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Possible deletion of a developmentally regulated heavy-chain variable region gene in autoimmune diseases

(rheumatoid arthritis/systemic lupus erythematosus/autoantibodies/fetal antibody repertoire/antibody network)

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ABSTRACT **Several autoantibody-associated variable region (V) genes are preferentially expressed during early ontogenic development, suggesting strongly that they are of developmental and physiological importance. As such, it is possible that polymorphisms in one or more of these genes may alter susceptibility to autoimmune disease. We have searched extensively for a probe related to a developmentally regulated V gene that has the power to differentiate among highly homologous V genes in human populations. Using such a probe (i.e., Humhv3005/P1) related to both anti-DNA and anti-IgG autoantibodies, we studied restriction fragment length polymorphisms in patients with rheumatoid arthritis and systemic lupus erythematosus and found an apparent heavy-chain V (V_H) gene deletion that was nearly restricted to the autoimmune patients. These data suggest that deletions of physiologically important V_H genes may increase the risk of autoimmunity through indirect effects on the development and homeostasis of the B-cell repertoire.**

Autoimmune diseases represent the pathological consequence of an abnormal expression of immune function. As such, it is logical to suspect that polymorphisms in the immune system's three major gene families—the major histocompatibility complex antigen (MHC) genes, the T-cell antigen receptor genes, and the immunoglobulin genes—may influence disease susceptibility. Ample evidence has established the association between small MHC gene polymorphisms and autoimmunity (1, 2). As an example, recent data indicated that a single amino acid change in DQ β chains may dramatically influence susceptibility to insulin-dependent diabetes mellitus (3).

Autoantibodies are the hallmarks of systemic autoimmune diseases and may contribute to tissue damage (4, 5). However, certain autoantibodies, such as those against nucleic acids and against IgG (rheumatoid factor, RF), are also found in a low concentration in the serum of healthy individuals and may serve some physiological functions (6–9). At the molecular level, the accumulated data have demonstrated that these two autoantibodies can be encoded directly by germline variable region (V) genes without any somatic modification and that such genes are conserved in populations (10–18). Furthermore, sequence comparisons of anti-DNA and anti-IgG autoantibodies with the small subset of immunoglobulin V genes that are preferentially expressed in human fetal liver have revealed extensive overlap (14, 17, 19–21). These results indicate that autoreactive V genes not only are inherited in most individuals but also comprise a significant proportion of the antibody repertoire during early ontogenic development. Similar results have been obtained in mice (8, 22, 23).

The finding that certain V genes encoding autoantibodies are preferentially expressed during fetal life suggests that they may influence the programmed development of the immune system. If so, then it is probably the abnormal expression and diversification of autoreactive V genes that relates in some way to the pathogenesis of systemic autoimmune diseases. Genetically, this implies that either amplification, deletion, or mutation of one or many autoantibody-associated V genes could predispose to autoimmune reactions.

Before this hypothesis can be tested directly, genetic polymorphisms in autoantibody V regions must be defined precisely. Previously, we cloned a human germ-line heavy-chain V (V_H) gene (Humhv3005) that encodes an amino acid sequence identical to that of the 56P1 cDNA. The 56P1 V_H gene is of particular interest, since it is the most abundantly expressed V_H gene in a 130-day fetal liver (24) and is highly homologous to the heavy chains of both anti-DNA and RF autoantibodies. In the present experiment, a series of probes from the hv3005 gene and its flanking regions were used to analyze DNA from patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and normal controls. The results showed that the hv3005, or a closely related gene(s), was frequently deleted in the autoimmune disease patients.

MATERIALS AND METHODS

Preparation and Characterization of the Regional Specific Humhv3005–4005 Probes. A recombinant phage, containing the Humhv3005 V_H3 gene and the Humhv4005 V_H4 gene, was isolated from a human genomic library with a V_H3 cDNA probe (56P1; from positions −124 to +408 in the μ-chain constant region), kindly provided by H. W. Schroeder and R. M. Perlmutter (19), as reported (24). Fig. 1 shows the restriction map of the Humhv3005–4005 phage clone and the sizes and locations of each probe. They include (*i*) Humhv3005/P1, a 466-base-pair (bp) *Bgl* II/*Pst* I fragment; (*ii*) Humhv3005/P2, an 802-bp *Pst* I fragment; (*iii*) Humhv3005/P3, a 435-bp *Pst* I fragment; (*iv*) Humhv3005/P4, a 581-bp *Pst* I fragment; (*v*) Humhv3005/B3.4, a 3.4-kilobase (kb) *Bam*HI fragment; (*vi*) Humhv3005/H5.2, a 5.15-kb *Hin*dIII fragment; (*vii*) Humhv3005/E4, a 3.95-kb *Eco*RI fragment; (*viii*) Humhv3005/B0.8, a 0.8-kb *Bam*HI fragment; (*ix*) Humhv3005/HE1.1, a 1.1-kb *Hin*dIII/*Eco*RI fragment; (*x*) Humhv4005/H5.7, a 5.65-kb *Hin*dIII fragment; (*xi*) Humhv3005/E1.6, a 1.6-kb *Eco*RI fragment; (*xii*) Humhv4005/EH3, a 2.95-kb *Eco*RI/*Hin*dIII fragment; (*xiii*)

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Abbreviations: RA, rheumatoid arthritis; RF, rheumatoid factor; RFLP, restriction fragment length polymorphism; SLE, systemic lupus erythematosus; V_H, heavy-chain variable region.

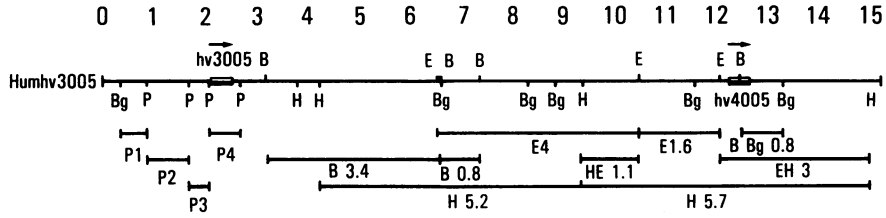

FIG. 1. Restriction map of the isolated Humhv3005–4005 fragment (5′ to 3′ from left to right) and the sizes and locations of 13 probes. The following enzymes were used: B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I. The *Pst* I sites were determined for the 2.8-kb *Bgl* II/*Bam*HI region only.

Humhv4005/BBg0.8, an 844-bp *Bam*HI/*Bgl* II fragment. The desired fragments were subcloned into pUC. After digestion of the plasmids with appropriate restriction enzymes, the DNA fragments of interest were isolated by electrophoresis in agarose gels.

Genomic DNA and Southern Blot Analyses. Peripheral blood leukocytes were prepared from 24 RA patients, 22 SLE patients, and 42 healthy individuals. Except for 3 RA patients, all were Caucasians. The RA patients satisfied the 1987 American Rheumatism Association (American College of Rheumatology) criteria for RA (25). All RA patients were seropositive for RF with latex titers of ≥1:60. The SLE patients fulfilled the 1982 American Rheumatism Association criteria for SLE (26).

Total genomic DNA from each leukocyte sample was digested separately with the indicated restriction enzymes, including *Bam*HI, *Bgl* II, and *Eco*RI. The lysates were loaded into wells of an agarose gel. After electrophoresis and transfer of DNA to nitrocellulose, the blots were hybridized with the indicated probes. Hybridizations were done in 5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) at 65°C, followed by washing twice in 0.1× SSC at 65°C.

Statistical Analyses. The genotype frequencies were compared in 2 × 2 tables, using the χ^2 test with Yates' correction. A P value of <0.05 was considered significant. The relative risk was calculated by the standard formula as follows: [(number of patients with the trait)/(number of patients without the trait)]/[(number of healthy individuals with the trait)/(number of healthy individuals without the trait)].

RESULTS

Characterization of the V_H Gene Locus Probes from the Humhv3005–4005 Region. Previously, we reported that Humhv3005/E1.6 is a regional specific probe that is highly informative for two V_H gene loci containing two autoantibody-associated V_H genes—namely, hv3005 *per se* and the closely related 1.9III gene (24, 27, 28). The 1.9III gene is identical in nucleotide sequence to the heavy chain of the Kim4.6 anti-DNA antibody (16), while the hv3005 differs by only one amino acid residue from the heavy chain of a B6 idiotype-positive RF (i.e., RF2) (29, 30). Hybridization of *Bam*HI-digested DNAs from different individuals with hv3005/E1.6 displayed five major restriction fragment length polymorphism (RFLP) patterns, 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 9 potential RFLP phenotypes: (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 the initial 34 unrelated individuals studied, 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 (27).

A comparison of the hv3005/E1.6 genotypes in patients with either RA or SLE and normal subjects showed no significant differences (results not shown). However, sequence analysis of the hv4005 gene that was contained in the original hv3005–4005 clone revealed that it was 99% homologous to both 1.9II and 12G1 (28, 31, 32) and suggested that the hv3005/E1.6-defined V_H region might actually consist of three or more closely related V_H gene loci. Hence, it was possible that the hv3005/E1.6 probe was too imprecise to discriminate between autoimmune patients and controls. Instead, we needed a probe that was both regionally specific and that could differentiate all three genes (i.e., hv3005, 1.9III, and the 56P1 corresponding germ-line V_H gene) in conjunction with the appropriate restriction enzyme. By sequence analysis and gene counting of Southern blots, it has been estimated that the human V_H gene locus contains 25–30 V_H3 genes and 10 or more V_H4 genes (28, 31, 32). Generally, members of a V_H family share 80–99% nucleotide sequence homology in their coding regions but a lower homology in their flanking regions. However, there is no rule to predict the exact area(s) around a V_H gene that is most specific for it. Accordingly, in addition to hv3005/E1.6, we prepared a series of other V_H locus probes from the whole isolated hv3005–4005 region, and we used each fragment to probe Southern blots that contained three sets of five DNA samples from unrelated individuals. Each set was digested with either *Bam*HI, *Bgl* II, or *Eco*RI. For simplicity, Fig. 2 displays the representative results from a single individual.

We first assessed the suitability and the specificity of each probe for Southern blot analysis of genomic DNA. Of the 13 probes (including hv3005/E1.6) tested, 7 hybridized to one to five major bands in each lane and thus appeared to be good probes for studying the human V_H gene locus (Fig. 2). The other 6 probes were unsuitable for the following reasons. Probes hv3005/P2, hv3005/HE1.1, and hv4005/H5.7 gave high background hybridization over the whole lane, indicating that they may contain some repetitive sequences. Probes hv3005/E4 and hv4005/EH3 showed specific hybridization, but with very high background. Probe hv3005/H5.2 contains six sites of our selected restriction enzymes and thus is not very useful. Among the 7 good probes, 3 were eliminated for the following reasons: hv3005/B3.4 hybridized to six bands with relatively similar intensities in *Bam*HI-digested DNA; hv3005/B0.8 hybridized only weakly to the expected 0.8-kb band in *Bam*HI-digested DNA (probably because of inefficient transfer of the small DNA fragment); hv4005/BBg0.8 was discontinued because we were more interested in V_H3 genes related to at least two autoantibody V_H genes. In addition, comparing the hybridization patterns of hv3005/P1, -3, and -4 in Fig. 2, it is clear that hv3005/P1 was the most specific probe. Accordingly, we narrowed the original 13 probes down to the initial hv3005/E1.6 probe and the new hv3005/P1 probe.

As summarized above, probing *Bam*HI-digested DNA with hv3005/E1.6 revealed two major bands that represented at least two highly homologous V_H3 genes. Thus, the single major hv3005/E1.6 hybridizing band in both *Bgl* II- and

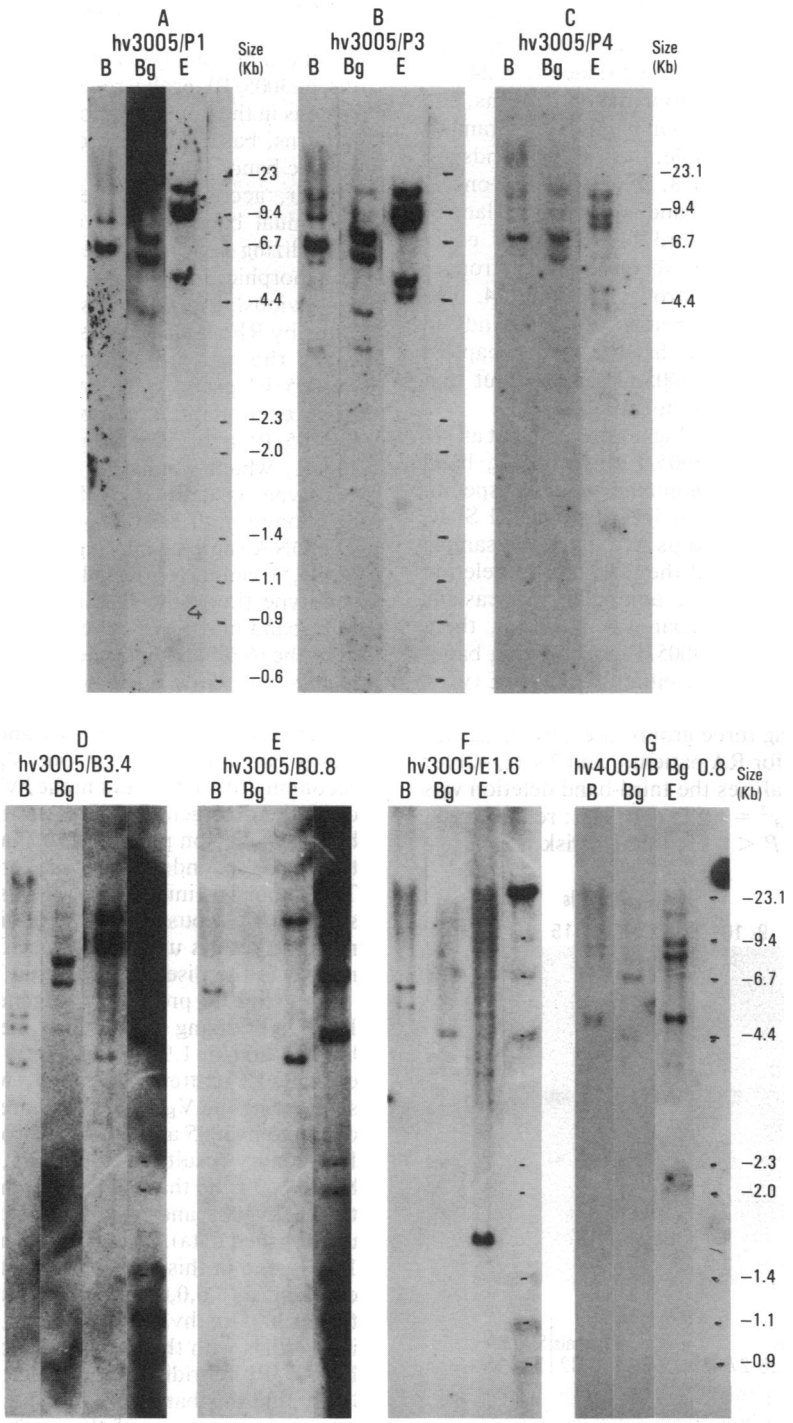

FIG. 2. Southern blot analyses of human genomic DNA. The genomic DNA from one normal individual was digested with the indicated enzymes. After gel electrophoresis and DNA transfer, the blots were hybridized with the indicated probes. The enzymes used are the same as in Fig. 1.

*Eco*RI-digested DNAs indicated that hv3005/E1.6 could not differentiate between the two known homologous V_H regions, at least when these enzymes are used. On the other hand, whereas hv3005/P1 also identified two major bands in *Bam*HI-digested DNA, it hybridized to three major bands in *Bgl* II-digested DNA and to four major bands in *Eco*RI-digested DNA. The latter four bands all reacted with the hv3005/P3 and hv3005/P4 probes, indicating that they each contain a V_H3 gene that is highly homologous to the hv3005 and/or the 1.9III genes. Combining these data, we concluded that hv3005/P1 is the regional specific probe that has the best differential power to detect V_H gene polymorphisms in hv3005-related genes. Therefore, the hv3005/P1 probe was used to compare the RFLP patterns in patients with SLE and RA and in healthy controls.

The hv3005/P1 Probe Defines a RFLP Found in SLE and RA Patients. Genomic DNAs from patients and controls were digested separately with *Eco*RI, and the resultant blots were hybridized with the hv3005/P1 probe. As can be seen from the initial results shown in Fig. 3, there are four major hybridizing bands of about 16, 10, 9.4, and 5.2 kb. Based on the presence or absence of these four bands, there are at least four distinctive RFLP patterns, consisting of (*i*) all four bands present (lanes 1–3, 5, 7, 8, 10, 14–23, 25, 26, 29, 30, 32, and

34), (*ii*) the second band from the top is missing (lanes 6, 12, and 13), (*iii*) the top two bands are missing (lanes 11 and 31), (*iv*) the third band from the top is missing (lanes 4, 9, 24, 27, 28, and 33). In addition to these four major patterns, the hybridization intensities of these four bands vary dramatically among individuals. For example, the top two bands are of equal intensity in lanes 2 and 3, while the top one is stronger in lane 4 and the second band is stronger in lane 5. Similarly, the second and the third bands are of equal intensity in lanes 3 and 8, while the second band is stronger in lane 2 and the third band is stronger in lane 14. The relatively equal hybridization intensities of four bands in many individuals suggest not only that the human haploid genome contains at least four hv3005-like genes, but that some of these genes are deleted frequently.

Significantly, the initial results in Fig. 3 revealed that all six individuals lacking the third hv3005/P1 hybridizing band (i.e., the 9.4-kb band) were autoimmune patients; specifically, the 9.4-kb band was absent in 5/24 RA and 1/1 SLE, but in 0/9 controls. This prompted us to expand the sample size to examine the significance of the 9.4-kb band deletion in autoimmune patients. To date, among 85 Caucasians analyzed (excluding 3 non-Caucasian RA patients), there were 12 who lacked the third hv3005/P1 hybridizing band. Eleven of these 12 individuals have either RA or SLE ($\chi^2 = 7.62$; $P < 0.01$; relative risk, 14.1). The frequencies of the 9.4-kb band deletion among three groups are 27% (6/22) for SLE patients, 24% (5/21) for RA patients, and 2% (1/42) for control subjects. By χ^2 analyses the third-band deletion was significant for both SLE ($\chi^2 = 6.81$; $P < 0.01$; relative risk, 15.4) and RA ($\chi^2 = 5.18$; $P < 0.05$; relative risk, 12.8).

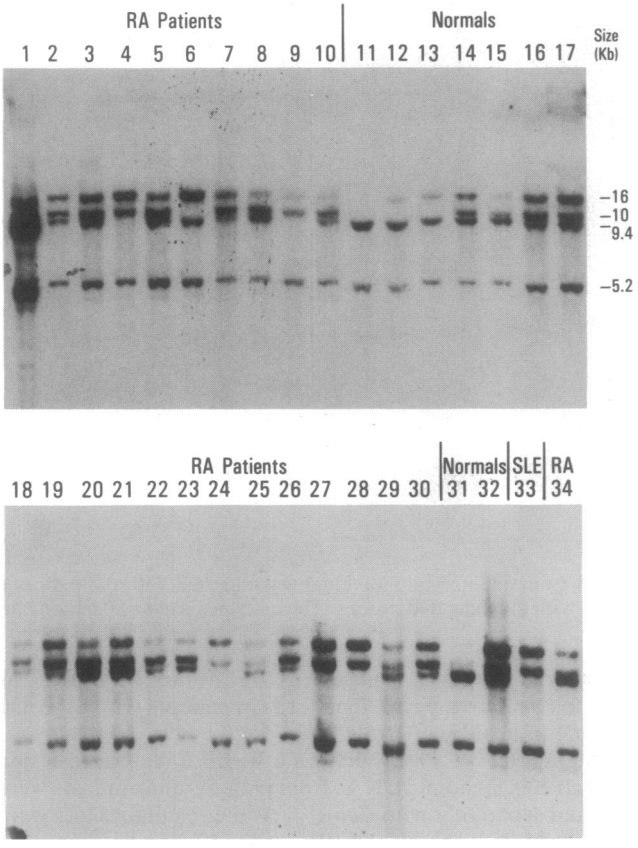

FIG. 3. Population studies with the Humhv3005/P1 probe. Genomic DNAs from 34 unrelated individuals, with or without autoimmune diseases, were designated with *Eco*RI and the lysates were loaded onto a 0.7% agarose gel. The resultant blots were hybridized with hv3005/P1.

DISCUSSION

Southern blot analyses of *Eco*RI-digested human DNA with the hv3005/P1 probe revealed a set of highly polymorphic regions in the human V_H locus. There were four major RFLP patterns, based on the presence or absence of four characteristic bands. These four major patterns could be classified further, according to the hybridization intensities of the individual bands. These results indicate that the hv3005-hybridizing regions in the V_H locus are both complex and polymorphic.

Previously, we defined several genotypes in the human V_H locus by RFLP analysis using the hv3005/E1.6 probe. However, the patterns obtained with the closely related hv3005/P1 probe were entirely different. This is apparent from even limited comparison of the hv3005/E1.6 and hv3005/P1 hybridization patterns. For example, lane 11 in Fig. 1, which is missing the top bands, has the (0,0,5,5) genotype as defined by hv3005/E1.6, implying the likely deletion of both 1.9III V_H3 gene loci in the diploid genome. The DNA samples missing the second band have either the (6,0,5,5) genotype (i.e., lanes 12 and 13) or the (6,6,5,0) genotype (i.e., lane 6). Similarly, the samples missing the third band may have either the (6,6,5,0) genotype (i.e., lane 4) or the (6,6,0,0) genotype (i.e., lane 33). The fact that there is no simple correlation between the RFLPs defined by two regional specific probes that are only 10 kb apart suggests that either some of the hv3005/P1 and hv3005/E1.6 hybridizing bands contain more than one V_H gene, or that there is a recombinational hot spot in the hv3005–4005 region. In either case, the divergent RFLP patterns obtained with two neighboring V_H region probes show that, unlike the HLA system, there is no extended haplotype for the human V_H gene locus. The important implication of this result is that V_H linkage studies of various autoimmune diseases will probably yield negative results unless the specific probes that are directly related to the disease-associated V_H genes are used.

To delineate precisely the genetic basis of each hv3005/E1.6 hybridizing band, it is necessary to sequence the hv3005- and/or 1.9III-like gene(s) from individuals who have each RFLP pattern. Recently, we have been cloning and sequencing the V_H3 genes that are identical or highly homologous to hv3005 and/or 1.9III from two chosen individuals. Preliminary results from a (0,0,5,5) individual show that he has a V_H3 gene that is highly homologous, but not identical, to the hv3005 and the 56P1 sequences (T.O. and P.P.C., unpublished data). Importantly, an exhaustive search for the 1.9III gene in this subject did not yield a positive result. In contrast, a (6,6,0,0) individual has the 1.9III gene but lacks the hv3005 or hv3005-like genes. Considering that all five individuals with the (6,6,0,0) genotype do not have the third hv3005/P1 hybridizing band (e.g., lane 33 in Fig. 1), it is very likely that this band contains the hv3005 and/or hv3005-like genes. At least one of these genes corresponds to the expressed 56P1 sequence that is likely to encode some RF heavy chains and that is preferentially expressed in fetal liver. Thus, the results strongly suggest that developmentally regulated V_H genes may be deleted in autoimmune patients.

Recently, Coutinho and his colleagues (33) proposed a "network" model to explain the programmed expression of some autoreactive V genes (such as the 56P1-corresponding germ-line V_H gene) during early ontogenic development. They hypothesized that, in the sterile fetal environment, either stimulation by autoantigens and/or idiotype–anti-idiotype interactions selectively expand B cells expressing autoreactive V genes to form an initial, self-sustaining network (34, 35). With subsequent continual exposure to exogenous antigens, the self-reactive B-cell pool diminishes in size and represents only ≈20% of the adult B-cell repertoire. The remaining 80% of B cells, which are not connected to the

network, typically respond to exogenous antigens. Through somatic mutation, gene conversion, secondary gene rearrangement/replacement, and other diversification mechanisms, these B cells mature and produce classic high-affinity antibodies (36, 37). At the same time, the residual autoreactive B cells interact with each other and with the nonconnected B cells and thus maintain the homeostasis of the overall B-cell repertoire.

According to this model, deletion of both copies of a critical autoreactive V gene in a diploid genome (such as the 56P1-related genes that may be missing in the 9.4-kb hv3005/P1 hybridizing band) may lead to abnormal B-cell development and antibody repertoire. This, in turn, may alter the humoral immune response to bacterial and/or viral infection and lead to the overproduction of unusual antibodies that contribute to the development and/or sustenance of autoimmunity. A corollary of this model is that the association of immunoglobulin V gene polymorphisms with the "inherited" cases of autoimmune diseases should be inherited as a recessive characteristic. Accordingly, it is important for us to follow the current population study with family studies in individuals with 9.4-kb deletions.

Moreover, as mentioned earlier, there are three gene families (i.e., HLA, T-cell receptor, and immunoglobulin genes) that may influence susceptibility to autoimmune diseases. The immunoglobulin gene family itself consists of three distinct loci (i.e., heavy chain, and κ and λ light chains) on three different chromosomes. Furthermore, within the V_H gene locus, there may be as many as nine developmentally regulated V_H genes related to autoantibodies. If a defect involving both copies of a gene is necessary to increase the risk of autoimmunity, then it can be expected that any single defect will be responsible for only a small subset of patients.

In sum, systematic studies of several probes from a developmentally regulated autoantibody V_H gene led to the identification of an apparent deletion that is nearly confined to patients with RA and SLE. The results are consistent with a model in which deletion of a critical V_H gene alters B-cell development and the adult B-cell repertoire and thereby increases the likelihood of autoimmune diseases. Family studies of involved patients will be needed to determine the exact association of this defect with systemic autoimmune disease. In the future, it seems likely that the ability to define precisely all the potential deletions of autoantibody V_H genes may provide a profile of the immunoglobulin gene abnormalities that predispose to autoimmunity and may lead to the logical development of specific antibody therapy.

Note. After this work was completed, we used polymerase chain reaction to sequence directly the Humhv3005-like and the 1.9III-like genes in some selected individuals. The results show that 3/3 individuals lacking the 9.4-kb band had only 1.9III-like genes and no hv3005-like genes (T.O. and P.P.C, unpublished data).

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1. Nepom, G. T., Hansen, J. A. & Nepom, B. S. (1987) *J. Clin. Immunol.* **7**, 1–7.
2. Bell, J. I., Todd, J. A. & McDevitt, H. O. (1989) *Adv. Hum. Genet.* **18**, 1–41.
3. Morel, P. A., Dorman, J. S., Todd, J. A., McDevitt, H. O. & Trucco, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8111–8115.
4. Theofilopoulos, A. N. (1987) in *Basic and Clinical Immunology*, eds. Stites, D. P., Stobo, J. D. & Wells, J. W. (Appleton & Lange, East Norwalk, CT), pp. 128–158.
5. Carson, D. A., Chen, P. P., Fox, R. I., Kipps, T. J., Jirik, F., Goldfien, R. D., Silverman, G., Radoux, V. & Fong, S. (1987) *Annu. Rev. Immunol.* **5**, 109–126.
6. Guilbert, B., Dighiero, G. & Avrameas, S. (1983) *J. Immunol.* **128**, 2779–2787.
7. Chen, P. P., Fong, S. & Carson, D. A. (1987) *Rheum. Dis. N. Am.* **13**, 545–568.
8. Zouali, M., Stollar, B. D. & Schwartz, R. S. (1988) *Immunol. Rev.* **105**, 137–158.
9. Casali, P. & Notkins, A. L. (1989) *Annu. Rev. Immunol.* **7**, 513–535.
10. Chen, P. P., Albrandt, K., Orida, N. K., Radoux, V., Chen, E. Y., Schrantz, R., Liu, F.-T. & Carson, D. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8318–8322.
11. Radoux, V., Chen, P. P., Sorge, J. A. & Carson, D. A. (1986) *J. Exp. Med.* **164**, 2119–2124.
12. Chen, P. P., Fong, S., Goni, F., Silverman, G. J., Fox, R. I., Liu, M.-F., Frangione, B. & Carson, D. A. (1988) *Springer Semin. Immunopathol.* **10**, 35–55.
13. Goni, F. R., Chen, P. P., McGinnis, D., Arjonilla, M. L., Fernandez, J., Carson, D., Solomon, A., Mendez, E. & Frangione, B. (1989) *J. Immunol.* **142**, 3158–3163.
14. Chen, P. P., Liu, M., Sinha, S. & Carson, D. A. (1988) *Arthritis Rheum.* **31**, 1429–1431.
15. Dersimonian, H., Schwartz, R. S., Barrett, K. J. & Stollar, B. D. (1987) *J. Immunol.* **139**, 2496–2501.
16. Cairns, E., Kwong, P. C., Misener, V., Ip, P., Bell, D. A. & Siminovitch, K. A. (1989) *J. Immunol.* **143**, 685–691.
17. Sanz, I., Dang, H., Takei, M., Talal, N. & Capra, J. D. (1989) *J. Immunol.* **142**, 883–887.
18. Siminovitch, K. A., Misener, V., Kwong, P. C., Song, Q.-L. & Chen, P. P. (1989) *J. Clin. Invest.* **84**, 1675–1678.
19. Schroeder, H. W., Jr., Hillson, J. L. & Perlmutter, R. M. (1987) *Science* **238**, 791–793.
20. Chen, P. P., Liu, M.-F., Glass, C. A., Sinha, S., Kipps, T. J. & Carson, D. A. (1989) *Arthritis Rheum.* **32**, 72–76.
21. Siminovitch, K. A. & Chen, P. P. (1990) *Int. Rev. Immunol.* **5**, 265–277.
22. Avrameas, S., Guilbert, B., Mahana, W., Matsiota, P. & Ternynck, T. (1988) *Int. Rev. Immunol.* **3**, 1–15.
23. Bona, C. A. (1988) *Annu. Rev. Immunol.* **6**, 327–358.
24. Chen, P. P. (1990) *Scand. J. Immunol.* **31**, 257–267.
25. Arnett, F. C., Edworthy, S. M., Bloch, D. A., McShane, D. J., Fries, J. F., Cooper, N. S., Healey, L. A., Kaplan, S. R., Liang, M. H., Luthra, H. S., Medsger, T. A., Jr., Mitchell, D. M., Neustadt, D. H., Pinals, R. S., Schaller, J. G., Sharp, J. T., Wilder, R. L. & Hunder, G. G. (1988) *Arthritis Rheum.* **31**, 315–324.
26. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N. & Winchester, R. J. (1982) *Arthritis Rheum.* **25**, 1271–1277.
27. Chen, P. P., Siminovitch, K. A., Olsen, N. J., Erger, R. A. & Carson, D. A. (1989) *J. Clin. Invest.* **84**, 706–710.
28. Berman, J. E., Mellis, S. J., Pollock, R., Smith, C. L., Suh, H., Heinke, B., Kowal, C., Surti, U., Chess, L., Cantor, C. R. & Alt, F. W. (1988) *EMBO J.* **7**, 727–738.
29. Crowley, J. J., Mageed, R. A., Silverman, G. J., Chen, P. P., Kozin, F., Erger, R. A., Jefferis, R. & Carson, D. A. (1990) *Mol. Immunol.* **27**, 87–94.
30. Natvig, J. B., Pascual, V., Capra, J. D., Sioud, M., Forre, O., Randen, I. & Thompson, K. (1989) *Int. Cong. Immunol., 7th*, 83-51 (abstr.).
31. Chen, P. P. & Yang, P.-M. (1990) *Scand. J. Immunol.* **31**, 593–599.
32. Lee, K. H., Matsuda, F., Kinashi, T., Kodaira, M. & Honjo, T. (1987) *J. Mol. Biol.* **195**, 761–768.
33. Coutinho, A., Grandien, A., Faro-Rivas, J. & Mota-Santos, T. A. (1988) *Ann. Inst. Pasteur/Immunol.* **139**, 599–607.
34. Pennell, C. A., Mercolino, T. J., Grdina, T. A., Arnold, L. W., Haughton, G. & Clarke, S. H. (1989) *Eur. J. Immunol.* **19**, 1289–1295.
35. Lundkvist, I., Coutinho, A., Varela, F. & Holmberg, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5074–5078.
36. Alt, F. W., Blackwell, T. K. & Yancopoulos, G. D. (1987) *Science* **238**, 1079–1087.
37. Kocks, C. & Rajewsky, K. (1989) *Annu. Rev. Immunol.* **7**, 537–559.