Favored Use of Immunoglobulin V_{H}4 Genes in AIDS-Associated B-Cell Lymphoma

By Alberto Bessudo, Vladimir Cherepakhin, Todd A. Johnson, Laura Z. Rassenti, Ellen Feigal, and Thomas J. Kipps

We examined the Ig heavy chain variable region genes (Ig V_{H} genes) expressed in biopsy specimens of 10 patients with acquired immunodeficiency syndrome (AIDS)-associated lymphoma. Eight expressed Ig V_{H} genes of the V_{H}4 group, indicating a bias toward expression of Ig V_{H} genes of this subgroup. Sequence analyses of Ig V_{H} genes isolated from any one lymphoma did not reveal evidence for intraclonal diversity. However, some lymphomas express Ig V_{H} genes that apparently have undergone somatic diversification and selection. In addition, we found that the sequence encoding each examined third complementarity determining region most likely resulted from D-D fusion, a process that ordinarily contributes to the generation of a relatively small proportion of the Ig heavy chain genes expressed by normal adult B cells. The noted restriction in the use of Ig V_{H} genes by AIDS-associated B-cell lymphomas suggests that antigenic stimulation contributes to lymphomagenesis in patients with AIDS.

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PATIENTS WITH acquired immunodeficiency syndrome (AIDS) have a high incidence of B-cell lymphoma. Multiple factors are hypothesized to contribute to this high incidence, including opportunistic viral infections, chronic antigenic stimulation, an acquired tendency for chromosomal translocations, and the human immunodeficiency virus (HIV) itself. Persons infected with HIV typically develop a persistent antibody response to external viral glycoproteins and internal core antigens. Moreover, patients with AIDS often have B-lymphoid hyperplasia and/or hypergammaglobulinemia. Finally, HIV-infected individuals can develop B cells with an altered surface-antigen phenotype or an impaired in vitro response to polysaccharides or pokeweed mitogens, suggesting that these patients may acquire intrinsic B-cell abnormalities that also may play a pathogenic role.

Structural analysis of the Ig genes expressed in AIDS-associated lymphoma can provide insight into the mechanisms involved in B-cell neoplasia. During B-cell development, Ig genes are formed by recombination and assembly of several genes selected from a larger pool of related gene segments. To generate an Ig heavy chain, rearrangements involving a heavy chain variable region gene (Ig V_{H} gene), a diversity (D) segment(s), and a heavy chain joining (J_{H}) segment must occur. There are ~50 functional Ig V_{H} genes in the human haploid genome that are grouped into seven Ig VH gene subgroups based on similarities in primary structure. Ig V_{H} genes of the Ig V_{H}3 subgroup encode the largest portion of Ig in the normal human adult repertoire, in part because of the relatively large number of functional Ig V_{H}3 genes in the human haploid genome. There also may be nonstochastic use of certain Ig V_{H} genes because of advantageous accessibility of particular Ig V_{H} genes to recombine, enhanced transcriptional activation of particular Ig V_{H} genes, and/or homology directed joining, as reviewed. Selection for or against particular Ig encoded by certain Ig V_{H} genes also may alter the distribution of Ig VH gene subgroups expressed in the Ig repertoire. For this reason, finding an abnormal distribution of Ig V_{H} genes expressed in AIDS-associated lymphomas could reveal selective forces acting on the expressed Ig repertoire that may contribute to the development of lymphoid neoplasia.

In addition, some Ig V_{H} genes innately encode antibodies that may have binding activity for HIV glycoproteins. HIV gp120, for example, has been found to bind B cells that express Ig encoded by a subset of Ig V_{H}3 genes. However, patients infected with HIV can develop anti-gp120 antibodies encoded by Ig V_{H} genes of other Ig V_{H} gene subgroups, as well as make antibodies to other HIV glycoproteins, such as gp41 and p24. Currently, it is not known whether the repertoire of Ig expressed in AIDS-associated lymphomas is biased, let alone biased toward or against Ig V_{H}3 genes that encode antibodies that innately have binding activity for HIV glycoproteins. This study was performed to evaluate the Ig V_{H} genes expressed by random cases of AIDS-associated B-cell lymphoma.

MATERIALS AND METHODS

Patient material. We obtained residual tissue from fresh frozen biopsy specimens of lymph nodes or extra-lymphatic lymphoid tissue from AIDS patients with clinical and pathologic diagnosis of high grade lymphoma. The tissue samples were embedded in Tissue-Tek Optimal Cutting Temperature (OCT) compound 4583 (Miles Inc, Elkhart, IN) and preserved at ~80°C until DNA and RNA was extracted, as described.

DNA hybridization. Restriction enzyme digested genomic DNA from each lymphoma specimen or from normal human placenta were applied to separate wells of a 0.8% agarose gel for electrophoresis. Electrophoretically size-separated DNA fragments were transferred onto nylon (Amer sham, Arlington Heights, IL) for Southern blot analyses, as described. Heavy chain Ig gene rearrangements were detected by hybridizing the filters with a 32P-radiolabeled human J_{H} gene segment probe, as described. Samples were considered to have detectable clonal Ig heavy chain gene rearrangements when they had one or more bands hybridizing with the J_{H} gene probe that were distinct from the expected germine band(s) present in enzyme-restricted normal human placental DNA.

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Polymerase chain reaction (PCR). Genomic DNA from lymphoma specimens having detectable Ig heavy chain gene rearrangements were used as templates for PCR, as described.\textsuperscript{27} Consensus Ig\textsubscript{H} antisense oligonucleotide primer 5'-ggggaattcACC-TGAGGACCRCTGACC-3' was used in combination with one of the following Ig\textsubscript{V}\textsubscript{H} subgroup-specific leader sense primers: 5'-ggagaatcAGTGTCTACAAGTGACC-3' (Ig\textsubscript{V}\textsubscript{H1}); 5'-gggaatcAGTGGAGTGCCGTCAGCTG-3' (Ig\textsubscript{V}\textsubscript{H2}); 5'-ggaggaatcAGTGGAGTTGCCGTGACC-3' (Ig\textsubscript{V}\textsubscript{H3}); 5'-gggaatcAGTGGAGTTGCCGTGGCTGACC-3' (Ig\textsubscript{V}\textsubscript{H4}); 5'-ggcgaattcAGTGGAGTTGCCGTGGCTGACC-3' (Ig\textsubscript{V}\textsubscript{H5}); 5'-ggggaatcAGTGGAGTTGCCGTGGCTGACC-3' (Ig\textsubscript{V}\textsubscript{H6}).

**RESULTS**

Southern blot analyses. We examined the biopsy tissue of 10 patients with AIDS-associated lymphoma (samples AL1 through AL10) for Ig heavy chain gene rearrangements by Southern blot analyses. Three had evidence for Ig gene rearrangements involving both alleles (patients AL4, AL5, and AL7), whereas the remaining 7 samples each had only one Ig gene rearrangement and a nonrearranged Ig gene fragment similar to that seen in enzyme restricted human placental DNA (data not shown).

Analysis of Ig\textsubscript{V}H gene subgroups expressed by AIDS-associated lymphomas. RNA was extracted from tissue samples AL1, AL2, AL4, AL5, AL7, AL9, and AL10, for use in an anchored RT-PCR/ELISA, as described.\textsuperscript{31} AL1, AL2, AL4, AL5, AL9, and AL10, generated a PCR product identified only by an oligonucleotide specific for rearranged Ig\textsubscript{V}H genes of the Ig\textsubscript{V}H4 subgroup, but not with probes for rearranged Ig\textsubscript{V}H genes of any one of the other Ig\textsubscript{V}H gene subgroups. Similarly, RT-PCR of AL7 generated a product that exclusively hybridized with an oligonucleotide probe specific for Ig\textsubscript{V}H genes of the Ig\textsubscript{V}H3 subgroup.

The Ig\textsubscript{V}H genes expressed by cases AL3, AL6, and AL8, were examined by PCR on isolated genomic DNA. PCR with DNA of AL3 or AL6 generated a 660 bp PCR product using oligonucleotides specific for Ig\textsubscript{V}H and the Ig\textsubscript{V}H4 subgroup, but not with primers for Ig\textsubscript{V}H4 and any one of the other 6 Ig\textsubscript{V}H gene subgroups. Similarly, PCR with the DNA of AL8 generated a 660 bp product only when the Ig\textsubscript{V}H4 primer was used in conjunction with sense-strand primers specific for Ig\textsubscript{V}H5 gene consensus leader sequence. The Ig\textsubscript{V}H4 gene subgroup was verified by Southern blot analyses of the PCR products using probes specific for each of the Ig\textsubscript{V}H4 gene subgroups (data not shown). Collectively, these studies indicated that only 1 of the 10 AIDS-associated lymphoma samples used Ig\textsubscript{V}H3 genes. On the other hand, 8 of the remaining samples each used Ig\textsubscript{V}H genes of the Ig\textsubscript{V}H4 subgroup.

Structural analysis of the rearranged Ig variable region genes. The PCR products of samples AL1, AL3, AL4, AL5, AL6, AL7, and AL8, were each cloned for double stranded DNA sequence analysis. This revealed each to have a rearranged Ig\textsubscript{V}H gene of the Ig\textsubscript{V}H4 (AL1, AL3, AL4, AL5, and AL6), Ig\textsubscript{V}H3 (AL7), or Ig\textsubscript{V}H5 (AL8) subgroup (Figs 1 and 2). Analyses of at least 2 separate clones of each PCR product did not reveal significant nucleotide sequence variation (less than 0.33% variation in each case). Nucleic acid sequence comparison of each cloned Ig\textsubscript{V}H gene with known germline Ig\textsubscript{V}H genes revealed that the clones from
A. \( \text{VH}_3 \)

<table>
<thead>
<tr>
<th>AL7.0</th>
<th>R</th>
<th>R</th>
<th>DD-A-H*</th>
<th>G</th>
<th>W</th>
<th>-59</th>
<th>G</th>
<th>L</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
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<td>R</td>
<td>R</td>
<td>DD-A-H*</td>
<td>G</td>
<td>W</td>
<td>-59</td>
<td>G</td>
<td>L</td>
<td>K</td>
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**B. \( \text{VH}_4 \)**

<table>
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<tr>
<th>AL4</th>
<th>V-V-N-P</th>
<th>*</th>
<th>T</th>
<th>*</th>
<th>D</th>
<th>*</th>
<th>R</th>
<th>V</th>
<th>V</th>
<th>L</th>
<th>T</th>
<th>H</th>
<th>R</th>
<th>G</th>
<th>HV-GCCTCNSLA</th>
<th>S</th>
<th>P</th>
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<tbody>
<tr>
<td>AL6</td>
<td>R</td>
<td>I</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AL2</td>
<td>I</td>
<td>K</td>
<td>T</td>
<td>*</td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

**C. \( \text{VH}_5 \)**

| AL6   | W  | A  | W  | A  | W  | W  | A  | W  | A  | W  | A  | W  | A  | W  | | | |
|-------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| AL2   | T  | K  | T  | *  | *  | | | | | | | | | | | | |

**Fig 2.** Amino acid sequences deduced from the Ig \( \text{VH} \) genes of AIDS-associated lymphomas. * - indicate sequence homology and asterisks are used to introduce gaps that maximize sequence homology. Related germline sequences and patient samples are indicated as in Fig 1.

AL1, AL4, and AL6, each had the highest homology with \( \text{VH}_4-59 \) (V71-4, DPW-71). Clones from AL3 had the highest homology with \( \text{VH}_4-34 \), clones from AL5 had the highest homology with \( \text{VH}_4-61 \) (V71-2), clones from AL7 were most homologous to \( \text{VH}_3-9 \), and AL8 clones had the highest homology with \( \text{VH}_5-51 \) (Fig 1 and Table 1).

We determined the DNA sequence of the Ig \( \text{VH} \) gene expressed by sample AL2 (Fig 1), AL9, and AL10, by direct dsDNA sequence analysis of the RT-PCR product in the region contiguous to the deduced CDR1. This confirmed that AL2, AL9, and AL10 each expressed an Ig \( \text{VH} \) gene of the Ig \( \text{VH}_4 \) subgroup (data not shown). Moreover, the deduced sequence of AL2 or AL10 have highest homology with \( \text{VH}_4-59 \) (V71-4) whereas AL9 has highest homology with germline \( \text{VH}_4-61 \) (V71-2) (Fig 1, and data not shown).

Comparison of the sequences with those reported in GenBank revealed that the Ig \( \text{VH} \) genes used by some AIDS-associated lymphomas have relatively high homology to certain Ig \( \text{VH} \) genes noted to encode anti-HIV antibodies of HIV-seropositive donors (Figs 1 and 2). For example, the Ig \( \text{VH} \) gene encoding the anti-gp120 antibody F105, displayed 87, 96, 88, or 96, percent nucleic acid sequence homology with AL1, AL2, AL4, or AL6, respectively. However, the degrees of homology noted between the lymphoma-derived Ig \( \text{VH} \) genes invariably were higher with their putative germline counterpart than with these rearranged and functional Ig \( \text{VH} \) genes. As some sites, there are base differences from the putative germline gene that are shared between certain members of these expressed Ig \( \text{VH} \) genes. For example, compared to \( \text{VH}_4-59 \) there is a nonconservative shared substitution in the third position of codon 29 of AL1, AL3, AL4, F105, or 268-D (Fig 1), resulting in a valine → isoleucine substitution (Fig 2). Such nonconserved shared substitutions most likely are due to genetic polymorphism in the Ig \( \text{VH} \) genes, rather than somatic selection.

All cloned genes had functional Ig gene rearrangements, except for the Ig \( \text{VH} \) isolated from the genomic DNA of AL7. Although this Ig \( \text{VH} \) gene had 99.6% nucleic sequence homology with a functional germline Ig \( \text{VH}_3-9 \) gene, \( \text{VH}_3-9 \), sequence analyses revealed a termination codon in the region.

**Fig 1.** Ig \( \text{VH} \) gene nucleotide sequences of the rearranged Ig \( \text{VH} \) genes cloned from AIDS-associated lymphoma tissue samples. The deduced CDR3 and FR4 regions are presented in Fig 3. The clones are designated with the prefix AL and are assigned the number corresponding to the patient number provided in the text. Sequence number AL7.0 is the sequence of the genomic PCR product of patient 7 that contains a stop codon in the deduced CDR3 (Fig 3). Sequence AL7.1 is the sequence of the RT-PCR product of the expressed Ig \( \text{VH} \) gene from the same patient sample. Each sequence is compared to that of the most closely homologous germline Ig \( \text{VH} \) sequence. (A) Ig \( \text{VH}_3 \): DPB2.97 and V3-3.28 (B) Ig \( \text{VH}_4 \): 5-69.57 and 6-61.87; and (C) Ig \( \text{VH}_5 \): 5-51.85. Also listed for comparison are the sequences of anti-HIV antibody heavy chains F105 and V268-D.43 (−) indicate nucleotide substitutions and (+) are used to introduce gaps that maximize sequence homology. The numbering of amino acids is according to Kabat et al.77
this patient had Ig gene rearrangements involving both al-

cles. Because of this, we deduced that AL7.1 also contained an Ig vH3 gene. However, this Ig

gene frequently found to encode IgM cold agglutinins. In-


tained the highest sequence homology with another


gene ordinarily encode most of the Ig expressed in the


gene that had the highest homology to lymphoma isolated sequences are presented using the revised nomenclature for germline Ig vH genes. The column marked "% Homology" provides the percent nucleotide sequence homology between the expressed Ig vH gene and its putative germline counterpart. FR labels the columns providing the analysis of segments FR1, FR2 and FR3, and CDR labels the columns providing the analysis of CDR1 and CDR2. Columns marked "R," "R:S," or "R:S," respectively, indicate the number of deduced silent mutations, replacement mutations, or ratio of replacement to silent mutations in each region. The columns labeled "Innate R:S" provide the total possible replacement mutations to total possible silent mutations for the codons in the specified regions, as calculated by Chang and Casali.

Abbreviation: ND, not determined.

Table 1. Structural Analysis of the Ig vH Genes in AIDS-Associated Lymphomas

<table>
<thead>
<tr>
<th>Sample</th>
<th>Germline vH Gene</th>
<th>% Homology</th>
<th>FR</th>
<th>CDR</th>
</tr>
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<tbody>
<tr>
<td>AL1</td>
<td>V4-59</td>
<td>89.7</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>AL3</td>
<td>V4-34</td>
<td>85.6</td>
<td>19</td>
<td>23</td>
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<tr>
<td>AL4</td>
<td>V4-61</td>
<td>83.0</td>
<td>6</td>
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</tr>
<tr>
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<td>V4-61</td>
<td>84.6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AL6</td>
<td>V4-59</td>
<td>99.0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>AL7.1</td>
<td>DP58</td>
<td>92.8</td>
<td>3</td>
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</tr>
<tr>
<td>AL8</td>
<td>V5-51</td>
<td>91.7</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>F105</td>
<td>V4-59</td>
<td>95.9</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>28B-D</td>
<td>V4-59</td>
<td>86.1</td>
<td>9</td>
<td>18</td>
</tr>
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</table>

Listed for comparison is the analysis of anti-HIV antibody heavy chains F105 and V4-28B-D. The germline Ig vH genes having the highest homology to lymphoma isolated sequences are presented using the revised nomenclature for germline Ig vH genes. The column marked "% Homology" provides the percent nucleotide sequence homology between the expressed Ig vH gene and its putative germline counterpart. FR labels the columns providing the analysis of segments FR1, FR2 and FR3, and CDR labels the columns providing the analysis of CDR1 and CDR2. Columns marked "R," "R:S," or "R:S," respectively, indicate the number of deduced silent mutations, replacement mutations, or ratio of replacement to silent mutations in each region. The columns labeled "Innate R:S" provide the total possible replacement mutations to total possible silent mutations for the codons in the specified regions, as calculated by Chang and Casali.

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We examined the Ig vH genes expressed in 10 cases of AIDS-associated lymphoma. We found that such B-cell lymphomas expressed a highly skewed repertoire of Ig vH genes. First, despite the finding that a subset of Ig vH3 genes can encode antibodies that innately have binding activity for HIV gp120, only one of the ten samples examined (sample AL7.1) expressed Ig vH genes of this subgroup. This proportion is significantly higher than what would be expected if the repertoire of Ig vH genes in AIDS-associated lymphoma reflected the repertoire of Ig vH genes expressed by normal B cells, with a χ-square of 6.9, P = .009, df = 1. Furthermore, the overall distribution of Ig vH genes in AIDS-associated lymphoma compared to normal adults is significantly different with a χ-square of 27.4 and a P value of .0001, df = 6.

The noted frequent expression of Ig vH4 genes in AIDS-associated lymphomas appears similar to that recently reported for large cell lymphomas of patients not infected with HIV. Hsu and Levy noted that large cell lymphomas frequently express Igs encoded by Ig vH4-34, an Ig vH4 gene frequently found to encode IgM cold agglutinins. Indeed, two AIDS-associated lymphomas found to produce cold agglutinins were noted to express Ig encoded by this particular Ig vH gene. However, in our series of 10 AIDS-associated lymphomas not selected for antigen binding activity, only one expressed an Ig vH4 gene that had the highest homology to Ig vH4-34 germline gene (AL3) (Fig 1).

DISCUSSION

We examined the Ig vH genes expressed in 10 cases of AIDS-associated lymphoma. We found that such B-cell lymphomas expressed a highly skewed repertoire of Ig vH genes. First, despite the finding that a subset of Ig vH3 genes can encode antibodies that innately have binding activity for HIV gp120, only one of the ten samples examined (sample AL7.1) expressed Ig vH genes of this subgroup. Ig vH3 genes ordinarily encode most of the Ig expressed in the normal adult B-cell repertoire. Some groups have noted HIV-infected patients may have a selective depletion of B cells expressing certain Ig vH3 genes and a concomitant increase in the relative use of Ig vH1 genes. However, using a quantitative RT-PCR/ELISA to examine the distribution of expressed Ig vH genes, we have not noted significant reduction in the relative expression of Ig vH3 genes by blood B cells of patients infected with HIV compared to that of normal adults. That only one of the lymphoma samples examined expressed Ig vH genes of this subgroup is significantly less than what would be expected if the repertoire of Ig vH genes in AIDS-associated lymphoma reflected the repertoire of Ig vH genes expressed by normal B cells, with a χ-square of 1.7 and a P value of .4 (df = 1).

Furthermore, the examined B-cell lymphomas predominantly expressed Ig vH genes of the Ig vH4 subgroup. Ordinarily, Ig vH4 genes encode between 12% to 22% (average 18%) of the Igs expressed by normal adult B cells. However, we found that eight of ten samples expressed Ig vH genes of this subgroup. This proportion is significantly higher than what would be expected if the repertoire of Ig vH genes in AIDS-associated lymphoma reflected the repertoire of Ig vH genes expressed by normal B cells, with a χ-square of 6.9, P = .009, df = 1. Furthermore, the overall distribution of Ig vH genes in AIDS-associated lymphoma compared to normal adults is significantly different with a χ-square of 27.4 and a P value of .0001, df = 6.

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Ig V<sub>H</sub> GENES EXPRESSED IN AIDS-ASSOCIATED LYMPHOMAS

34. Five samples (AL1, AL2, AL4, AL6, and AL9) expressed Ig V<sub>H</sub> genes that each had the highest nucleic acid sequence homology with V<sub>H</sub>4-59 (V71-4), and two additional cases (AL5 and AL10) used an Ig V<sub>H</sub> with highest homology with V<sub>H</sub>4-61 (V71-2) (Fig 1). Ig encoded by these Ig V<sub>H</sub> genes generally have a high degree of structural similarity, as shown by the fact that they frequently share cross-reactive idiotypic determinants, such as those detected by the anti-idiotypic monoclonal antibody Lcl.27 Future studies should examine for the frequent expression of this supratypic cross-reactive idiotype in AIDS-associated lymphoma specimens.

The observed restriction in the Ig V<sub>H</sub> genes expressed by AIDS-associated lymphomas may be because of selection for B cells that express Ig with certain binding specificities. Evidence for this may be derived from the structural analyses of the Ig V<sub>H</sub> genes expressed by the AIDS-associated lymphomas. First, we deduce that most of the AIDS lymphomas express Ig V<sub>H</sub> genes that have incurred somatic mutations. Second, several of these Ig V<sub>H</sub> genes have relatively high ratios of deduced replacement mutations (R) to silent mutations (S) in regions that encode part of the antigen combining site(s) (namely CDR1 and CDR2) (Table 1). Moreover, the deduced R:S ratios of the segments encoding the FR portion of the antibody variable region are consistently lower than those of CDR1 and CDR2, and lower than the average R:S ratio of 2.9 calculated for random codons.28 Higher R:S ratios in the CDR1 and CDR2 compared to those in the FR are observed commonly in Ig V genes expressed by B cells selected in a secondary antigen-driven immune response.29,30,32,35-37

However, Chang and Casali observed that the codons for the CDR of many germline Ig V genes are more prone to replacement mutations than are those used in most other proteins.26 Conversely, the codons used in the FR of many germline V genes are slightly less prone to replacement mutations than are random codons.28 As such, finding higher R:S ratios for the CDR than for the FR segments could result from random nonselected nucleotide base substitutions. Nevertheless, the Ig V<sub>H</sub>4-59 genes used by AL1, AL4, and AL8, still have deduced R:S ratios in the CDR1/CDR2 that exceed that expected from random base substitutions. Furthermore, most of the Ig V<sub>H</sub> genes characterized in this study have lower R:S ratios than that expected for random base substitutions in the FR (Table 1).
It should be noted that anti-HIV antibodies from patients with AIDS may be encoded by Ig V_H genes that have deduced somatic mutations resulting in R:S ratios that are less than that expected for random base substitutions in the CDR (eg, Table 1, and data not shown). This may reflect a reduced capacity of B cells to undergo antibody affinity maturation in the HIV-infected host. If found to be the case, then the immune status of patients with AIDS may favor use of Ig V_H genes in immune responses that selectively can incur advantageous replacement mutations in the CDR with minimal numbers of somatic mutations. Conceivably, this could enhance the expression of such Ig V_H genes in AIDS-associated lymphomas. In this regard it is noteworthy that the V_H genes that we found frequently expressed in AIDS-associated lymphomas, namely V_H_4-59 and V_H_4-61, have among the highest average innate R:S ratios for codons within CDR1/CDR2, namely 4.486 and 4.613, respectively. The mean innate R:S ratio for CDR1/CDR2 of all Ig V_H genes is 4.152 (±0.449 SD). Moreover, these two Ig V_H genes have the lowest average innate R:S ratio for segments encoding the antibody FR of all characterized germline Ig V_H genes, namely 2.593 (The mean innate R:S ratio for FR of all Ig V_H genes is 2.845 (±0.169 SD). Analyses of the CDR3 of the Ig heavy chains expressed in AIDS-associated lymphomas provides additional evidence for selection. The heavy chain CDR3 of Ig genes expressed by all lymphomas except AL6 have nonconservative base differences from the deduced D and J_H gene segments that encode the CDR3 (Fig 3). Furthermore, the length of each CDR3 ranged from 10 to 22 amino acids (m = 15 ± 4). This is longer than the noted range of 8 to 18 amino acids (m = 13 ± 4) for the CDR3 of the Ig heavy chains used by normal adult B cells. The probable number of N base additions in the CDR3 (range 4-18, m = 10, Fig 3) appears similar to that reported for IgS expressed by normal adult blood B cells. However, we deduce that each CDR3 except AL1 probably is encoded by 2 or 3 different D genes segments (Fig 3). The length of each AL1 probably represents D-D fusion of DIR genes, genes of approximately 170 bp in length with irregular recombination signal sequences that are present in multiple copy in the human haploid genome. In contrast, only approximately 10% of Ig heavy chains expressed by normal adult B cells have CDR3 with evidence for D-D fusion. In this regard, it is noteworthy that the characterized anti-HIV antibodies of patients infected with HIV each has a heavy chain CDR3 that also probably results from D-D fusion. Moreover, the heavy chain CDR3 of recombinant antibodies selected for binding to HIV from combinatorial libraries also display features similar to the CDR3 of the lymphoma-derived immunoglobulin genes described in this study. Conceivably, such D-D fusions may enhance the expression probability for antibodies capable of binding HIV glycoproteins. However, we note that the nonproductive allele of AL7 (Fig 3), also has evidence for having been formed through D-D fusion. As the nonsense mutation is the only identified defect in this Ig gene, it is possible that it was acquired during a process of Ig somatic mutation. Alternatively, the D-D fusion deduced for this nonfunctional gene may reflect an enhanced propensity of B cells from patients infected with HIV to undergo D-D fusion. Further studies on the functional and nonfunctional Ig gene rearrangements of normal B cells from patients with AIDS are necessary to resolve this issue.

Persons infected with HIV develop a persistent humoral immune response to HIV. It is noteworthy that several previously characterized anti-HIV-1 antibodies made by HIV-seropositive donors are similar in primary structure to those described in this study (Figs 1 and 2, and data not shown). Furthermore, characterized antibodies from cDNA of HIV-seropositive donors also have identified Ig V_H genes similar to those described in this study. Finally, Ng et al recently reported that cell lines established by cloning single cells from biopsies obtained from two AIDS patients with Burkitt’s lymphoma produced IgM antibodies reactive with HIV gp160. Collectively, these studies and the findings reported here support a model proposing that chronic antigenic stimulation in HIV-infected individuals can lead to selective proliferation of B cells that ultimately may undergo neoplastic transformation.

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