A highly informative probe for two polymorphic Vh gene regions that contain one or more autoantibody-associated Vh genes.

P P Chen, … , R A Erger, D A Carson

J Clin Invest. 1989;84(2):706-710. https://doi.org/10.1172/JCI114218.

Research Article

Efforts to determine the role of specific Ig variable region (V) genes in human autoimmune responses have been hampered by the lack of suitably polymorphic probes. Recently we isolated a heavy chain V (Vh) gene, designated Humhv3005, that is 99% homologous to the 1.9III Vh gene and can encode an anti-DNA antibody. To study the relation between these two genes, different DNA fragments from the isolated Humhv3005 clone were used to probe Southern blots of human genomic DNA. A 1.6-kb Eco RI fragment (designated hv3005/E1.6) was found to hybridize with only one band in Eco RI-digested DNA, and with two major bands in Bam HI-digested DNA. Importantly, the sizes of the latter two bands were indistinguishable from the corresponding Bam HI fragment sizes of the isolated hv3005 clone and the isolated 1.9III clone, respectively. Population and family studies with the hv3005/E1.6 probe revealed five different hybridization patterns of these two characteristic bands, which defined nine possible genotypes for two human Ig Vh gene loci. Together the data demonstrate that hv3005/E1.6 is a highly informative probe for an autoantibody-associated Vh gene(s), and should prove useful in elucidating the role of Ig Vh genes in autoimmune diseases.

A Highly Informative Probe for Two Polymorphic Vh Gene Regions That Contain One or More Autoantibody-associated Vh Genes

Pojen P. Chen, Katherine A. Siminovitch,* Nancy J. Olsen,‡ Rochelle A. Erger, and Dennis A. Carson
*Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic, La Jolla, California 92037; *Department of Medicine, University of Toronto, Toronto, Ontario, Canada M5T 2S8; and ‡Department of Medicine, Vanderbilt University, Nashville, Tennessee 37232*

Abstract

Efforts to determine the role of specific Ig variable region (V) genes in human autoimmune responses have been hampered by the lack of suitably polymorphic probes. Recently we isolated a heavy chain V (Vh) gene, designated Humhv3005, that is 99% homologous to the 1.9III Vh gene and can encode an anti-DNA antibody. To study the relation between these two genes, different DNA fragments from the isolated Humhv3005 clone were used to probe Southern blots of human genomic DNA. A 1.6-kb Eco RI fragment (designated hv3005/E1.6) was found to hybridize with only one band in Eco RI-digested DNA, and with two major bands in Bam HI-digested DNA. Importantly, the sizes of the latter two bands were indistinguishable from the corresponding Bam HI fragment sizes of the isolated hv3005 clone and the isolated 1.9III clone, respectively. Population and family studies with the hv3005/E1.6 probe revealed five different hybridization patterns of these two characteristic bands, which defined nine possible genotypes for two human Ig Vh gene loci. Together the data demonstrate that hv3005/E1.6 is a highly informative probe for an autoantibody-associated Vh gene(s), and should prove useful in elucidating the role of Ig Vh genes in autoimmune diseases.

Introduction

A major aim of immunopathology research is to identify the genetic factors that promote abnormal or sustained autoantibody production. Although autoantibodies were first discovered in patients with various autoimmune diseases, they have also been detected among healthy individuals (1–3). While the inherited structural genes for Ig light and heavy chains dictate the potential antibody repertoire of any individual, Ig genes also undergo extensive somatic diversification during life (4, 5). Variations in the number and structure of inherited Ig genes, as well as somatic changes, may therefore influence the generation of autoantibodies, and in some cases predispose individuals to autoimmune diseases. To dissect the relative contribution of these two factors, one must first identify the autoantibody-associated Ig gene segments and then determine their polymorphisms in human populations.

During the last several years we and others have sought to isolate human autoantibody-associated Ig variable region (V)[1] genes. To date, two kappa light chain V (Vk) genes (i.e., Humkv325 and Humkv328) and one heavy chain V (Vh) gene (i.e., Vh 783) that can encode rheumatoid factors have been characterized (6–8). In addition, we have identified a Vh gene (i.e., Vh 26) corresponding to the 16/6 crossreactive idiotype on anti-DNA antibodies (9). Similarly, Cairns et al. (10) and Makar et al. (11) reported that the Vh nucleotide sequences for three autoantibodies against DNA, Sm, and insulin, respectively, were identical to three reported Vh genes.

Recently, in an effort to delineate the underlying mechanisms for the preferential expression of certain autoantibody-associated human Ig V genes during early ontogeny, we isolated a Vh 3 gene, termed Humhv3005.[2] It encodes an amino acid sequence identical to the 56P1 sequence, which was most frequently found in a 130-d fetal liver (12). Interestingly, the hv3005 sequence is also 99% homologous to that of the 1.9III gene, which is identical to the Vh nucleotide sequence of an anti-DNA antibody (i.e., kim 4.6[10]; reference 13). These results suggested that hv3005 and 1.9III either were alleles of a single human Vh gene locus, or were distinct but homologous V genes in the haploid human genome. Accordingly, we used fragments from the Humhv3005 clone to analyze Southern blots of human genomic DNA. Population and family studies provided evidence for polymorphism in two Vh gene regions, containing at least one anti-DNA autoantibody-associated Vh gene.

Methods

Isolation and characterization of the Humhv3005-containing clone.[2] The Humhv3005 was recently isolated from a human genomic library

This is publication 5809-BCR from the Research Institute of Scripps Clinic.

Address correspondence to P. P. Chen, Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

Received for publication 14 March 1989 and in revised form 25 April 1989.

J. Clin. Invest.

0021-9738/89/08/0706/05 $2.00
Volume 84, August 1989, 706–710

1. *Abbreviations used in this paper:* Ig V gene, Ig variable region gene; RFLP, restriction fragment length polymorphism; Vh gene, heavy chain V gene; Vk gene, kappa light chain V gene.
2. Chen, P. P. 1989. Structural analyses of the human developmentally regulated Vh3 genes. Manuscript submitted for publication.

(Y79, kindly provided by Dr. W. Lee, University of California, San Diego, CA) that was screened with a Vh 3 cDNA probe (56P1; from nucleotide positions −124 in the V region to 408 in the Cu region, donated by Dr. H. W. Schroeder and Dr. R. M. Perlmutter, University of Washington, Seattle, WA) (12, 14). The purified hv3005 clone was mapped by single and double restriction enzyme digestions of the isolated recombinant phage, and by subsequent Southern blot analyses of these lysates. After subcloning into M13, the nucleotide sequence of hv3005 was determined by the chain termination method.

Genomic DNA and Southern blot analyses. Genomic DNAs were prepared from peripheral blood polymorphonuclear cells of unrelated subjects or members of two Jewish families involved in the study of Gaucher's disease. Of the 32 unrelated subjects, 19 were patients with rheumatoid arthritis and 1 was a patient with systemic lupus erythematosus. Regarding their ethnic origins, 25 were Caucasians, 3 were Hispanics, and 2 were American blacks. DNA samples were digested separately with Bam HI and Eco RI, according to the manufacturer's directions (New England Biolabs, Beverly, MA). The lysates were loaded into wells of a 0.7% agarose gel. After electrophoresis and transfer of DNA to nitrocellulose, the blots were hybridized with the indicated probes, including a 1.6-kb Eco RI fragment of the Humhv3005 clone, and Humkv328/pl (a 1.4-kb Hind III fragment of Humkv328) (15). Hybridizations were done in 5× standard saline citrate (SSC; 1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) at 65°C, followed by washing twice in 1× SSC at 65°C.

Results

Characterization of the Humhv3005/1.6-kb Eco RI fragment. Fig. 1 *a* shows the relevant restriction enzyme sites of the Humhv3005 clone, from 2 kb upstream to 12 kb downstream of the coding region. In an effort to identify suitable probes for the hv3005 and 1.9III Vh gene polymorphisms, a series of fragments from the isolated Humhv3005 clone were used to analyze Southern blots of human genomic DNA.

As illustrated in Fig. 1 *b*, the Humhv3005/1.6-kb Eco RI fragment (designated hv3005/E1.6) hybridized with only a single 1.6-kb band in human genomic DNA digested with Eco RI. This result indicated that hv3005/E1.6 was a very specific probe for the hv3005 locus. When the same human DNA was digested with Bam HI and analyzed with the hv3005/E1.6, two major bands of ~ 5.2 and 6 kb, respectively, were visualized (Fig. 1 *b*). Based on the map of the hv3005 clone, the 5.2-kb band represents the hv3005 locus. In addition, the reported map of the homologous Vh 1.9III gene showed that there was a Bam HI fragment of ~ 6 kb in a position similar to the 5.2-kb region in the hv3005 clone (13). Thus, the 6-kb hybridizing band was likely to represent the Vh 1.9III locus. Together these results showed that hv3005/E1.6 is a regional-specific probe that identifies two diagnostic bands representing the hv3005 and the Vh 1.9III gene loci.

Restriction fragment length polymorphism (RFLP) analyses with the hv3005/E1.6 probe. To analyze genetic variation in the hv3005 and 1.9III loci, genomic DNA from 18 unrelated individuals was digested with Bam HI, electrophoresed, blotted, and hybridized with the hv3005/E1.6. The results revealed variability among individuals not only with regard to the presence of the 6- and 5.2-kb bands, but also with regard to the hybridization intensities of these two restriction fragments. No allelic counterparts were detectable for either the 6- or 5.2-kb fragments, but five patterns of band combinations were apparent (Fig. 2 *a*). They consisted of: (*a*) a 6-kb band alone (lanes *3*, *12*, and *16*); (*b*) a 5.2-kb band alone (lane *1*); (*c*) both 5.2- and 6-kb bands of approximately equal intensity (lanes *5–7*, *13*, and *14*); (*d*) a strong 6-kb band with a weak 5.2-kb band (lanes *2*, *8–11*, *15*, *17*, and *18*); and (*e*) a strong 5.2-kb band and a weak 6-kb band (lane *4*). It is possible that the last two patterns might have been due to the partial digestion of the DNA samples. To address this question, the same blot was hybridized with a Vk gene probe (Humkv328/pl) that had been characterized extensively in our laboratory. Only one hybridizing band of expected size was detected in each genomic DNA sample (Fig. 2 *b*). In addition, we digested the left-over DNA (used in Fig. 2 *a*) with an additional aliquot of Bam HI overnight, and obtained an essentially identical result (data not shown).

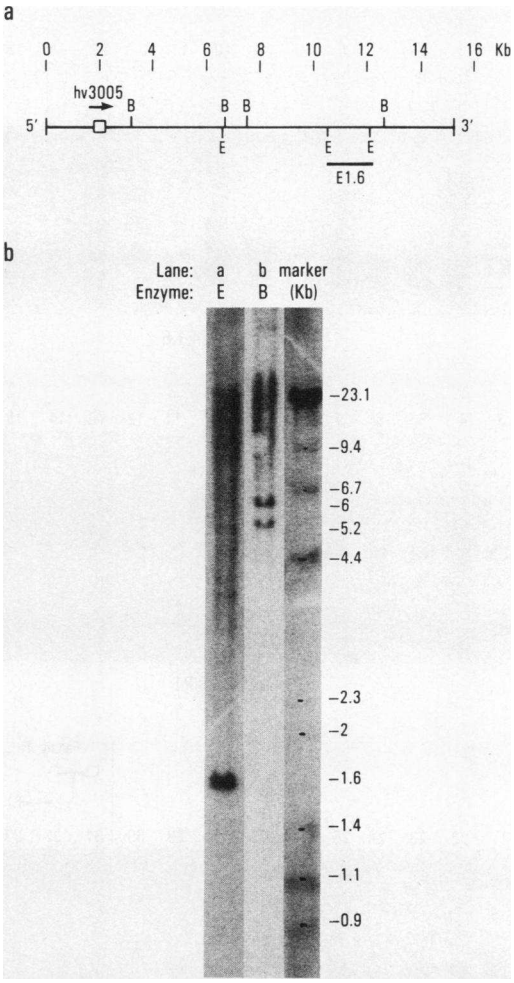

Figure 1. Characterization of the Humhv3005/1.6-kb Eco RI fragment. (*a*) The restriction map of the Humhv3005 clone, 5′ to 3′ from left to right. The enzymes used are *B*, Bam HI, and *E*, Eco RI. (*b*) Southern blot analyses of human genomic DNA with the hv3005/E1.6 probe. DNA was digested separately with Eco RI (lane *a*) and Bam HI (lane *b*), and the lysates were loaded onto a 0.7% agarose gel. The resultant blot was hybridized with hv3005/E1.6.

Subsequently, DNA from members of two families were analyzed. Fig. 2 *c* shows that in the first family the father had both bands of equal intensity, the mother had a 6-kb band alone, and the son had a strong 6-kb band with a weak 5.2-kb band. In the second family, the father had a 6-kb band alone, the mother had a strong 6-kb band with a weak 5.2-kb band, and the daughter had a 6-kb band alone. The result from the second family indicates that the mother had the (6,6,5,0) genotype, where the two "6s" and one "5" imply that there are two

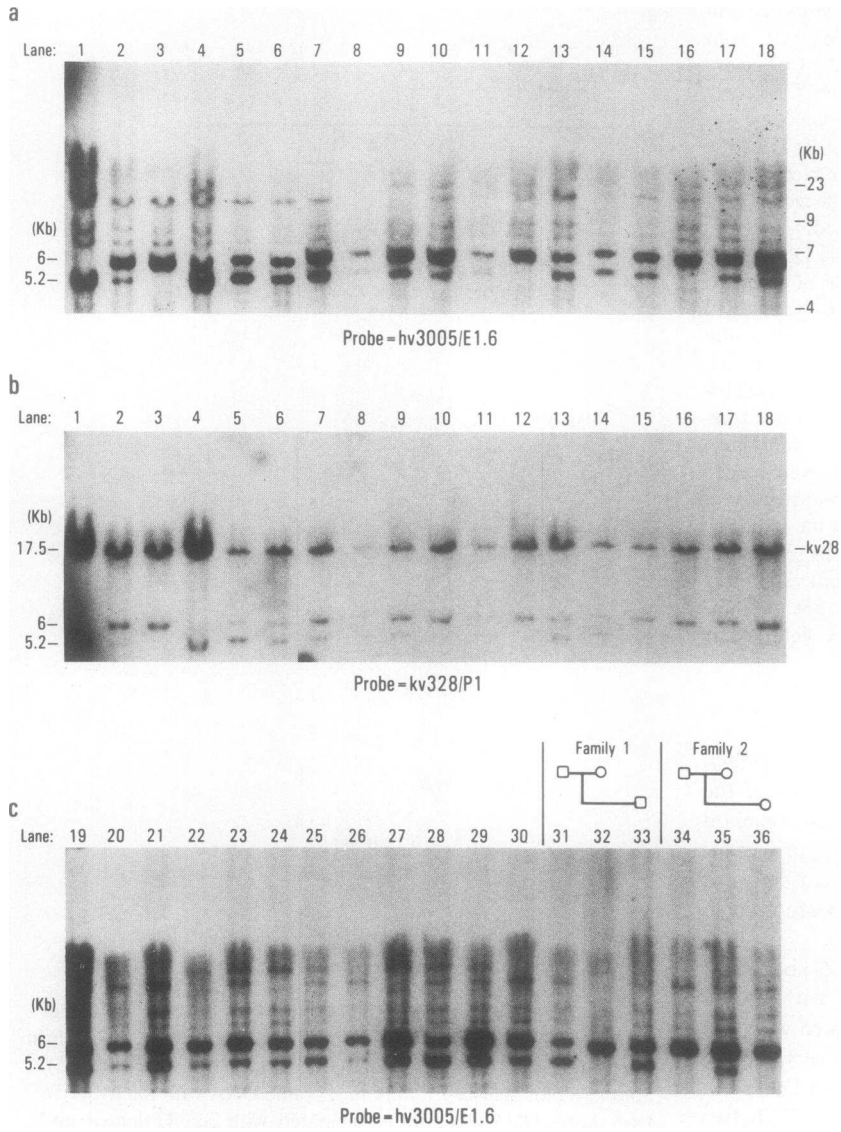

Figure 2. Population and family studies with the hv3005/E1.6 probe. Genomic DNAs from 30 unrelated individuals (lanes *1–30*) and members of two unrelated families (lanes *31–36*) were digested with Bam HI and the lysates were loaded onto a 0.7% agarose gel. The resultant blot was hybridized sequentially with hv3005/E1.6 (*a* and *c*) and Humhv328/pl (*b*).

copies of the 6-kb/1.9III region and one copy of the 5.2-kb/hv3005 region per diploid genome. From the RFLP pattern we deduced that the mother had the (6,5) and the (6,0) haplotypes. Accordingly, the hv3005 and the 1.9III loci are likely to represent two different Vh gene loci in a haploid genome.

To differentiate between the (6,6,5,5) and (6,0,5,0) genotype for the father in the first family, the same blot was hybridized simultaneously with hv3005/E1.6 and a 6-kb Hind III fragment of the kappa constant region (16). A comparison of the intensities of the three bands indicated that the father had the (6,6,5,5) genotype (data not shown).

Discussion

In this report we describe the identification of RFLPs in the vicinity of two highly homologous human Vh genes, namely, Humhv3005 and 1.9III. These Bam HI polymorphisms are defined by either the presence or absence of hybridizing bands at the 6-kb/1.9III region and the 5.2-kb/hv3005 region, and by the intensity of each hybridizing band, which reflects homozygosity or heterozygosity for the respective locus. Thus, there are nine potential genotypes: (6,6,5,5), (6,6,5,0), (6,6,0,0), (6,0,0,0), (6,0,5,5), (6,0,5,0), (0,0,5,5), (0,0,5,0), and (0,0,0,0). Among 34 unrelated individuals studied here (excluding two children from the family studies), the major (6,6,5,0) type accounted for 50% of the observed genotypes, whereas the (6,6,5,5), (6,6,0,0), (6,0,5,0), (6,0,5,5), and (0,0,5,5) types each accounted for 18, 15, 12, 6, and 6%, respectively. The (6,0,0,0), (0,0,5,0), and (0,0,0,0) types were not observed.

Human Ig Vh gene polymorphisms defined by RFLP analysis have been described previously (17–20). However, the probes used in those studies hybridized to multiple bands in genomic DNA, yielding complex RFLP patterns. Moreover, the identities of the hybridizing Vh genes are generally not known. Compared with the previously used Vh probes, the hv3005/1.6 probe is very specific, hybridizing to two major Vh loci under conditions of medium stringency (1× SSC wash at 55–65°C). More importantly, probing Bam HI digested genomic DNA with hv3005/E1.6 generates highly informative and yet simple patterns of two bands. This renders it possible to use the hv3005/E1.6 probe for large disease-linkage studies in clinical laboratories. Finally, since the identities of the two Vh

genes detected by the hv3005/1.6 probe are known, and one of these is related to anti-DNA antibodies, population and family studies using this probe may be particularly useful in autoimmune and/or lymphoproliferative diseases.

Extensive studies in mice have revealed that autoantibodies may be encoded by different Ig V genes derived from most (if not all) murine V gene families, and that at the gross level there is no unique RFLP pattern in the V genes of mouse strains with genetic forms of systemic autoimmune disease (21–23). These results indicate that polymorphisms in one or a few V genes are unlikely to exert a predominant influence on autoantibody production. However, these studies do not exclude a role for qualitative and/or quantitative differences in autoantibody-associated Ig V genes in heterogeneous human populations with sporadic autoimmune disease. To date, this issue has not been addressed because of the lack of suitable informative Ig V gene probes.

In humans, certain Ig constant region allotypes have been associated with some autoimmune diseases and/or with autoantibody formation (24–26). Since the Ig variable and constant region gene segments are linked, and the V genes ultimately dictate the potential antibody repertoire of any individual, the autoantibody-associated allotypes probably reflect differences in Ig V gene repertoires. Accordingly, it is essential to define the major autoantibody-related Ig V region gene elements, and to elucidate their relationship, if any, to autoantibody production and to the autoimmune diseases.

Programmed expression of Vh genes during B cell development has been clearly documented in mice (27, 28). Murine genes of the 7183-Vh family are preferentially expressed early in ontogeny. Interestingly, the same 7183-Vh genes are commonly used by antibodies reactive with self determinants (29). Recently, Schroeder et al. found similar patterns of developmentally restricted Vh gene expression in humans (12). One of the nine Vh genes that is predominant in fetal liver (i.e., 30P1), is identical to the Vh sequence of an anti-DNA antibody, while another (i.e., 51P1) is likely to encode rheumatoid factors that express the G6 crossreactive idiotype (8, 9). In addition, Makar et al. showed that a third Vh gene expressed in fetal liver (i.e., 20P1/M26) has an identical sequence to that of an anti-Sm antibody (11). By analogy, it seems likely that Humhv3005, which encodes an amino acid sequence identical to the frequently expressed 56P1 gene, is likely to encode an autoantibody. Thus, the currently characterized polymorphic Vh regions may actually contain more than one autoantibody-associated Vh gene.

Accumulated data from two laboratories indicate that the total human Vh gene number is likely to be > 120, spread over a region of 1,500–2,000 kb (13, 30). In the future it may prove necessary to generate a panel of other probes that are specific for Vh locations at 300–500-kb intervals, and that identify autoantibody-associated Vh genes. Used together, such probes would permit the linkage analysis of autoimmune disease to the Vh region and the possible identification of disease-associated Ig gene segments. The current report represents our first step in this direction.

Acknowledgments

We thank Dr. W. Lee for the human genomic library, Dr. H. W. Schroeder and Dr. R. M. Perlmutter for the Vh3 cDNA clone 56P1 and the preprint (20), and Dr. E. Beutler for DNA samples from two families. The technical assistance of S. Sinha is gratefully acknowledged. We thank Ms. Nancy Noon and the Basic and Clinical Research Word Processing Center for help in preparing this manuscript.

Funding for this research is supported in part by grants AR-39039, AR-33489, AR-25443, RR-00833, and BRSG 2S07-RR-05424 from the National Institutes of Health.

References

1. Schwartz, R. S., and B. D. Stollar. 1985. Origins of anti-DNA autoantibodies. *J. Clin. Invest.* 75:321–327.

2. Cohen, I. R., and A. Cooke. 1986. Natural autoantibodies might prevent autoimmune disease. *Immunol. Today.* 7:363–364.

3. Chen, P. P., S. Fong, and D. A. Carson. 1987. Rheumatoid factor. *Rheum. Dis. Clin. North Am.* 13:545–568.

4. Rajewsky, K., and T. Takemori. 1983. Genetics, expression, and function of idiotypes. *Annu. Rev. Immunol.* 1:569–607.

5. Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature (Lond.).* 302:575–581.

6. Chen, P. P., K. Albrandt, T. J. Kipps, V. Radoux, F.-T. Liu, and D. A. Carson. 1987. Isolation and characterization of human VkIII germline genes: implications for the molecular basis of human VkIII light chain diversity. *J. Immunol.* 139:1727–1733.

7. Chen, P. P., D. L. Robbins, F. R. Jirik, T. J. Kipps, and D. A. Carson. 1987. Isolation and characterization of a light chain variable region gene for human rheumatoid factors. *J. Exp. Med.* 166:1900–1905.

8. Chen, P. P., M.-F. Liu, C. A. Glass, S. Sinha, T. J. Kipps, and D. A. Carson. 1989. Characterization of two Ig Vh genes which are homologous to human rheumatoid factors. *Arthritis Rheum.* 32:72–76.

9. Chen, P. P., M. Liu, S. Sinha, and D. A. Carson. 1988. A 16/6 idiotype positive anti-DNA antibody is encoded by a conserved Vh gene with no somatic mutation. *Arthritis Rheum.* 31:1429–1431.

10. Cairns, E., P. C. Kwong, V. Misener, P. Ip, D. A. Bell, and K. A. Siminovitch. 1989. Analysis of variable region genes encoding a human anti-DNA antibody of normal origin: implications for the molecular basis of human autoimmune responses. *J. Immunol.* In press.

11. Makar, R., I., Sanz, J. W. Thomas, and J. D. Capra. 1988. A structural basis for human cross-reacting idiotypes. *Ann. Inst. Pasteur Immunol.* 139:651–657.

12. 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.

13. Berman, J. E., S. J. Mellis, R. Pollock, 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 VH locus: definition of three new VH families and linkage to the Ig CH locus. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:727–738.

14. Lee, W.-H., R. Bookstein, F. Hong, L.-J. Young, J.-Y. Shew, and Y.-H. P. Lee. 1987. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science (Wash. DC).* 235:1394–1399.

15. Liu, M.-F., D. L. Robbins, J. J. Crowley, S. Sinha, F. Kozin, T. J. Kipps, D. A. Carson, and P. P. Chen. 1989. Characterization of four homologous light chain variable region genes which are related to 6B6.6 idiotype positive human rheumatoid factor light chains. *J. Immunol.* 142:688–694.

16. Hieter, P. A., E. E. Max, J. G. Seidman, Jr., J. V. Maizel, and P. Leder. 1980. Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments. *Cell.* 22:197–207.

17. Johnson, M. J., A. M. Natali, H. M. Cann, T. Honjo, and L. L. Cavalli-Sforza. 1984. Polymorphisms of a human variable heavy chain gene show linkage with constant heavy chain genes. *Proc. Natl. Acad. Sci. USA.* 81:7840–7844.

18. Turnbill, I., O. Bernard, K. S. Sriprakash, and J. D. Mathews. 1987. Human immunoglobulin variable region genes: a new VH sequence used to detect polymorphism. *Immunogenetics.* 25:184–192.

19. Walter, M. A., and D. W. Cox. 1988. Analysis of genetic variation reveals human immunoglobulin VH-region gene organization. *Am. J. Hum. Genet.* 42:446–451.

20. Van Dijk, K. W., H. W. Schroeder, Jr., R. M. Perlmutter, and E. C. B. Milner. 1989. Heterogeneity in the human immunoglobulin V-H locus. *J. Immunol.* 142:2547–2554.

21. Painter, C., M. Monestier, B. Bonin, and C. A. Bona. 1986. Functional and molecular studies of V genes expressed in autoantibodies. *Immunol. Rev.* 94:75–98.

22. Kofler, R., R. M. Perlmutter, D. J. Noonan, F. J. Dixon, and A. N. Theofilopoulos. 1985. Ig heavy chain variable region gene complex of lupus mice exhibits normal restriction fragment length polymorphism. *J. Exp. Med.* 162:346–351.

23. Trepicchio, W., A. Maruya, and K. J. Barrett. 1987. The heavy chain genes of a lupus anti-DNA autoantibody are encoded in the germ line of a nonautoimmune strain of mouse and conserved in strains of mice polymorphic for this gene locus. *J. Immunol.* 139:3139–3145.

24. Schur, P. H., J. P. Pandey, and J. A. Fedrick. 1985. Gm allotypes in white patients with systemic lupus erythematosus. *Arthritis Rheum.* 28:828–830.

25. Demaine, A. G., D. H. Taube, R. W. Vaughan, L. A. P. Kerr, and K. I. Welsh. 1986. Immunoglobulin heavy chain switch region restriction fragment length polymorphisms are associated with renal disease. *Clin. Exp. Immunol.* 66:406–413.

26. Gaiser, C. N., M. J. Johnson, G. DeLange, L Rassenti, L. L. Cavalli-Sforza, and L. Steinman. 1987. Susceptibility to multiple sclerosis associated with an immunoglobulin gamma 3 restriction fragment length polymorphism. *J. Clin. Invest.* 79:309–313.

27. Alt, F. W., T. K. Blackwell, and G. D. Yancopoulos. 1987. Development of the primary antibody repertoire. *Science (Wash. DC).* 238:1079–1087.

28. Perlmutter, R. M. 1987. Programmed Development of the antibody repertoire. *Curr. Top. Microbiol. Immunol.* 135:96–109.

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

30. Kodaira, M., T. Kinashi, I. Umemura, F. Matsuda, T. Noma, Y. Ono, and T. Honjo. 1986. Organization and evolution of variable region genes of the human immunoglobulin heavy chain. *J. Mol. Biol.* 190:529–541.