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# Favored Use of Immunoglobulin $V_H4$ 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 $V_H4$ 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.[1-4] 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.[5-8] Persons infected with HIV typically develop a persistent antibody response to external viral glycoproteins and internal core antigens.[9-11] Moreover, patients with AIDS often have B-lymphoid hyperplasia and/or hypergammaglobulinemia.[12] Finally, HIV-infected individuals can develop B cells with an altered surface-antigen phenotype[13] or an impaired in vitro response to polysaccharides or pokeweed mitogens,[14,15] 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 $V_H$ gene subgroups based on similarities in primary structure.[16,17] Ig $V_H$ genes of the Ig $V_H3$ subgroup encode the largest portion of Ig in the normal human adult repertoire,[18] in part because of the relatively large number of functional Ig $V_H3$ genes in the human haploid genome.[19] There also may be nonstochastic use of certain Ig $V_H$ genes[20,21] because of advantageous accessibility of particular Ig $V_H$ genes to recombinase, enhanced transcriptional activation of particular Ig $V_H$ genes, and/or homology directed joining, as reviewed.[22] Selection for or against particular Ig encoded by certain Ig $V_H$ genes also may alter the distribution of Ig $V_H$ 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 $V_H3$ genes.[23] However, patients infected with HIV can develop anti-gp120 antibodies encoded by Ig $V_H$ genes of other Ig $V_H$ gene subgroups,[24] as well as make antibodies to other HIV glycoproteins, such as gp41 and p24.[25,26] Currently, it is not known whether the repertoire of Ig expressed in AIDS-associated lymphomas is biased, let alone biased toward or against Ig $V_H3$ 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 RNA and/or DNA was extracted, as described.[27]

*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 (Amersham, Arlington Heights, IL) for Southern blot analyses, as described.[28] Heavy chain Ig gene rearrangements were detected by hybridizing the filters with a $^{32}$P-radiolabeled human $J_H$ gene segment probe, as described.[29] 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 germline 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.[27] Consensus $J_H$ antisense oligonucleotide primer 5′-ggcgaattcACC-TGAGGAGACRGTGACC-3′ was used in combination with one of the following Ig $V_H$ subgroup-specific leader sense primers: 5′-gtggaattcaagcttGTTCHTCACCATGGACTG-3′ (Ig $V_H1$); 5′-gtgaagcttATGGACATACTTTGTTCCACG-3′ (Ig $V_H2$); 5′-gtg-ctgcagaagcttATGGAGTTKGGGCTGAGCTGG-3′ (Ig $V_H3$); 5′-gtggaattcaagcttATGAAACAYCTGTGGTTCTTC-3′ (Ig $V_H4$); 5′-gcaagcttatcgatCCATCATGGGGTCAACCG-3′ (Ig $V_H5$); 5′-gtggaattcaagcttCATGTCTGTCTCCTTCC-3′ (Ig $V_H6$) (NC-IUB nomenclature,[30] linkers are shown in lower case).

*RNA isolation, cDNA synthesis, and reverse transcription (RT)-PCR.* Total cellular RNA was isolated from cryopreserved AIDS-associated B-cell lymphoma specimens using RNAzol B (TEL-TEST, Inc, Friendswood, TX). First strand cDNA was synthesized from 5 $\mu$g total RNA using an oligo-dT primer and Superscript II reverse transcriptase (Life Technologies, Grand Island, NY). RT was performed for 1 hour at 42°C. Afterwards, the mixture was heated to 70°C for 15 minutes, cooled on ice 10 minutes, and then incubated with RNase H (Life Technologies) at 37°C for 20 minutes. Five microliters of this reaction mixture was used for a 100 $\mu$L total volume PCR reaction, as previously described. Primers consisted of an Ig heavy chain constant region oligonucleotide and Ig $V_H$ subgroup oligonucleotides. Where indicated the RT-PCR product was size separated via agarose gel electrophoresis, blotted onto nylon, and hybridized with an Ig heavy chain variable region third framework (FR3) oligonucleotide specific for each of the different $V_H$ subgroups.

*Anchored RT-PCR enzyme linked immunosorbent assay (ELISA).* An anchored RT-PCR assay was performed to assess the relative levels of different Ig $V_H$ genes expression in blood or lymphoid tissue, as described.[31] dG tailed first strand cDNA is subjected to primary aPCR amplification followed by a second step "nested PCR," using 5′ biotinylated primers. The primers consists of an anchored primer and $C\mu$ antisense oligonucleotides. The antisense biotin labeled $V_H$ gene is added to an avidin coated ELISA plate. The presence of specific $V_H$ genes is probed by adding digoxigenenin labeled oligonucleotides specific for the different $V_H$ gene leaders and developed by adding antidigoxigenin bound to peroxidase to produce a colored reaction.

*Sequence analysis of Ig $V_H$ genes.* Amplified Ig $V_H$ DNA fragments were ligated into Bluescript (Stratagene, San Diego, CA) that subsequently was used to transform XL1-Blue strain of *Escherichia coli* (Stratagene). Screening of transformed bacterial colonies and sequencing of the Ig variable region inserts were performed as described,[32] using dideoxy chain termination (Version 2 DNA sequencing kit; USB, Cleveland, OH). We determined the Ig variable region nucleotide sequence of at least two colonies of transformed bacteria. Where indicated, direct dsDNA sequencing of the amplified PCR product was performed using the *fmol* DNA Sequencing System (Promega, Madison, WI), as per manufacturer's instructions.

All sequences were analyzed using the GenBank database and the Sequence Analysis Software Package, 1991 (Genetics Computer Group, Inc, Madison, WI). Ig $V_H$ gene sequences were aligned with the published germline Ig $V_H$ gene sequences having the highest homology using the local homology algorithm of Smith and Waterman.[33] Differences between the $V_H$ gene sequence and its putative germline counterpart were defined as being a replacement (R) mutation if the observed difference results in a change in the deduced amino acid sequence. Nucleotide differences that do not result in an amino acid substitution were defined as being silent (S). Calculations of the expected ratio of R mutations to S mutations (R:S ratio) for any given codon were derived from analyses of the actual codons used in the putative germline $V_H$ gene sequence. This takes into consideration the observation that codons encoding the first or second complimentary determining region (CDR) generally are more susceptible to having replacement mutations from any given single nucleotide change than codons within the framework regions.[34]

*CDR3 analysis.* Each sequence was searched for homology against all published D genes available in the GenBank database. To best-fit D genes we accepted reverse homology, and gave priority to the homology of the $J_H$ segment. We avoided introducing gaps and base additions to the alignment and selected D segments that had the highest homology and the longest stretch of shared nucleic acid sequence.

## 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 $V_H$ 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.[31] AL1, AL2, AL4, AL5, AL9, and AL10, generated a PCR product identified only by an oligonucleotide specific for rearranged Ig $V_H$ genes of the Ig $V_H4$ gene subgroup, but not with probes for rearranged Ig $V_H$ genes of any one of the other Ig $V_H$ gene subgroups. Similarly, RT-PCR of AL7 generated a product that exclusively hybridized with an oligonucleotide probe specific for Ig $V_H$ genes of the Ig $V_H3$ subgroup.

The Ig $V_H$ 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 $J_H$ and the Ig $V_H4$ subgroup, but not with primers for $J_H$ and any one of the other 6 Ig $V_H$ gene subgroups. Similarly, PCR with the DNA of AL8 generated a 660 bp product only when the $J_H$ primer was used in conjunction with sense-strand primers specific for Ig $V_H5$ gene consensus leader sequence. The Ig $V_H$ gene subgroup was verified by Southern blot analyses of the PCR products using probes specific for each of the Ig $V_H$ gene subgroups (data not shown). Collectively, these studies indicated that only 1 of the 10 AIDS-associated lymphoma samples used Ig $V_H3$ genes. On the other hand, 8 of the remaining samples each used Ig $V_H$ genes of the $V_H4$ 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 $V_H$ gene of the Ig $V_H4$ (AL1, AL3, AL4, AL5, and AL6), Ig $V_H3$ (AL7), or Ig $V_H5$ (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 $V_H$ gene with known germline Ig $V_H$ genes revealed that the clones from

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## A. VH3

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       +-----------------------LEADER------------------------+(---------------------------------------------INTERVENING SEQUENCE---------------------------------------------)
DP58   ATGGAGTTGGGGCTGTGCTGGGTTTTCCTTGTTGCTATTTTAGAAGGTGATTCATGGAAAACTAGAGAGATTTAGTGTGTGTGGATATGAGTGAGAGAAACAGTGGATATGTGTGGCAGTTTCTGACCTTGGTGTCTCTTTGTTTGCAGGT
AL7.1  ···········(------------------------------------------- Intron deleted------------------------------------------)

AL7.0  ··············A····A········T·G·········A··············G··A··········G······A····C···************······T·····························G··········
VH3-9  ··········A··A····A········T·G·········A··············G··A··········G······A····C···************······T·····························G··········

       1                          10                           20                           30 +-----CDR1----+36          40
       GluValGlnLeuValGluSerGlyGlyGlyLeuValGlnProGlyGlySerLeuArgLeuSerCysAlaAlaSerGlyPheThrPheSerSerTyrGluMetAsnTrpValArgGlnAlaProGlyLysGlyLeuGluTrpV
DP58   GTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGTTATGAAATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG
AL7.1  ················T························································································A·C··A·C······G····T·····························

AL7.0  ··········A·····················C···············CA··························································TGA·GA·····CC···C·········G··A·············C·········
VH3-9  ··········A·····································CA··························································TGA·GA·····CC···C·········G··A·············C·········

       49 +---------52A--------------CDR2------------------+66          70                                        80    82 A  B  C                              90           94
       alSerTyrIleSerSerSerGlySerThrIleTyrTyrAlaAspSerValLysGlyArgPheThrIleSerArgAspAsnAlaLysAsnSerLeuTyrLeuGlnMetAsnSerLeuArgAlaGluAspThrAlaValTyrTyrCysAlaArg
DP58   TTTCATACATTAGTAGTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTTTATTACTGTGCGAGA
AL7.1  ····················T··T··TG··················································································T·······························C··········

AL7.0  ·C···GGT······T·G·A·A··G···G····GG···T··G·····················································C·····················T········T···········CT·G···········A·A·
VH3-9  ·C···GGT······T·G·A·A··G···G····GG···T··G·····················································C·····················T········T···········CT·G···········A·A·
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## B. VH4

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                                                                                                                                                        1
         +-----------------------LEADER----------------------+(--------------------------------------INTERVENING SEQUENCE----------------------------------)      GlnVal
VH4-59   ATGAAACATCTGTGGTTCTTCCTTCTCCTGGTGGCAGCTCCCAGATGTGAGTATCTCAGGGATCCAGACATGGGGATATGGGA*GGTGCCTCTGATCCCAGGGCTCACTGTGGGTCTCTCTGTTCACAGGGGTCCTGTCCCAGGTG
AL1      ·······························A··············G························*·························································
AL2                                                                                                                                                ·············
AL4      ·······C·························································G······················A······*··································A·························
AL6      ·······C····································································································································
F105     ········································(------------------------------- Intron deleted-----------------------------------)·····················
268-D                                                                                                                                                      ······

AL5      ··················C························G·····················G·······*··························································
VH4-61   ·······C··········C························G·····················G·······*··························································

AL3      ··················C························G·······A··G·G··TG··AA···C·TA··T··························T·T··············C···T··T··AA·A
VH4-34   ·······C··········C························G·······A··G·G··T···AA·····A··T·C·························T···························

                  10                        20                        30 +--------CDR1-------+36       40                    49
         GlnLeuGlnGluSerGlyProGlyLeuValLysProSerGluThrLeuSerLeuThrCysThrValSerGlyGlySerValSerSerTyrTyrTrpSer******TrpIleArgGlnProProGlyLysGlyLeuGluTrpIleGly
VH4-59   CAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCGTCAGTAGTTACTACTGGAGC******TGGATCCGGCAGCCCCCAGGGAAGGGACTGGAGTGGATTGGG
AL1      ·································T··············A··C··AC·C········CG******··························A·······CA
AL2      ························AC·····························A···C···········T··******····G·············
AL4      ·······A·················A·······················T···A···CC·A····T·····******··········A·G················C···········
AL6      ······················G···························A·······················******····································
F105     ···························································A·····A········C··············******··········T·················C·········A
268-D    ···T·····T···································C··A···A··A·GC········CA******········A················G··A····ACC····A

AL5      ···························································C·············C·A·ACTAGT·ACTA·TGGAGC································
VH4-61   ··············································································C···GGTAGT·ACTA·TGGAGC································

AL3      ···T·AA·AC·A·G···G········C···A··C··················AA··TG····T·A······GC··C·····G····T········T******········A···T·TA····AGG······C·········C··A
VH4-34   ·····A··AC···G····G········T······························G······A·····G···T·····G··················******········C··············G···············

         +---------------------CDR2-------------------+66       70                              80    82 A  B  C                             90          94
         TyrIleTyrTyrSerGlySerThrAsnTyrAsnProSerLeuLysSerArgValThrIleSerValAspThrSerLysAsnGlnPheSerLeuLysLeuSerSerValThrAlaAlaAspThrAlaValTyrTyrCysAlaArg
VH4-59   TATATCTATTACAGTGGGAGCACCAACTACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGA
AL1      ·······C·G··········AG·····GC····TT·T·····················T·TT·····C·G··············G·············G·····CG·······G·················G···A·····T····T··AG
AL4      ························GA·················G·················GT·······C········A··········T···············T··C·A··············C··A········G···A···············
AL6      ·····························································································································
F105     ········C·····················G·······································G····································C····A·················································C··
268-D    ···G·····C·T·C····AGT··········T··················C··GC·········A··A···············G····G······A······C·GC····AG·T··················T·········A·····T········

AL5      ·······T··························································A··························································
VH4-61   ·····························································································································

AL3      G·A···A··G·TG·C··AG··········T··GT·G··T······G··················GA·C····T····T····CT··A····T····G·A···C··············C··········TACA····T··················
VH4-34   G·A···A··C·T·····A······················G·························································C··········T··················
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## C. VH5

```
                                                                                                                                                     1
         +-----------------------LEADER----------------------+(--------------------------------------INTERVENING SEQUENCE----------------------------------)      GluVal
VH5-51   ATGGGGTCAACCGCCATCCTCGCCCTCCTCCTGGCTGTTCTCCAAGGTCAGTCCTGCCGAGGGCTTGAGGTCACAGAGGAGAACGGGTGGAAAGGAGCCCCTGATTCAAATTTTGTGTCTCCCCCACAGGAGTCTGTGCCGAGGTG
AL8      ··························································A·························A················A··································

                          10                        20                       30 +-----CDR1----+36          40                           49 +--
         GlnLeuValGlnSerGlyAlaGluValLysLysProGlyGluSerLeuLysIleSerCysLysGlySerGlyTyrSerPheThrSerTyrTrpThrGlyTrpValArgGlnMetProGlyLysGlyLeuGluTrpMetGlyIle
VH5-51   CAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGACCGGCTGGGTGCGCCAGATGCCCGGGAAAGGCTTGGAGTGGATGGGGATC
AL8      ·····························G············G··········C···T··T········C···C··T·C·CG····································································

         ------52A---------------CDR2-------------------+66        70                              80    82 A  B  C                             90          94
         IleTyrProGlyAspSerAspThrArgTyrSerProSerPheGlnGlyGlnValThrIleSerAlaAspLysSerIleSerThrAlaTyrLeuGlnTrpSerSerLeuLysAlaSerAspThrAlaMetTyrTyrCysAlaArg
VH5-51   ATCTATCCTGGTGACTCTGATACCAGATACAGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATCAGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGA
AL8      ·········C············TT········T·········G······G··········T··········C·····A···························GATG·····················A················
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A. VH3
                                                                                                   N-D-N           JH
                                                                                                 Segments        Segments
         1        10        20        29+CDR1-+36 40       48+-----CDR2------+66              82ABC    90 +---------CDR3--------++---FR4---+
DP58     EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYEMN**WVRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
AL7.1    ···························NLNT····•*···················VIM·········································VLRPPNDQRDCGGGCYSP¯YFDCWGQGTLVTVSS

AL7.0    ·········R·····R·············DD·A·H**··············G··WNSGS·G·································L····KDTVGAYTAMASYIII·················
VH3-9    ···············R·············DD·A·H**··············G··WNSGS·G·································L····K··································

B. VH4
         1        10        20        29+CDR1-+36 40       48+-----CDR2------+66              82ABC    90 +---------CDR3--------++---FR4---+
VH4-59   QVQLQESGPGLVKPSETLSLTCTVSGGSVSSYYWS**WIRQPPGKGLEWIGYIY*YSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
AL1      ······················S·····ITNS··T**·············A··S*D··K·KH·S······SL·L····S····R·T··S····G····VKMGD·KRAG·RSET------G··I····M·····
AL4      ···························V·ITN·F··**····T·····D······*·····R·····V····V··L··T··H·····R·······G······HV·GGRLGDLSSA---------S··P········
AL6      ············R···············I·······**···················*···································································DNKFLR------------A··I····M·····
F105     ····························I··H···•*····S·····Q······*········S···········E··········T·M·············GPV·AVFYG·YR--------L·P··········
268-D    ·····V······················PINNA··T**············YL··V·*HT·V·········L··TI···RK·L··S·KF·····S·······EWAEDG·FG---------NA·HV·····M·A···

AL5      ·······························NT···WS·················F*·············Q·······································DTVLVIGAAG--------W···L··R········
VH4-61   ··································WS··················*·································································

         1        10        20        29+CDR1-+36 40       48+-----CDR2------+66              82ABC    90 +---------CDR3--------++---FR4---+
VH4-34   QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWS**WIRQPPGKGLEWIGEIN*HSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
AL2      ·I··K··············K······PL·······**····ST·G··········*DG·G···KS···G·····I·M··T··F·E·R·········T·F···GRDYY·HTTLTQEFT-------T····N······

C. VH5
         1        10        20        29+CDR1-+36 40       48+--A--CDR2------+66              82ABC    90 +---------CDR3--------++---FR4---+
VH5-51   EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIG**WVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCAR
AL7      ··················R···QVF··T··TR···**··················A···I······E·R······T·N········DV······I·····HWGDGYNYKT---------WS··L··R·········
```

**Fig 2.** **Amino acid sequences deduced from the Ig $V_H$ 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 $V_H$4-59 (V71-4, DP-71), clones from AL3 had the highest homology with $V_H$4-34, clones from AL5 had the highest homology with $V_H$4-61 (V71-2), clones from AL7 were most homologous to $V_H$3-9, and AL8 clones had the highest homology with $V_H$5-51 (Fig 1 and Table 1).[35]

We determined the DNA sequence of the Ig $V_H$ 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 $V_H$ gene of the Ig $V_H$4 subgroup (data not shown). Moreover, the deduced sequence of AL2 or AL10 have highest homology with $V_H$4-59 (V71-4) whereas AL9 has highest homology with germline $V_H$4-61 (V71-2) (Fig 1, and data not shown).

Comparison of the sequences with those reported in GenBank revealed that the Ig $V_H$ genes used by some AIDS-associated lymphomas have relatively high homology to certain Ig $V_H$ genes noted to encode anti-HIV antibodies of HIV-seropositive donors (Figs 1 and 2). For example, the Ig $V_H$ gene encoding the anti-gp120 antibody F105,[24] 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 $V_H$ genes invariably were higher with their putative germline counterpart than with these rearranged and functional Ig $V_H$ genes. As some sites, there are base differences from the putative germline gene that are shared between certain members of these expressed Ig $V_H$ genes. For example, compared to $V_H$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 nonconservative shared substitutions most likely are due to genetic polymorphism in the Ig $V_H$ genes, rather than somatic selection.[36]

All cloned genes had functional Ig gene rearrangements, except for the Ig $V_H$ isolated from the genomic DNA of AL7. Although this Ig $V_H$ gene had 99.6% nucleic sequence homology with a functional germline Ig $V_H$3 gene, $V_H$3-9, sequence analyses revealed a termination codon in the region

←

**Fig 1.** **Ig $V_H$ gene nucleotide sequences of the rearranged Ig $V_H$ 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 $V_H$ gene from the same patient sample. Each sequence is compared to that of the most closely homologous germline Ig $V_H$ sequence. (A) Ig $V_H$3: DP58[17] and V3-9,[35] (B) Ig $V_H$4: 4-59[35,67] and 4-61[35,67]; and (C) Ig $V_H$5: 5-51.[35,68] Also listed for comparison are the sequences of anti-HIV antibody heavy chains F105[24] and $V_H$268-D.[60] (·) indicate nucleotide sequence homology and (*) are used to introduce gaps that maximize sequence homology. The numbering of amino acids is according to Kabat et al.[37]**

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**Table 1. Structural Analysis of the Ig $V_H$ Genes in AIDS-Associated Lymphomas**

| Sample | Germline $V_H$ Gene | % Homology | FR S | FR R | FR R:S | FR Innate R:S | CDR S | CDR R | CDR R:S | CDR Innate R:S |
|---|---|---|---|---|---|---|---|---|---|---|
| AL1 | V4-59 | 89.7 | 8 | 14 | 1.8 | 2.6 | 2 | 12 | 6.0 | 4.6 |
| AL3 | V4-34 | 85.6 | 19 | 23 | 1.2 | 2.7 | 4 | 7 | 1.8 | 4.5 |
| AL4 | V4-59 | 93.0 | 6 | 15 | 2.5 | 2.6 | 0 | 5 | 5.0 | 4.6 |
| AL5 | V4-61 | 98.6 | 1 | 1 | 1.0 | 2.6 | 0 | 4 | 4.0 | 4.5 |
| AL6 | V4-59 | 99.3 | 0 | 2 | 2.0 | 2.6 | 0 | 0 | 0.0 | 4.6 |
| AL7.1 | DP58 | 92.8 | 3 | 2 | 0.6 | ND | 2 | 6 | 3.0 | ND |
| AL8 | V5-51 | 91.7 | 4 | 13 | 3.2 | 3.0 | 1 | 7 | 7.0 | 3.5 |
| F105 | V4-59 | 95.9 | 2 | 6 | 3.0 | 3.0 | 1 | 2 | 2.0 | 4.6 |
| 268-D | V4-59 | 86.1 | 9 | 18 | 2.0 | 3.0 | 3 | 11 | 3.6 | 4.6 |

Listed for comparison is the analysis of anti-HIV antibody heavy chains F105[24] and $V_H$268-D.[60] The germline Ig $V_H$ genes having the highest homology to lymphoma isolated sequences are presented using the revised nomenclature for germline Ig $V_H$ genes.[69] The column marked "% Homology" provides the percent nucleotide sequence homology between the expressed Ig $V_H$ 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 "S," "R," 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.[58]

Abbreviation: ND, not determined.

deduced to encode the CDR3 (Fig 3). As mentioned earlier, this patient had Ig gene rearrangements involving both alleles, making it possible that this clone contained the Ig $V_H$ gene of the nonexpressed allele. Because of this, we determined the nucleic acid sequence of the RT-PCR product of tissue sample 7, designated AL7.1 (Fig 1). We found that AL7.1 also contained an Ig $V_H3$ gene. However, this Ig $V_H3$ gene differed from that obtained through genomic DNA PCR, and had the highest sequence homology with another germline Ig $V_H3$ gene, DP58 (Fig 1). Consistent with this clone representing the expressed allele, AL7.1 had a functional Ig $V_H$ gene rearrangement (Fig 2).

The CDR3 and FR4 were deduced for each clone, as per Kabat et al[37] (Fig 3). Each variable region gene encoded a long CDR3 of 10 to 22 amino acid residues. Comparison of the CDR3 with known germline D segments revealed that each probably arose through D-D fusion (Fig 3). By comparing the CDR3 and FR4 with known $J_H$ gene segments, we deduce that AL5 and AL8 probably use $J_H2$, AL1 and AL6 use $J_H3b$, AL3 and AL7.1 use $J_H4b$, whereas sample AL4 most likely uses $J_H5b$ (Fig 3).[38-43]

## DISCUSSION

We examined the Ig $V_H$ genes expressed in 10 cases of AIDS-associated lymphoma. We found that such B-cell lymphomas expressed a highly skewed repertoire of Ig $V_H$ genes. First, despite the finding that a subset of Ig $V_H3$ genes can encode antibodies that innately have binding activity for HIV gp120,[23] only one of the ten samples examined (sample AL7.1) expressed Ig $V_H$ genes of this subgroup. Ig $V_H3$ genes ordinarily encode most of the Ig expressed in the normal adult B-cell repertoire.[18,44-47] Some groups have noted HIV-infected patients may have a selective depletion of B cells expressing certain Ig $V_H3$ genes[48] and a concomitant increase in the relative use of Ig $V_H1$ genes.[49] However, using a quantitative RT-PCR/ELISA to examine the distribution of expressed Ig $V_H$ genes,[31] we have not noted significant reduction in the relative expression of Ig $V_H3$ genes by blood B cells of patients infected with HIV compared to that of normal adults.[50] That only one of the lymphoma samples examined expressed Ig $V_H$ genes of this subgroup is significantly less than what would be expected if the repertoire of Ig $V_H$ genes in AIDS-associated lymphoma reflected the repertoire of Ig $V_H$ genes expressed by normal B cells, $\chi$-square of 4.17, and a $P$ value of .04 (df = 1).

Furthermore, the examined B-cell lymphomas predominantly expressed Ig $V_H$ genes of the Ig $V_H4$ subgroup. Ordinarily, Ig $V_H4$ genes encode between 12% to 22% (average 18%) of the Igs expressed by normal adult B cells.[18,44-47] However, we found that eight of ten samples expressed Ig $V_H$ genes of this subgroup. This proportion is significantly higher than what would be expected if the repertoire of Ig $V_H$ genes in AIDS-associated lymphoma reflected the repertoire of Ig $V_H$ genes expressed by normal B cells, with a $\chi$-square 6.8, $P$ = .009, df = 1. Furthermore, the overall distribution of Ig $V_H$ genes in AIDS-associated lymphoma compared to normal adults is significantly different with a $\chi$-square of 27.4 and a $P$ value of .0001, df = 6.

The noted frequent expression of Ig $V_H4$ genes in AIDS-associated lymphomas appears similar to that recently reported for large cell lymphomas of patients not infected with HIV.[51] Hsu and Levy[51] noted that large cell lymphomas frequently express Igs encoded by Ig $V_H4$-34, an Ig $V_H4$ gene frequently found to encode IgM cold agglutinins.[52] Indeed, two AIDS-associated lymphomas found to produce cold agglutinins were noted to express Ig encoded by this particular Ig $V_H$ gene.[53] However, in our series of 10 AIDS-associated lymphomas not selected for antigen binding activity, only one expressed an Ig $V_H4$ gene that had the highest homology to Ig $V_H4$-34 germline gene (AL3) (Fig 1).

Rather, most AIDS-associated lymphomas we examined used a sub-subgroup of Ig $V_H4$ genes distinct from Ig $V_H4$-

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```
                    N - D - N                                                              JH  Segment
                    Segments
         |--------------------------------CDR3-----------------------------------||---------------FR4--------------|  CDR3     N
                                                                                                                      length  additions
         MetGlyAspProLysArgAlaGlyArgArgSerGluThr                         GlyPheAspIleTrpGlyGlnGlyThrMetValThrValSerSer
AL1      ATGGGAGATCCGAAAAGGGCGGGCCGTCGAAGTGAAACT                         GGTTTTGATATCTGGGGCCAAGGGACAATGGTCACTGTCTCCTCA     17       7
DIR       ····T·T····                                                    ·C··························              JH3b
DIR                  ·C······CC······G·T·

         GlyArgAspTyrTyrAsnHisThrThrLeuThrGlnGluPheThr                      PheAspThrTrpGlyGlnGlyAsnLeuValThrValSerSer
AL3      GGCCGTGATTACTACAATCACACGACTCTGACTCAGGAATTCACT                      TTTGACACATGGGGCCAGGGAAACTTGGTCACCGTCTCCTCA     18      15
DLR4-C    ··T·GT·C·········                                                 ······TAC·············C·C··               JH4b
DIR                               ···G······G··

         HisValArgGlyGlyArgLeuGlyAspLeuSerSerAla                              AspSerTrpGlyProGlyThrLeuValThrValSerSer
AL4      CATGTGAGGGGGGGGCGCTTGGGGGACTTATCCTCGGCG                              GACTCCTGGGGCCCGGGAACCCTGGTCACCGTCTCCTCA     15      10
D6        ····TC····                                                          ·············A··········                  JH5b
D21/10                ··T········G····G··

         AspThrValLeuValIleGlyAlaAlaGly                                TrpTyrPheAspLeuTrpGlyArgGlyThrLeuValThrValSerSer
AL5      GACACTGTACTAGTGATTGGCGCGGCAGGC                                TGGTATTTCGATCTCTGGGGCCGTGGCACCCTGGTCACCGTCTCCTCA     15      12
DLR1B    ··T·T······G···G·                                             ································               JH2
DN1                          ··A····

         AspAsnLysPheLeuArg                                               AlaPheAspIleTrpGlyGlnGlyThrMetValThrValSerSer
AL6      GATAATAAATTTTTGCGT                                               GCTTTTGATATCTGGGGCCAAGGGACAATGGTCACTGTCTCCTCA     10       4
Dxp'1-C  ·······                                                          ···························               JH3b
Dxp5           ·······

         AspThrValGlyAlaTyrThrAlaMetAlaSerTyrIleIleIleEnd
AL7.0    GATACTGTCGGGGCTTATACAGCTATGGCTTCGTATATCATCATCTAGTACGCGGTCCCGAGGTGTACA    ACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCA
DIR-C          ········                                                            ·································· JH5b
DK4                     ············T·A·
DN1                                     ·····G··G··G··G····

         ValLeuArgProProAsnAspGlnArgAspCysGlyGlyGlyCysTyrSerPro          TyrPheAspCysTrpGlyGlnGlyThrLeuValThrValSerSer
AL7.1    GTTCTTAGGCCCCCGAATGACCAAAGAGATTGTGGTGGTGGTTGCTATTCGCCC          TACTTTGACTGCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA     22      18
DIR                ·····T···G··                                          ··········A··················             JH4b
DLR3                               ·T············A·········

         HisTrpGlyAspGlyTyrAsnTyrLysThr                                TrpSerPheAspLeuTrpGlyArgGlyThrLeuValThrValSerSer
AL8      CATTGGGGAGATGGCTACAATTATAAAACT                                TGGTCCTTCGATCTCTGGGGCCGCGGCACCCTGGTCACTGTCTCCTCA     15      10
D6         ·C······                                                    ····A·················T·········               JH2b
DK1                     ·······G····
```

**Fig 3. CDR3 and FR4 regions. Lines one and two display the amino acid and nucleotide sequence of the CDR3 of each Ig of AIDS-associated lymphomas. Lines 3 and 4 are the germline $J_H$ and diversity (D) segments that have the highest homology to that of the lymphoma-derived Ig gene. (·) Indicate sequence homology and asterisks are used to introduce gaps that maximize sequence homology. The CDR3 and FR4 is defined as per Kabat et al.[37] Indicated for sample AL7.0 is the termination codon in the genomic DNA PCR product. AL7.1 is the RT-PCR sequence of the RNA isolated from the same patient. The underlined portion of the $J_H$ segment identifies the sequence of the oligonucleotide primer.**

34. Five samples (AL1, AL2, AL4, AL6, and AL9) expressed Ig $V_H$4 genes that each had the highest nucleic acid sequence homology with $V_H$4-59 (V71-4), and two additional cases (AL5 and AL10) used an Ig $V_H$ with highest homology with $V_H$4-61 (V71-2) (Fig 1). Ig encoded by these Ig $V_H$4 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 Lc1.[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 $V_H$ 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 $V_H$ genes expressed by the AIDS-associated lymphomas. First, we deduce that most of the AIDS lymphomas express Ig $V_H$ genes that have incurred somatic mutations. Second, several of these Ig $V_H$ 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.[54] 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.[16,22,55-57]

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.[58] Conversely, the codons used in the FR of many germline V genes are slightly less prone to replacement mutations than are random codons.[58] 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 $V_H$ 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 $V_H$ genes characterized in this study have lower R:S ratios than that expected for random base substitutions in the FR (Table 1).

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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 $V_H4$ 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).[58] 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).[58]

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 ($m = 15 \pm 4$). This is longer than the noted range of 8 to 18 amino acids ($m = 13 \pm 4$) for the CDR3 of the Ig heavy chains used by normal adult B cells.[43] 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.[43] However, we deduce that each CDR3 except AL1 probably is encoded by 2 or 3 different D genes segments (Fig 3). The CDR3 of 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.[59] In contrast, only approximately 10% of Ig heavy chains expressed by normal adult B cells have CDR3 with evidence for D-D fusion.[43] 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.[24,60,61] 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.[62,63] 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.[9-11,64] 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).[9-11,64] Furthermore, characterized antibodies from cDNA of HIV-seropositive donors also have identified Ig $V_H$ genes similar to those described in this study.[9-11,63-66] Finally, Ng et al[20] 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.

## REFERENCES

1. Ziegler JL, Beckstead JA, Volberding PA, Abrams DI, Levine AM, Lukes RJ, Gill PS, Burkes RL, Meyer PR, Metroka CE, Mouradian J, Moore A, Riggs SA, Butler JJ, Cabanillas FC, Hersh E, Newell GR, Laubenstein LJ, Knowles D, Odajnyk C, Raphael B, Koziner B, Urmacher C, Clarkson BD: Non-Hodgkin's lymphoma in 90 homosexual men. Relation to generalized lymphadenopathy and the acquired immunodeficiency syndrome. N Engl J Med 311:565, 1984

2. Levine AM, Meyer PR, Begandy MK, Parker JW, Taylor CR, Irwin L, Lukes RJ: Development of B-cell lymphoma in homosexual men. Ann Intern Med 100:7, 1984

3. Ioachim HL, Cooper MC, Hellman GC: Lymphomas in men at high risk for acquired immune deficiency syndrome (AIDS). Cancer 56:2831, 1985

4. Serraino D, Salamina G, Franceschi S, Dubois D, La Vecchia C, Brunet JB, Ancelle-Park RA: The epidemiology of AIDS-associated non-Hodgkin's lymphoma in the World Health Organization European Region. Br J Cancer 66:912, 1992

5. Cremer KJ, Spring SB, Gruber J: Role of human immunodeficiency virus type 1 and other viruses in malignancies associated with acquired immunodeficiency disease syndrome. J Natl Cancer Inst 82:1016, 1990

6. Laurence J, Astrin SM: Human immunodeficiency virus induction of malignant transformation in human B lymphocytes. Proc Natl Acad Sci USA 88:7635, 1991

7. Montefiori DC, Zhou J, Shaff DI: CD4-independent binding of HIV-1 to the B lymphocyte receptor CR2 (CD21) in the presence of complement and antibody. Clin Exp Immunol 90:383, 1992

8. Karp JE, Broder S: The pathogenesis of AIDS lymphomas: a foundation for addressing the challenges of therapy and prevention. Leuk Lymphoma 8:167, 1992

9. Sarngadharan MG, Popovic M, Bruch L, Schupbach J, Gallo RC: Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 224:506, 1984

10. Robey WG, Safai B, Oroszlan S, Arthur LO, Gonda MA, Gallo RC, Fischinger PJ: Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. Science 228:593, 1985

11. Weber JN, Clapham PR, Weiss RA, Parker D, Roberts C, Duncan J, Weller I, Carne C, Tedder RS, Pinching AJ: Human

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immunodeficiency virus infection in two cohorts of homosexual men: Neutralising sera and association of anti-gag antibody with prognosis. Lancet 1:119, 1987
12. Levine AM, Liebman HA: The acquired immunodeficiency syndrome (AIDS), in Beutler E, Lichtman MA, Coller BS, Kipps TJ (eds): Williams Hematology, New York, NY, McGraw-Hill, 1995, p 975
13. Scott ME, Landay AL, Lint TF, Spear GT: In vivo decrease in the expression of complement receptor 2 on B-cells in HIV infection. AIDS 7:37, 1993
14. Martinez-Maza O, Crabb E, Mitsuyasu RT, Fahey JL, Giorgi JV: Infection with the human immunodeficiency virus (HIV) is associated with an in vivo increase in B lymphocyte activation and immaturity. J Immunol 138:3720, 1987
15. Miedema F, Petit AJ, Terpstra FG, Schattenkerk JK, De Wolf F, Al BJ, Roos M, Lange JM, Danner SA, Goudsmit J, Schellekens PTA: Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. J Clin Invest 82:1908, 1988
16. Pascual V, Capra JD: Human immunoglobulin heavy-chain variable region genes: Organization, polymorphism, and expression. Adv Immunol 49:1, 1991
17. Tomlinson IM, Walter G, Marks JD, Llewelyn MB, Winter G: The repertoire of human germline VH sequences reveals about fifty groups of VH segments with different hypervariable loops. J Mol Biol 227:776, 1992
18. Guigou V, Cuisinier AM, Tonnelle C, Moinier D, Fougereau M, Fumoux F: Human immunoglobulin VH and VK repertoire revealed by in situ hybridization. Mol Immunol 27:935, 1990
19. Walter MA, Surti U, Hofker MH, Cox DW: The physical organization of the human immunoglobulin heavy chain gene complex. EMBO J 9:3303, 1990
20. Ng VL, Hurt MH, Fein CL, Khayam-Bashi F, Marsh J, Nunes WM, McPhaul LW, Feigal E, Nelson P, Herndier BG: IgMs produced by two acquired immune deficiency syndrome lymphoma cell lines: Ig binding specificity and VH-gene putative somatic mutation analysis. Blood 83:1067, 1994
21. Suzuki I, Pfister L, Glas A, Nottenburg C, Milner EC: Representation of rearranged VH gene segments in the human adult antibody repertoire. J Immunol 154:3902, 1995
22. Stewart AK, Schwartz RS: Immunoglobulin V regions and the B cell. Blood 83:1717, 1994
23. Berberian L, Goodglick L, Kipps TJ, Braun J: Immunoglobulin VH3 gene products: Natural ligands for HIV gp120. Science 261:1588, 1993
24. Marasco WA, Bagley J, Zani C, Posner M, Cavacini L, Haseltine WA, Sodroski J: Characterization of the cDNA of a broadly reactive neutralizing human anti-gp120 monoclonal antibody. J Clin Invest 90:1467, 1992
25. Wang H, Muller S, Zolla-Pazner S, Kohler H: Human monoclonal and polyclonal anti-human immunodeficiency virus-1 antibodies share a common clonotypic specificity. Eur J Immunol 22:1749, 1992
26. Boudet F, Girard M, Theze J, Zouali M: Antibodies of HIV-1 positive subjects and experimentally immunized primates and rodents bind to sequence divergent regions of the third variable domain (V3) of gp120. Int Immunol 4:283, 1992
27. Pratt LF, Szubin R, Carson DA, Kipps TJ: Molecular characterization of a supratypic cross reactive idiotype associated with IgM autoantibodies. J Immunol 147:2041, 1991
28. Southern EM: Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503, 1975
29. Rassenti LZ, Kipps TJ: Lack of extensive mutations in the VH5 genes used in common B cell chronic lymphocytic leukemia. J Exp Med 177:1039, 1993
30. Nomenclature Committee of the International Union of Biochemistry (NC-IUB): Nomenclature for incompletely specified bases in nucleic acid sequences. Recommendations 1984. Eur J Biochem 150:1, 1985
31. Rassenti LZ, Kohsaka H, Kipps TJ: Analysis of immunoglobulin $V_H$ gene repertoire by an anchored PCR-ELISA. Ann NY Acad Sci 764:463, 1995
32. Kipps TJ, Duffy SF: Relationship of the CD5 B cell to human tonsillar lymphocytes that express autoantibody-associated cross reactive idiotypes. J Clin Invest 87:2087, 1991
33. Smith TF, Waterman MS: Overlapping genes and information theory. J Theor Biol 91:379, 1981 (letter)
34. Chang B, Casali P: The CDR1 sequences of a major proportion of human germline Ig VH genes are inherently susceptible to amino acid replacement. Immunol Today 15:367, 1994
35. Matsuda F, Shin EK, Nagaoka H, Matsumura R, Haino M, Fukita Y, Taka-ishi S, Imai T, Riley JH, Anand R: Structure and physical map of 64 variable segments in the 3′0.8-megabase region of the human immunoglobulin heavy-chain locus. Nat Genet 3:88, 1993
36. Van der Maarel S, Van Dijk KW, Alexander CM, Sasso EH, Bull A, Milner EC: Chromosomal organization of the human VH4 gene family. Location of individual gene segments. J Immunol 150:2858, 1993
37. Kabat E, Wu TT, Perry HM, Gottesman KS, Foeller C: Sequences of Proteins of Immunological Interest (ed 5). Bethesda, MD, U.S. Department of Health and Human Services, 1991
38. Ravetch JV, Siebenlist U, Korsmeyer S, Waldmann T, Leder P: Structure of the human immunoglobulin mu locus: Characterization of embryonic and rearranged J and D genes. Cell 27:583, 1981
39. Schroeder HW Jr, Hillson JL, Perlmutter RM: Early restriction of the human antibody repertoire. Science 238:791, 1987
40. Schroeder HW Jr, Wang JY: Preferential utilization of conserved immunoglobulin heavy chain variable gene segments during human fetal life. Proc Natl Acad Sci USA 87:6146, 1990
41. Bird J, Galili N, Link M, Stites D, Sklar J: Continuing rearrangement but absence of somatic hypermutation in immunoglobulin genes of human B cell precursor leukemia. J Exp Med 168:229, 1988
42. Pascual V, Randen I, Thompson K, Sioud M, Førre Ø, Natvig J, Capra JD: The complete nucleotide sequences of the heavy chain variable regions of six monospecific rheumatoid factors derived from Epstein-Barr virus-transformed B cells isolated from the synovial tissue of patients with rheumatoid arthritis. Further evidence that some autoantibodies are unmutated copies of germ line genes. J Clin Invest 86:1320, 1990
43. Yamada M, Wasserman R, Reichard BA, Shane S, Caton AJ, Rovera G: Preferential utilization of specific immunoglobulin heavy chain diversity and joining segments in adult human peripheral blood B lymphocytes. J Exp Med 173:395, 1991
44. Huang C, Stewart AK, Schwartz RS, Stollar BD: Immunoglobulin heavy chain gene expression in peripheral blood B lymphocytes. J Clin Invest 89:1331, 1992
45. Huang C, Stollar BD: A majority of Ig H chain cDNA of normal human adult blood lymphocytes resembles cDNA for fetal Ig and natural autoantibodies. J Immunol 151:5290, 1993
46. Fumoux F, Guigou V, Blaise D, Maraninchi D, Fougereau M, Schiff C: Reconstitution of human immunoglobulin VH repertoire after bone marrow transplantation mimics B-cell ontogeny. Blood 81:3153, 1993
47. Logtenberg T, Young FM, van Es J, Gmelig-Meyling FH, Berman JE, Alt FW: Frequency of VH-gene utilization in human

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EBV-transformed B-cell lines: The most JH-proximal VH segment encodes autoantibodies. J Autoimmun 2:203, 1989

48. Berberian L, Valles-Ayoub Y, Sun N, Martinez-Maza O, Braun J: A VH clonal deficit in human immunodeficiency virus-positive individuals reflects a B-cell maturational arrest (published erratum appears in Blood 78:1899, 1991). Blood 78:175, 1991

49. David D, Demaison C, Bani L, Zouali M, Theze J: Selective variations in vivo of VH3 and VH1 gene family expression in peripheral B cell IgM, IgD and IgG during HIV infection. Eur J Immunol 25:1524, 1995

50. Bessudo A, Rassenti L, Feigal E, Kipps TJ: Immunoglobulin $V_H$ gene repertoire of patients with AIDS. Blood 86:929a, 1995 (suppl 1)

51. Hsu FJ, Levy R: Preferential use of the $V_H4$ Ig gene family by diffuse large-cell lymphoma. Blood 86:3072, 1995

52. Schutte ME, Van Es JH, Silberstein LE, Logtenberg T: VH4.21-encoded natural autoantibodies with anti-i specificity mirror those associated with cold hemagglutinin disease. J Immunol 151:6569, 1993

53. Riboldi P, Gaidano G, Schettino EW, Steger TG, Knowles DM, Dalla-Favera R, Casali P: Two acquired immunodeficiency syndrome-associated Burkitt's lymphomas produce specific anti-i IgM cold agglutinins using somatically mutated VH4-21 segments. Blood 83:2952, 1994

54. Jukes TH, King JL: Evolutionary nucleotide replacements in DNA. Nature 281:605, 1979

55. Shlomchik MJ, Marshak-Rothstein A, Wolfowicz CB, Rothstein TL, Weigert MG: The role of clonal selection and somatic mutation in autoimmunity. Nature 328:805, 1987

56. Rajewsky K, Förster I, Cumano A: Evolutionary and somatic selection of the antibody repertoire in the mouse. Science 238:1088, 1987

57. Weiss U, Zoebelein R, Rajewsky K: Accumulation of somatic mutants in the B cell compartment after primary immunization with a T cell-dependent antigen. Eur J Immunol 22:511, 1992

58. Chang B, Casali P: A sequence analysis of human germline Ig VH and VL genes. The CDR1s of a major proportion of VH, but not VL, genes display a high inherent susceptibility to amino acid replacement. Ann N Y Acad Sci 764:170, 1995

59. Sanz I, Wang SS, Meneses G, Fischbach M: Molecular characterization of human Ig heavy chain DIR genes. J Immunol 152:3958, 1994

60. Andris JS, Johnson S, Zolla-Pazner S, Capra JD: Molecular characterization of five human anti-human immunodeficiency virus type 1 antibody heavy chains reveals extensive somatic mutation typical of an antigen-driven immune response. Proc Natl Acad Sci USA 88:7783, 1991

61. Moran MJ, Andris JS, Matsumato Y, Capra JD, Hersh EM: Variable region genes of anti-HIV human monoclonal antibodies: Non-restricted use of the V gene repertoire and extensive somatic mutation. Mol Immunol 30:1543, 1993

62. Bagley J, Dillon PJ, Rosen C, Robinson J, Sodroski J, Marasco WA: Structural characterization of broadly neutralizing human monoclonal antibodies against the CD4 binding site of HIV-1 gp120. Mol Immunol 31:1149, 1994

63. Barbas CF, Collet TA, Amberg W, Roben P, Binley JM, Hoekstra D, Cababa D, Jones TM, Williamson RA, Pilkington GR: Molecular profile of an antibody response to HIV-1 as probed by combinatorial libraries. J Mol Biol 230:812, 1993

64. Robinson WEJ, Kawamura T, Gorny MK, Lake D, Xu JY, Matsumoto Y, Sugano T, Masuho Y, Mitchell WM, Hersh E: Human monoclonal antibodies to the human immunodeficiency virus type 1 (HIV-1) transmembrane glycoprotein gp41 enhance HIV-1 infection in vitro. Proc Natl Acad Sci USA 87:3185, 1990

65. Ditzel HJ, Barbas SM, Barbas CF, Burton DR: The nature of the autoimmune antibody repertoire in human immunodeficiency virus type 1 infection. Proc Natl Acad Sci USA 91:3710, 1994

66. Andris JS, Capra JD: The molecular structure of human antibodies specific for the human immunodeficiency virus. J Clin Immunol 15:17, 1995

67. Kodaira M, Kinashi T, Umemura I, Matsuda F, Noma T, Ono Y, Honjo T: Organization and evolution of variable region genes of the human immunoglobulin heavy chain. J Mol Biol 190:529, 1986

68. Shen A, Humphries C, Tucker P, Blattner F: Human heavy-chain variable region gene family nonrandomly rearranged in familial chronic lymphocytic leukemia. Proc Natl Acad Sci USA 84:8563, 1987

69. Cook GP, Tomlinson IM, Walter G, Riethman H, Carter NP, Buluwela L, Winter G, Rabbitts TH: A map of the human immunoglobulin VH locus completed by analysis of the telomeric region of chromosome 14q. Nat Genet 7:162, 1994

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