Isolation of Germinal Centerlike Events from Human Spleen RNA

Somatic Hypermutation of a Clonally Related $V_{H6}DJ_H$ Rearrangement Expressed with IgM, IgG, and IgA

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Abstract

12 rearranged human V_{H6} immunoglobulin heavy chain genes arising from the same rearrangement were isolated without preselection from the RNA of a fragment of human spleen. The 12 clones were isolated from a pool of 31 unique V_{H6} clones arising from 18 unique rearrangements. 2 of the 12 related clones were expressed with IgM, 2 with IgG, and 8 with IgA1. All the clones, including those expressing IgM, showed extensive somatic mutation of germline bases (5.6%), which was consistent with antigen-driven activation of these V_{H6}-expressing clones with recruitment into the immune repertoire. On the basis of significant sharing of somatic mutations between the IgM clones and clones expressing the other isotypes (six mutations shared with IgG clones and eight mutations shared with IgA clones), it was apparent that the IgM-expressing precursor in this diversified family had undergone extensive antigen-driven somatic mutation prior to isotype switching. This family of related clones suggests that a germinal centerlike event had been sampled. The highly mutated IgM clones suggest that there may exist memory B cells capable of further somatic mutation and differential isotype-switching depending on the specific antigenic stimulus. (*J. Clin. Invest.* 1993. 91:1838–1842.)
Key words: V_{H6} • somatic hypermutation • clonal diversification • isotype switching • immunoglobulins

Introduction

Germinal centers of murine lymphoid tissues are sites where somatic mutations of variable (V) region genes of activated B lymphocytes can be found to accumulate (1–5) and presumably where affinity maturation and selection (5) as well as isotype switching occur (6). B cells isolated from germinal centers of mice immunized with a specific hapten demonstrate clonal relatedness as well as diversification through the accumulation of somatic mutations of their V genes (4, 5). Examination of V genes for evidence of somatic mutation may be confounded by the extensive polymorphism present in some V_H gene families (7). The smaller human V_H gene families, however, lend themselves to such evaluation because polymorphism is less extensive and the germline genes have been better characterized (8). The single member human V_H gene family, V_{H6}, has a highly conserved germline nucleotide sequence (7–10) and thus is well suited for the identification of somatic mutations.

Using rearrangements of the V_{H6} gene as a model, we examined somatic diversification and isotype switching in human spleen-derived $V_{H6}DJ_HC_H$ transcripts. 12 clones isolated from RNA derived from a fragment of a human spleen had undergone somatic mutation, showed differences in heavy chain constant region gene expression, and arose from the same $V_{H6}DJ_H$ rearrangement. The results suggest that germinal centerlike events can be isolated from human spleen cells and that a high degree of somatic mutation can occur in IgM-expressing B cells prior to isotype switching. Mutated IgM–expressing lymphocytes may serve as memory B cells which could undergo further mutation and isotype switching upon restimulation by antigen.

Methods

Isolation of $V_{H6}DJ$ rearrangements expressed with Cα and Cγ was performed as previously described with modification (10A). Briefly, cDNA was prepared with random hexamers from total cellular RNA derived from a single gram fragment of spleen from an 11-yr-old who underwent splenectomy in preparation for a heterologous bone marrow transplant for chronic myelogenous leukemia. She had received one year of maintenance therapy with hydroxyurea. Absolute lymphocyte counts ranged from 10^3 to $10^4/\mu l$ (average $4.5 \times 10^3/\mu l$) over the course of the year and were normal at the time of splenectomy. Pneumococcal and *H. influenzae* type b polysaccharide vaccines were given 1 mo before splenectomy. Amplification by PCR was carried out with the following oligonucleotide primers: V_{H6}(A), a V_{H6} FR1 specific "sense" primer, corresponding to codons 2–8, with a HindIII restriction site at the 5'end, and Cα, a Cα CH1-specific "antisense" primer, corresponding to codons 153-163 of both Cα isotypes with a BamHI restriction site at the 5'end. The thermal profile consisted of an initial denaturation for 5 min at 94°C, 20 cycles with annealing at 55°C for 1 min, elongation at 72°C for 1 min, and denaturation at 94°C for 1.5 min. This was followed by a final annealing step at 55°C for 2 min and elongation at 72°C for 8 min. Amplification was performed with a thermal cycler (Tempcycler Model 50; Coy Laboratory Products, Ann Arbor, MI). The product was reamplified using the same primers and thermal parameters for an additional 35 cycles. Overlapping nucleotides were filled in using Klenow fragment (Bethesda Research Laboratories, Gaithersburg, MD), the inserts were cut with HindIII and BamHI (Bethesda Research Laboratories) and ligated into M13, mp18 and mp19, predigested with these same enzymes. $V_{H6}DJ_HC\gamma$ sequences were derived in a similar fashion from independently prepared mRNA from the same spleen fragment but with a Cγ CH1-specific

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3′-amplification primer corresponding to codons 117–124. $V_{H6}DJ_HC\mu$ clones were derived independently but with a $C\mu$ CH1-specific 3′-amplification primer, corresponding to codons 116-122 and only one round of 30 cycles.

Competent mid-log phase JM103 *E. coli* were transformed and cloned. $V_{H6}DJ_HC\mu$ clones were picked, grown, and screened by PCR amplification with V_{H6}(B), a nested V_{H6} FR1 specific "sense" primer, corresponding to codons 24-30, and the $C\alpha$ "antisense" primer. 10 $V_{H6}DJ_HC\gamma$ clones were picked at random and sequenced. Screening of $V_{H6}DJ_HC\mu$ clones was carried out by PCR with the V_{H6}(B) primer and V_{H6}(C), an FR3 specific antisense primer, corresponding to codons 86-92. Single-stranded DNA was prepared and the nucleotide sequence determined for each clone on an Applied Biosystems 370A automated DNA sequencer by the University of Rochester Core Nucleic Acid Laboratory. Sequences were analyzed using the Seqed, GAP, Translate, and Pile-Up programs of the Sequence Analysis Software Package of the Genetic Computer Group (Madison, WI) (11), on the VAX at the University of Rochester.

We previously demonstrated identity of the germline sequence of the V_{H6} gene segment of this patient to the published V_{H6} germline sequence and showed a degree of fidelity of the PCR process of only one error per 318 bases after 80 cycles of amplification (10A), a frequency similar to that reported for comparable PCR experiments (3, 12).

Results

Following one round of PCR amplification only a weak single ethidium bromide stained band was visualized by gel electrophoresis with IgA and IgG constant region gene antisense primers, in contrast to the IgM primer. Thus, gel purified IgA and IgG primary PCR products were reamplified. 12 clones that expressed the same V_{H6}-N-Dxp′1-N-J_{H4b} rearrangement were isolated without selection from different preparations of RNA from the same spleen fragment (Fig. 1). Eight were expressed with IgA1, two with IgG, and two with IgM heavy chain constant region genes. Of interest, the eight IgA-expressing clones were isolated from a pool of only 11 randomly selected and sequenced unique clones. Similarly, the two IgM-expressing clones were identified in 14 sequenced unique clones and the two IgG-expressing clones from six sequenced unique clones. These 31 clones represented 18 unique rearrangement events.

All 12 clones (8-IgA, 2-IgM, 2-IgG) had an open reading frame and showed mutations away from the germline V_{H6} sequence (Fig. 2). There were on average 15 somatic mutations per clone (range 7 to 20). No base insertions or deletions were found in the V_{H6} segments. Of the 3,302 total nucleotides sequenced in the V_{H6} segments of the 12 clones, 5.6% represented mutations away from the germline sequence (IgM–4.3%, IgA–5.9%, IgG–6.6%). The frequency of mutations was highest in the CDR vs. the FR: FR1 (3.7%), CDR1 (18%), FR2 (0.8%), CDR2 (8.7%), and FR3 (4.5%). The overall frequency of replacement mutations was 4.1% and of silent mutations 1.5%.

Since mutations shared between the 12 clones may have arisen from single mutation events in common precursors, they were considered as a single mutation in the following calculations. The overall ratio of replacement to silent mutations (R/S) for the 12 clones was 2.6. The highest R/S was seen in CDR1 (14), FR1 (3.0), and CDR2 (3.0), while the lowest R/S occurred in FR2 and FR3 (1.0 and 1.7, respectively). Up to six mutations were shared between the IgM and IgG clones (M41 and G8) and up to eight mutations were shared between the IgM and IgA clones (M34 and A5.219). The number of shared mutations between isotypes may actually have been even higher since certain nucleotide changes may represent secondary mutations at the same position. For example, the apparent germline adenine at codon 87 in FR3 of clone M34 could represent a back mutation from the mutation to guanine seen in all other clones at that position (Fig. 2). Similar examples in which several different mutations occurred at the same nucleotide position are evident throughout Fig. 2. The location of the replacement mutations is appreciated from the derived translated sequences shown in Fig. 3.

Discussion

The human V_{H6} family contains a single highly conserved gene and is the most J_H proximal human immunoglobulin heavy chain variable region gene segment (7–10). A V_{H6} gene which differs from the human sequence in only 2-4 nucleotides has also been identified in several higher primate species (17), and V_{H6}-like sequences have been described in the mouse (17, 18). Expression of V_{H6} rearrangements is found, along with several other preferentially rearranged V_H genes, early in fetal development (14, 19–21). It has been suggested that the early expressed fetal V_H repertoire may be programmed to shape the developing fetal immune network via a high degree of self connectivity (22). Many of these same early-expressed germline V_H genes encode self-reactive antibodies (23–26). Indeed, germline encoded V_{H6} rearrangements expressed in IgM antibodies bind to self antigens (27). In contrast to the situation in

	DXP′1 GTATTACTATGGTTCGGGGAGTTATTATAAAC	ACTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA	J_{H4b}	C
A2.618	TGGAAGACATGCAAC-G-A----------C--G	-a-C---t----------Ttt----------	A2.618	α1
A2.418	TGGAAGACATGCAAC-G-A----------C--G	-a-C---t----------	A2.418	α1
A1.219	TGGAAGACATGCAAC-G-A----------C--G	-a-C---t----A----------	A1.219	α1
A1.118	TGGAAGACATGCAAC-G-A----------C--G	-g-C---t----------	A1.118	α1
A5.219	TGGAAGACATGCAAC-G-A----------C--G	-a-C---t----------	A5.219	α1
A2E218	TGGAAGACATGCAATTG-A-------C-------C--G	-a-X---t---X----------X----	A2E218	α1
A5.319	TGGAAGACATGCAATTG-G----------C--G	-c-C---t----------g------A----------	A5.319	α1
A5.419	TGGAAGACATGCAATTG-G----------C--G	-c-C---t----------g----------	A5.419	α1
M34	TGGAAGACATGCAATTG-A----------C--G	-a-C---t-----X----------	M34	μ
M41	TGGAAGACATGCAAT-G-A----------C--G	-a-C---t----------	M41	μ
G6	TGGAAGACATCTAAT-G-A-C----------C--G	-a-C---t----------	G6	γ
G8	TGCAAGACATGCAAT-G-A----------c---C---	-a-G-C-t----------	G8	γ

Figure 1. D_H, N, and J_H region sequences of $V_{H6}DJ_HC\alpha$, $V_{H6}DJ_HC\mu$, and $V_{H6}DJ_HC\gamma$ related clones. Comparison is shown to the published Dxp′1 (13) and J_{H4b} (14, 15) germline sequences. The N-D-N region was considered to begin at the most 5′ nucleotide of codon 95 (16) not encoded by the germline V_{H6} sequence. The most 3′D/N region nucleotide was considered to be the last unmutated nucleotide before the sequence could be identified as a particular J_H sequence (16). Heavy chain isotype is indicated for each clone. Dashes (-) indicate sequence identity with the germline sequence, lower case letters represent silent nucleotide substitutions, capital letters represent nucleotide substitutions leading to an amino acid replacement. × indicates an indeterminate nucleotide. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers L04762–L04773.

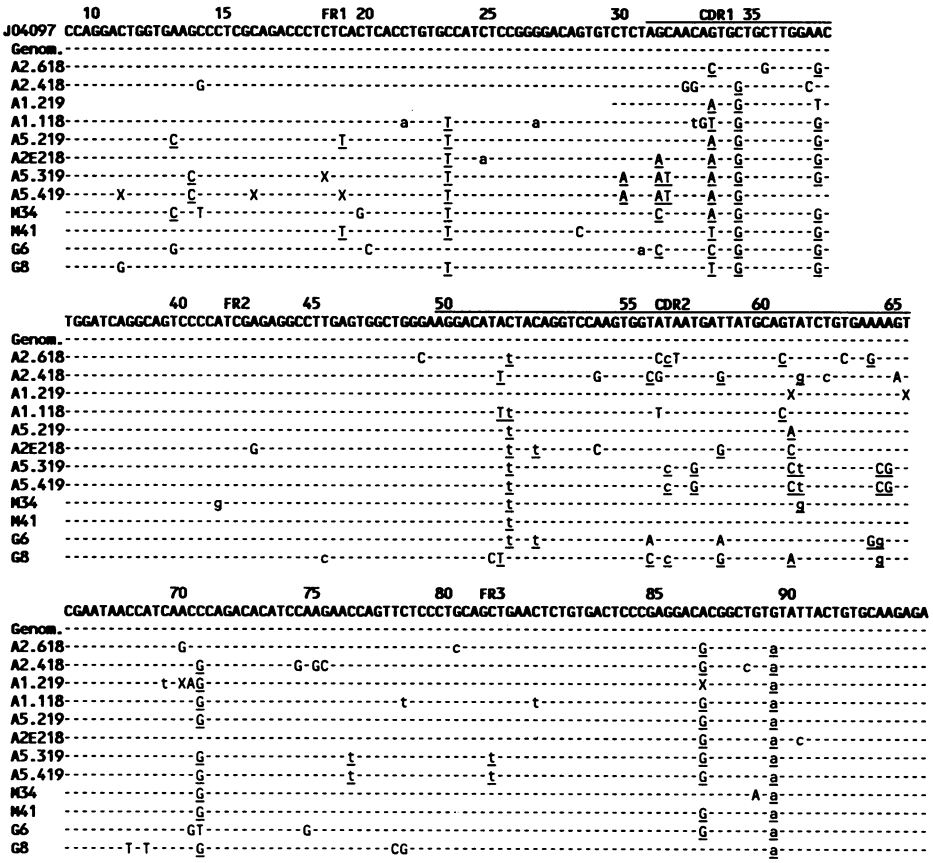

Figure 2. V_{H6} sequences of $V_{H6}DJ_HC\alpha$, $V_{H6}DJ_HC\mu$, and $V_{H6}DJ_HC\gamma$ related clones. Nucleotide sequences of V_{H6} expressing clones are compared with the published V_{H6} genomic sequence, J04097 (10), and to the genomic sequence of the spleen donor. The sequences start immediately 3′ of the 5′PCR primer. Codons are numbered per Kabat et al. (16). Underlined nucleotides are shared between clones. Framework (FR) and complementarity determining regions (CDR) are indicated above the germline sequence. Other symbols are as in Fig. 1 legend.

the fetus, evidence for the participation of V_{H6} in the mature, expressed immune repertoire has, until recently (10A), been lacking. Thus, it was not surprising that two rounds of PCR amplification were required to demonstrate V_{H6} expression with IgG and IgA in the spleen fragment we examined. That V_{H6} expressed with IgM could be found after only 30 cycles of amplification suggests that these transcripts were present in greater abundance in this spleen fragment than the V_{H6} IgG or IgA transcripts. This could result from a greater number of V_{H6} IgM-expressing cells or a higher level of immunoglobulin transcripts in the V_{H6} IgM-expressing cells compared to the other isotypes.

The highly conserved nature of the V_{H6} germline gene, along with the demonstration of its use in the immune repertoire, makes this V gene an ideal substrate for the study of somatic hypermutation. The high degree of somatic hypermutation found in the clones studied and the distribution of the replacement and silent mutations with targeting of replacement mutations to the CDR are evidence for the recruitment of the antibodies encoded by these V_{H6} rearrangements into the immune repertoire by antigenic selection. The high frequency of somatic mutation (5.6%) is similar to the mutation frequency we found in $V_{H6}DJ_HC\gamma$ containing transcripts,[1] and is among the highest reported (28).

While it is conceivable that the subject's underlying malignancy and treatment with hydroxyurea may have altered her B cell repertoire to permit over-representation of V_{H6} expressing cells, this seems unlikely since chronic myelogenous leukemia affects myeloid precursors, not the lymphoid lineage. Lymphocyte precursors appeared normal by bone marrow biopsy, and while she was transiently lymphopenic early in the course of her disease, the absolute lymphocyte count was normal at the

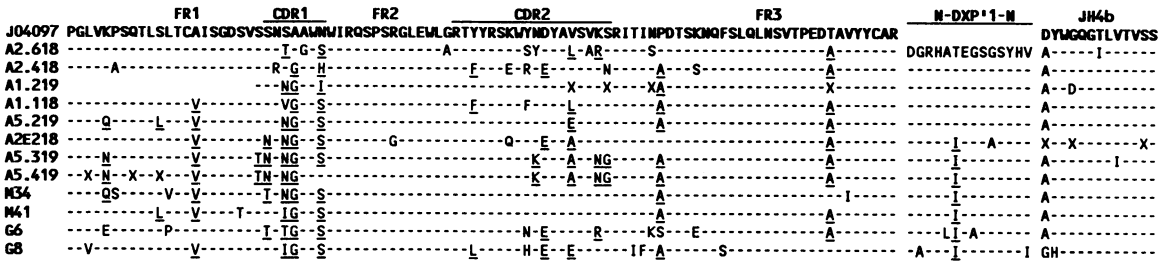

Figure 3. Translated amino acid sequences of $V_{H6}DJ_HC\alpha$, $V_{H6}DJ_HC\mu$, and $V_{H6}DJ_HC\gamma$ related clones. The single letter amino acid designation is used. Amino acid substitutions are indicated. × indicates an indeterminate amino acid. Underlined amino acid replacements are shared between clones. Other symbols as in Fig. 1 legend.

time of splenectomy. In addition, we have isolated numerous V_{H6} rearrangements from spleens of subjects with neither malignancy nor autoimmune disease (unpublished data).

The findings also argue against restriction of V_{H6} expression in the postnatal period as may occur in the J-proximal murine V_H gene V_H 81X which is preferentially expressed early in murine ontogeny and does not appear to encode antibodies in the adult mouse (29). Indeed, in this experiment from RNA from this one spleen fragment, we have isolated 31 unique clones representing 18 unique rearrangement events, and, in addition, we have isolated in other experiments 22 other clones representing another 10 rearrangements from RNA from the same spleen (unpublished data). Therefore, a total of 28 unique V_{H6} rearrangement events have been isolated from this spleen with three rearrangements giving rise to multiple unique isolated members. The fact that none of the 12 clones described here were isolated more than once suggests that the pool of related V_{H6} containing transcripts had not been completely sampled in our experiments.

These findings suggest that active proliferation, diversification, and recruitment into the expressed immune repertoire is occurring with the human V_{H6} gene. The fact that the V_{H6} sequence has been highly conserved with evolution along with the finding that V_{H6} is expressed with antigenically stimulated B cells that have isotype switched to IgA and IgG, suggest that the V_{H6} gene may encode antibodies important for host defense. Germline V_{H6} is known to bind DNA when expressed with various light chains (27). The specificity of our recombinant derived somatically mutated clones is unknown. It is interesting to note, however, that anti-DNA associated idiotypes have been described on human antibodies to pneumococcal polysaccharide (30).

The fixation of shared mutations leading to amino acid replacements in the majority of these V_{H6} clones suggests that these changes may have been important for increasing affinity for the eliciting antigen. The high amino acid replacement to silent mutation ratio as well as the high degree of sharing of mutations between clones in CDR1 suggests that the CDR1 mutations may have been selected on the basis of imparting higher affinity. The amino acids in CDR3, and to a lesser extent, CDR2, remained relatively unaltered, suggesting that these regions, and in particular CDR3, may have been important in the initial clonal selection by antigen.

Isolation without selection of 12 related clones, based on sharing the same rearrangement and N insertions from the spleen fragment, may reflect recent antigenic stimulation and expansion of this rearrangement event and is reminiscent of the findings in murine germinal centers (1–5). Germinal centers are sites in lymphoid organs where B cells undergo rapid proliferation in proximity to antigen retained and presented on follicular dendritic cells and to T cells (1, 2). Immunoglobulin rearrangements in germinal centers are oligoclonal (31) reflecting their derivation from as few as one to three B cells (32, 33). The B cells in germinal centers are undergoing somatic hypermutation (4, 5) although it is argued that the initial stimulation of the B cell and activation of the mutation process may have begun prior to germinal center formation (5). It is thought that those B cells with mutations that do not lead to higher affinity for the stimulating antigen undergo apoptotic cell death. In contrast, B cells with mutated higher affinity antibody are selected and become memory or antibody-forming cells (34, 35).

Of special interest was the high degree of mutation in the IgM M34 and M41 clones. These IgM clones were highly mutated (14 and 10 somatic mutations per V_{H6} segment in the M34 and M41 clones, respectively) demonstrating a dissociation between somatic hypermutation and isotype switching, as previously described (36, 37). These two IgM clones were not unique in having mutations because the 14 other IgM clones also were mutated to a similar extent (unpublished data). While somatic mutation of IgM V_H genes has been described in both the mouse (38–41) and in man (12, 42), the average frequencies of mutation have not been as high as we found for these two IgM clones (4.3%). The fact that these IgM clones were amplified for only 30 cycles, which generated a fidelity of one error in 644 nucleotide sequences in our laboratory, makes PCR artifact an unlikely cause of this high mutation frequency. The high frequency in these IgM clones of total and especially of silent mutations, which cannot be selected by antigen, suggests that the precursor of these clones may have undergone repeated rounds of somatic hypermutation (43, 44). The high degree of sharing of mutations between the IgM and the IgA and IgG clones indicates that a high degree of mutation, if not the majority for some clones, may occur before isotype switching. Such clonal expansion and accumulation of somatic mutations in murine IgM-expressing B cells prior to switching has been recently postulated (45). In addition, the unique somatic mutations in the IgM clones indicate that these IgM clones have continued to undergo somatic mutation even after their precursor gave rise to cells that underwent isotype switching. Similarly, mutations appeared to have accumulated in the IgG and IgA clones following switching. Mutation following isotype switching from μ to γ heavy chains has been demonstrated by others in murine hybridomas (36, 37). The results imply that mutated IgM bearing B lymphocytes could persist in lymphoid tissues as memory B cells, as suggested by others (46), which could then be restimulated after antigenic stimulation to undergo further mutation and isotype switching.

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References

1. MacLennan, I. 1991. The centre of hypermutation. *Nature (Lond.).* 354:352–353.

2. Nossal, G. J. V. 1992. The molecular and cellular basis of affinity maturation in the antibody response. *Cell.* 68:1–2.

3. Jacob, J., and G. Kelsoe. 1992. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. II. A common clonal origin for periarteriolar lymphoid sheath-associated foci and germinal centers. *J. Exp. Med.* 176:679–687.

4. Jacob, J., G. Kelsoe, K. Rajewsky, and U. Weiss. 1991. Intraclonal generation of antibody mutants in germinal centres. *Nature (Lond.).* 354:389–392.

5. Berek, C., A. Berger, and M. Apel. 1991. Maturation of the immune response in germinal centers. *Cell.* 67:1121–1129.

6. Kraal, G., I. L. Weissman, and E. C. Butcher. 1982. Germinal centre B cells: antigen specificity and changes in heavy chain class expression. *Nature (Lond.).* 298:377–379.

7. Willems van Dijk, K., H. W. Schroeder, Jr., R. M. Perlmutter, and E. C. B. Milner. 1989. Heterogeneity in the human Ig VH locus. *J. Immunol.* 142:2547–2554.

8. Sanz, I., P. Kelly, C. Williams, S. Scholl, P. Tucker, and J. D. Capra. 1989. The smaller human V_H gene families display remarkably little polymorphism. *EMBO. (Eur. Mol. Biol. Organ.) J.* 8:3741–3748.

9. Berman, J. E., S. J. Mellis, R. Pollack, C. L. Smith, H. Suh, B. Heinke, C. Kowal, U. Surti, L. Chess, C. R. Cantor, and F. W. Alt. 1988. Content and organization of the human Ig V_H locus: definition of three new V_H families and linkage to the Ig C_H locus. *EMBO. (Eur. Mol. Biol. Organ.) J.* 7:727–738.

10. Schroeder, H. W., M. A. Walter, M. H. Hofker, A. Ebers, K. Willems van Dijk, L. C. Liao, D. W. Cox, E. C. B. Milner, and R. M. Perlmutter. 1988. Physical linkage of a human immunoglobulin heavy chain variable region gene segment to diversity and joining segments. *Proc. Natl. Acad. Sci. USA.* 85:8196–8200.

10a. Varade, W. S., E. Marin, A. M. Kittelberger, and R. A. Insel. 1993. Use of the Jh-Proximal Human Immunoglobulin Heavy Chain Variable Region Gene, V_{H6}, in the Expressed Immune Repertoire. *J. Immunol.* 150: In press.

11. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.

12. Huang, C., A. K. Stewart, R. S. Schwartz, and B. D. Stollar. 1992. Immunoglobulin heavy chain gene expression in peripheral blood B lymphocytes. *J. Clin. Invest.* 89:1331–1343.

13. Ichihara, Y., H. Matsuoka, and Y. Kurosawa. 1988. Organization of human immunoglobulin heavy chain diversity gene loci. *EMBO. (Eur. Mol. Biol. Organ.) J.* 13:4141–4150.

14. Schroeder, H. W., Jr., J. L. Hillson, and R. M. Perlmutter. 1987. Early restriction of the human antibody repertoire. *Science (Wash. DC).* 238:791–793.

15. Yamada, M., R. Wasserman, B. A. Reichard, S. Shane, A. J. Caton, and G. Rovera. 1991. Preferential utilization of specific immunoglobulin heavy chain diversity and joining segments in adult human peripheral blood B lymphocytes. *J. Exp. Med.* 173:395–407.

16. Kabat, E. A., T. T. Wu, M. Reid-Miller, H. M. Perry, and K. S. Gottesman. 1987. Sequences of proteins of immunological interest. 4th ed. Pub. Hlth. Serv. Washington, D.C.

17. Meek, K., T. Eversole, and J. D. Capra. 1991. Conservation of the most J_H proximal Ig V_H gene segment (V_HVI) throughout primate evolution. *J. Immunol.* 146:2434–2438.

18. Clarke, S., R. Rickert, M. K. Wloch, S. Staudt, W. Gerhard, and M. Weigert. 1990. The BALB/c secondary response to the Sb site of influenza virus hemagglutinin. Nonrandom silent mutation and unequal numbers of VH and Vk mutations. *J. Immunol.* 145:2286–2296.

19. Berman, J. E., K. G. Nickerson, R. R. Pollock, J. E. Barth, R. K. B. Schuurman, D. M. Knowles, L. Chess, and F. W. Alt. 1991. V_H gene usage in humans: biased usage of the V_H6 gene in immature B lymphoid cells. *Eur. J. Immunol.* 21:1311–1314.

20. Cuisinier, A.-M., V. Guigou, L. Boubli, M. Fougereau, and C. Tonnelle. 1989. Preferential expression of V_H5 and V_H6 immunoglobulin genes in early human B-cell ontogeny. *Scand. J. Immunol.* 30:493–497.

21. Schroeder, H. W., Jr., and J. Y. Wang. 1990. Preferential utilization of conserved immunoglobulin heavy chain variable gene segments during human fetal life. *Proc. Natl. Acad. Sci. USA.* 87:6146–6150.

22. Kearney, J. F., and M. Vakil. 1986. Idiotype-directed interactions during ontogeny play a major role in the establishment of the adult B cell repertoire. *Immunol. Rev.* 94:39–50.

23. Pascual, V., and J. D. Capra. 1991. Human immunoglobulin heavy-chain variable region genes: organization, polymorphisms, and expression. *Adv. Immunol.* 49:1–70.

24. Bona, C. A. 1988. V genes encoding autoantibodies: molecular and phenotypic characteristics. *Annu. Rev. Immunol.* 6:327–358.

25. Chen, P. P., R. W. Soto-Gil, and D. A. Carson. 1990. The early expression of some human autoantibody-associated heavy chain variable region genes is controlled by specific regulatory elements. *Scand. J. Immunol.* 31:673–678.

26. Kipps, T. J., B. A. Robbins, and D. A. Carson. 1990. Uniform high frequency expression of autoantibody-associated crossreactive idiotypes in the primary B cell follicles of human fetal spleen. *J. Exp. Med.* 171:189–196.

27. Logtenberg, T., F. M. Young, J. H. Van Es, F. H. J. Gmelig-Meyling, and F. W. Alt. 1989. Autoantibodies encoded by the most J_H-proximal human immunoglobulin heavy chain variable region gene. *J. Exp. Med.* 170:1347–1355.

28. Berek, C., and C. Milstein. 1988. The dynamic nature of the antibody repertoire. *Immunol. Rev.* 105:5–26.

29. Kearney, J. F., J. Bartels, A. M. Hamilton, A. Lehuen, N. Solvason, and M. Vakil. 1992. Development and function of the early B cell repertoire. *Int. Rev. Immunol.* 8:247–257.

30. Grayzel, A., A. Solomon, C. Aranow, and B. Diamond. 1991. Antibodies elicited by pneumococcal antigens bear an anti-DNA-associated idiotype. *J. Clin. Invest.* 87:842–846.

31. Razzeca, K. J., E. Pillemer, I. L. Weissman, and R. V. Rouse. 1986. In situ identification of idiotype-positive cells participating in the immune response to phosphorylcholine. *Eur. J. Immunol.* 16:393–399.

32. Kroese, F. G. M., A. S. Wubbena, H. G. Seijen, and P. Nieuwenhuig. 1988. The de novo generation of germinal centres is an oligoclonal process. *Adv. Exp. Med. Biol.* 237:245–250.

33. Jacob, J., R. Kassir, and G. Kelsoe. 1991. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. I. The architecture and dynamics of responding cell populations. *J. Exp. Med.* 173:1165–1175.

34. Leanderson, T., E. Kallberg, and D. Gray. 1992. Expansion, selection and mutation of antigen-specific B cells in germinal centers. *Immunol. Rev.* 126:47–61.

35. MacLennan, I. C., Y-J Liu, and G. D. Johnson. 1992. Maturation and dispersal of B-cell clones during T cell-dependent antibody responses. *Immunol. Rev.* 126:144–161.

36. Manser, T. 1989. Evolution of antibody structure during the immune response. *J. Exp. Med.* 170:1211–1230.

37. Shan, H., M. Shlomchik, and M. Weigert. 1990. Heavy-chain class switch does not terminate somatic mutation. *J. Exp. Med.* 172:531–536.

38. Rudikoff, S., M. Pawlita, J. Pumphrey, E. Mushinski, and M. Potter. 1983. Galactan-binding antibodies. Diversity and structure of idiotypes. *J. Exp. Med.* 158:1385–1400.

39. Rudikoff, S., M. Pawlita, J. Pumphrey, and M. Heller. 1984. Somatic diversification of immunoglobulins. *Proc. Natl. Acad. Sci. USA.* 81:2162–2166.

40. Claflin, J. L., J. Berry, D. Flaherty, and W. Dunnick. 1987. Somatic evolution of diversity among anti-phosphocholine antibodies induced with Proteus morganii. *J. Immunol.* 138:3060–3068.

41. Chien, N. C., R. R. Pollock, C. Desaymard, and M. D. Scharff. 1988. Point mutations cause the somatic diversification of IgM and IgG2a antiphosphorylcholine antibodies. *J. Exp. Med.* 167:954–973.

42. Harindranath, N., I. S. Goldfarb, H. Ikematsu, S. E. Burastero, R. L. Wilder, A. L. Notkins, and P. Casali. 1991. Complete sequence of the genes encoding the V_H and V_L regions of low- and higher-affinity monoclonal IgM and IgA1 rheumatoid factors produced by CD5+ B cells from a rheumatoid arthritis patient. *Int. Immunol.* 3:865–875.

43. Rada, C., S. K. Gupta, E. Gherardi, and C. Milstein. 1991. Mutation and selection during the secondary response to 2-phenyloxazolone. *Proc. Natl. Acad. Sci. USA.* 88:5508–5512.

44. Berek, C., and C. Milstein. 1987. Mutation drift and repertoire shift in the maturation of the immune response. *Immunol. Rev.* 96:23–41.

45. Berek, C. 1992. The development of B cells and the B-cell repertoire in the microenvironment of the germinal center. *Immunol. Rev.* 126:5–19.

46. Gray, D. 1992. The dynamics of immunological memory. *Seminars in Immunology.* 4:29–34.