heavy chain genes, the corresponding Ig light chain genes showed significant deviation from known germline gene sequences. The large proportion of V_H3-expressing lymphomas as well as the high degree of somatic deviation from germline suggest that these EBV-negative lymphomas might arise from antigen-selected expanded B-cell clones before transformation. Further support for this hypothesis is provided by sequential Ig sequence analysis in 1 patient with large-cell lymphoma. It was shown that 3 years before the diagnosis of axillary lymphoma, there existed several B-cell clones in this patient's bone marrow. One of these clones present in the bone marrow expressed the same rearranged V_H gene as the axillary lymphoma. Taken together, the current findings from Ig gene analyses suggest that activation of B cells in the early phase of HIV infection may be a predisposing factor for subsequent B-cell transformation.

BESIDES KAPOSI SARCOMA, non-Hodgkin's lymphoma (NHL) is one of the most frequently occurring neoplasms in acquired immune deficiency syndrome (AIDS) patients. With the prolonged survival of AIDS patients due to advances in antiretroviral treatment and better management of opportunistic infections, the incidence of the AIDS-related lymphoma (ARL) cases has increased during the last several years. Because of its extremely poor prognosis, ARL is becoming one of the major causes of death in AIDS patients. Currently, there is a little understanding of the pathogenesis of ARL. Very aggressive, disseminated progression of ARL with frequent involvement of the extranodal sites and their nearly exclusive B-cell origin distinguish ARLs from lymphomas occurring in the general population.[1,2] Two thirds of ARLs are high-grade lymphomas and one third of cases are of intermediate grade. Histologically, the high-grade ARLs are classified as immunoblastic or small noncleaved lymphoma (Burkitt's and non-Burkitt's type) and the intermediate-grade ARLs are classified as diffuse large-cell tumors.[2,3] The high-grade immunoblastic ARLs are frequently associated with Epstein-Barr virus (EBV) infection and morphologically resemble lymphomas developing in immunosupressed patients.[2,4,5] In these cases, EBV-dependent proliferation and immortalization seem likely to be factors predisposing B cells for subsequent malignant transformation. Nevertheless, the majority of ARLs show no human immunodeficiency virus (HIV) or EBV infection. The prevalence of c-myc oncogene rearrangement in ARL is variable, ranging from 35% to 90%.[6-8] However, a recent study suggested that c-myc is rearranged in virtually all lymphoma cases of the small noncleaved type.[9] Lymphomas of this histologic subtype are often EBV-negative; therefore, c-myc deregulation may be an important pathogenetic factor in the multistep process of lymphomagenesis in EBV-negative tumors. Chronic activation by HIV has also been postulated to be a predisposing factor in lymphoma development. For example, follicular hyperplasia of multiple lymph nodes and oligoclonal hypergammaglobulinemia often develop in HIV-infected individuals and are speculated to precede lymphomagenesis.[10-12] Moreover, activation of B cells by HIV could be shown in vitro[13] and Ig variable heavy chain gene family (V_H3) products have been reported to be a natural ligand for HIV gp120.[14] V_H3-expressing B cells represent the majority of circulating B cells in healthy individuals[15,16] and in HIV-infected patients during the early phase of infection.[17-19] In contrast, the number of V_H3-expressing B cells in peripheral blood markedly decreases in the later stages of infection.[17-19] Consequently, it may be postulated that, if ARLs originate from (activated) B cells in the early phase of infection, one would find a high frequency of V_H3 genes in ARL. On the other hand, if ARLs arise from B cells present in the later phases of HIV infection, a bias against V_H3-expressing lymphomas could be expected.

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Table 1. EBV-Negative AIDS-Related Lymphomas

	Clonality*	Histology	CD4
1	Mono	SNCL	60
2	Poly	SNCL	10
3	Mono	DLCL	ND
4	Mono	SNCL	ND
5	Mono	DLCL	185
6	Mono	DLCL	123
7	Mono	SNCL	129
8	Mono	DLCL	285
9	Mono	SNCL	0
10	Mono	SNCL	125
11	ND	DLCL	170
12	ND	SNCL	440
13	Mono	DLCL	ND
14	Mono	SNCL	101
Σ		SNCL-8	148
		DLCL-6	

Abbreviations: SNCL, small noncleaved lymphoma; DLCL, diffuse large-cell lymphoma; Mono, monoclonal; Poly, polyclonal; ND, not done; CD4, number of $CD4^+$ T lymphocytes per 1 μL of peripheral blood.

* Clonality determined by Southern blot with JH probe.

To gain insight into the process of lymphomagenesis in AIDS patients, we analyzed Ig heavy chain (IgH) and Ig light chain (IgL) genes expressed in 14 EBV-negative ARLs. In particular, we were interested in the pattern of Ig V gene usage and the degree of somatic diversification as evidence for B-cell activation. In further support for a role of B-cell activation in ARL development, we were able to show in 1 case that the lymphoma developed from an expanded B-cell clone present in the bone marrow 3 years before the diagnosis of peripheral lymphoma.

MATERIALS AND METHODS

Patient samples. In this study, 14 EBV-negative ARLs were investigated. In 13 cases, cryopreserved tissue samples were available, and in 1 case a paraffin-embedded sample was used. As determined by histology, 8 specimens were of the small noncleaved cell type and 6 were of the diffuse large-cell type (Table 1).

The case ARL 11 had a serum paraprotein 3 years before presentation with a diffuse large-cell lymphoma. Although this patient was diagnosed as having multiple myeloma based on clinical and laboratory criteria, his disease course was very mild and the patient was not compliant with follow-up clinic visits. Consequently, he received no chemotherapy before his presentation with peripheral lymphoma. Thus, HIV infection is the most likely ethiologic factor for lymphoma development in this patient.

Southern blot analysis. High molecular weight DNA was isolated from cryopreserved lymphoma samples by standard techniques. Ten micrograms of genomic DNA was digested with *Bam*HI, *Hin*dIII, or *Eco*RI or with both *Eco*RI and *Bam*HI. DNA fragments were size-separated in a 0.7% agarose gel and transferred onto nylon membranes (Zeta-Probe; Bio-Rad, Richmond, CA). Filters were hybridized with a J_H probe, a 3.3-kb genomic fragment containing 4 of the 6 germline heavy chain joining segments,[20] and with a 1.9-kb EBV-specific probe containing the unique DNA sequence 5′ from the tandem repeats.[21]

Polymerase chain reaction (PCR). Total RNA was extracted from cryopreserved lymphoma samples using a guanidinium thiocyanate-phenol-chloroform method (Trizol; Life Technologies, Inc, Gaithersburg, MD). Five micrograms of total RNA were reverse-transcribed using oligo d(T) primer and SuperScript II reverse transcriptase (Life Technologies, Inc). PCR of 50 μL total volume was performed with 1 μL of 20 μL total cDNA reaction; 400 nmol/L of each primer; 200 μmol/L each of dATP, dCTP, dGTP, and dTTP (Perkin Elmer Cetus, Norwalk, CT); 1.5 U Taq polymerase; and PCR buffer containg 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 0.001% (wt/vol) gelatin. After 3 minutes of denaturation at 94°C, 30 PCR cycles were performed, with each cycle consisting of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute, and extension at 72°C for 1 minute in a DNA Thermal-Cycler (Coy Inc, Grass Lake, MI). The final extension was performed at 72°C for 10 minutes. For seminested analysis, 1 μL of PCR products was further amplified for 25 cycles with a seminested 3′ primer under the same conditions. A negative control was included in each PCR reaction. PCR products were analyzed by 2% agarose gel electrophoresis containing ethidium bromide. The nucleotide sequences of the primers used are listed in Table 2.

DNA extraction from the paraffin-embedded samples. Genomic DNA was extracted from 10-μm sections of paraffin-embedded lymphoma sample as described by Greer et al.[22] A single section was placed in the 1.5-mL Eppendorf tube. To deparaffinize the sample, 1 mL of xylene was added and mixed for 10 minutes. After centrifugation for 5 minutes in a microcentrifuge, the solvent was removed and the pellet was washed with 98% ethanol. The pellet was dried and suspended in 200 μL digestion buffer (50 mmol/L Tris-HCl [pH 8.5], 1 mmol/L EDTA, 0.5% Tween 20). One hundred micrograms of proteinase K was added and the sample was incubated overnight at 55°C. Two microliters of the crude DNA preparation was used for PCR. PCR was performed using as a 5′ primer FR1,3,5; FR2; or FR4,6; and a 3′ JH primer under the same conditions as described for cDNA analysis. For seminested analysis, 1 μL of PCR product was amplified for 25 cycles with the seminested 3′ JHbio primer.

Direct sequencing of PCR products. PCR products for sequencing were obtained as described above using seminested, biotinylated

Table 2. Primer Sequences

IgH gene	
5′ Primers	
FR1VH1,3,5:	CAG GTG CAG CTG GTG CAG TCT GG
FR1VH2:	CAG GTC AAC TTA AGG GAG TCT GG
FR1VH4,6:	CAG GTG CAG CTG CAG GAG TCG GG
LVH1:	ATG GAC TGG ACC TGG AGG GTC
LVH2:	ATG GAC ATA CTT TGT TCC AC
LVH3:	ATG GAG TTT GGG CTG AGC TGG
LVH4:	ATG AAA CAC CTG TGG TTC TT
LVH5:	ATG GGG TCA ACC GCC ATC CT
LVH6:	ATG TCT GTC TCC TTC CTC AT
3′ Primers	
Cμ:	CGA GGG GGA AAA GGG TTG GGG C
Cγ:	CAG GGG GAA GAC CGA TGG GC
J_H:	GCT CTA GAC TTA CCT GAG GAG ACG GTG ACC
JHbio:	TGA GGA GAC GGT GAC CAG GGT
IgL gene	
5′ Primers	
LVK1:	ATG GAC ATG AG(GA) GTC C
LVK2:	ATG AGG CTC GCT CAG CTC CTG
LVK3:	ATG GAA ACC CCA GCN CAG CTT CTC
LVK4:	ATG GTG TTG CAG ACC CAG GTC TTC
3′ Primers	
Cκbio:	AGA TGG CGG GAA GAT GAA GAC

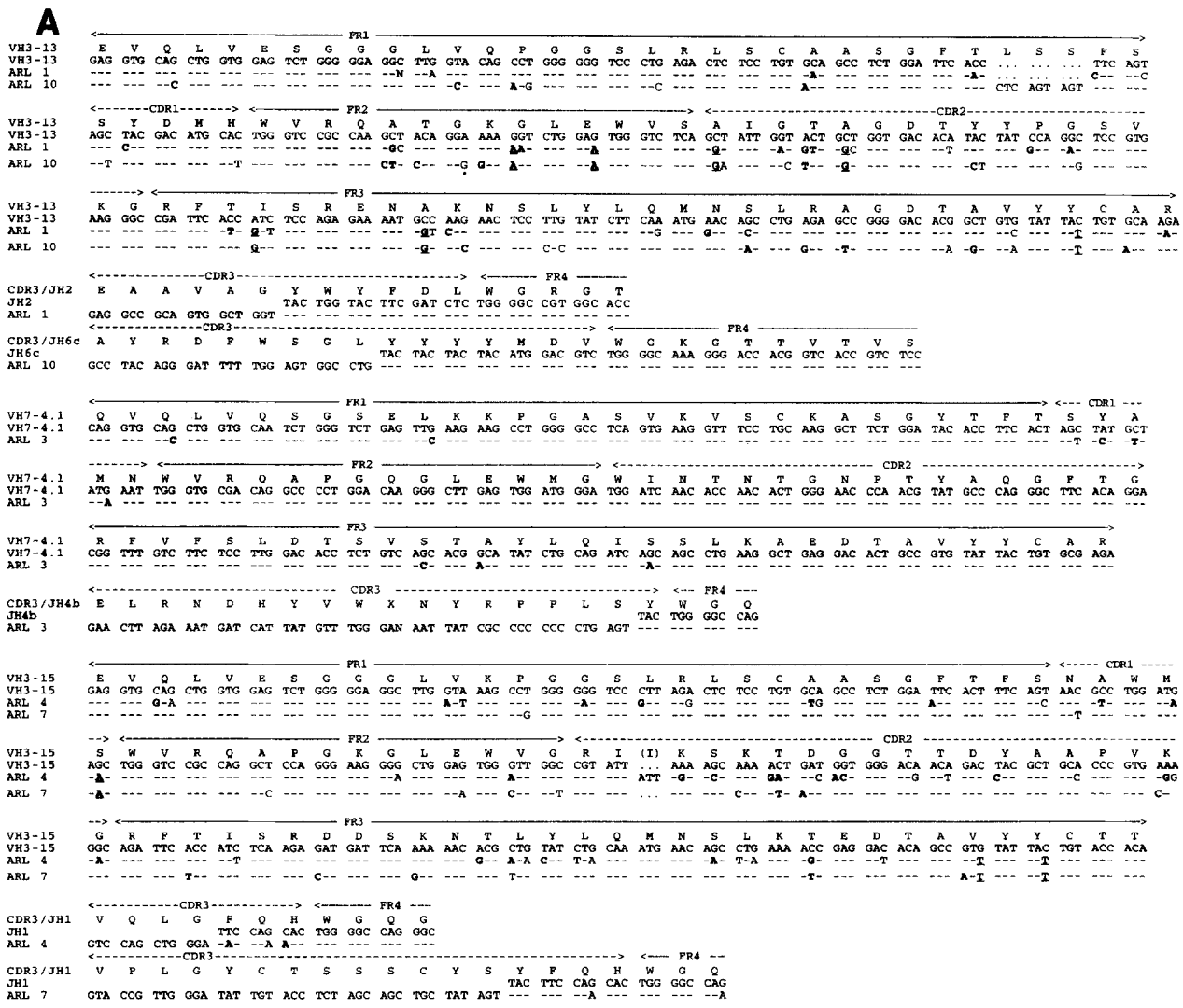

Fig 1. Nucleotide sequence alignment of the ARL-related IgH genes with their best matching germline V_H genes. The deduced amino acid sequence of the germline V_H gene is given. V_H genes were named according to the nomenclature proposed by Matsuda et al.[27] FR, framework region; CDR, complementarity determining region; JH, joining region. The nucleotide substitutions encoding for replacement mutations are printed in bold. Shared nucleotide differences present in the different samples are underlined.

Cκbio or JHbio primer. Single-stranded DNA was prepared using magnetic beads (Dynabeads) and the solid support purification method (Dynal, Lake Success, NY). Dideoxynucleotide chain termination sequencing reaction was performed with Sequenase Version 2.0 DNA sequencing kit (US Biochemicals, Cleveland, OH), as recommended by the supplier. Ten picomoles of one of the two PCR primers was used for sequencing. αS35 dATP (DuPont, NEN, Boston, MA; specific activity, 1,000 Ci/mmol) was used as the radioactive label. Both DNA strands were sequenced.

Cloning and sequencing of PCR products. PCR-amplified rearranged IgH genes were gel purified using the Micropure DNA purification kit (Amicon, Beverly, MA). After phosphorylation with T polynucleotide kinase (New England Biolabs, Beverly, MA) PCR products were blunt end ligated into the *Sma* I restriction site of the phagemid pBluscript II KS (+) (Stratagene, La Jolla, CA) and electoporated into the XL-1 Blue *Escherichia coli* strain (Stratagene). Plasmid DNA was extracted from single clones using the QIAGEN-tip 100 kit (QIAGEN, Chatsworth, CA). Nucleotide sequencing was performed with Sequenase Version 2.0 DNA sequencing kit (US Biochemicals), as described above. Plasmid DNA (0.5 pmol) and 10 pmol of the −40 sequencing primer were used for sequencing.

IgG cDNA repertoire analysis of bone marrow cells. Total RNA was extracted from cryopreserved bone marrow sample and 5 μg of RNA was reverse-transcribed using oligo d(T) primer as described above. PCR was performed with each of six leader amplimers as 5′ primers and a Cγ amplimer as the 3′ primer. The amplified products were cloned into the pBluescript cloning vector and bacterial clones representing the various V_H families were randomly picked for sequencing.

N-terminal amino acid sequencing. The p24-specific IgG paraprotein of ARL case no. 11 was affinity purified as previously reported.[23] Approximately 50 pmol of purified protein was then subjected to sequence analysis using automated Edman degradation, as previously published.[24,25]

Homology search. Obtained nucleotide sequences were ana-

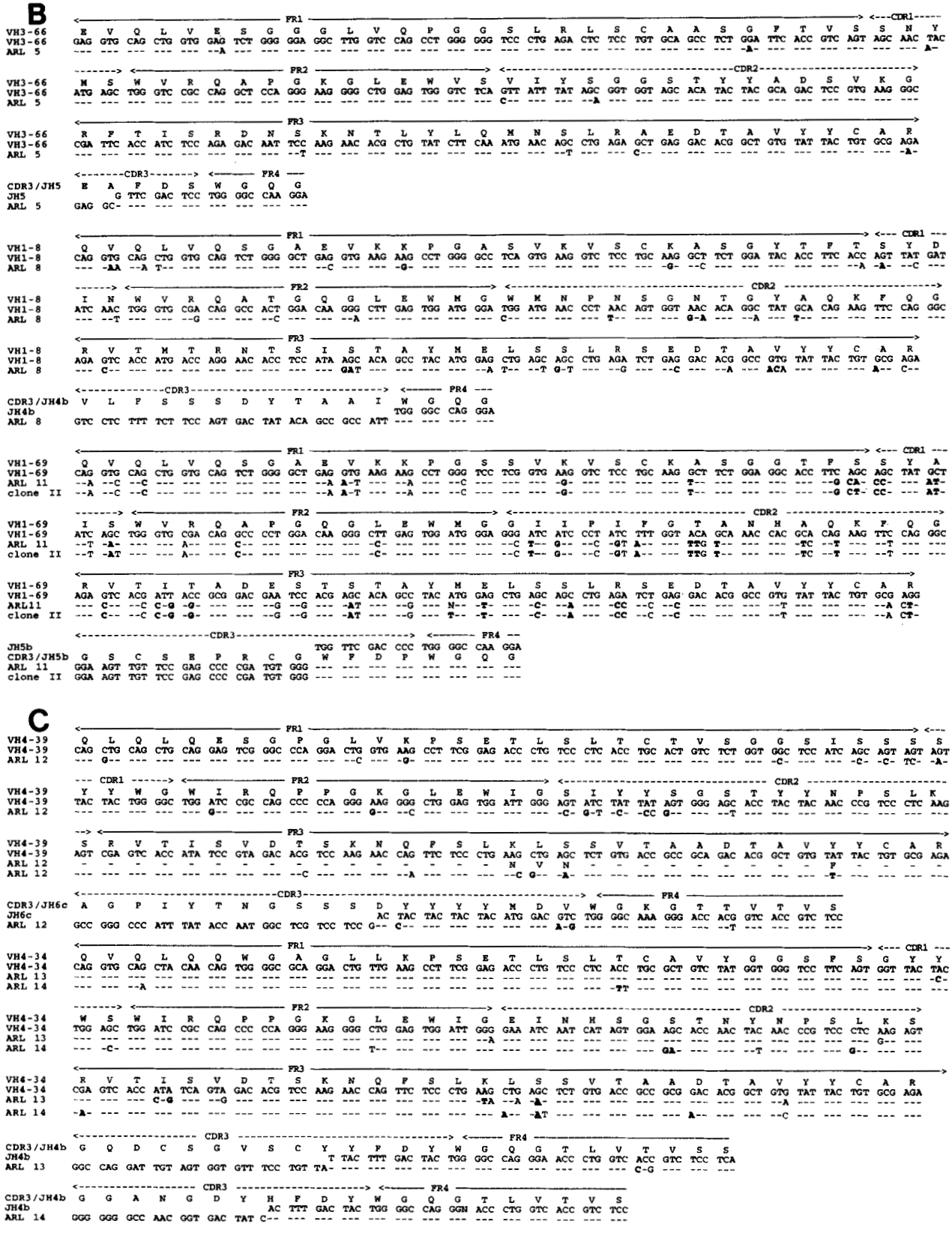

Fig 1 (cont'd).

Table 3. V_H Analysis of EBV-Negative AIDS-Related Lymphoma

	V_H Gene	Homology	Isotype
1	V_H 3-13	89.0%	ND
2	Poly	Poly	Poly
3	V_H 7-4.1	96.6%	IgM
4	V_H 3-15	84.5%	IgG
5	V_H 3-66	96.9%	IgM
6	NA	NA	NA
7	V_H 3-15	93.7%	IgM
8	V_H 1-8	87.1%	IgM
9	NA	NA	NA
10	V_H 3-13	85.7%	IgM
11	V_H 1-69	80.3%	ND
12	V_H 4-39	91.2%	IgM
13	V_H 4-34	96.2%	IgM
14	V_H 4-34	94.8%	IgM
Σ		90.5%	IgM: 8; IgG: 1

Abbreviations: NA, not amplifiable; Poly, polyclonal; ND, not done.

lyzed for their homology to germline V_H genes using the BLAST (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD) homology search program.[26]

RESULTS

IgH gene usage by AIDS-related lymphomas. To determine the tumor-derived Ig V_H gene, 5 μg of total mRNA was reverse-transcribed using an oligo-d(T) primer. One twentieth of the obtained cDNA was used for PCRs with one of the FR1 primers and either Cμ or Cγ primers. Because lymphoma tissue often contains normal lymphocytes in addition to tumor tissue, we obtained in some of the cases PCR products with more than one primer combination. However, when the amplified products were size-separated on a 10% polyacrylamide gel, the intense and sharp tumor-related band could easily be distinguished from the diffuse bands of different sizes corresponding to the polyclonal lymphocytes in the lymph node specimen. All monoclonal PCR products and some of PCR products showing diffuse bands were re-amplified with the nested JHbio primer and directly sequenced. Although the sequence analysis of the monoclonal bands represented a single nucleotide sequence, the diffuse polyclonal bands represented, as expected, multiple sequences within the third complementarity determining region (CDR3; data not shown).

Using this approach, the IgH gene expressed by the malignant clone was identified in 11 cases (see Table 3). In 2 cases, the monoclonal IgH gene could not be amplified, possibly due to somatic mutations at the primer binding site. Eight cases expressed IgM and 1 case expressed IgG. In 2 cases, DNA was used for PCR and the Ig class could not be determined. V_H3 genes were found in 5 cases, V_H4 in 3 cases, V_H1 in 2 cases, and V_H7 in 1 case.

ARLs do not show intraclonal diversity. To determine whether AIDS lymphomas exhibit intraclonal diversity, PCR products from selected ARL cases were cloned into pBluescript plasmid. Altogether, we sequenced 7 randomly picked clones from ARL 4, 2 clones from ARL 7, and 8 clones from ARL 10. All sequenced inserts were related to the previously identified lymphoma sequences. No intraclonal diversity was found. All the ARL 10 clones were identical. Two of seven ARL 4 sequences and one of two ARL 7 sequences differed by one nucleotide, most likely due to Taq polymerase infidelity.

V_H genes expressed by AIDS-related lymphomas appear somatically mutated from germline. The nucleotide sequences of the V_H genes and their best matching germline counterparts are shown in Fig 1. V_H genes were named according to the nomenclature proposed by Matsuda et al.[27] None of the expressed V_H genes showed a high degree of homology with a known germline V_H gene sequence; the homology ranged from 79.6% to 96.9% (Table 3). Two cases (4 and 10) appear to contain inframe insertions (3 nucleotides in case no. 4 and 9 nucleotides in case no. 10). Finally, it is noteworthy that the expressed V_H genes of cases no. 1 and 10 shared 7 nucleotide differences when compared with the most homologous germline gene V_H3-13. Five of these seven

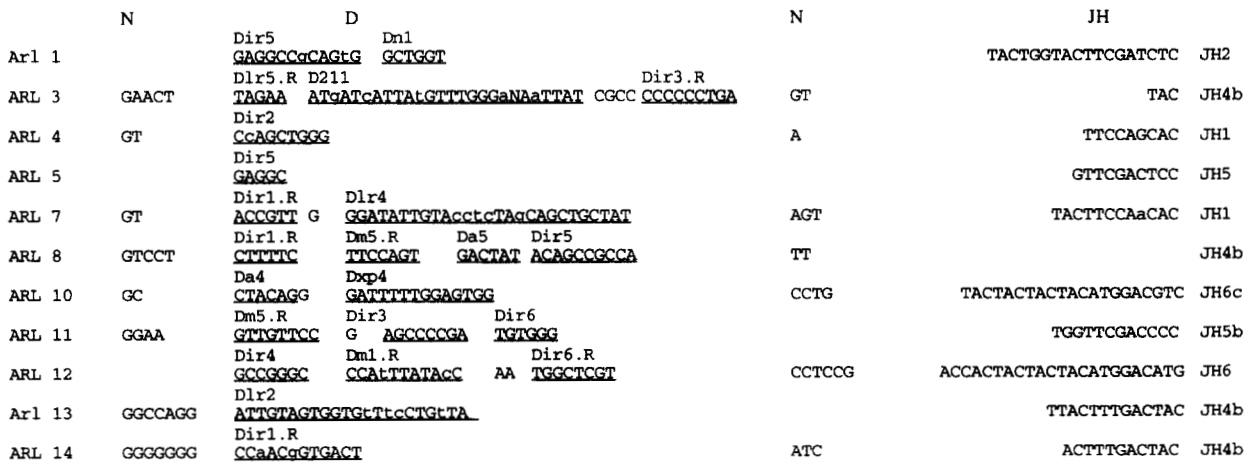

Fig 2. Analysis of the CDR3. N, N-nucleotides; D, diversity segments; JH, part of the CDR3 encoded by a joining region; .R, indicates diversity segment in reverse orientation. Diversity segments are underlined. Small letters indicate a mismatch with published D sequence.

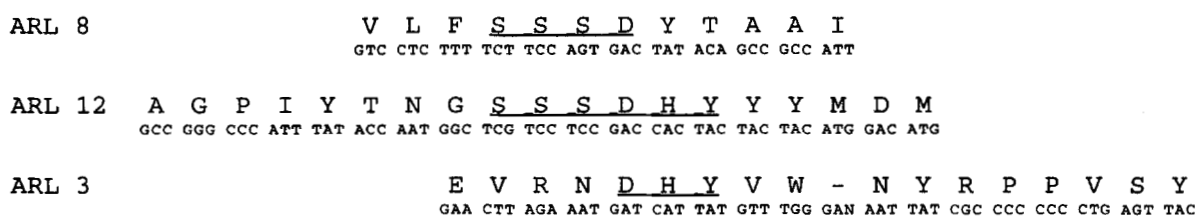

Fig 3. Amino acid homology analysis of the CDR3 regions. Nucleotide sequence and the deduced amino acid sequence are shown. Homologous regions are underlined. –, amino acid not determined .

nucleotide substitutions predict the same amino acid replacement. This observation raises the possibility that these two tumors may express Ig V_H germline genes that have not yet been reported. Alternatively, the expressed V_H genes are indeed mutated in a similar fashion, possibly reflecting their ability to bind similar ligands.

CDR3 analysis. As shown in Fig 2, the CDR3 regions were very different. The length ranged from 15 to 54 nucleotides. The CDR3 region was analyzed for D segment usage by comparison to D_H sequences from the GenBank database. No predominant usage of D_H segments was observed. The deduced amino acid sequences of the different CDR3 regions also were compared with each other and with published CDR3 sequences from cell lines producing anti-HIV antibodies.[28,29] Whereas no homology was found when comparing the AIDS lymphoma Igs to the anti-HIV Igs produced by cell lines, we did observe small stretches of amino acid homology in 3 ARL cases (Fig 3). ARL case no. 12 showed a four amino acid match with case no. 8 and a three amino acid match with ARL case 3. In all 3 ARL cases, the homologous portion of CDR3 was contributed by different diversity (D) genes and therefore differed at the nucleotide level. This observation suggests that these somatic changes may have resulted from selection by similar ligands.

Presence of a B-cell clone in the bone marrow 3 years before diagnosis of axillary lymphoma. ARL case no. 11 was obtained from a patient with diffuse large-cell lymphoma who presented with a p24-specific IgG paraprotein 3 years earlier.[23] Sequence analysis of 45 cloned reverse transcriptase-PCR (RT-PCR) products from the bone marrow showed at least 7 expanded B-cell clones (Table 4). Of 32 cloned RT-PCR products obtained with the LVH1 primer and Cγ primer, B-cell clone I was represented by 8 bacterial clonal inserts (25%); B-cell clones II, III, and IV by 4 bacterial clonal inserts each (12%); B-cell clones V and VI by 3 clonal inserts (9%); and the remaining 6 of the V_H1 clonal inserts contained unique sequences. Ten cloned amplification products obtained with LVH3 primer and Cγ primer were all unique. The repeated isolation of a certain VDJ rearrangement suggests either the presence of an expanded B-cell clone or alternatively the repeated sequences are derived from multiple copies of mRNA from one cell. Although the latter can not be excluded with certainty, we think it is less likely because, when a similar IgG cDNA analysis was performed on the bone marrow samples of two healthy individuals, repeat VDJ sequences were not obtained (Kraj et al, manuscript in preparation). B-cell clone II had the same V_H-CDR3 sequence as the diffuse lymphoma of ARL case no. 11, except for 2 nucleotide differences in the V_H region that are likely due to Taq polymerase infidelity.

Interestingly, the p24-specific paraprotein was encoded by a V_H4 family gene. The partial amino acid sequence of the p24-specific paraprotein was obtained by NH_2 amino acid sequencing. Amino acid residues 2 through 16 of the paraprotein matched entirely to the same deduced amino acid residues of first framework region (FR1) of the V_H4-30.2 gene expressed by B-cell clone VII that was derived from cloned V_H4 inserts (Fig 4).

IgL gene usage by AIDS-related lymphomas. Because of the lack of the D segments, the size of the rearranged Ig light chain genes do not differ sufficiently to determine the clonality by polyacrylamide gel electrophoresis. Therefore, all obtained PCR products were directly sequenced. In each case, only one, usually the most intense band, contained a single, clonal nucleotide sequence. Seven Ig light chain genes were sequenced. Three cases expressed a Vκ1 gene (ARL cases no. 3, 7, and 11), 2 cases a Vκ3 (ARL cases no. 9 and 10), and 2 cases a Vκ4 gene (ARL cases no. 4 and 13).

Somatic diversification of the IgL genes. Similar to IgH genes, the nucleotide sequence of the IgL genes differed

Table 4. IgG cDNA Analysis of Bone Marrow of ARL Case No. 11 3 Years Before the Diagnosis of Lymphoma

B-Cell Clone	Frequency
V_H1 family	
I	8/32 (25%)
II*	4/32 (12%)
III	4/32 (12%)
IV	4/32 (12%)
V	3/32 (9%)
VI	3/32 (9%)
Unique	6/32 (19%)
V_H3 family	
Unique	10/10 (100%)
V_H4 family	
VII†	2/3 (67%)
Unique	1/3 (33%)

* B-cell clone II has the same V-D-J rearrangement as the lymphoma clone.

† The deduced amino acid residues 2-16 of FR1 of B-cell clone VII matched entirely with the same amino acid residues of the predominant circulating p24-specific IgG paraprotein.[23]

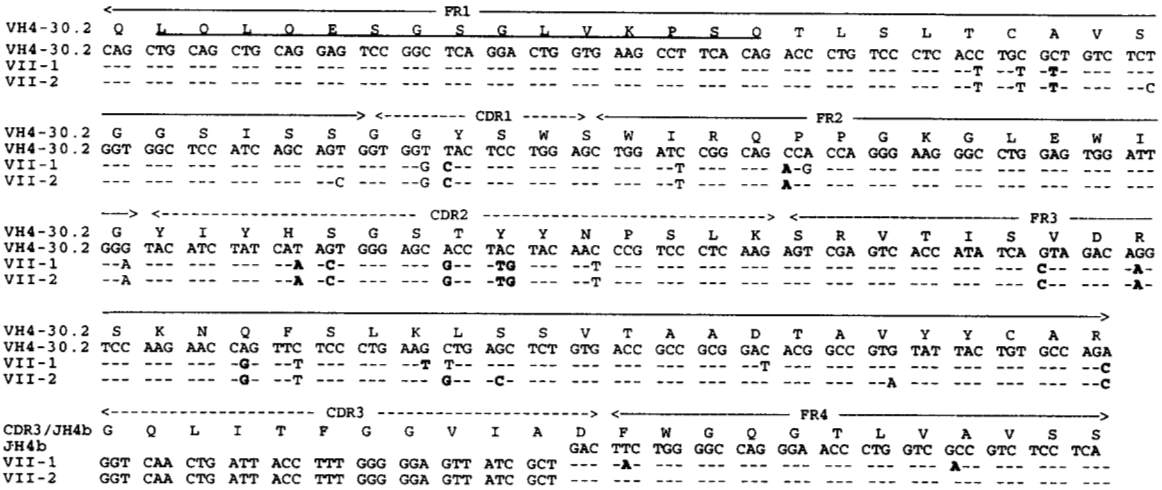

Fig 4. Comparison of the nucleotide sequence of the IgH gene expressed by the B-cell clone VII detected in the bone marrow with a partial amino acid sequence of the anti-p24 paraprotein from the serum in case ARL 11. VII-1 and VII-2, nucleotide sequences of clone VII obtained from two different bacterial clones; V_H4-30.2, germline V_H gene showing the highest homology to the clone VII (according Matsuda et al[27]). FR, framework region; CDR, complementarity determining region; JH, joining region. Partial amino acid sequence of the anti-p24 serum paraprotein is underlined. The nucleotide substitutions encoding for replacement mutations are printed in bold.

markedly from known germline Vκ genes. In 3 cases (no. 4, 8, and 6), the homology to published germline Vκ genes was quite low (86% to 89.4%), whereas, in the remaining cases, the sequence homology ranged between 92% and 98.2% (Table 5). In 1 case (ARL case no. 10), the expressed Vκ1 gene showed a 97.2% sequence homology to a published monoclonal antibody specific to gp41.[30]

DISCUSSION

In the current study, we investigated Ig genes expressed in 14 EBV-negative ARLs. By histology, 8 of the specimens were of the small noncleaved cell type and 6 consisted of the large diffuse cell type. No immunoblastic lymphomas were observed in our cohort of patients, which is in concordance with studies reporting a strong association between immunoblastic type ARL and EBV infection.[4,9] Immunoblastic lymphomas also frequently occur in immunosuppressed transplant recipients. In both situations, there appears to be a strong correlation between lymphoma development and the degree of immunosuppression. In fact, the CD4 count in EBV-positive immunoblastic lymphomas is generally less than 100 cells/μL.[31,32] In contrast, the relationship between degree of immunosuppression/stage of HIV infection and lymphoma development was not as clear in the EBV-negative lymphomas. In our series, the CD4 count ranged between 0 and 440 cells/μL, suggesting that other or additional pathogenetic factors are important in the EBV-negative lymphomas.

Table 5. V_L Analysis of EBV-Negative AIDS-Related Lymphoma

	V_L Gene	Homology	Isotype
3	Vκ1-A20	97.5%	κ PCR
4	Vκ4	89.4%	κ PCR
7	Vκ1-26	98.2%	κ PCR
8	Vκ1	88.8%	κ PCR
9	Vκ3-35	92.0%	κ PCR
10	Vκ3-41	86.0%	κ PCR
12	ND	ND	λ FC
13	Vκ4	89.4%	κ PCR
14	ND	ND	κ FC
Σ		91.6%	κ: 8 λ: 1

Abbreviations: PCR, isotype determined by polymerase chain reaction; FC, isotype determined by flow cytometry; ND, not done.

In 11 cases, the expressed Ig genes could be amplified by PCR and further analyzed. One case could not be further analyzed because of its polyclonal origin and 2 cases could not be amplified probably due to somatic mutations occurring at primer binding sites. Except for 1 case, all EBV-negative lymphomas of this series expressed the IgM isotype. The significance of this finding to the pathobiology of ARL is not clear. It is noteworthy that all of these cases expressed somatically diversified V_H and VL genes (see below), which is unusual when compared with IgM-expressing B cells from the peripheral blood of healthy subjects.[15,16] Five of 11 ARL cases expressed V_H3 genes, 3 expressed V_H4 genes, 2 expressed V_H1 genes, and 1 expressed a V_H7 gene.

The distribution of V_H family genes expressed by ARLs in our series corresponds to V_H gene usage in peripheral blood B lymphocytes from healthy individuals (Fig 5). Similar patterns of V_H gene expression have also been reported in follicular lymphoma and Burkitt's lymphoma not associated with HIV infection,[33,34] although in these instances there appears to be a 20% shift from V_H1 to V_H3 genes (Fig 5). In contrast, one series of diffuse large-cell lymphoma preferentially expressed V_H4 genes (88%) with a strong bias of the V_H4-34 gene (65%)[35] (Fig 5). In our series, we did not observe the predominant usage of V_H4 family genes in the diffuse large-cell ARLs and only 1 of the 5 diffuse large-cell lymphomas expressed the V_H4-34 gene. In addition to

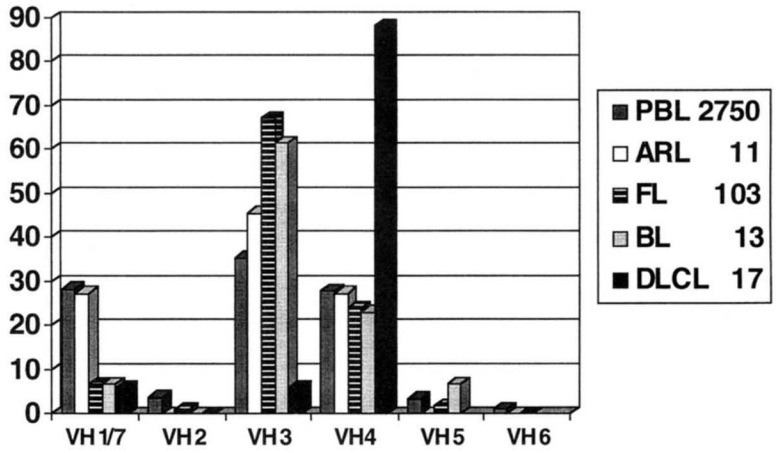

Fig 5. V_H family genes usage in ARL, peripheral blood B-lymphocytes (PBL),[16] and NHLs not associated with the HIV infection: follicular lymphoma (FL),[33] Burkitt's lymphoma (BL),[34] and diffuse large-cell lymphoma (DLCL).[35] The y-axis delineates the percentage of V_H family gene usage. The number of investigated cases is indicated in the box on the right.

our current study, there exist Ig gene sequence data from three ARL-derived cell lines reported by Riboldi et al[36] and two ARL-derived cell lines reported by Ng et al.[37] Three of these cell lines were EBV-negative and all of them expressed the V_H4-34 gene. Although the data are limited, the observed prevalence of the V_H4-34 gene among ARLs may simply reflect the overrepresentation of the V_H4-34 gene in peripheral blood B cells of healthy individuals.[16]

Both in EBV-negative lymphoma and in HIV-infected individuals during the early phases of infection, the frequency of V_H3-expressing B cells approximates 50%. This relatively high frequency of V_H3 genes contrasts with the reported V_H3 clonal deficit in HIV-infected individuals during late stages of infections.[17-19] One potential explanation for this difference in V_H3 expression is that, during early stages of infection, V_H3-expressing B cells in particular are activated and in the later stages these cells are lost due to the induction of apoptotic processes. Because the current series of EBV-negative ARLs express V_H3 genes at high frequency, one could speculate that ARLs may arise preferentially from B cells that are activated during earlier phases of HIV infection. This hypothesis is further supported by the case of B-cell lymphoma that developed in a HIV-positive patient who presented with a p24-specific paraprotein 3 years earlier. At that time, the patient had no evidence of lymphoma, but Ig cDNA repertoire studies of the bone marrow suggested the presence of at least seven B-cell clones. After 3 years, one of these B-cell clones, potentially representing 12.5% of the V_H1-expressing bone marrow B cells (4/32 sequenced V_H1 bacterial clones), developed into a B-cell lymphoma of an axillary node. This observation is unique in that it provides evidence for the hypothesis suggested by others[38] that B-cell activation leading to clonal expansion and dominance precedes lymphomagenesis in HIV-infected individuals.

Sequence analysis of the IgH and IgL genes expressed by the ARLs showed many nucleotide differences when compared with the closest known germline Ig genes. The average homology to known germline genes was approximately only 90%, indicating significant somatic diversification of the expressed Ig genes. This high degree of somatic diversification is likely to be the result of antigenic stimulation, as argued by several experimental systems.[39]

Thus, the Ig gene studies reported here show several aspects that suggest that antigenic stimulation (by HIV or other environmental antigens) might play an important role in AIDS-related lymphomagenesis. These aspects include a relatively high frequency of V_H3-expressing tumors (compared with a V_H3 deficit in circulating B cells in later stages of HIV infection), significant somatic diversification from germline Ig genes, and the amino acid sequence homology in CDR3 of 3 ARL cases. Furthermore, in one instance, we found evidence for an expanded tumor-related B-cell clone in the bone marrow that developed into a peripheral diffuse large-cell lymphoma 3 years later.

The risk of developing NHL in AIDS patients has been estimated as 10% per every survived year,[40,41] whereas the incidence of NHLs in the normal population is 13.6 in 100,000.[42] The nearly exclusive B-cell origin of ARLs and the almost 1,000 times higher incidence when compared with the lymphoma incidence in the normal population suggest that certain factors predisposing and/or accelerating B-cell tumor development exist in HIV-infected individuals. Our findings suggest that B-cell activation, potentially occurring in the early rather than later stages of infection, may be a predisposing factor for subsequent B-cell transformation. As mentioned previously, a likely source of B-cell activation in AIDS patients is the HIV itself. Studies are currently in progress to determine whether in fact the Ig expressed by the present series of EBV-negative lymphomas recognize HIV-related epitopes. If indeed this is the case, it would be of interest to determine whether the mechanisms of binding involves conventional (CDR-dependent) or nonconventional (FR-dependent) interactions.

NOTE ADDED IN PROOF

Since the submission of this article, it has been brought to our attention that there exists a study reporting V_H3 family gene expression in two of three cases of AIDS-related Burkitt's lymphoma. Similarly to that of our cohort, these cases showed a high number of nucleotide exchanges when compared with that of their germline counterparts.[43]

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