ISOLATION AND CHARACTERIZATION OF A LIGHT CHAIN VARIABLE REGION GENE FOR HUMAN RHEUMATOID FACTORS

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Rheumatoid factors (RFs) are anti-IgG autoantibodies, and are found in patients with rheumatoid arthritis and normal individuals (reviewed in reference 1). Previously (2, 3), we prepared two antiidiotypic antibodies by immunization with two synthetic peptides (PSL2 and PSL3), corresponding to the second and the third complementarity determining regions (CDRs) of the Wa crossreactive idiotype (CRI)-positive RF Sie light chain. When a total of 24-25 human monoclonal IgM RFs were analyzed, anti-PSL2 and anti-PSL3 reacted with 20 and 15 RFs, respectively. Furthermore, amino acid sequence analysis of 9 PSL2 and PSL3 CRI* RF light chains revealed that they share 89-96 of 96 amino acid residues (4). These results suggested that the CRI* positive RF light chains were encoded by a single conserved V, gene. This contention was supported by the recent isolation from normal human placenta of a V, gene (designated V,RF/Humav325) that encodes the exact 96 amino acids found on four separate RF light chains (4, 5).

Recently, a rearranged κ light chain gene (designated ka31es) was cloned from the malignant B lymphocytes of a patient (Les) with an IgM RF paraprotein, (6). The deduced amino acid sequence of ka31es is homologous to the amino acid sequence of the Po CRI* RF Pom light chain (2, 7). Here we report the isolation and characterization of a germline V, gene (Humav31es/Humav328) whose deduced amino acid sequence is very homologous to both the RF Les and Pom light chains. Our data suggest that Humav328 represents a second germline V, gene that is used for RF light chain synthesis.

Materials and Methods

Genomic DNA, Probes, and Southern Blotting. Germline genomic DNA was prepared from the peripheral blood granulocytes of the patient Les. The specific probe for identifying the Humav31es/Humav328 was a 378-bp Sac I–Kpn I fragment from 272 to 106 of the ka31es, designated ka31es/1 (6). Probe Humav305/1 is a 865-bp Sau 3A1 fragment from a human VκIII gene (4). Southern blot analysis was done by hybridizing...
the blot 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.

**Library Construction and Screening.** Eco RI fragments of 6–20 kb long were cloned into the Eco RI site of the phage EMBL4. The recombinants were screened with the \( \text{Ka}3\text{les} \) probe in 2× SSC at 65°C, followed by washing in 1× SSC at 65°C. Additionally, positive clones were characterized by hybridizing with two synthetic oligonucleotides corresponding to the diagnostic sequence of the \( \text{Ka}3\text{les} \) in its first and third framework regions (FRs). They consist of GGGTTGCTGGAGACTG (from positions 31 to 15) and CGTAGGGTCGGTCCA (from positions 187 to 173). Hybridization and washing conditions for oligomers were as described previously (8).

**DNA Sequencing.** Sau 3A I fragments of the isolated recombinants that contained the putative coding regions were subcloned into the phage M13mp8, and resultant recombinant phages were sequenced by the dideoxynucleotide chain-termination method. In addition to the universal sequencing primer, a 17-mer oligonucleotide (CAGGCTCCT-CATCTATG, from position 135 to 151) was synthesized and used in sequencing.

**Results**

**Identification and Isolation of the Hum\( \text{Kv}3\text{Ies} / \text{Hum} \text{Kv}3\text{28} \) Gene.** To date, eight human V\( \text{k} \)III germline genes have been isolated and sequenced (reviewed in reference 9). Moreover, comparison of the deduced amino acid sequences of these genes with the reported amino acid sequences of all human V\( \text{k} \)III light chains suggests that at least one additional V\( \text{k} \)III gene exists in the germline, which encodes \( \text{Ka}3\text{les} \) and three other V\( \text{k} \)III light chains (9). These four light chain variable regions have characteristic amino acids at positions 4 (Met), 9 (Ala), 13 (Val), and a deletion of Tyr at position 32. Among the isolated human V\( \text{k} \)III genes, the \( \text{Ka}3\text{les} \) gene is ~90% homologous with \( \text{Hum} \text{Kv}3\text{05}, \text{Hum} \text{Kv}3\text{25}, \text{Hum} \text{Kv}3\text{g}, \) and \( \text{Hum} \text{Kv}3\text{h} \) (renamed from Vg, and Vh, respectively) (10). When Eco RI-digested Les germline DNA was probed with either \( \text{k}3\text{ales} / \text{l} \) or \( \text{Hum} \text{Kv}3\text{05}/1 \), each hybridized to the same three bands of ~14, 7.5, and 5.3 kb in size (data not shown). Our previous experiments (5) showed that the 5.3-kb band contains both \( \text{Hum} \text{Kv}3\text{05} \) and \( \text{Hum} \text{Kv}3\text{25} \) genes. Together, these data suggested that the corresponding germline gene for \( \text{Ka}3\text{les} \) came from either the 14-kb band or the 7.5-kb band.

To isolate the putative \( \text{Hum} \text{Kv}3\text{Ies} \) gene, Les germline DNA was digested with Eco RI, and fragments of 6–20-kb size were cloned into the phage EMBL4. \( 2 \times 10^5 \) recombinant phages were screened with the \( \text{Ka}3\text{les} \) probe, and seven positive clones were isolated. Among these seven isolates, only four remained strongly positive with the \( \text{Ka}3\text{les} \) probe after washing at 0.1× SSC, and hybridized with two \( \text{Ka}3\text{les} \)-specific oligonucleotides under conditions that identify only clones containing perfect complementary sequences. Restriction enzyme analyses of these four clones with six different enzymes failed to differentiate them. Thus, Sau 3A fragments containing the V\( \text{k} \) coding regions from each of these four clones were subcloned and sequenced. The results showed that two isolates contained an identical V\( \text{k} \)III pseudogene, while the remaining two contained an identical functional V\( \text{k} \)III gene, that was designated \( \text{Hum} \text{Kv}3\text{Ies} \) or \( \text{Hum} \text{Kv}3\text{28} \).

**Characterization of the Hum\( \text{Kv}3\text{28}/ \text{Hum} \text{Kv}3\text{Ies} \) Gene.** Fig. 1 shows the genomic structure of the \( \text{Hum} \text{Kv}3\text{28} \) gene, together with \( \text{Ka}3\text{les} \) and three homologous human V\( \text{k} \)III germline genes. Fig. 2 compares the amino acid sequences of k328, \( \text{Ka}3\text{les} \), three other RF light chains, and four homologous V\( \text{k} \)III germline genes.
Genomic structure of the *HumKv3.1es/HumKv3.28* gene. Both the nucleotide sequence and the deduced amino acid sequence are given. The sequences of the rearranged *ka3les* gene, and three most closely related *VaIII* germ line genes are included for comparison. The figure only depicts nucleotides at the positions where the latter genes differ from the *kv328* sequence. All nucleotide sequences are first aligned for maximum homology and the introduced gaps are marked by dots. The *kv3g* and *kv3h* are renamed from Vg and VA reported previously by Pechet et al. (10). Nucleotides are numbered according to the translated amino acid sequence. The 7-mer and 9-mer are the consensus sequences for gene rearrangement. These sequence data have been submitted to the EMBL/GenBank under accession number Y00640.
As can be seen, $\kappa_{32}$ and $\kappa_{31}$ have 92 identical amino acids among a total of 95 residues. At the nucleotide level, $\kappa_{32}$ and $\kappa_{31}$ share 369 of 380 nucleotides. In addition, $\kappa_{32}$ shares with $\kappa_{31}$ the distinct characteristics of having Val (instead of Leu) at the position 13, and of lacking Tyr at the position 32 in the first CDR. The relatively conserved Leu and Tyr residues are present in most reported V$\times$III light chain protein sequences and in all functional V$\times$III germline genes that have been sequenced previously (9). Together, these data strongly suggest that $\kappa_{32}$ is the corresponding germline gene for the rearranged $\kappa_{31}$ gene that encodes a human RF light chain.

As shown in Fig. 1, all eleven nucleotides by which $\kappa_{31}$ differs from $\kappa_{32}$ are absent in any of the three most closely related human V$\times$III germline genes that have been isolated. Thus, it is likely that they are due to single base somatic mutations, or to allelic differences present in Les genomic DNA. The latter hypothesis would imply that the patient Les is heterozygous for $\kappa_{32}$, and that $\kappa_{31}$ is encoded by the allelic form of $\kappa_{32}$, but not $\kappa_{32}$ itself.

Discussion

Using a specific probe the $\kappa_{31}$ from human RF-secreting cells, we isolated and characterized the corresponding germline V$\times$ gene ($Hum\kappa_{32}$). In addition to the RF Les light chain, $Hum\kappa_{32}$ is identical to the RF Pom light chain from positions 44 to 95, and to the RF Cla and She light chains from positions 1 to 23/26 (Fig. 2). The amino acid residues found at position 9 of both Cla and She were Ala and Gly (11). These data suggest that these three RF light chains are also encoded by the $\kappa_{32}$, or by a very closely related gene. Moreover, both the Les and Pom light chains react with an mAb (6B6.6) that identifies a CRI on human RFs (reference 12; and Crowley, J., S. Fong, R. Schrohenloher, W. Koopman, and D. Carson, unpublished data).

To explain the molecular basis for autoantibody production, Giusti et al. (13) showed that a single A-to-C transversion in the heavy chain variable region of the murine S107 antibody, changed the antibody specificity from phosphocho-
line-binding to dsDNA binding. This result indicated that autoantibody synthesis may occur because of mutations in V genes encoding antibodies against foreign antigens. However, it has also been observed (14) that A/J mice use an unmuted V gene to generate DNA-binding antibodies, and use the same V gene with some somatic diversification to produce antiarsonate antibodies. These latter findings suggested that in certain instances autoantibodies may be directly encoded by germline Ig gene segments that serve as the precursor genes for antibodies against exogenous antigens.

In humans, *Humvc325* is identical with the light chains of four IgM RFs as well as an antibody reactive with intermediate filaments (4, 15). The same V gene with some somatic changes encodes the light chains of other RFs, and one autoantibody against low-density lipoprotein (4, 15, 16). Very recently, a human IgG anticytomegalovirus (CMV) antibody was found to express both PSL2 and PSL3 CRI markers (Newkirk, M., P. P. Chen, D. A. Carson, and J. D. Capra, unpublished data). Collectively, these observations demonstrate that antibodies to both self and non-self epitopes may derive from the same Ig gene segments, either with or without somatic diversification.

It is well established that the antigen binding site of an antibody molecule consists of CDRs of both heavy and light chains. However, recent analyses of many murine RF light chains revealed that their CDR sequences were heterogeneous, while their second and third FRs were more homologous than could be explained by chance (17). These results led the authors to postulate that the k light chain FRs also contribute in some way to the IgG binding site. In light of this, it is noteworthy that the two human germline genes that can be used for RF synthesis (*Humvc325* and *Humvc328*) have homologous sequences in their second FRs and second CDRs (Fig. 2). It is thus conceivable that the second FR, as well as the second CDR, of RF light chains plays a role in IgG binding.

**Summary**

Previously, we isolated a V* gene (*Humvc325*) from a human placenta that encodes RF light chains bearing the PSL2 and PSL3 CRI markers. Here we report the isolation and characterization of a second human V* gene (*Humvc328*) that can be used for RF synthesis. This V* gene probably encodes at least two 6B6.6 CRI+ RF light chains (Les and Pom) from unrelated subjects, and thus may be related to the light chain–associated 6B6.6 CRI.

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**References**

1. Carson, D. A., P. P. Chen, R. I. Fox, T. J. Kipps, F. Jirik, R. D. Goldfien, G. Silverman, V. Radoux, and S. Fong. 1987. Rheumatoid factors and immune networks. *Annu. Rev. Immunol.* 5:109.
2. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973.
Cross idiotypic specificity among monoclonal IgM proteins with anti-gamma globulin activity. J. Exp. Med. 137:331.

3. Chen, P. P., S. Fong, F. Goni, R. A. Houghten, B. Frangione, F. Liu, and D. A. Carson. 1987. Analyses of human rheumatoid factors with antiidiotypes induced by synthetic peptides. Monogr. Allergy. In press.

4. Chen, P. P., K. Albrandt, N. K. Orida, V. Radoux, E. Y. Chen, R. Schrantz, F.-T. Liu, and D. A. Carson. 1986. Genetic basis for the cross-reactive idiotypes on the light chains of human IgM anti-IgG autoantibodies. Proc. Natl. Acad. Sci. USA. 83:8318.

5. Radoux, V., P. P. Chen, J. A. Sorge, and D. A. Carson. 1986. A conserved human germline Vκ gene directly encodes rheumatoid factor light chains. J. Exp. Med. 164:2119.

6. Jirik, F. R., J. Sorge, S. Fong, J. G. Heitzmann, J. G. Curd, P. P. Chen, R. Goldfien, and D. A. Carson. 1986. Cloning and sequence determination of a human rheumatoid factor light-chain gene. Proc. Natl. Acad. Sci. USA. 83:2195.

7. Jirik, F. R., J. Sorge, S. Fong, J. G. Heitzmann, J. G. Curd, P. P. Chen, R. Goldfien, and D. A. Carson. 1986. Cloning and sequence determination of a human rheumatoid factor light-chain gene. Proc. Natl. Acad. Sci. USA. 83:2195.

8. Radoux, V., P. P. Chen, J. A. Sorge, and D. A. Carson. 1986. A conserved human germline Vκ gene directly encodes rheumatoid factor light chains. J. Exp. Med. 164:2119.

9. Pech, M., and H. G. Zachau. 1984. Immunoglobulin genes of different subgroups are interdigitated within the Vκ locus. Nucleic Acids Res. 12:9229.

10. Schroenloher, R. E., and W. J. Koopman. 1986. An idiotypic common to rheumatoid factors from patients with rheumatoid arthritis identified by a monoclonal antibody. Arthritis Rheum. 29:S28.