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V_HDJ_H Gene Sequences and Antigen Reactivity of Monoclonal Antibodies Produced by Human B-1 Cells:

Evidence for Somatic Selection^{1,,2}

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Abstract

To understand whether the distinct V_HDJ_H gene utilization by natural polyreactive Abs reflects the developmentally restricted Ig V_HDJ_H rearrangements putatively expressed by B-1 cells, we generated 11 (8 IgM, 1 IgG3, 2 IgA1), 7 (6 IgM, 1 IgG1), and 7 (2 IgM, 3 IgG1, 2 IgG3) mAb-producing lines using B-1a (surface CD5⁺, CD45RA^{low}), B-1b (surface CD5⁻, CD45RA^{low}, CD5 mRNA⁺), and B-2 (surface CD5⁻, CD45RA^{high}, CD5 mRNA⁻) cells, respectively, sorted from adult human peripheral blood. Most B-1a and B-1b, but no B-2, cell-derived mAbs were polyreactive; i.e., they bound different self and foreign Ags with different affinities. B-1a and B-2 mAbs preferentially utilized V_H4 ($p = 0.003$) and V_H3 ($p = 0.010$) genes, respectively. All three mAb populations utilized DXP, DLR, DN D_H genes, and J_H6, but no mAb utilized *DHQ52*. There were fewer unencoded nucleotide (N) additions in the V_HDJ_H junctions of B-1b (3.00 ± 2.52 , mean \pm SD) than of B-1a (12.45 ± 3.93 , $p = 1.23 \times 10^{-5}$) or B-2 (8.29 ± 4.75 , $p = 0.020$) mAbs. Partly due to the fewer N additions and a paucity of D-D fusions, the B-1b mAb CDR3s were significantly shorter than the B-1a mAb CDR3s ($p = 0.013$), which contained a nonrandom Tyr distribution ($p = 0.003$). Finally, all but two B-1 cell-derived mAbs were mutated, in a fashion similar to that of the Ag-selected B-2 mAbs. Thus, in the human adult, B-1 cells that make natural polyreactive Abs may not be representative of the predominantly B-1 developmental waves of colonization of the fetal and neonatal B cell repertoires, and are somatically selected.

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Numerous studies have suggested that in the mouse B-1a, B-1b, and B-2 cells originate from distinct developmental waves of B cell precursors (1–3). Murine B-1a cells (formerly known as Ly-1⁺ B cells), and, perhaps, the related B-1b cells (formerly known as Ly-1 sister B cells), arise early in ontogeny. They would persist in the adult as self-replenishing cells that derive from the replication of other surface (s)Ig⁺ B cells rather than from bone marrow sIg⁻ progenitors. Along with their B-1b counterparts, they are highly represented in the peritoneal cavity. B-2 cells or “conventional” B cells develop late during ontogeny, emerge from adult bone marrow sIg⁻ cells, and are the predominant clonotypes in the adult spleen and lymph nodes. Perhaps reflecting their distinct origins and/or clonal amplification due to somatic selection, B-1 (B-1a and B-1b) cells may express a selected repertoire of Ig H chain V, D, and/or J genes that differs from that expressed by B-2 cells (1–3).

The early murine B cell Ig H chain gene repertoire is the product of preferential rearrangements of members of J_H-proximal V_H-families in the absence of terminal deoxynucleotidyl transferase. The resulting absence of unencoded nucleotide (N) additions in the Ig V_HDJ_H junctions creates a repertoire of limited diversity and restricted length (4, 5). The primarily germline restricted repertoire of expressed Ig V_HDJ_H genes encode for Ag-binding sites with distinctive functional features. Most fetal and neonatal Abs appear capable of binding two or more Ags, including self and exogenous Ags, even highly different in nature (polyreactive Abs) (6). A similar pattern of polyreactivity has also been noted in Abs derived from human fetal B lymphocytes (7). Polyreactive Abs account for the vast majority of the Ig that have been traditionally referred to as natural autoantibodies or Abs. Because of their reactivity with self Ags, natural Abs would provide the core of the interclonal network of “connectivity” crucial in shaping the early B cell repertoire development, and may also provide the templates for autoantibodies arising later in life (8, 9). Because of their reactivity with multiple exogenous, particularly bacterial and viral, Ags, natural Abs may play a major role in the initial response to infection (8, 9). In both early and adult life, natural Abs are mainly the product of B-1 cells (3, 10).

In the human, first and early second trimester fetal B cells are mainly surface CD5⁻ (11). They express IgM genes that frequently use V_H3 family genes and contain H chain complementarity-determining region (CDR)3⁸ sequences similar in length to those found in the mouse in spite of the presence of some N additions (12). Generation of short H chain CDR3s is abetted by frequent use of the short *DHQ52* gene segment and the absence of the long J_H6 gene segment (12). Neonatal B cells are mostly surface CD5⁺ (3, 10, 13). They express mainly IgM that frequently use V_H3 genes with longer CDR3 sequences, which result from increased frequency of D-D joins, increased N addition, use of J_H6, and use of D_H gene segments that are longer than *DHQ52* (14, 15). These longer V_HDJ_H junctional sequences encode H chain CDR3 structures not seen in the mouse. Human adult B cells are mainly CD5⁻ but also include a large CD5⁺ subpopulation. Unselected human adult peripheral blood B cells express mainly V_H3 family genes with an IgM H chain CDR3 composition and length similar to those of the Ig described in the neonate (16).

⁸Abbreviations used in this paper: CDR, complementarity-determining region; FR, framework region; K_d, Ab dissociation constant for Ag; N additions, unencoded nucleotide additions; R, replacement (mutation); S, silent (mutation); H chain, heavy chain; L chain, light chain; TI, thymus independent.

The human adult B cell repertoire comprises three cell subsets similar to those seen in the mouse: B-1a, B-1b, and B-2 cells (17–20). B-1a cells, which are surface CD5⁺CD45RA^{low}, account for 15 to 30% of the circulating, splenic, and tonsillar B lymphocytes in the adult, and constitute the major B lymphocyte population in the fetus and neonate. B-1b cells are surface CD5⁻ and CD45RA^{low}, but express CD5 mRNA at levels comparable with those of B-1a cells, and account for 2 to 6% of adult peripheral B lymphocytes. B-2 cells are surface CD5⁻ and CD45RA^{high}, include memory B cells, and constitute the major population of the adult B cell repertoire. Both B-1a and B-1b cells produce mainly IgM naturally occurring Abs, the majority of which are polyreactive, although polyreactive IgG and IgA have also been isolated (18–20). In contrast, B-2 cells produce mainly Ag-induced monoreactive IgG (18, 20).

We addressed the issue as to whether the utilization of distinct V_HDJ_H gene rearrangements by polyreactive Abs reflects the developmentally restricted Ig V_HDJ_H rearrangements putatively expressed by circulating human B-1 cells. We generated polyreactive IgM, IgG, and IgA mAbs from sorted human adult peripheral blood B-1a and B-1b cells, and monoreactive IgG mAbs from sorted B-2 cells. By cloning and sequencing the V_HDJ_H gene segments, we found that the V_H and J_H gene utilization, and/or the patterns of junctional V_HDJ_H gene sequences of the mAbs derived from B-1a and, to a lesser extent, B-1b cells differ from those of the mAbs derived from “conventional” B-2 cells, and therefore those expressed by the human adult B cell repertoire as a whole. We also found that the gene selection and junctional pattern of the V_HDJ_H sequences of the natural polyreactive mAbs analyzed do not reflect those thought to be characteristic of the B-1 cell repertoire at large, as previously defined by the analysis of neonatally expressed Ig genes. These differences suggest that the polyreactive B-1 cell-derived Ab repertoire in the human adult is generated through somatic expansion, possibly dependent on Ag, of a minor clonotypic B cell population, rather than resulting from the mere germline encoded natural reactivity. Our conclusion is further supported by the high load of putative replacement (R) mutations in the B-1a and B-1b polyreactive Ig-binding sites with preferential segregation of the R mutations within the V_H segment CDRs. These results should prompt a further re-evaluation of the forces that shape the composition of the adult human B cell repertoire in both normal and disease states.

Materials and Methods

Generation of mAb-producing cell lines from sorted human B-1a, B-1b, and B-2 cells

The generation and features of 18 of the 25 B-1a, B-1b, or B-2 cell-derived mAb-producing cell lines have been reported (20–23). The remaining seven cell lines (cell lines 417.F1.1.35, 417.7.3.1, 417.32.F4.8.5, 417.32.F24.7, 417.F33.3, 417.F32.36, and 417.F32.26) were generated for the purpose of these studies. B lymphocytes were enriched by T cell and monocyte depletion from the PBMCs of healthy donors A (cell line 416.F2.2.34) (20), B (cell lines 417.F25, 417.F9.5, 417.F21.5, 417.F14, 417.F22, 417.F23, 417.F29, 417.F28, 417.F1.1.35, 417.7.3.1, 417.32.F4.8.5, 417.32.F24.7, 417.F33.3, 417.F32.36, 417.F32.26) (20), C (cell line 418.F63.35) (20), D (cell line 18) (22), E (cell line 21) (22), F (cell line 17) (22), G (cell line 44) (22), H (cell line 26) (22), and the rheumatoid arthritis patient I (cell

lines 63, 65, and 67) (23). Enriched B cells were reacted with specific mouse mAbs to CD20, CD5, and CD45RA (Coulter Immunology, Hialeah, FL) and applied to an EPICS Elite FACS (Coulter Immunology) to yield purified B-1a, B-1b, and B-2 cells (20, 24). In some experiments, only CD5⁺ (B-1a) cells were sorted using mAbs to CD20 and CD5 (19, 22). Sorted B-1a, B-1b, and B-2 cells were infected with EBV for 1 h and then immediately distributed into 96-well plates (18–24). The Igs produced by these microcultures were analyzed using appropriate ELISA (18–24). Microcultures originally seeded with B-1a or B-1b cells were selected for production of natural IgM Abs binding to self and/or foreign Ags, including IgG Fc fragment, ssDNA, insulin, thyroglobulin, actin, phosphorylcholine, tetanus toxoid, *Escherichia coli* β -galactosidase, and/or *E. coli* LPS (18–24). Microcultures originally seeded with B-2 cells were selected for the production of mainly IgG, irrespective of their Ag-binding activity. The B-1b cell-derived IgG mAb 417.F1.1.35, and all B-2 cell-derived IgM and IgG mAbs did not bind any of the above nine Ags tested, and were operationally defined as monoreactive Abs, specific for Ags yet to be identified. EBV-transformed B cells were cloned by sequential subculturing (18–24), and then, with the exception of the cell line 417.7.3.1, fused with human-mouse heterohybridoma F3B6 cells (25). The Ag-binding activities of the mAbs were analyzed in specific dose-saturable binding and competitive inhibition ELISAs using the nine Ags listed above, and the dissociation constant (K_d) for Ag was calculated as reported (18–27). The K_d for the different Ags were different in each mAb; the K_d for the same Ag were different in the different mAbs, and ranged from 10^{-5} to 10^{-8} M. EBV-transformed cells and/or EBV-transformed B cell hybrids were used for mAb V_HDJ_H gene cloning and CD5 mRNA analysis.

Analysis of CD5-specific mRNA expression

The levels of cellular CD5 mRNA were analyzed using the PCR method we devised ad hoc (20, 24, 26). Briefly, poly(A)⁺ RNA from each mAb-producing cell line (0.3 μ g), freshly isolated T cells (0.3–0.009 μ g), and F3B6 cells (0.3 μ g) were purified by affinity absorption to oligo(dT)-cellulose using the Mini RiboSep ULTRA mRNA Isolation Kit (Becton Dickinson Labware, Bedford, MA), and then reverse transcribed using M-MLV reverse transcriptase (SuperScript Preamplification System for First Strand cDNA Synthesis, Life Technologies, Gaithersburg, MD) in conjunction with a poly(dT)_{12–18} primer. The cDNA were used as templates in PCR amplifications utilizing the β -actin-specific sense (5'-GTACCACTGGCATCGTGATGGACT-3') and antisense (5'-ATCCACACGGAGTACTTGCCTCA-3') oligonucleotide primers, or the CD5-specific sense (5'-AGACGGATGGCACATGGTTT-3') and antisense (5'-TTGTCCTGGGCTCATAGCT-3') oligonucleotide primers. When amplifying cDNA, the β -actin-specific and CD5-specific sense and antisense primers yielded ~0.6 kb and ~0.45 kb products, respectively. Each PCR was performed in a 50- μ l vol and consisted of 25 cycles of: denaturation, 94°C for 1 min; annealing, 60°C for 1 min; and extension, 72°C for 2 min. The amplified cDNA was applied to a 1.2% agarose gel containing 1 μ g/ml of ethidium bromide, fractionated, and transferred onto a nylon membrane (GeneScreen Plus, Du Pont, NEN, Boston, MA). Hybridization with the internal β -actin specific oligonucleotide, (5'-GACTGACTACCTCATGAAGATCCT-3') or the internal CD5-specific oligonucleotide (5'-CCAGAAGACAACACCTCCAA-3') probe, labeled with [γ -³²P]ATP (NEN), was

performed overnight at 43°C. The filters were washed twice with 2× SSC/0.1% SDS at room temperature for 30 min, and twice with 1× SSC/0.1% SDS at 52°C for 30 min. Autoradiography was performed overnight using Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY).

Cloning and sequencing of the mAb V_HDJ_H gene segments

mRNA was isolated from the mAb-producing cell lines and reverse transcribed as detailed above. Second-strand cDNA synthesis and amplification were performed by PCR using 100 ng of first-strand cDNA as template in a 50- μ l reaction volume. The degenerate sense oligonucleotide primers consisted of sequences encompassing an area of the leader region of the different V_H gene families plus an *Eco*RI site, as follows: V_H1 (5'-GGGAATTCATGGACTGGACCTGGAGG(AG)TC(CT)TCT(GT)C-3'); V_H2 (5'-GGGAATTCATGGACATACT(GT)TG(GT)T(CT)CACGCT(CT)CT(GC)C-3'); V_H3 (5'-GGGAATTCATGGAG(CT)TTGGGCTGA(CG)CTGG(CG)TTT(CT)T-3'); V_H4 (5'-GGGAATTCATGAA(AG)CA(TC)CGTGGTTCTT(CT)(AC)T(CT)CT(CG)C-3'); V_H5 (5'-ATGGGGTCAACCGCCATCCTCGCCCT-3'); and V_H6 (5'-GGGAATTCATGTCTGTCTCCTTCCTCATCTTCC-3'). The antisense C μ (5'-CCGAATTCAACGAGGGGGAAAAGGGT-3') and C γ (5'-CCGAATTCTAGTCCTTGACCAGGCAGCC-3') oligonucleotide primers (27) consisted of the reverse complements of the 5' portion of the C μ and C γ sequences (20 nucleotides), respectively, plus an *Eco*RI site. V_HDJ_H cDNA was amplified by 30 cycles of PCR in a 50- μ l vol. Each cycle consisted of: denaturation, 94°C for 1 min; annealing, 52°C for 1 min; and extension, 72°C for 2 min. Amplified cDNA products were purified by fractionation in 1.2% Ultra Pure Low Melting Point Agarose (Life Technologies), and then ligated into the pCR II plasmid vector (Invitrogen Corp., San Diego, CA). Sequencing was performed by the dideoxynucleotide chain termination method using [α -³⁵S]dATP (NEN) and the TaqTrack Sequencing System (Promega Corp., Madison, WI). Each V_HDJ_H gene sequence was derived from the analysis of at least four independent recombinant clones. Differences in nucleotide sequences among different recombinant clones were rarely observed (<0.0005/base, which is consistent with the error rate of the Taq polymerase). The BLAST algorithm, as found in the NCBI World Wide Web home page accessed through the Netscape Navigator, was used to analyze the GenBank DNA sequence database. The MacVector version 5.0 sequence analysis software (International Biotechnologies, New Haven, CT) was used to analyze the current human Ig gene V-BASE database (MRC Centre for Protein Engineering, Cambridge, U.K.).

Statistical methods

The observed frequencies of V_H or J_H gene utilization by the mAbs produced by B-1a, B-1b, and B-2 cells were compared with the expected frequencies using the χ^2 goodness-of-fit test (28). When the overall differences were significant at $p < 0.050$, the frequencies of V_H or J_H gene utilization were compared with those expected using the exact binomial distribution (28). The expected frequencies were based on the genomic complexity and distribution of the 7 different V_H gene families or six J_H genes. The adopted Ig gene terms of comparison were based on the family genomic complexities reported by Walter et al. (29), Matsuda et al. (30), Cook et al. (31), and Cook and Tomlinson (32), who place the numbers of the

functional genes for the seven different V_H families as follows: V_H1 , 11 members; V_H2 , 3 members; V_H3 , 22 members; V_H4 , 11 members; V_H5 , 2 members; V_H6 , 1 member; and V_H7 , 1 member. The J_H gene terms of comparison were based on an equal representation of the genomic J_H genes, as reported by Ravetch et al. (33), and/or on reported frequencies of use of individual gene segments at different stages of ontogeny. The reported p values for the significant comparisons were based on the two-tailed t test.

To test whether the V_H and J_H genes utilized by the mAbs from the different B cell subsets were different, the χ^2 test for homogeneity was performed (28). When the overall differences were significant at $p < 0.050$, the frequencies of V_H and J_H gene utilization by the mAbs produced by B-1a, B-1b, and B-2 cells were compared between populations using the χ^2 test for independent proportions (28). Exact methods were applied when sample sizes were small. The reported p values for the significant comparisons were based on the two-tailed t test. The χ^2 test for homogeneity and the χ^2 test for independent proportions were applied to test whether the total number of putative R + silent (S) mutations and the total number of putative R mutations in the mAb V_H genes of the three B cell subsets were significantly different. The comparison of the total number of R + S mutations in the CDRs and in the FRs of the mAb V_H genes of the three B cell subsets was also assessed by the same statistical analysis method. The average number of unencoded N nucleotides found at the V_HDJ_H junctions, and the average number of amino acids comprising the CDR3 of the mAbs produced by B-1a, B-1b, and B-2 cells were compared between the three populations by analysis of variance (28). Pairwise comparisons were evaluated using the Tukey method for multiple comparisons at an $\alpha = 0.05$ level (28). Nonparametric analyses, based on rank-transformed data, were also performed; but no significant differences were observed. The reported p values for the significant comparisons were based on the two-tailed t test.

Results

Generation and characterization of human mAbs from B-1a, B-1b, and B-2 cells

We generated 11, 7, and 7 mAbs from B-1a, B-1b, and B-2 cells, respectively, sorted from the peripheral blood of nine adult subjects. The cell origin, the H chain isotype, the L chain type, and the Ag-binding activity of these mAbs are listed in Table I. Eight of the B-1a cell-derived mAbs were IgM, one IgG3, and two IgA1. Six of the B-1b cell-derived mAbs were IgM, and one IgG1. Two of the B-2 cell-derived mAbs were IgM, and five IgG (three IgG1, and two IgG3). All B-1a and B-1b cell-derived mAbs—with the exception of mAb 417.F1.1.35, for which a specific Ag was not determined, and mAb 417.F9.5, which bound to ssDNA only—were polyreactive. They bound with different affinities to nine different self- and foreign Ags, as detailed in *Materials and Methods*. None of the B-2 cell-derived mAbs bound to any of the nine Ags tested. These mAbs were therefore operationally defined as monoreactive to an unidentified Ag (20). Thus, the classes and the Ag reactivity of the mAbs generated were representative of the predominant classes and functional features of the Abs produced by each B cell subset.

CD5 mRNA expression by the mAb-producing B-1a, B-1b, and B-2 cell-derived lines

We have shown that B-1a and B-1b cells, but not their B-2 counterparts, express high levels of CD5 mRNA (20, 24, 26), and that the density of CD5 on the surface of B-1a cells is directly proportional to the level of cellular CD5 mRNA expression (20). To further verify the origin of the mAb-producing cell lines generated using sorted B cell subsets, we analyzed the level of CD5 mRNA expression in three B-1a, three B-1b, and four B-2 cell-derived mAb-producing EBV-transformed lines progenitors of the respective somatic cell hybrids, as well as three B-1b cell-derived somatic hybrids. Poly(A)⁺ RNA were individually extracted from each of the 12 mAb-producing cell lines, from the negative control F3B6 cells, and from freshly purified T cells, and independently reverse transcribed. cDNA was utilized as a template for CD5 or β -actin specific DNA amplification. The reverse transcription of the different poly(A)⁺ samples yielded similar amounts of cDNA, as indicated by the PCR-amplification of comparable amounts of β -actin cDNA (Fig. 1A). The PCR amplification of CD5 mRNA-derived cDNA was dose dependent as shown by the amplification of increasing amounts of cDNA from T cells (Fig. 1C). The validity of analyzing a hybridoma rather than an EBV transformant in the cases of the cell lines 417.F14, 417.F1.1.35, and 418.F63.35 was indicated by 1) the consistent failure to amplify CD5 mRNA from the negative control F3B6 cells (Fig. 1B), and 2) the detection of comparable amounts of CD5 mRNA in the mAb 417.F14-producing EBV-transformed B cell hybrid and its EBV-transformed clonal progenitor (Fig. 1B). All of the B-1a and B-1b tested, but none of the B-2 cell-derived mAb-producing cell lines, expressed significant amounts of CD5 mRNA (Fig. 1B), thus confirming the respective B cell origin of the different cell lines.

V_H genes of the mAbs produced by B-1a, B-1b, and B-2 cells

The V_H gene sequences of mAb 18, mAb 21, mAb 17, mAb 44, mAb 63, mAb 65, mAb 67, and mAb 26 have been previously reported (22, 23). Their respective GenBank accession numbers are listed in Table I. The V_H genes of the mAbs produced by the remaining three B-1a, seven B-1b, and seven B-2 cell-derived lines were cloned and sequenced for the purpose of these studies and are available in the GenBank data base under accession numbers L23555 through L23571 (Table I). The deduced amino acid sequences of all 25 mAb V_H genes are depicted in Figure 2. The degree of identity of the mAb V_H segment nucleotide and deduced amino acid sequences to those of the closest reported germline V_H genes ranged from 79% (mAb 417.F28 V_H gene sequence compared with that of the germline V3-23 gene) to 100.0% (mAb 63 and mAb 418.F63.35 V_H gene sequences compared with those of the germline V4-34 and V3-33 genes, respectively) and are summarized in Table I. The mAbs produced by B-1a and B-2 cells utilized genes of the V_H4 and V_H3 families, respectively, at significantly higher frequency than that theoretically expected ($p = 0.003$ and $p = 0.010$, χ^2 goodness-of-fit and exact binomial distribution), while the mAbs produced by B-1b cells did not show any significant bias in V_H family utilization ($p = 0.240$). The mAbs produced by B-1a and B-1b cells differed significantly in utilization of V_H1 ($p = 0.010$, χ^2 test for homogeneity and the χ^2 test for independent proportions) and V_H4 genes ($p = 0.050$); those produced by the B-1a and B-2 cell subsets differed significantly in utilization of V_H4 genes ($p = 0.009$); and those produced by the

B-1b and B-2 cell subsets did not differ significantly in utilization of the V_H genes ($p = 0.135$).

D and J_H genes and junctional V_HDJ_H sequences of the mAbs produced by B-1a, B-1b, and B-2 cells

The nucleotide sequences of the D genes of the mAbs produced by the B-1a, B-1b, and B-2 cells were compared with those of the reported germline D gene segments (34–38) (Fig. 3). The best fit D gene segments were identified based on the following criteria: 1) priority was given to identity with a V_H or J_H gene sequence when V_H and D, or D and J_H region sequences overlapped (16); 2) if more than one candidate D gene was identified, the germline D gene sequence displaying the longest stretch of identity (with a minimum match of six nucleotides in a stretch of 7 bp or with a minimum match of five nucleotides in a row) (16) to that of the mAb D gene was assigned (16). In those gene segments with more than one D gene segment, the germline position of each gene segment relative to its partner was taken into account. D gene segments were identified in all 25 mAb sequences.

The mAb D gene utilization is listed in Table I. The degree of identity of the mAb gene sequences to those of the putative D genes ranged from 75 (mAb 21) to 100% in seven mAbs (mAb 417.7.3.1, mAb 417.F23, mAb 417.F28, mAb 417.F29, mAb 417.F32.36, mAb 417.F33.3, and mAb 26). Fifteen, seven, six, five, and two mAb D gene sequences could be assigned to portions of those of members of the germ-line DXP, DLR, DN, DM, and DK families, respectively. The mAbs produced by the B-1a, B-1b and B-2 cells did not differ significantly in the utilization of the DXP family ($p = 0.53$, χ^2 test for homogeneity and the χ^2 test for independent proportions). Within the DXP family, six, four, three, and two mAb D segment sequences could be assigned to portions of those of the germ-line *DXP4*, *DXP1'*, *DXP D21/9*, or *DXP1* genes, respectively. Within the DLR family, two, two, one, one, and one mAb D gene segment sequences could be assigned to portions of those of germline *DLR4*, *DLR1*, *DLR3*, *D4*, and *D6* genes, respectively. Within the DN family, three, two, and one mAb D gene segment sequences could be assigned to portions of germline *DN2*, *DNI*, and *DN4* genes, respectively. Within the DM family, three and two sequences could be assigned to portions of those of the germline *DMX* and *DMI* genes, respectively. Finally, within the DK family, one mAb D gene sequence could be assigned to portions of the *DK1* gene segment, and a second mAb D sequence gene segment sequence could be assigned to portions of the *DK4* gene segment. No mAb D segment bore resemblance to sequences of the germline *DA* or *DHQ52* genes.

Sixteen mAbs utilized a single germline D gene; eight utilized two different germline D genes, likely as D-D gene fusions (e.g., mAb 65); and one mAb sequence (mAb 67) utilized three different D genes (Table I and Fig. 3). In five mAbs, the D segments could be accounted for by the utilization of portions of the reverse complements of germline D genes (e.g., mAb 18). All five of the mAbs with inverted D segments utilized two or more D genes, including two sequences with two inverted D gene segments. The mAbs produced by B-1a cells differed from the mAbs produced by B-1b and B2 cells in the use of multiple D gene segments (7 of 11 vs 1 of 7 and 1 of 7, respectively, $p = 0.04$, χ^2 test for independent proportions). Thus, the B-1a, B-1b, and B-2 cell-derived mAbs utilized a heterogeneous

selection of germline D genes in conventional, fused, or inverted configurations, but the B-1a-derived Abs differed from the B-1b and B-2 Abs in the frequent use of more than one D gene segment.

The germline J_H repertoire consists of six functional gene segments and three pseudogenes (33). The sequences of the mAb J_H genes were compared with those of the germline J_H genes and their-allelic forms (16). The region of the mAb J_H gene sequences depicted in Figure 3 spans from the 5' end of the open reading frame of the J_H gene to the first TGG triplet, coding for the invariant Trp. Nine mAbs (three from B-1a, three from B-1b, and three from B-2) utilized J_H genes in full-length form, each with zero to six mutations. Seven, four, and four mAbs from B-1a, B-1b, and B-2 cells, respectively, utilized J_H genes in a truncated form, each with zero to six mutations. Consistent with the findings of Yamada et al. (16) and Schroeder et al. (39), the sites of truncation for the 15 mAb J_H genes were at or 5' of the first TG doublet (Fig. 3). In the germline J_H3b gene sequence, the first TG doublet appears at position 2, and correspondingly, the J_H genes utilized by mAb 416.F2.2.34 (B-1a), mAb 417.F23 (B-1b), and mAb 417.F32.26 (B-2) were truncated by zero to three nucleotides. In the J_H1 , J_H2 , J_H5b , and J_H4b germline genes, the first TG doublet appears at nucleotide positions 3, 4, 6, and 8, respectively, and thus the J_H genes of mAbs utilizing the J_H1 , J_H2 , J_H5b , and J_H4b gene segments were truncated by a maximum of zero, one, eight, and eight nucleotides, respectively (e.g., mAb 417.F32.36 from B-2, mAb 26 from B-1a, mAb 417.7.3.1 from B-1b, and mAb 417.F21.5 from B-1b cells, respectively). The first TG doublet in the J_H6b germline gene appears at position 22, and is consistent with the wide heterogeneity of truncation sites in the expressed J_H6b segments. For example, the J_H gene of mAb 417.F22 (B-1b) was untruncated, whereas that of mAb 44 (B-1a) was truncated by 16 nucleotides. The structure of the 3' sequences of the mAb J_H genes (beginning with the invariant TGG codon) was highly conserved. The J_H genes of 18 mAbs were unmutated in this region, while those of mAb 416.F2.2.34 (B-1a), mAb 417.F9.5 (B-1a), mAb 65 (B-1a), mAb 417.F21.5, (B-1b), mAb 418.F63.35 (B-1b), mAb 417.7.3.1 (B-1b), and mAb 417.32.F24.7 (B-2) displayed one or two somatic point mutations. The mAbs from B-1a cells utilized the J_H6b gene three times, the J_H4b gene five times, the J_H3b gene once, and the J_H2 gene twice; those from B-1b cells utilized the J_H6b , J_H5b , and J_H4b genes each twice, and the J_H3 gene once; and those from B-2 cells utilized the J_H6b gene four times, and the J_H5b , J_H3b , and the J_H1 genes each once (Table I). The mAbs produced by B-1a and B-2 cells utilized at significantly higher frequency than that theoretically expected J_H4b ($p = 0.020$, χ^2 goodness-of-fit test and exact binomial distribution analyses) and J_H6b ($p = 0.009$) genes, respectively, while the mAbs produced by B-1b cells did not show any apparent bias in J_H gene utilization ($p = 0.550$). In addition, J_H gene utilization by the mAbs produced by the B-1a and B-1b ($p = 0.731$, χ^2 test for homogeneity), the B-1b and B-2 ($p = 0.170$), and the B-1a and B-2 ($p = 0.120$) subsets was not significantly different.

The junctional V_HDJ_H nucleotide sequences of the mAbs produced by B-1a, B-1b, and B-2 cells are depicted in Figure 3. All the residues that could not be attributed to the sequence of D genes according to the aforementioned criteria were considered unencoded nucleotide (N) additions. No N additions were found in the V_HD junctions of B-1b cell-derived mAb 417.F21.5 and mAb 417.F32, and the DJ_H junctions of mAb 417.F2.2.34 and mAb 417.F14,

but a minimum of two N nucleotides were found in all of the sequences. In the B-1a cell-derived mAbs, the total number of unencoded V_HDJ_H junction nucleotides ranged from 7 (mAb 21) to 45 (mAb 18) (15.5 ± 10.5 , mean \pm SD); in the B-2 cell-derived mAbs, it ranged from 9 (mAb 417.F32.36) to 19 (mAb 417.32.F26) nucleotides (13.4 ± 3.3); and in the B-1b cell-derived mAbs it ranged from 2 (mAb 417.F21.5) to 20 (mAb 417.F14) (7.7 ± 5.9). Although there was a trend for fewer N additions in the V_HDJ_H junctions of the mAbs produced by B-1b cells, such a trend failed to achieve statistical significance (B-1b vs B-1a, $p = 0.142$; B-1b vs B-2, $p = 0.059$; and B-1a vs B-2, $p = 0.886$, one way analysis of variance).

The difference in the mean number of N nucleotide additions between the mAbs generated from the different B cell subsets was in part due to the N nucleotides added during D-D gene fusion, but differences were also detected in the V_HD and the DJ_H gene junctions. In the V_HD junctions, the average number of N nucleotide additions was: B-1a, 5.9 ± 1.3 (mean \pm SD); B-1b, 4.6 ± 1.7 ; and B-2, 6.3 ± 1.7 . In the DJ_H junctions the average number of N nucleotide additions was: B-1a, 8.1 ± 1.7 ; B-1b, 2.9 ± 2.2 ; and B-2, 5.9 ± 2.2 . However, these differences did not reach statistical significance.

H chain CDR3 structure of the mAbs from B-1a, B-1b, and B-2 cells

The deduced amino acid sequences of the DJ_H genes of the mAbs produced by B-1a, B-1b, and B-2 cells were segregated into CDR3 and FR4 (Fig. 4), as outlined in the compilation by Kabat et al. (40). The FR4 sequences displayed minimal codon diversity. In contrast, the CDR3 sequences were highly divergent in composition and length, ranging from 11 (mAb 416.F2.2.34) to 26 (mAb 18) (17.64 ± 4.25 , mean value \pm SD) codons for B-1a, 6 (mAb 417.F21.4) to 17 (mAb 417.F22) (12.00 ± 4.00) codons for B-1b, and 12 (mAb 417.32F.4.8.5) to 18 (mAb 417.F33.3) (14.86 ± 2.12) codons for B-2 cell-derived mAbs. Partially due to the paucity of D-D fusions, and to fewer N segment additions, the H chain CDR3s of the mAbs produced by B-1b cells were significantly shorter than those of the mAbs produced by B-1a ($p = 0.013$, analysis of variance and Tukey method for multiple comparisons), although they were not shorter than those of the mAbs from B-2 cells ($p = 0.120$). The H chain CDR3s of the mAbs produced by B-1a and B-2 cells did not significantly differ in length ($p = 0.130$).

J_H6 differs from the other J_H elements in the presence of a series of Tyr residues in the CDR3 portion of the gene product, enriching the carboxyl terminal portion of the CDR3 for an amino acid whose combined properties of hydrophilicity, hydrophobicity, space-filling shape, and flexibility allow it to play a key role in a number of Ag-Ab interactions (41, 42). We compared the distribution of Tyr to Gly in the amino and carboxyl termini of the H chain CDR3s of the B-1a-, B-1b-, and B-2-derived mAbs. Each H chain CDR3 was divided in half (when present, the odd residue was attributed to the amino terminus) and the number of Gly and Tyr residues in each portion of the CDR3 determined. Among the B-2 cell-derived mAbs, there were 4 Gly and 3 Tyr in the amino terminus, and 3 Gly and 11 Tyr in the carboxyl terminus (7 of which derived from the J_H6 gene segment). These differences in amino acid distribution were not statistically significant ($p = 0.6$, χ^2 test for homogeneity). Among the B-1b cell-derived mAbs, there were 6 Gly and 4 Tyr in the amino terminus, and

2 Gly and 11 Tyr in the carboxyl terminus (seven of which were derived from J_H6 gene segments). These differences in amino acid distribution just failed to reach statistical significance ($p = 0.074$, χ^2 test for homogeneity). However, among the B-1a cell-derived mAbs, there were 16 Gly and 9 Tyr in the amino terminus, and only 5 Gly with 21 Tyr (7 derived from the J_H6 gene segment) in the carboxyl terminus. The probability that this biased amino acid distribution was not due to chance alone was highly significant ($p = 0.003$, χ^2 test for homogeneity), consistent with the positive selection in the H chain CDR3 structure of an amino acid (Tyr) that is known for the promiscuity of its molecular interactions.

Somatic point mutations in the mAb V_H gene segments

The analysis of the B-1 cell-derived murine CH lymphoma has shown that these cells express Ig genes in unmutated configuration and has led to the view that some if not all B-1 cells may be equipped with a primordial, possibly ineffective mutational machinery (43). As a result of the affinity maturation process which entails enhancement of the Ag-binding potential and preservation of the overall structure of the combining site, nucleotide differences yielding amino acid replacements, R mutations, accumulate in Ab V segment CDRs rather than FRs, resulting in high and low CDR R:S mutation ratios, respectively (44). To verify whether human B-1 cells mutate less effectively their expressed Ig genes and, therefore, differ in their ability to sustain a process of Ag-driven selection as compared to their “conventional” B-2 counterparts, we analyzed the number and distribution of the mAb V_H gene R and S mutations using the respective putative germline V_H gene sequences as references. All but one (mAb 63) of the B-1a, all but one (mAb 418.F63.35) of the B-1b, and all B-2 cell-derived mAbs were mutated (Table I). Furthermore, 1) the mAbs produced by B-1a and B-1b cells displayed a load of total (R + S) mutations (4.3×10^{-2} and 3.5×10^{-2} change/base, respectively) comparable with that of the mAbs produced by B-2 cells (4.9×10^{-2} change/base) (B-1a vs B-2, $p = 0.779$; B-1b vs B-2, $p = 0.460$; B-1a vs B-1b, $p = 0.485$, χ^2 test for homogeneity); 2) the mAbs produced by B-1a and B-1b cells displayed a load of R mutations (2.5×10^{-2} and 2.4×10^{-2} R change/base, respectively) comparable with that of the mAbs produced by B-2 cells (3.6×10^{-2} R change/base) (B-1a vs B-2, $p = 0.450$; B-1b vs B-2, $p = 0.431$; B-1a vs B-1b, $p = 0.941$, χ^2 test for homogeneity); and 3) the mAbs produced by B-1a and B-1b cells displayed a preferential distribution of R mutations within the CDRs as compared with the FRs (B-1a, 5.3×10^{-2} vs 1.6×10^{-2} R change/base, $p = 1.26 \times 10^{-7}$; B-1b, 3.7×10^{-2} vs 2.0×10^{-2} R change/base, $p = 0.001$, χ^2 test for homogeneity) and high CDR R:S mutation ratios (Table I), in a fashion similar to that of the mAbs derived from B-2 cells (5.8×10^{-2} vs 2.9×10^{-2} R change/base, $p = 2.73 \times 10^{-7}$). Thus, the B-1a and B-1b cells mutated thoroughly their expressed Ig V genes, and likely sustained a process of Ag-driven selection similar to that of their “conventional” B-2 counterparts.

Discussion

To gain insight into the mechanisms that underlie the generation of the human B-1 cell-derived polyreactive Ab repertoire, we generated 11 (8 IgM, 1 IgG3, 2 IgA1), 7 (6 IgM, 1 IgG1), and 7 (2 IgM, 3 IgG1, 2 IgG3) mAb-producing EBV-transformed cell lines using

B-1a, B-1b, and B-2 cells, respectively, sorted from adult peripheral blood. We have previously shown that B-1 cells are transformed by EBV as effectively as their B-2 counterparts (18–21). Consistent with the discrete functional features of the Ig produced by the different B cell subsets, most B-1a and B-1b cell-derived mAbs were polyreactive, whereas all B-2 cell-derived mAbs were monoreactive. The origin of the mAb-producing cells was confirmed by the expression of CD5 mRNA in three B-1a and five B-1b cell-derived lines, including a B-1b cell line expressing IgG1; and the lack thereof in four B-2 cell-derived lines, including two cell lines that expressed IgM. Thus, the fidelity of CD5 mRNA expression continued after transformation. The V_H , D, and J_H gene sequence analysis suggested that natural polyreactive Abs derived from adult human B-1a and B-1b cells are produced by a selected assortment of clonotypes that not only differs from that of their monoreactive Ab-producing “conventional” B-2 counterparts, but that also bears little resemblance to the clonotypic composition characteristic of the fetal or neonatal B repertoire, where B-1 cells predominate. It also showed that in addition to their distinctive clonotypic makeup, the natural polyreactive Ab-producing B-1a and B-1b cells analyzed here expressed Ig V_HDJ_H genes that contain a load of R mutations that is comparable in magnitude and distribution with that of the V_HDJ_H genes expressed by the monoreactive Ab-producing “conventional” B-2 cells, and that is consistent with positive clonal selection by Ag.

Preferential use of specific V_H gene families by fetal and neonatal B lymphocytes in the mouse would yield an Ab repertoire containing Ag-binding sites supported by similar FR structures (12, 42). Due to the lack of N additions, fetal Ig H chains that contain the same V_H gene segment often share H chain CDR3s of similar germline-encoded sequence and structure, thereby restricting the diversity of the repertoire (4–6, 45, 46). The absence of N additions limits not only the diversity, but also the range of CDR3 lengths that can be created. Thus, Ag-binding structures that are common in the adult may be rare or may not even exist in the fetus (12). It has been suggested that the structural limitations imposed on fetal H chain CDR3s may play a critical role in establishing the polyreactivity that is characteristic of the fetal Ab repertoire (42). Molecular modeling indicates that limitations in the size of H chain CDR3 results in a relatively “concave” or “flat” Ab-binding site (12, 41). Such a “flat” site could maximize the number of different interactions possible between the residues of the Ag-binding site and potential Ags, allowing for the binding of different Ags, as characteristic of the natural (polyreactive) Ig population expressed in the fetal and neonatal B cell repertoires. It might also prevent a tight conformational fit between Ag and Ab, consistent with the observed low to moderate affinity also characteristic of the fetal and neonatal Ig repertoires. The preferential use of J_H -proximal V_H family genes, limited or absent N additions in the V_HDJ_H gene junctions, and an absence or relative paucity of somatic mutations similar to those seen in fetal and neonatal Ig have been documented in the natural autoantibodies or Abs that occur in adult murine life and that, like those in early life, are mainly polyreactive and represent the products of B-1 cells (6). Thus, the identity and/or strong similarities of V_HDJ_H gene sequences expressed by B-1 cells in the adult to those expressed early in murine life to encode for natural Ab-binding sites support the results of cell transfer experiments suggesting that adult B-1 cells are self-replenishing from sIg⁺ lymphocytes that first emerged during fetal life (1, 3, 5). Indeed, should the mature B

lymphocyte population be ablated by experimental means, only under exceptional circumstances can B-1 cells regenerate in the adult mouse (3).

The results of the present V_H , D , and J_H gene analyses, as summarized in Figure 5, suggest that like their murine equivalents, human B-1a and, perhaps, B-1b cells that are committed to the production of natural polyreactive Abs consist of a clonotypic population distinct from that of their monoreactive Ab-producing “conventional” B-2 counterparts. The natural mAb-producing B-1a cells studied here preferentially utilized V_{H4} and J_{H4} genes, while the monoreactive mAb-producing B-2 cells preferentially utilized V_{H3} and J_{H6} genes. The fact that the biased Ig V_H and J_H gene expression by the $CD5^-$, $CD45RA^{high}$ cells analyzed here closely reflects the biased Ig V_H and J_H gene expression that is characteristic of the adult human B cell repertoire at large is consistent with notion that B-2 cells, as defined by surface $CD5^-$, $CD45RA^{high}$ lymphocytes, are representative of the predominant population in the human adult B cell repertoire (10, 20), and further emphasizes the distinctiveness of the B-1 cell population. The natural polyreactive B-1b cell-derived mAbs may also utilize selected V_H and/or J_H genes, but not to an extent that is significantly different from B-1a or B-2 cell-derived mAbs.

The present findings also support the contention that adult B-1 cells that are committed to the production of natural polyreactive Abs are not representative of the predominantly B-1 developmental waves of colonization of the fetal and/or neonatal B cell repertoire. Major defining characteristics of Abs generated during the fetal life of the human are a preferential use of members of the V_{H3} family, biased usage of D_H and J_H gene segments and limits in the extent of N additions, especially between D_H and J_H (12). DH_{Q52} , which is both the most J_H -proximal and the shortest D_H gene segment, is used in up to 40% of first and second trimester fetal Ig transcripts, but in less than 1% of adult Ig rearrangements (12, 47). Members of the DXP , DLR and DN families, which are the longest conventional D_H gene segments, predominate in the adult repertoire. Fetal Ig transcripts rarely contain more than one D_H gene segment, whereas approximately 10 to 15% of cord blood and the adult peripheral blood transcripts contain multiple D s. J_{H4} is the most commonly used J_H gene segment at all stages of ontogeny. The short J_{H3} is the second most commonly used J_H gene segment in the fetus; J_{H6} , the longest human J_H gene segment, is rarely utilized in fetal Ig transcripts but is the second most commonly used J_H gene in the adult B cell repertoire. Finally, although DH_{Q52} rearrangements from 8 weeks of gestation on display extensive N additions between D and J_H , V_HDJ_H rearrangements that use other D_H gene segments, such as DXP , DN , and DLR , often lack N additions between D and J_H . Similar gene rearrangements, however, display extensive N additions between D and J_H in the expressed adult B cell repertoire.

In the fetus, differences in D and J_H gene utilization, and N addition lead to a repertoire of Ig H chain CDR3 intervals that is distinctly shorter than that found in the adult. Few H chain CDR3 intervals of greater than 16 amino acids have been described and the average CDR3 interval in the fetus measures 10.5 ± 2.5 residues (mean \pm SD). In cord blood, the average is 13.4 ± 4.5 residues; and in the adult, approximately 50% of CDR3 intervals are 16 amino acids or longer. The distribution of CDR3 lengths and the lesser number of N additions in the DJ_H junctions of the mAbs of the B-1b repertoire is similar to that seen in fetal life, but

neither the B-1b nor B-1a natural Ig repertoires match the pattern of D_H or J_H utilization seen in second trimester fetal liver. Thus, although some of the natural mAbs analyzed here displayed features reminiscent of those of the early B cell repertoire, e.g., short CDR3 intervals with minimal N additions in the DJ_H junctions of the Abs expressed by B-1b cells, neither the B-1a nor the B-1b cell-derived Abs were encoded by selections of Ig V_HDJ_H gene sequences overlapping with those of the fetal or neonatal human B cell repertoire.

The feature of the B-1a and B-1b cell-derived mAbs most reminiscent of the fetal repertoire is polyreactivity. Analysis of the mAbs reported here suggests that germline-encoded limitations in the sequence and in the length of H chain CDR3, as seen in B-1b cell-derived Ig, cannot per se account for polyreactivity, as polyreactivity is also a feature of the B-1a cell-derived mAbs. The B-1a mAbs frequently used multiple D segments, and contained, on average, more unencoded nucleotide additions in their V_HDJ_H junctions (17.0 ± 10.5 , mean \pm SD) than the B-1b cell-derived mAbs did (7.7 ± 5.9). The preferential use of multiple D elements by the B-1a cell-derived mAbs and the relative lack of N additions in the B-1b cell-derived mAbs resulted in significant differences in CDR3 length, with the H chain CDR3s of B-1a cell-derived mAbs, on average, five to six amino acids larger than those found in B-1b cell mAbs. The use of multiple D-D joins leaves open the possibility that the polyreactivity of B-1a-derived Abs reflects fine differences in the mechanisms of Ig gene rearrangement inherent to different human B cell lineages or populations. Accordingly, preferential usage of selected V_H and and/or J_H genes could merely reflect preferential rearrangement of given family members or genes by precursors of different populations. Alternatively, restricted expression of given V_H family genes may be brought about or magnified by selection by a “superantigen” for unconventional Ag-binding sites encoded partially or entirely by the FR structures that define the family (48), as seen in the binding of *Staphylococcus* protein A by most human V_H3 family gene products (49), and in the binding of the I/i Ag by the V_H4 family V4-34 gene product in human cold agglutinins (50). Such broad selection processes are likely to have a major impact on the assortment of the B cell repertoire at different stages of ontogeny. However, amplification of a highly restricted population of B cell clonotypes is a typical feature of the mechanism of Ag selection and depends on driving forces that impact primarily on somatically generated structures, and mainly the H chain CDR3, as suggested by the crystallographic analysis of different Ag-Ab complexes (51). A primary role of the H chain CDR3 in providing the main structural correlates for polyreactivity, and therefore Ag selection in B-1 cell expansion is suggested by in vitro human Ig V(D)J gene shuffling and site mutagenesis experiments by us and others (52–56). Expansion of clonotypes bearing polyreactive but functionally discrete surface $F(ab')_2$ receptors for Ag could be driven by a heterogeneous assortment of naturally occurring Ags, as suggested by the structural heterogeneity of the H chain CDR3s of the present B-1 cell-derived natural mAbs, and that of other human polyreactive IgM, IgA, and IgG mAbs (8, 19–27, 57–59) with multiple but yet discrete Ag-binding activities. This contention is also consistent with the asymmetrical enrichment for Tyr residues in the present mAb H chain CDR3s. Preference for a promiscuous Ag-binding residue in the Ag-binding site could likely occur as a result of selection by Ag.

The hypothesis that, as suggested for murine B-1 cells (60–61),⁹ Ag receptor-based selection plays an important role in shaping the B-1 repertoire and may be at the basis of the emergence and/or expansion of most natural polyreactive Ab-producing cell clonotypes in the human adult is further supported by the high degree of somatic diversification of the B-1 cell-derived polyreactive mAb V_HDJ_H segments analyzed here. Somatic diversification is underpinned by cellular events that are triggered by Ag selection and that lead to Ab affinity maturation. All but 2 (1 from B-1a and 1 from B-1b cells) of the 18 B-1 cell-derived mAbs sequenced displayed a somatic R mutation load comparable with that found in the putatively monoreactive B-2 cell-derived mAbs, with preferential distribution of R mutations within the V_H segment CDRs as compared with the FRs (4.97×10^{-2} vs 2.10×10^{-2} R change/base, respectively, $p = 0.004$), and high CDR R:S mutation ratios (Table I). In selection by Ag of “conventional” B-2 cells, Ig somatic mutation and class-switching are in general concurrent although mechanistically independent processes. The presence of somatic mutations in the majority of B-1 cell-derived IgM (present data), IgG and IgA (58, 59) strongly suggests that these B cells can undergo a process of clonal expansion and selection mediated by occupancy of the surface F(ab')₂ receptor for Ag. Such an Ag-dependent clonal selection would be consistent with the oligoclonality detected in human adult B-1a cells by other investigators (62).

Although limited in scope, the present findings suggest that in the adult human, natural Ab-producing B-1a cell precursors are distinct, and possibly derive through Ag-driven selection from a restricted set of clonotypes that accounts for a negligible proportion of the early or adult B cell repertoire. The latter possibility is at variance with the early ontogenic origin of B-1a cells, as defined by many experiments in the mouse (3, 5), but would be supported by the recent suggestion—also in the mouse—that adult bone marrow B cells can progress along two discrete differentiation pathways, B-1 or B-2, in response to different stimuli (2, 61): 1) B cells induced to proliferate by cross-linking of surface IgM by soluble anti- μ F(ab')₂ fragments express surface CD5; 2) whereas B cells induced to proliferate in response to thymus-dependent inductive signals, e.g., CD40L, display high density of surface CD23 and no CD5. Cross-linking of surface IgM in absence of engagement of CD40 by CD40L on Th cells is characteristic of thymus-independent type 2 (TI-2) Ags, including ubiquitous self Ags or commonly occurring bacterial Ags, and would be highly effective in B cells expressing Ig genes encoding for natural polyreactive autoantibodies. A TI Ab response, defined as a response that can be induced in vivo in absence of T cells, should arise in the absence of Ig somatic diversification. However, like the polyreactive B-1a and B-1b cell-derived mAbs analyzed here, Abs to prototypic TI-2 Ags, as those on certain human pathogenic bacteria, e.g., *Haemophilus influenzae* type B polysaccharide, have been shown to undergo extensive somatic mutation and diversification (63, 64). Accordingly, murine Ab responses to similar formally TI-2 Ags, e.g., *Pneumococcus* polysaccharides, have been shown to be under the influence of regulatory T cells (65). Thus, most of the B-1 cells found in human adults may derive from the expansion of limited clonotypes selected through TI-2 mechanisms.

⁹M. Nouri-Shirazi and A. M. Stall. CD40 signaling induces lineage-specific B cell differentiation in neonatal mice. *Submitted for publication.*

Taken collectively, the present findings suggest that in the adult human, B-1 cells are distinct from their monoreactive “conventional” B-2 counterparts, and likely emerge through Ag-dependent expansion of a minor clonotypic B cell population, possibly through a somatic diversification mechanism similar to the one that drives the affinity maturation of a “conventional” B-2 Ab response. They, however, cannot provide any indication as to whether the restricted B cell clonotypes to which antigenic pressure is applied are part of the predominantly B-1 developmental waves of colonization of the fetal and neonatal B cell repertoire or emerge through the B-1 differentiation pathway that is putatively available to a mature virgin B cell in the adult bone marrow, as discussed above. These two hypothetically distinct patterns of B-1 cell developmental selection are not mutually exclusive, and would rely on a common stimulus for their initiation, i.e., the engagement of the polyreactive surface F(ab')₂ receptor for Ag by structures borne on self-components or common microbial agents. The nature of the selection factors impinging on B lymphocytes and eventually leading to the expansion of natural Ab-producing B-1 cell precursors remains to be elucidated.

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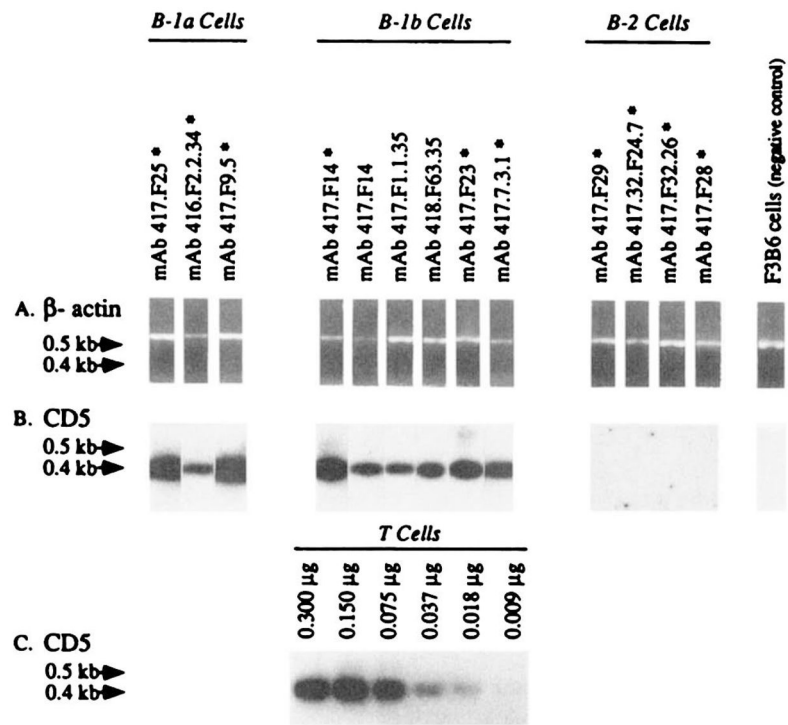
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**FIGURE 1.**

Expression of CD5 mRNA by the B-1a, B-1b, and B-2 cell-derived mAb-producing lines. Poly(A)⁺ RNA (0.300 μ g) extracted from mAb-producing EBV-transformed cell lines (asterisks), mAb-producing EBV-transformed somatic cell hybrid lines, freshly isolated T cells, or from the human-mouse heterohybridoma F3B6 cells, were individually reverse transcribed. The cDNA was individually amplified by PCR using β -actin-specific or CD5-specific oligonucleotide primers and fractionated on a 1.2% agarose gel (see *Materials and Methods*). A, Ethidium bromide-stained gel containing amplified β -actin DNA (~0.6 kb); B, Hybridization of the ³²P-labeled “internal” CD5-specific oligonucleotide probe with fractionated CD5 DNA amplified from cDNA reverse transcribed from the mAb-producing cell lines or the fusion partner F3B6 cells; C, hybridization of the ³²P-labeled “internal” CD5-specific oligonucleotide probe with fractionated CD5 DNA amplified from cDNA individually reverse transcribed from different amounts (0.009 to 0.300 μ g) of purified T cell mRNA.

B-1a cell-derived mAbs

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V3-23      FR1          CDR1          FR2          CDR2          FR3
mAb 18     EVQLVESGGGLVQPGGSLRLSCAASGFTFSYAMSWRQAPGKLEWVAISGGSGSTYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAK 98
mAb 21     -----L-N-----A-----GTG-R-D-N-----Q-M-S-----T-V-----K

V3-33      FR1          CDR1          FR2          CDR2          FR3
mAb 417. F25 QVQLVESGGGVQPGSLRLSCAASGFTFSYGMHWVWVQAPGKLEWVAIWDYGSNKYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAR 98
-----V-----I-----NE-----

V4-34      FR1          CDR1          FR2          CDR2          FR3
mAb 17     QVQLQQMGAGLLKFSSETLSLTCVAVYGGSPFYTWIRQPPGKLEWIGEINHSGSTYINPDLKSRVTVISVDTSKNQPSLKLSSVTAADTAVYYCAR 97
mAb 44     -----T-----G-----E-----A-----
mAb 63     -----T-----G-----E-----A-----

V4-39      FR1          CDR1          FR2          CDR2          FR3
mAb 416. F2. 2. 34 QVQLQESGGGLVQPGSLRLSCTVSGGSISSSYGMWIRQPPGKLEWIGSIYSGSTYINPDLKSRVTVISVDTSKNQPSLKLSSVTAADTAVYYCAR 99
-----D-----R-----A-----T-----D-----I-----T-----

VIV-4      FR1          CDR1          FR2          CDR2          FR3
mAb 417. F9. 5 QVQLQESGGGLVQPGSLRLSCTVSGGSISSSYGMWIRQPPGKLEWIGRIYTSGSTYINPDLKSRVTVISVDTSKNQPSLKLSSVTAADTAVYYCAR 97
-----N-----K-----R-----D-----R-----T-----

V4-31      FR1          CDR1          FR2          CDR2          FR3
mAb 65     QVQLQESGGGLVQPGSLRLSCTVSGGSISSGYMHWIRQPPGKLEWIGYIYSGSTYINPDLKSRVTVISVDTSKNQPSLKLSSVTAADTAVYYCAR 99
E-----R-----F-----T-----K-----D-----F-----S-----L-----Q-----R-----R-----S-----

V4-4       FR1          CDR1          FR2          CDR2          FR3
mAb 67     QVQLQESGGGLVQPGSLRLSCTVSGGSISSSMWVWVQPPGKLEWIGEIYHSGSTYINPDLKSRVTVISVDTSKNQPSLKLSSVTAADTAVYYCAR 98
-----A-----

V4-28      FR1          CDR1          FR2          CDR2          FR3
mAb 26     QVQLQESGGGLVQPGSLRLSCTVSGGSISSSMWVWVQPPGKLEWIGYIYSGSTYINPDLKSRVTVISVDTSKNQPSLKLSSVTAADTAVYYCAR 98
-----Q-----G-----GDY-----S-----H-----AI-----N-----N-----RA-----T-----

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B-1b cell-derived mAbs

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V1-2       FR1          CDR1          FR2          CDR2          FR3
mAb 417. F21. 5 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWVWVQAPGQGLWGMHININSGSTYIAQKFGQVITMTRDTSTSTAYMELSLRSEDVAVYYCAR 98
mAb 417. F14  -----F-----L-----D-----R-----S-----
-----V-----T-----IA-----HV-----H-----F-----V-----A-----C-----V-----N-----

V1-46      FR1          CDR1          FR2          CDR2          FR3
mAb 417. F22 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWVWVQAPGQGLWGMHININSGSTYIAQKFGQVITMTRDTSTSTAYMELSLRSEDVAVYYCAR 98
-----S-----Q-----W-----P-----

V1-69      FR1          CDR1          FR2          CDR2          FR3
mAb 417. F1. 1. 35 QVQLVQSGAEVKKPGASVKVSCKASGYTFSYAMHWVWVQPPGQGLWGMHIIIPGTATYAKFGQVITMTRDTSTSTAYMELSLRSEDVAVYYCAR 98
-----A-----P-----D-----S-----S-----

V3-33      FR1          CDR1          FR2          CDR2          FR3
mAb 418. F63. 35 QVQLVESGGGVQPGSLRLSCTVSGGSISSYGMHWVWVQAPGKLEWVAIWDYGSNKYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAR 98
-----V-----I-----NE-----

H11       FR1          CDR1          FR2          CDR2          FR3
mAb 417. F23 QVQLVESGGGLVQPGGSLRLSCTVSGGSISSYGMHWVWVQAPGKLEWVSRINSDGSGTSTYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAR 98
-----V-----E-----H-----ERC-----K-----R-----

V4-59      FR1          CDR1          FR2          CDR2          FR3
mAb 417. 7. 3. 1 QVHLQESGGGLVQPGSLRLSCTVSGGSISSYGMHWVWVQPPGKLEWIGYIYSGSTYINPDLKSRVTVISVDTSKNQPSLKLSSVTAADTAVYYCAR 97
-----Q-----N-----S-----D-----R-----DT-----R-----R-----

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B-2 cell-derived mAbs

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DP-3       FR1          CDR1          FR2          CDR2          FR3
mAb 417. 32. F4. 8. 5 EVQLVQSGAEVKKPGATVWISCKVSGYFTFDYMHMHWVQAPGKLEWGLVDFEDGETTIAEKFGQVITMTRDTSTSTAYMELSLRSEDVAVYYCAT 98
-----R-----V-----V-----A-----P-----I-----V-----W-----A-----

V3-30      FR1          CDR1          FR2          CDR2          FR3
mAb 417. F33. 3 QVQLVESGGGVQPGSLRLSCTVSGGSISSYGMHWVWVQAPGKLEWVAIWDYGSNKYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAR 98
-----V-----L-----Q-----L-----S-----

V3-9       FR1          CDR1          FR2          CDR2          FR3
mAb 417. 32. F24. 7 EVQLVESGGGLVQPGSLRLSCTVSGGSISSYGMHWVWVQAPGKLEWVSGISWNSGSIYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAK 98
-----T-----GN-----P-----H-----D-----V-----

V3-33      FR1          CDR1          FR2          CDR2          FR3
mAb 417. F29 mAb 417. F32. 36 QVQLVESGGGVQPGSLRLSCTVSGGSISSYGMHWVWVQAPGKLEWVAIWDYGSNKYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAR 98
-----L-----DT-----R-----T-----

V3-30.3    FR1          CDR1          FR2          CDR2          FR3
mAb 417. F32. 26 QVQLVESGGGVQPGSLRLSCTVSGGSISSYAMHWVWVQAPGKLEWVAIWDYGSNKYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAR 98
-----N-----Q-----H-----LH-----T-----T-----D-----R-----XT-----R-----

V3-23      FR1          CDR1          FR2          CDR2          FR3
mAb 417. F28 EVQLVESGGGLVQPGGSLRLSCTVSGGSISSYAMSWRQAPGKLEWVAISGGSGSTYADSVKGRFTISRDNKNTLYLQNSLRAEDTAVYYCAK 98
-----V-----S-----R-----RG-----GIN-----F-----GF-----RNKAVGAE-----A-----R-----S-----D-----VA-----V-----VKTD-----TR

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FIGURE 2. Deduced amino acid sequences of the V_H genes utilized by the mAbs produced by B-1a, B-1b, and B-2 cells. In each cluster, the top sequence is given for comparison and represents the reported germ-line V_H gene displaying the highest degree of identity to the expressed V_H genes. The V3-23, V3-30, V3-33, H11, and V3-9 genes are members of the V_H3 gene family. The V4-34, V4-39, V_{IV}-4, V4-31, V4-4, V4-28, and V4-59 genes are members of the V_H4 gene family. The V1-2, V1-46, V1-69, and DP-3 genes are members of the V_H1 gene family. Dashes indicate identities. Solid lines on the top of each sequence depict CDR. These sequence data are available from EMBL/GenBank/DBJ under the accession numbers listed in Table I.

	FR3	CDR3	FR4
	V _H	D	J _H
<i>B-1a cell-derived mAbs</i>			
mAb 18	TOT GCGAAG GTGGGTTG	DIE4* AGCTGG CATCAAC	DMK* AAGATCAT CCTCTATCTGGGACTCAACCCAATCAGA TACTGGTACTTGGATCTC TGG
mAb 21	TOT GCAAAAG TTGCG	DIE1* AGTTTCAGT* T	DMK* TCTTCCGAT C ACTACTACTAAGGTATGGACGTC TGG
mAb 417.F25	TOT GCGAGAG TAG	DIE3 TGTGTTGGTATGTC* C	CCCCGGTTAC TACTTTGACTAC TGG
mAb 17	TOT GCGA AGTGGCG	D6 ATTACTATG CT	DIE1 AAGATATGATCAATGGTGTATGC GACCCCGG CTTTGATAC TGG
mAb 44	TOT GCGAG GGCA	DIE-1 CGGCGAG ATTTAGG	DIE1 ATGTTTAAATGGTATGC GCAGATCC CGGTATGGACGTC TGG
mAb 63	TOT GCGAGAG GGGATCG	DMK4 GTATTAAGATTTTGGAGTGGTATAT* G	TCCCGCT TTTGACTAC TGG
mAb 416.F2.2.34	TOT GCGAGAG GGG	DIE1 GGGTACG GTCCC	DIE1 TGGTGG A TTTTGATGTC TGG
mAb 417.F9.5	TOT GCGAGAG GCCCAGC	DIE1 GGGTACG A	DIE1 TGGG TTTGACTAC TGG
mAb 65	TOT GCGAG GTGG	DIE4 GGTATTA CA	DIE18 AAGATATGATCA CTCCCGTGT CTACTACTCTACGGTATGGACGTC TGG
mAb 67	TOT GCGAGAG T	DIE1 GGGTACG CCACA	DIE1 GGGTATGATATAT* TCCG ACTTTGACTAC TGG
mAb 26	TOT GCGA CTCCTGTCTCT	DIE18 GGTATGATGATGG C	DIE18 CCTTAAAC ACTGGTACTTGGATCTC TGG
<i>B-1b cell-derived mAbs</i>			
mAb 417.F21.5	TOT GCGAGAGA	DIE-1 TCCGGA A	DMK* AAGATTTT TGACTAC TGG
mAb 417.F14	TOT GCGAGAG TCTAGGTGGGGGAGCA	DIE4 AAGATTTT A	DMK* AAGATTTT GACTAC TGG
mAb 417.F22	TOT GCTAGAG G	DIE18 GGTATGATGATGAT A	DIE18 CCTT ATTACTACTACTACGGTATGGACGTC TGG
mAb 417.F1.1.35	TOT TCGAG GCA	DIE1 GGTATGATGATGAT C	DIE1 AAGATTTT TACTACTACGGTATGGACGTC TGG
mAb 418.F63.35	TOT GCGAGA CCA	DIE1 GGTATGATGATGATGAT A	DIE1 AAGATTTT AACTGGTGGACCC TGG
mAb 417.F23	TOT GCAAGAGA	DIE1 GGTATGATGATGAT GA	DIE1 TGGT GCTTTGATAC TGG
mAb 417.7.3.1	TOT GCGAG GTATA	DIE1 GGTATGATGATGAT ATT	DIE1 TGGT TTGGACCC TGG
<i>B-2 cell-derived mAbs</i>			
mAb 417.32.F4.8.5	TOT GC AGCAG	DIE1 GGTATGATGAT C	DIE1 AAGATTTT GGTATGGACGTC TGG
mAb 417.F29	TOT GCGAGAGA TCTCTTG	DIE1 GGTATGATGATGAT C	DIE1 AAGATTTT ACTATTACTACGGTATGGACGTC TGG
mAb 417.32.F24.7TOT	GCTAAAG CTCT	DIE1 GGTATGATGATGAT T	DIE1 AAGATTTT TACGGTTGGACGTC TGG
mAb 417.F33.3	TOT GCGAGAG GGGCGG	DIE1 GGTATGATGATGATGAT C	DIE1 AAGATTTT AACTGGTGGACCC TGG
mAb 417.F32.36	TOT GCGAGA ATGGAA	DIE1 GGTATGATGATGATGAT G	DIE1 AAGATTTT GACTACTTACGAC TGG
mAb 417.F32.26	TGT CTTCC	DIE1 GGTATGATGATGATGAT C	DIE1 AAGATTTT GCTTTTAAATC TGG
mAb 417.F28	TGT AGCAGACC	DIE1 GGTATGATGATGATGAT GAA	DIE1 AAGATTTT ATTCTACTACTACGGTATGGACGTC TGG

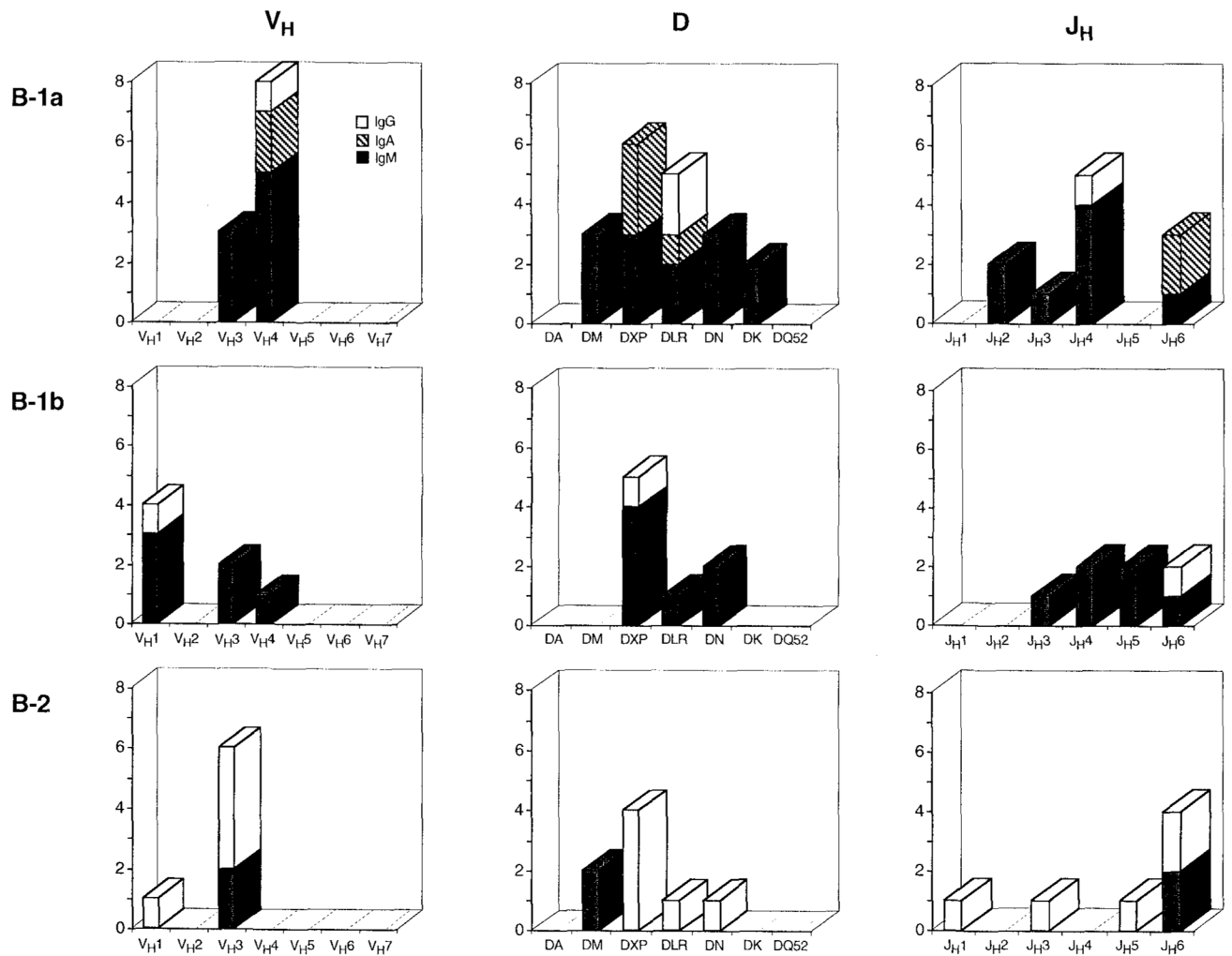
FIGURE 3.

Nucleotide sequences of the V_HDJ_H junctions (CDR3-FR4 areas) of the mAbs produced by B-1a, B-1b, and B-2 cells including unencoded nucleotides. The nucleotide sequences of the relevant portions of the germ-line D genes are given for comparison, and appear above the expressed mAb D segments. Dashes indicate identity with germline D segment sequences. Asterisks indicate the reverse complement of the known germline D segment. Unencoded nucleotide additions are between V_H and D, D and D, and D and J_H gene segments. The TGG triplet encoding the invariant Trp that marks the beginning of the 3' conserved portion of the J_H gene is listed under FR4. These sequences are available from EMBL/GenBank/ DDBJ under the accession numbers of the respective V_H genes as listed in Table I.

* Denotes a D gene that is utilized in an inverted position.

	FR3	CDR3	FR4
	TAVYYCAR		WGOGTLVTVSS
<i>B-1a cell-derived mAbs</i>			
mAb 18	-----K	GGVELASTKPSSIWRLNPIRYWYFDL	--R-----
mAb 21	-----K	VASYSFRRFRHYYYGMDV	-----T-----
mAb 417.F25	-----	VGCGDCSPVYYFDY	-----
mAb 17	--I----K	CAVITLLGYCANGDCDPGEY	-----
mAb 44	-----	ARGDLGVAMVYAQDPGMDV	-----T-----
mAb 63	-----	GGSVLRFLEWLLYPAFDY	-----
mAb 416.F2.2.34	-----	GGLRSLDGFVDV	-----M-----
mAb 417.F9.5	-----	GPTATTGTGFDY	-----P-----P
mAb 65	S-----	WGYNSNGSPLVYFYQGQNV	--P--T-----
mAb 67	-----	VTGSTFWSGYYTRGYFDY	-----
mAb 26	-----T	SVSSMIVVALNHWFYDL	--R-----
<i>B-1b cell-derived mAbs</i>			
mAb 417.F21.5	-----	DRRNDY	---A-----
mAb 417.F14	-----	VLGGGEQYVFDY	-----
mAb 417.F22	-----	GDNSGYRYYYYYYGMVDV	-----T-----
mAb 417.F1.1.35	-----S-	QVRGLLIKYYGMDV	-----T-----
mAb 418.F63.35	-----	PVLLWFRPEYNWFDP	-----
mAb 417.F23	-----	EAGEAGAAFDI	-----M-----
mAb 417.7.3.1	-----	YKQQIFDP	----I-----
<i>B-2 cell-derived mAbs</i>			
mAb 417.32F.4.8.5	--W----A	GLKIYPPTGMDV	-----T-----
mAb 417.F29	-----	DLLVTGTDHYYGMDV	-----T-----
mAb 417.32.F24.7	--L----K	ALSTSCYRVSYGLDV	-----IT-----
mAb 417.F33.3	-----	GAGITDFWVSGYVNWFDV	-----
mAb 417.F32.36	-----	MEYSSGWYGEYLQH	-----
mAb 417.F32.26	-----R-	PLHLAEVDFAFNI	-----M-----
mAb 417.F28	-----S-	PQLERRNFYYGMDV	-----T-----

FIGURE 4. FR3-CDR3-FR4 (deduced) amino acid sequences of the mAbs derived from B-1a, B-1b, B-2 cells. The FR3 and FR4 are depicted as consensus sequences. Dashes indicate identity.

**FIGURE 5.**

Graphic representation of the selection of V_H, D, and J_H genes utilized by the B-1a, B-1b, and B-2 cell-derived mAbs. IgM Abs are depicted by solid bars, IgA Abs are depicted by cross-hatched bars, and IgG Abs are depicted by white bars.

Table 1

Features of the mAbs derived from sorted human B-1a, B-1b, and B-2 cells

Clone	Chain		Donor	H	L	Ag Reactivity	V _H Gene ^d		Identify ^b	Nucleotide Differences				J _H Gene ^d	GenBank Accession No.
	Donor	H					L	Ag Reactivity		Family	Member	CDR	FR		
<i>B-1a cell-derived mAbs</i>															
mAb 18	D	μ	κ			Polyreactive	3	V3-23	98	2:0	0:1		DLR4, DMX	J _H 2	L23931
mAb 21	E	μ	κ			Polyreactive	3	V3-23	91	7:0	6:7		DM1, DMX	J _H 6b	L23932
mAb 417.F25	B	μ	λ			Polyreactive	3	V3-33	97	3:3	1:2		DLR3	J _H 4b	L23557
mAb 17	F	γ	κ			Polyreactive	4	V4-34	88	7:3	6:6		D6, DLR1	J _H 4b	L23930
mAb 44	G	α ₁	λ			Polyreactive	4	V4-34	96	2:1	2:5		DXP1, DLR1	J _H 6b	L23934
mAb 63	I	μ	λ			Polyreactive	4	V4-34	100	0:0	0:0		DXP4	J _H 4b	X54441
mAb 416.F2.2.34	A	μ	λ			Polyreactive	4	V4-39	96	4:0	3:6		DK1, DN2	J _H 3b	L23556
mAb 417.F9.5	B	μ	λ			Monoreactive ^e	4	V _H IV-4	96	3:1	4:3		DN1	J _H 4b	L23570
mAb 65	I	α ₁	λ			Polyreactive	4	V4-31	91	6:1	7:6		DXP4, D21-9	J _H 6b	X54443
mAb 67	I	μ	γ			Polyreactive	4	V4-4	99	0:0	1:0		DN4, DXP4, DK4	J _H 4b	X54445
mAb 26	H	μ	λ			Polyreactive	4	V4-28	87	4:4	11:12		D21-9	J _H 2	L23933
<i>B-1b cell-derived mAbs</i>															
mAb 417.F21.5	B	μ	κ			Polyreactive	1	V1-2	97	3:0	4:3		DXP1	J _H 4b	L23558
mAb 417.F14	B	μ	κ			Monoreactive ^f	1	V1-2	93	4:2	10:3		DXP4	J _H 4b	L23559
mAb 417.F22	B	μ	λ			Polyreactive	1	V1-46	96	2:3	2:3		D21-9	J _H 6b	L23560
mAb 417.F1.1.35	B	γ ₁	λ			Monoreactive ^g	1	V1-69	97	1:1	4:3		DXP1	J _H 6b	L23561
mAb 418.F63.35	C	μ	λ			Polyreactive	3	V3-33	100	0:0	0:0		DXP1	J _H 5b	L23571
mAb 417.F23	B	μ	λ			Polyreactive	3	H11	95	5:0	5:2		D4, DN2	J _H 3b	L23562
mAb 417.7.3.1 ^h	B	μ	λ			Polyreactive	4	V4-59	96	2:0	7:2		DN1	J _H 5b	L23563
<i>B-2 cell-derived mAbs</i>															
mAb 417.32F4.8.5	B	γ ₁	κ			Monoreactive ^g	1	DP-3	94	2:2	7:4		DXP1	J _H 6b	L23555
mAb 417.F29	B	μ	λ			Monoreactive ^g	3	V3-33	99	2:1	1:0		DM1	J _H 6b	L23564
mAb 417.32.F24.7	B	γ ₃	κ			Monoreactive ^g	3	V3-9	96	3:1	5:1		DLR4	J _H 6b	L23565

Clone	Chain		Donor	H	L	Ag Reactivity	V _H Gene ^a			Identity ^b	Nucleotide Differences		D Gene ^c	J _H Gene ^d	GenBank Accession No.
	H	L					Family	Member	CDR		FR	R:S			
mAb 417.F33.3	B	γ ₃	κ	Monoreactive ^g	3	V3-30	3	V3-30	97	2:2	4:1	DXP4	J _H 5b	L23566	
mAb 417.F32.36	B	γ ₁	κ	Monoreactive ^g	3	V3-33	3	V3-33	98	1:0	2:1	DN2	J _H 1	L23567	
mAb 417.F32.26	B	γ ₁	λ	Monoreactive ^g	3	V3-30.3	3	V3-30.3	93	4:1	8:3	DXP4, DXP1	J _H 3b	L23568	
mAb 417.F28	B	μ	κ	Monoreactive ^g	3	V3-23	3	V3-23	79	13:2	20:8	DMX	J _H 6b	L23569	

^aThe complete sequences of the genomic germ-line V_H genes are reported in References 29 to 32.

^bCompared with the genomic germline sequences.

^cThe complete sequences of the genomic germ-line D genes are reported in References 33 to 38.

^dThe complete sequences of the germ-line and expressed J_H genes are reported in References 16 and 33.

^eMonoreactive to SSDNA.

^fMonoreactive to β-galactosidase.

^gAg not identified.

^hmAb 417.7.3.1 was a cloned mAb-producing EBV-transformed B cell line. All other mAb-producing cell lines were B cell hybrids.