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Brief Definitive Report

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_κ gene. This contention was supported by the recent isolation from normal human placenta of a V_κ gene (designated V_κ(RF)/*Humκv325*) that encodes the exact 96 amino acids found on four separate RF light chains (4, 5).

Recently, a rearranged κ light chain gene (designated *κa31es*) was cloned from the malignant B lymphocytes of a patient (Les) with an IgM RF paraprotein, (6). The deduced amino acid sequence of *κa31es* 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_κ gene (Humκv31es/*Humκv328*) whose deduced amino acid sequence is very homologous to both the RF Les and Pom light chains. Our data suggest that *Humκv328* represents a second germline V_κ 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 Humκv31es/*Humκv328* was a 378-bp Sac I–Kpn I fragment from 272 to 106 of the *κa31es*, designated *κa31es/1* (6). Probe *Humκv305/1* is a 865-bp Sau 3A1 fragment from a human VκIII gene (4). Southern blot analysis was done by hybridizing

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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 *κa31es/1* 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 κa31es in its first and third framework regions (FRs). They consist of GGGTGGCTGGAGACTG (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 3A1 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 Humκv31es/Humκv328 Gene. To date, eight human VκIII 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 Vκ III light chains suggests that at least one additional VκIII gene exists in the germline, which encodes *κa31es* and three other VκIII 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 VκIII genes, the *κa31es* gene is ~90% homologous with *Humκv305*, *Humκv325*, *Humκv3g*, and *Humκv3h* (renamed from *Vg*, and *Vh*, respectively) (10). When Eco RI–digested Les germline DNA was probed with either *κ3ales/1* or *Humκv305/1*, 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 *Humκv305* and *Humκv325* genes. Together, these data suggested that the corresponding germline gene for κa31es came from either the 14-kb band or the 7.5-kb band.

To isolate the putative Humκv31es gene, Les germline DNA was digested with Eco RI, and fragments of 6–20-kb size were cloned into the phage EMBL4. 2×10^5 recombinant phages were screened with the *κa31es/1*, and seven positive clones were isolated. Among these seven isolates, only four remained strongly positive with the *κa31es/1* probe after washing at 0.1× SSC, and hybridized with two κa31es-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 Vκ coding regions from each of these four clones were subcloned and sequenced. The results showed that two isolates contained an identical VκIII pseudogene, while the remaining two contained an identical functional VκIII gene, that was designated Humκv31es or *Humκv328*.

Characterization of the Humκv328/Humκv31es Gene. Fig. 1 shows the genomic structure of the *Humκv328* gene, together with *κa31es* and three homologous human VκIII germline genes. Fig. 2 compares the amino acid sequences of *κv328*, *κa31es*, three other RF light chains, and four homologous VκIII germline genes.

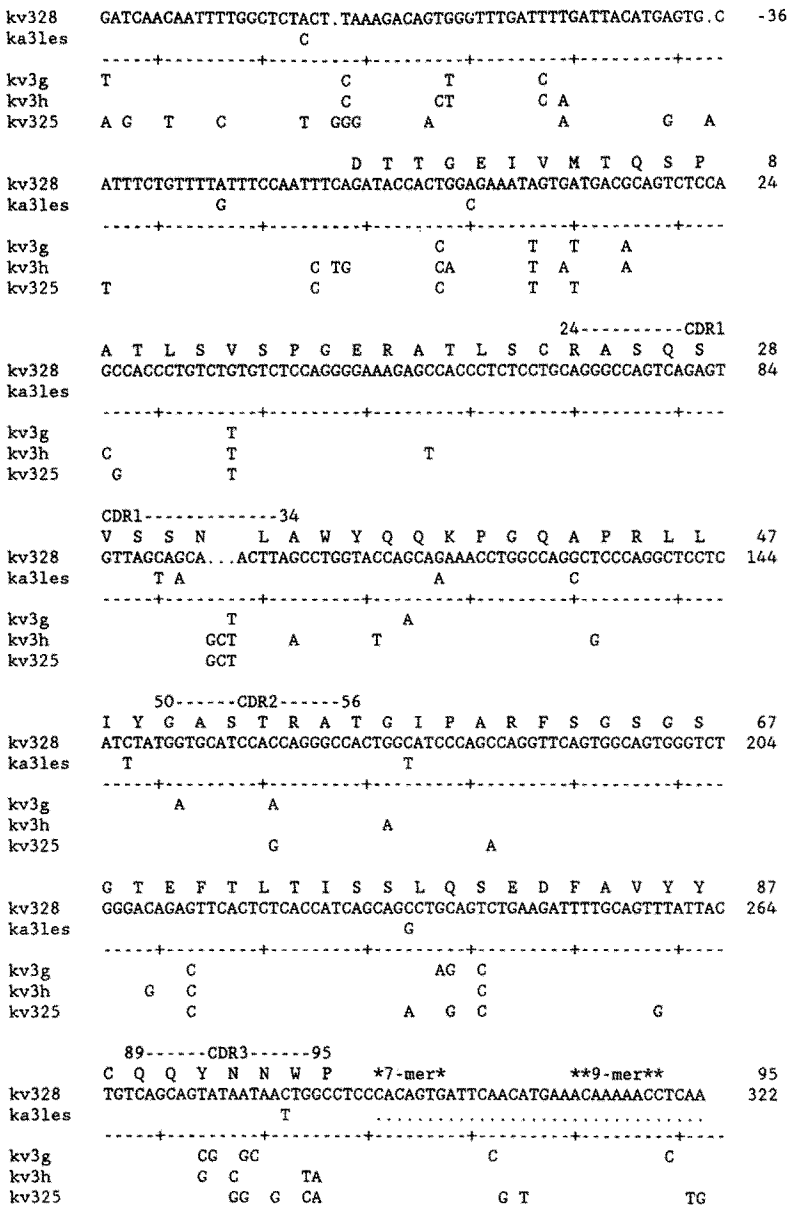

FIGURE 1. Genomic structure of the *Humκv31es/Humκv328* gene. Both the nucleotide sequence and the deduced amino acid sequence are given. The sequences of the rearranged *κa31es* gene, and three most closely related VκIII germline genes are included for comparison. The figure only depicts nucleotides at the positions where the latter genes differ from the *κv328* sequence. All nucleotide sequences are first aligned for maximum homology and the introduced gaps are marked by dots. The *κv3g* and *κv3h* are renamed from *Vg* and *Vh* reported previously by Pech 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.

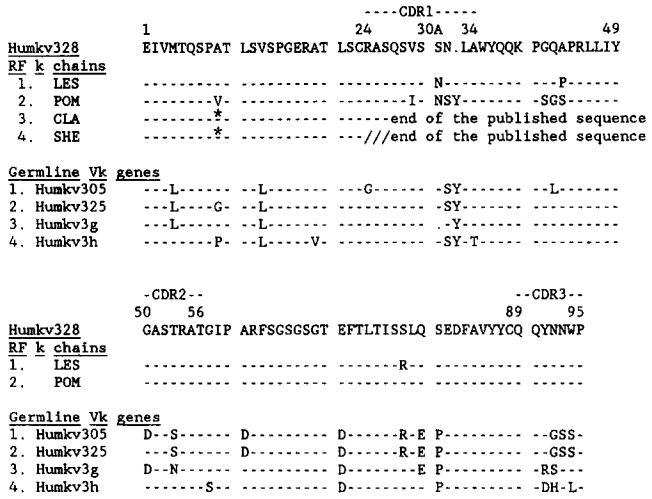

FIGURE 2. A comparison of the *Humκv328* amino acid sequence with the RF Les Pom, Cla, and She light chain sequences, as well as four closely related V_κ gene amino acid sequences (4, 5, 7, 10, 11). The asterisk at position 9 of the Cla and She indicates that the amino acids were determined to be Ala and Gly (11). The complete sequence of the *Humκv328* is given, and others are given only at positions where they differ from *κv328*. All sequences are first aligned for maximum homology, and the introduced gaps are marked by dots.

As can be seen, *κv328* and *κa31es* have 92 identical amino acids among a total of 95 residues. At the nucleotide level, *κv328* and *κa31es* share 369 of 380 nucleotides. In addition, *κv328* shares with *κa31es* 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 VκIII light chain protein sequences and in all functional VκIII germline genes that have been sequenced previously (9). Together, these data strongly suggest that *κv328* is the corresponding germline gene for the rearranged *κa31es* gene that encodes a human RF light chain.

As shown in Fig. 1, all eleven nucleotides by which *κa31es* differs from *κv328* are absent in any of the three most closely related human VκIII 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 *κv328*, and that *κa31es* is encoded by the allelic form of *κv328*, but not *κv328* itself.

Discussion

Using as a specific probe the *κa31es/1* from human RF-secreting cells, we isolated and characterized the corresponding germline V_κ gene (*Humκv328*). In addition to the RF Les light chain, *Humκv328* 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 *κv328*, 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 unmutated V_H gene to generate DNA-binding antibodies, and use the same V_H 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, *Humκv325* is identical with the light chains of four IgM RFs as well as an antibody reactive with intermediate filaments (4, 15). The same V_κ 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 κ 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 (*Humκv325* and *Humκv328*) 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_κ gene (*Humκv325*) 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_κ gene (*Humκv328*) that can be used for RF synthesis. This V_κ 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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