Gamma Heavy Chain Disease in Man

Genomic Sequence Reveals Two Noncontiguous Deletions in a Single Gene

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Abstract

A genomic clone was isolated from a human lymphoid cell line which synthesized an NH_2-terminally deleted gamma3 heavy chain disease protein. Nucleotide sequence analysis revealed a normal sequence from 310 bp 5′ to the initiator ATG through the codon for VH amino acid 14. Amino acid 15 was derived from the codon for the last J4 amino acid. Thus, the clone contained a deletion of the codons for the VH region beyond amino acid 14, as well as those for the entire D region and most of the J coding region. Some sequence abnormalities were observed in the 400 bp after the deletion. Beyond this, there was excellent homology to published J and intervening sequences, including those containing the enhancer elements. The 1,200-bp switch region was abruptly interrupted by a sequence corresponding to the 3′ one-third of CH1. Thus, a second deletion eliminated the acceptor splice site at the 5′ end of CH1. When splicing of the primary RNA transcript occurred, the truncated VH region was joined via the J4 donor splice site to the next available acceptor site 5′ to the first hinge exon. Hence, the aberrant serum protein was the product of two deletions and a splice correction as well as postsynthetic NH_2-terminal proteolysis.

Introduction

Heavy chain disease (HCD)[1] is a human lymphoproliferative disorder in which abnormally short monoclonal immunoglobulin (Ig) heavy (H) chains can be isolated from the patient's serum and/or urine. HCD proteins of four of the five Ig classes (mu, delta, gamma, and alpha) have been described (1–3). In contrast to the other classes, some mu HCD proteins have associated light (L) chains. The gamma, alpha, and delta proteins were identified by their unusual appearance on immunoelectrophoresis; i.e., the absence of light chain determinants from a monoclonal protein. In the cases in which the unusual proteins were subjected to amino acid sequence analysis, they showed typical structural features: normal V region initiation for the first few residues, followed by deletion of the remaining V region and the entire CH1 domain, with normal sequence resuming at the start of the hinge. The constancy of such findings suggests a relationship between the deletions and the lack of L chain synthesis. It has been established that the HCD proteins are primarily the products of aberrant biosynthesis. The identification of this family of similarly defective immunoglobulins suggested that the analysis of the associated gene abnormalities would add to our understanding of the processes involved in normal Ig synthesis.

The OMM HCD protein was of the gamma3 subclass. When isolated from the serum and analyzed, it showed an apparent NH_2-terminal deletion of the entire V and CH1 domain, with sequence starting within the hinge (4).

Our laboratory established a permanent cloned cell line from the peripheral blood of the patient OMM (5). The cell line synthesized and secreted only the deleted HCD protein. In contrast to the serum protein, which had no V region sequence, the molecule secreted by the cultured cells had a normal V region amino terminus (6). The mRNA coding for the HCD protein was shorter than that coding for a normal-sized heavy chain, and the short mRNA directed the synthesis in vitro of the short gamma heavy chain containing a hydrophobic NH_2-terminal leader sequence similar to that expected for a secretory protein.

A cDNA copy of the mRNA was cloned and sequenced (7). The mRNA coded for a protein containing a 19-amino acid leader sequence, a short V region containing 14 amino acids, and a 15th proline residue. We originally assigned the proline to the V region, although it must be noted that proline is not found at position 15 in any published human VH sequence so far described. The remainder of the sequence coding for the V region and the entire CH1 domain was missing from the OMM mRNA. Normal mRNA sequence resumed at the precise beginning of the hinge. The entire hinge region, the CH2, CH3, and 3′ untranslated regions were normal. To resolve the nature of the structural gene deletion in the OMM cells it was necessary to clone and sequence the gene coding for the HCD protein. The results are described in this report.

Methods

Cell cultures. Cells were maintained in RPMI 1640 with 5% FCS. For preparation of DNA and RNA, cells were grown to 5×10^5 cells/ml in spinner flasks and then frozen at −80°C.

Genomic cloning. DNA was extracted from the frozen cells by the method of Blin and Stafford (8). For cloning, DNA was digested with the restriction endonuclease Bcl I and size fractionated by electrophoresis through a 1% agarose gel prepared in the Bullseye electrophoresis apparatus (SE2020; Hoefer Scientific Instruments, San Francisco, CA). The fractions were collected and aliquots electrophoresed on an analytical 0.7% agarose gel. The DNA was transferred to nitrocellulose (9) and hybridized with the OMM cDNA probe, which recognized all four gamma subclasses. The fraction most enriched in the rearranged

This work was published in abstract form in 1986. *Clin. Res.* 34:667A.

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Received for publication 12 January 1988 and in revised form 18 May 1988.

1. *Abbreviation used in this paper:* HCD, heavy chain disease.

gamma band was selected for ligation to the lambda L47.1 Bam H1 arms prepared as described (10). The ligated DNA was packaged into phage particles using packaging extracts purchased from Promega Biotech Co. (Madison, WI). The recombinant phages were screened with H-chain variable (V) region, constant (C) region, and joining (J) probes prepared as described below, using the Benton-Davis procedure (11).

Southern blotting and hybridization. DNA was digested with restriction endonucleases purchased from New England Biolabs (Beverly, MA) and electrophoresed on 0.7 or 1% agarose gels. The DNA was transferred from the gels to nitrocellulose filter paper (Schleicher and Schuell, Keene, NH) by the method of Southern (9). Probes were labelled by nick translation with [^{32}P]dCTP (New England Nuclear, Boston, MA) to a specific activity of 10^8 cpm/μg. Southern blots and phage screening filters were hybridized overnight in 50% formamide at 43°C. Filters were washed at room temperature in 2× SSC with 0.1% SDS, then at 60°C with 1× SSC, 0.1% SDS followed by 0.1× SSC, 0.1% SDS at the same temperature.

Probes. The 1-kb gamma probe was derived from the OMM HCD cDNA (7) by subcloning a fragment containing the hinge, CH2 and CH3 regions into M13 (12). The 0.2-kb OMM V probe was isolated from the cDNA-containing plasmid and subcloned into M13. It included coding sequences for the 19-amino acid leader as well as the 15-amino acid V region. The 0.5-kb mu probe is a cDNA clone, encompassing the CH4 domain, which was constructed in pBR322 (13). The 3.3-kb J probe contained most of the H chain J region and was derived from the germline mu clone, H18, by subcloning into pBR322 (J. Buxbaum, unpublished results).

DNA sequencing. DNA fragments were cloned into M13 vectors (New England Biolabs, Beverly, MA) and sequenced by the dideoxy chain termination method (12, 14). DNA sequencing kits using ^{35}S-dATP were purchased from New England Nuclear.

Results

The total DNA extracted from the OMM cells was digested with several different restriction endonucleases, electrophoresed through 0.7% agarose gels and blotted onto nitrocellulose filter paper. The blots of each restriction digest were hybridized with probes for VH, JH, gamma, and mu as described in Methods. The restriction enzyme patterns obtained with Hind III and Bcl I are shown in Fig. 1.

When compared with the germline pattern of placental DNA, Hind III produced one rearranged OMM V band comigrating with the single rearranged J band (Fig. 1 *A*). The gamma probe revealed one rearranged band, the other gamma genes appearing grossly normal when compared with the germline genes. The sequences of all human germline gamma genes contain a Hind III site 215 bp 5′ to CH1 (15). The rearranged gamma band in the OMM Hind III digest was larger than the germline band, thus one Hind III site was missing. In view of the loss of CH1 in the OMM HCD mRNA, it was presumed that the rearranged band contained the OMM HCD gene and that the missing Hind III site was the one 5′ to CH1.

Bcl I digestion of OMM DNA shows one rearranged V gene that comigrates with the single rearranged J band and a

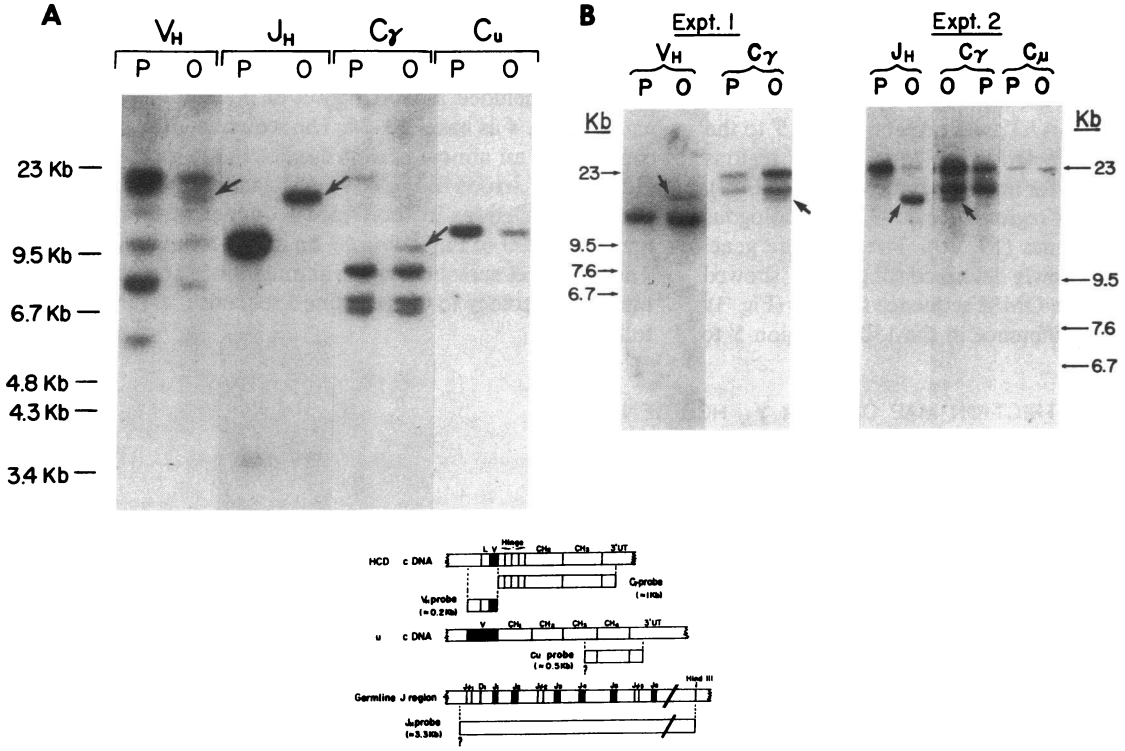

Figure 1. Southern blots of Hind III- (*A*) and Bcl I (*B*) -digested OMM DNA. *P,* human placental DNA. *O,* DNA extracted from the OMM HCD cells. 10 μg of DNA was used per lane. The arrows indicate the positions of the rearranged bands containing the HCD gene sequences. VH, JH, C-gamma, and C-mu refer to the hybridization probes used for each panel. In *B,* a different VH probe was used. This probe contained 5′ sequences from another mRNA that was artifactually joined to the OMM sequence during construction of the cDNA. The dark bands in the VH panel are due to hybridization of the 5′ sequence. The 5′ borders of the mu and J probes were not determined but were approximately as indicated on the maps.

rearranged gamma band (Fig. 1 *B*). The other gamma genes appeared normal and did not hybridize with either the JH or V probe.

Southern blots of OMM and placental DNA, digested with a series of different restriction enzymes (not shown), indicated that only one copy of the J region was present in OMM, the other being deleted. Similarly, the intensity of the mu band in OMM, when compared with that in placental DNA, suggested that only one copy of mu was present (Fig. 1 *A*). The OMM mu gene appeared unrearranged on digestion with Hind III, Bcl I (Fig. 1), Xba I, and Bgl II (not shown). Thus the mu constant region was normal by Southern blot analysis. Since there was only one copy of J region material, and these sequences seemed to be associated with the HCD gene, we concluded that the mu locus was rearranged on both chromosomes with the mu constant region being deleted from the expressed chromosome and J deleted from the other. In other Southern blot experiments there was no apparent deletion of any of the alpha genes (S. Rosen and J. Buxbaum, unpublished results).

The rearranged OMM gamma gene fragment, produced by Bcl I digestion, was cloned into the Bam HI site of the bacteriophage lambda L47.1. Based on our experience, Bcl I appeared to be a generally useful enzyme for the cloning of rearranged human gamma genes.

Six recombinant clones were positive with the gamma probe. Of these, five were also positive with the J probe and therefore expected to contain the rearranged OMM gene. Three J positive clones were studied further and all contained V sequences. One of these (clone 2B4-1) was chosen for detailed mapping and sequence analysis.

A partial restriction enzyme map of the OMM gene is shown in Fig. 2 and its sequence is shown in Fig. 3. The Bcl I cloning site was 310 bp 5′ to the initiator ATG. The conserved octamer sequence, ATGCAAAT, was present 105 bp 5′ to the ATG. This sequence is characteristically found 5′ to V regions and appears to be necessary for accurate transcription (16, 17).

The OMM leader and V region were 80–97% homologous to other sequenced VH genes (19, 20). The germline gene, VH71-4, a member of the newly described subgroup 4, showed the greatest similarity to the OMM sequence (20, 21) (Fig. 3). There was only one base difference in the 150 bp region 5′ to the initiation ATG. The leader coding sequence was identical in the two genes, although there were five differences in the 82 bp intervening sequence (IVS). The codons for the first 14 V region amino acids contained two single-base changes that resulted in amino acid differences. Beyond amino acid 14 there was no evidence of any sequence in OMM homologous to the remainder of the 71-4 V region. The 15th V amino acid in VH71-4 and in other closely related V regions was serine, coded by TCG, while the 15th OMM triplet was CCA, coding for proline. Assuming that the remainder of the 71-4 V region was equally homologous with the OMM V region before the deletion event, two base changes would have been necessary to convert the 71-4 V 15th serine to the OMM proline. Since a double-base change is an unlikely event in such a similar sequence, the 71-4 data suggest that the 15th proline is not V region derived.

A comparison of the OMM genomic sequence with its own cDNA sequence and with the germline J4 sequence provided an explanation for the unique proline at amino acid 15. The codons for the first 14 V region amino acids and the unusual 15th proline were present in the gene exactly as they appeared in the cDNA. 3′ to this, there was no evidence for sequence homology to any V region. Instead, a sequence homologous to the published J4 splice site and 3′ flanking sequence was found (22).

Fig. 4 shows the sequence surrounding the germline J4 splice site compared with the OMM sequence beyond the codons for amino acid 14. The two sequences are almost identical for the first 16 bp after the splice site. Presumably, the proline at residue 15 is partially encoded in the sequence immediately 5′ to the J4 splice. Beyond the initial region of homology, the OMM sequence showed several abnormalities when compared with the germline sequence over the next 400 bp. These included a short region of poor homology, designated in Fig. 4 as bases 52–74. The sequence from 132 to 207 consisted of an almost perfect duplication of a 37-bp region, indicated by arrows in Fig. 4. The two additional major anomalies were deletions in the OMM sequence of 54 bp beyond nucleotide 290 of Fig. 4 and 19 bp in the coding region of J5. The sequences surrounding the anomalous regions showed significant homology to the germline J sequence ranging from 70 to 80%.

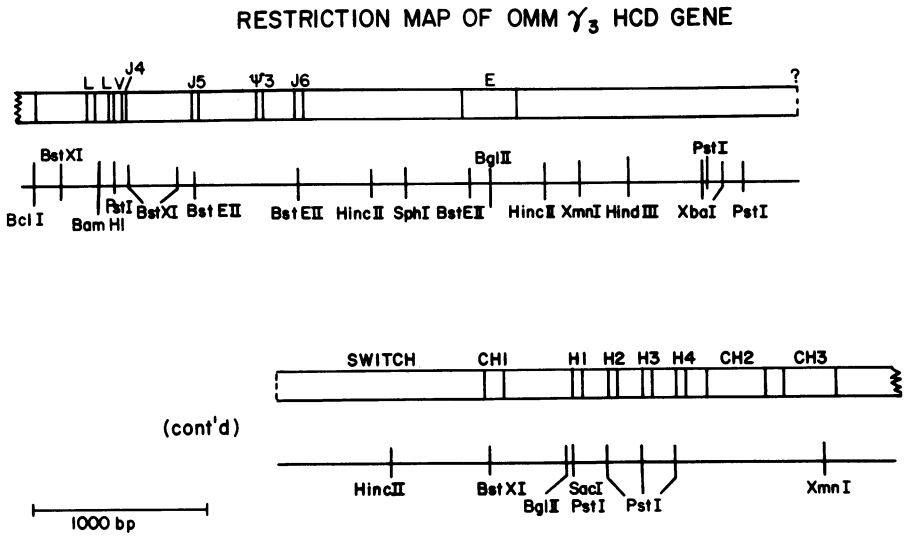

Figure 2. Restriction map of OMM gamma3 HCD gene. The ? indicates the approximate start of the switch region.

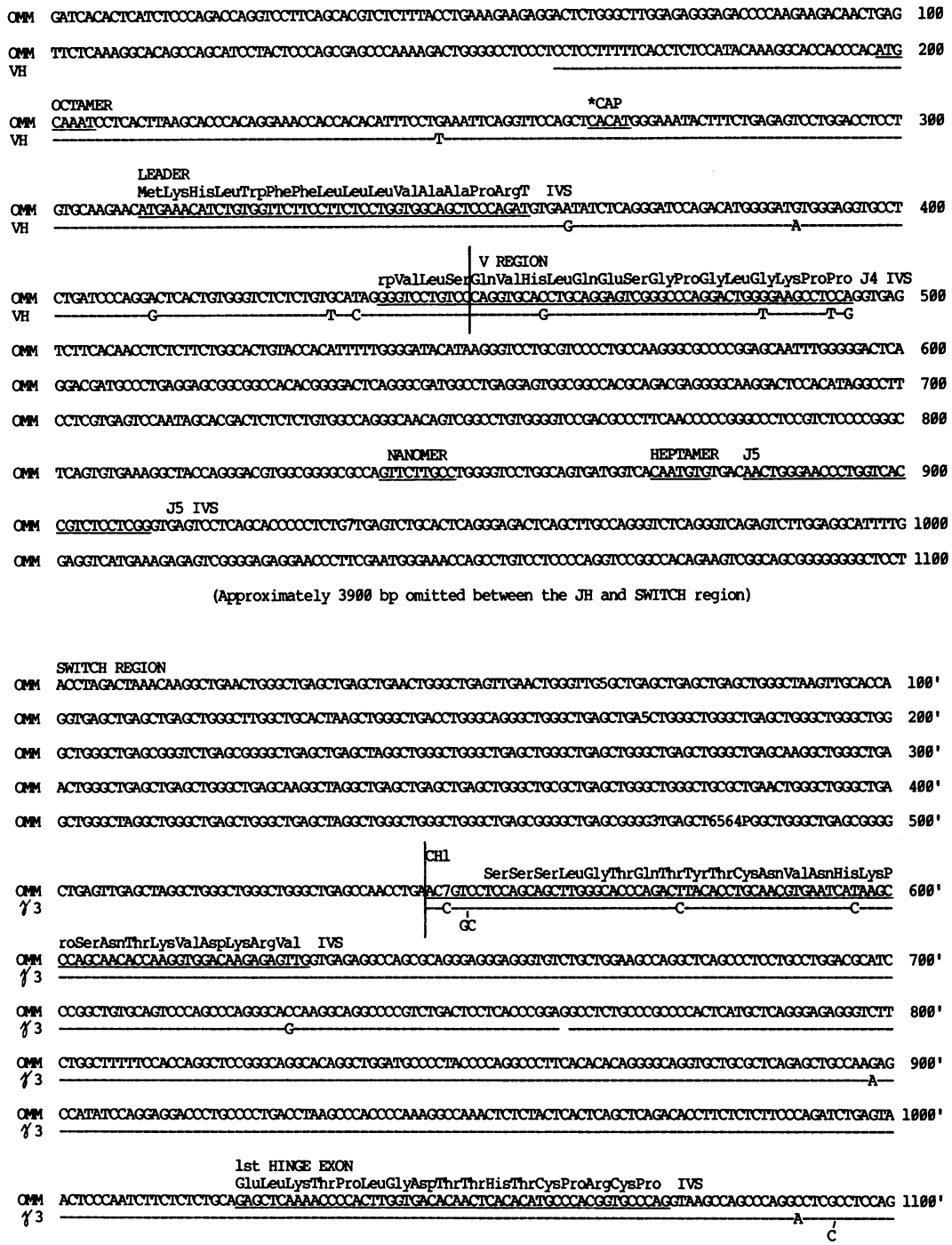

Figure 3. Sequence of the OMM gamma3 HCD gene. Approximately 3,900 bp of sequence from J6 to the switch region were omitted from the figure. Most of this sequence has been determined and is available from the authors. *IVS,* intervening sequence. *, position of the CAP site in the closely related VH gene, HIGl (18). *VH,* heavy chain variable region gene, 71–4 (20). *γ3,* human germline gamma3 gene (15). The dashes indicate sequence identity. Functional regions and coding sequences are underlined. Ambiguous nucleotides are indicated according to the convention used by Intelligenetics, Inc. as follows: 3, probably C (if not, then some unidentified base); 4, probably T (if not, then some unidentified base); 5, probably A (if not, then some unidentified base); 6, probably G (if not, then some unidentified base); 7, maybe C (if not, then no base); 9, maybe A (if not, then no base); 0, maybe G (if not, then no base); P, either A or G.

For the next 750 bp, homology was relatively constant at ~ 88%. Thus, it appeared that homology improved with increased distance from the V/J deletion. Interestingly, there was a duplication of 29 bp in the germline sequence 3′ to J6 that was not present in the OMM sequence (not shown). The heptamer-nanomer recombination signals and the splice signals associated with J5 and J6 in the OMM gene appeared normal.

A comparison with published restriction maps and limited sequence data indicated that the large intervening sequence beyond J6 was essentially normal (23, 24). The published sequence containing the immunoglobulin enhancer was identical to an appropriately located region in the OMM gene (24). The positions of the Hind III and Xba I sites were as expected from the published mapping data, except that the OMM sequence contained two Xba I sites separated by ~ 120 bp.

A region of ~ 1,000–1,200 bp showed homology to the human switch region. The sequence following this was homologous to the 3′ one-third of the CH1 domain (Fig. 3). Thus, an undetermined amount of switch region, as well as the 5′ end of CH1 have been deleted from the OMM gene.

In contrast to the interrupted homology seen after the V/J deletion, the sequence 3′ to the switch/CH1 deletion appeared to be normal, showing excellent homology to the CH1 and IVS of the germline gamma3 gene (15).

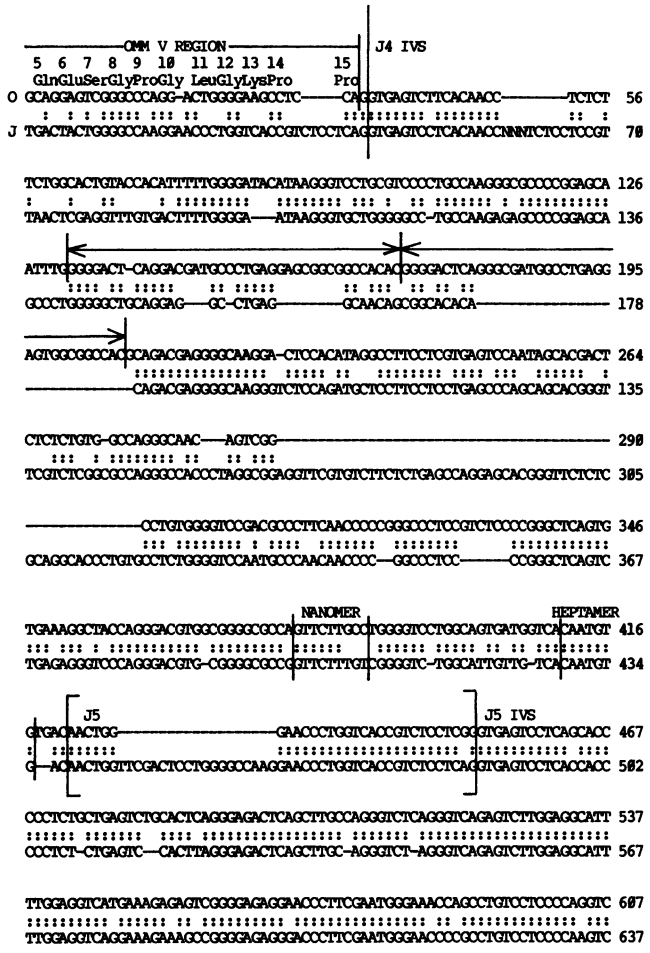

Figure 4. Computer-generated comparison of OMM sequences 3′ to the V region with germline J region sequences. The Bionet Ifind program was used for the comparison. The arrows designate the 37-bp OMM sequence that has undergone duplication. The dashed lines indicate regions that must be deleted to maximize homology. *IVS*, intervening sequence.

Discussion

Fig. 5 shows the structure of the OMM HCD gene compared with a normal rearranged gene of the gamma3 subclass. In the OMM gene, the two discrete DNA deletions caused both loss of coding sequence and alteration in the use of splice sites. When the large intervening sequence was removed from the primary RNA transcript the J4 donor splice site was used. Since there was no acceptor splice site 5′ to CH1, splicing occurred at the next available site, 5′ to the first hinge exon. All other splices were normal, yielding an mRNA containing a short V coding region, which was contiguous with the hinge. The fully spliced mRNA directed the synthesis of a protein containing a 19-amino acid leader sequence, 14 amino acids of *bona fide* V region, and a 15th proline derived partially from the 3′ end of J4. The following sequence was that of the hinge coding region. The leader was cleaved from the body of the molecule, yielding an internally deleted secretory protein that subsequently underwent limited proteolysis in the serum to produce the NH_2-terminally deleted HCD Ig.

Analyses of aberrant gene structures have been performed in two other cases of human HCD. In RIV, a gamma1 HCD

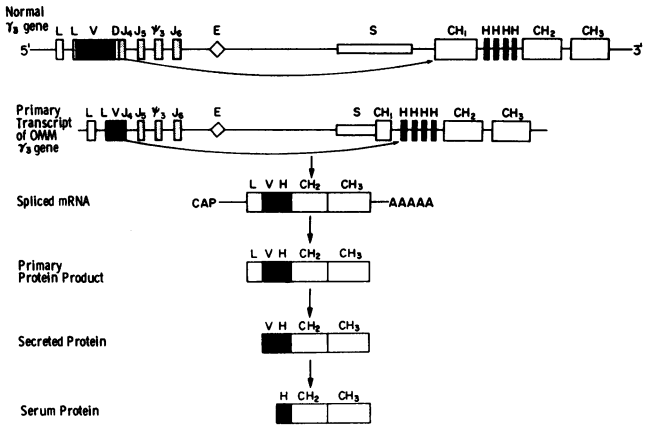

Figure 5. Comparison of a normal rearranged gamma3 gene with the primary RNA transcript of the OMM gamma3 HCD gene.

protein, the V and CH1 domains were absent from both the protein and the mRNA (28). Sequencing of the genomic DNA revealed a structure similar to OMM in that both genes contained deletions in the V/J and switch/CH1 regions (P. Guglielmi, personal communication). In the switch region, the OMM and RIV genes were abruptly interrupted by sequence showing almost perfect identity to the 3′ one-third of CH1. Thus, in both, deletion events left the 3′ flanking sequences intact. In contrast, the sequences 3′ to the V/J deletion were disrupted in both genes. However, the two genes differed in that OMM 5′ to the V/J deletion was completely normal (Fig. 3), while RIV showed aberrations on both sides of the deletion. The authors of the RIV study attributed the unusual sequences to insertion/deletion events. We believe that the OMM sequence 3′ to the V/J deletion reflects a more complex process, possibly involving inexact repair after the deletion event (see below). Due to destruction of the J donor splice site, splicing of the RIV primary RNA transcript was accomplished by joining the intact donor splice site of the leader region to the acceptor site of the hinge. Such splicing has occurred in other Ig mRNAs such as those coding for the mu HCD protein BW (44) and the light chain fragment found in the murine plasmacytoma MPC-11 (45).

In BW, a mu HCD protein with a deleted V region, was secreted into the patient's serum (44). DNA sequencing of the isolated gene showed that while normal VDJ4 joining had occurred, a single-base deletion generated three stop codons in the V region. In addition, an insertion/deletion event eliminated the J4 donor splice site. Splicing occurred between the 3′ end of the leader and the 5′ end of CH1. The entire V region was thus eliminated from the mature mRNA, and consequently did not appear in the protein. The four C-region domains were normal by restriction mapping, and appeared to be spliced correctly. As a group, the mu HCD proteins are heterogeneous in structure (1, 2). Some are missing only V region sequences and have associated L chains. Others resemble the gamma and alpha HCD proteins and are missing CH1 and CH2 sequences as well as V. The latter type of protein usually lacks L chains. It would not be surprising if the genes for such proteins contained switch as well as J deletions. The varied structure of the mu HCD proteins may reflect different underlying mechanisms of origin, possibly associated with different disease processes, or they may be due to common mechanisms acting on cells at different stages of maturation.

Approximately 50 gamma HCD proteins have been subclassed and some have been studied in detail. Those are shown in Fig. 6. In general, the proteins show internal deletions and deletions of whole domains. Often, they have normal V region NH_2 termini, followed by a deletion of the remainder of the V and the entire CH1 domain. Sequence commonly resumes at the beginning of the hinge, a region now known to be coded by at least one separate exon. In some proteins one or more hinge exons are deleted. Clearly, the resumption of normal sequence, at the precise beginning of domains, is due to the utilization of splice signals at the borders of coding regions when the mRNA template is generated from the long RNA transcript (42).

The polypeptide synthesized by the OMM cells showed similarities to the HCD protein WIS (32, 37). WIS began with a seven-amino-acid sequence showing some homology to VH subgroup 1, followed by four amino acids which were identical to the last four residues of the J regions. No CH1 was present, the J residues being followed immediately by the complete normal gamma3 hinge region. Thus the WIS gene must have contained at least two deletions in positions comparable to those in OMM. The first deletion left more of the 3′ end of the J coding region, and therefore J residues appeared in the WIS protein. Since no CH1 residues were present, a second defect must have occurred that altered or eliminated the splice site(s)

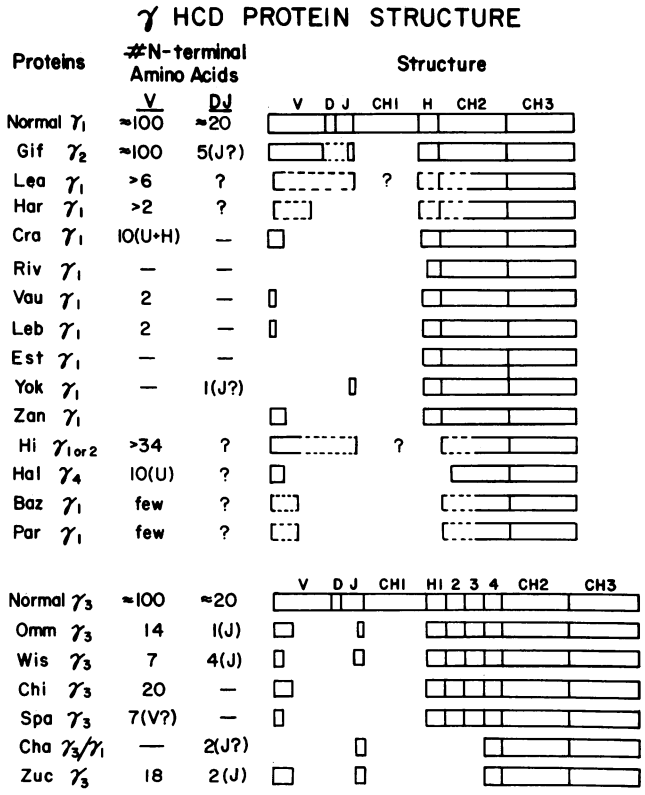

Figure 6. Gamma HCD protein structure. *U,* unusual amino acid sequence. *H,* heterogeneous amino acid sequence. Dashed lines indicate likely structures for which sequence data are missing. The presence of normal CH2 and CH3 regions was generally inferred from antigenic and chemical data and from partial amino acid sequencing. Thus, in some cases, small abnormalities in the constant region cannot be ruled out. Gif (25), Lea and Har (26), Cra (27), Riv (28), Vau and Leb (29), Est (30), Yok (31), Zan (32), Hi (33), Hal (34), Baz (35), Par (36), Wis (32, 37), Chi (38), Spa (39), Cha (40), Zuc (41).

of CH1. In other HCD proteins, ZUC (41), CHA (40), YOK (31), and GIF (25), the amino acids immediately preceeding the hinge resembled the residues found at the 3′ end of J coding regions. Thus, different proteins may retain remnants of J sequences in varying amounts, depending on the extent of the deletion. These observations, combined with the genetic data on OMM and RIV, suggest that most of the gamma HCD proteins have similar origins.

A number of proteins contain NH_2-terminal deletions with sequence starting within the hinge or CH2 domain (43). They have been omitted from Fig. 6. Since the OMM protein shows NH_2-terminal proteolysis superimposed on an internal deletion, we believe that the NH_2-terminally deleted proteins described arise in an analogous fashion.

An important question in considering mechanisms responsible for deletions in gamma HCD is whether the occurrence of two deletions and the absence of L chain are coincidental or obligatory. If the two deletions are random, one would also expect to see molecules containing single deletions; that is, those with intact V/J regions but missing CH1 regions and those with deleted V/J regions but intact CH1 regions. It has been suggested that normal H chains will not be secreted in the absence of L chains due to the attachment of a heavy chain–binding protein (Bip) at the CH1 domain (46). Mutant Igs that lack CH1 can be secreted without L chains due to their inability to bind Bip. Thus, H chains that contained only the V/J deletion and were unattached to L chains might be synthesized but would not exit the cell. Gamma HCD proteins missing CH1 but with intact V/J regions should be detectable in the serum. No such molecule has been isolated and definitively analyzed, however protein sequence data on GIF showed that most of the V region was present through at least residue 100 (25). The CH1 region was missing, but the hinge was present. Although sequence data was limited, the authors were able to identify the five amino acids immediately preceding the hinge. A comparison between these amino acids and the last five residues of J regions showed identity in three of the positions. Thus, it is possible that the entire V region was present. In two other cases, HI and LEA, the proteins contained normal V region NH_2-termini, and high molecular weights consistent with small deletions. The cited exceptions notwithstanding, the HCD proteins characteristically contain only 2–20 V-region amino acids. Thus, it seems that the frequent presence of two deletions cannot be easily dismissed as chance events.

In addition to the gamma3 HCD protein, OMM's serum contained a small amount of an apparently intact H_2L_2 monoclonal protein of the gamma3 H-chain subclass and lambda light-chain type (47). If the HCD cells were progeny or variants of the H_2L_2 cells, then the deletions and the loss of L chains would have occurred after the rearranged mu gene had undergone switching to the gamma3 subclass. In any event, it is likely that the switch/CH1 deletion, which eliminated the borders of both regions in the OMM and RIV genes, occurred after the mu to gamma switch.

The distribution of gamma subclasses among human myeloma proteins is approximately: gamma1, 77%; gamma2, 14%; gamma3, 6%; and gamma4, 3%, while the subclass representation in the gamma HCD proteins is approximately: gamma1, 72%; gamma2, 2.5–5%; gamma3, 23%; and gamma4, 5% (48, 49). The increased frequency of gamma3 proteins approaches statistical significance (P = 0.06–0.07) and should be reevaluated as more proteins are reported. One

explanation for the unusual class distribution in HCD was suggested by our data showing deletions in the OMM switch region. The immunoglobulin genes are ordered in the genome as follows: 5'—mu, delta, gamma3, gamma1, pseudoepsilon, alpha1, pseudogamma3, gamma2, gamma4, epsilon, alpha2—3'. There is evidence that when switching occurs, the intervening DNA is looped out and deleted (50). An examination of murine switch region sequences in genes that have switched from mu to one of the other classes indicates that the same cell may undergo sequential switching using constant region genes in a 5' to 3' direction (51). In humans, the genes for gamma3, gamma1, and alpha1 lie in a closely linked cluster 3' to the mu and delta genes, while gamma2, gamma4, and alpha2 are found in a cluster downstream from mu. If class switching is in whole or part due to a mechanism that functions sequentially, a structural defect in the switch region could freeze the process at an earlier stage and prevent the more 3' classes from being expressed. It is interesting in this regard that all alpha HCD proteins described to date have been of the alpha1 subclass, while IgA precursor cells show a subclass distribution of ~ 50:50 (52, 53). DNA sequencing has shown that both the OMM and RIV HCD genes have deleted part of the switch sequence. From the similar structures of the other gamma HCD proteins, we believe that extensive switch region deletions are characteristic of their genes. Such deletions in antibody producing B cells or plasma cells could prematurely terminate a sequential switching process, perhaps increasing the representation of subclasses close to mu (gamma3 and gamma1) and decreasing representation of the more 3' subclasses (gamma2 and gamma4). Although the gamma1 gene is situated 3' to gamma3 and is therefore further removed from mu, it is by far the most prevalent subclass in either the normal or mutant form. Thus, other factors must operate to influence subclass selection.

Cytogenetic studies of Burkitt's lymphoma cells show frequent translocations of the *myc* oncogene into an immunoglobulin locus on the unexpressed Ig chromosome (t8:14) (54). The two favored sites for *myc* insertion are the regions of the H-chain locus that normally undergo recombination, i.e., the J and switch regions. It has been suggested that these areas of chromatin are in a more open configuration during VDJ joining and switching, and that the openness affects both chromosomes sufficiently to allow *myc* translocation into the same relative regions on the unexpressed chromosome (55).

Southern blots of OMM DNA hybridized with several oncogene probes showed no evidence for translocation of *myc, mos, abl, bcr, bcl* 1, or *bcl* 2 (J. Buxbaum, unpublished results). Hence, the OMM genomic deletions are probably not associated with oncogene rearrangement to any Ig locus. The pattern of deletions seen in gamma HCD indicate that the affected regions are in the same areas that are the target for *myc* translocation. In HCD, however, both *myc* target regions contain deletions rather than translocations, and the affected chromosome is the one involved in active transcription. It is likely that the two deletion events do not occur simultaneously. It is not clear if one abnormality predisposes to the second.

In the OMM gene, the interrupted homology to J after the V/J deletion may be a clue to the deletion process. The retention of homology following the switch/CH1 deletion suggests that the two regions may have been deleted by different mechanisms. Alternatively, the different nature of the sequences 3' to the two deletions may reflect aberrations in the repair processes rather than in the primary event. The OMM sequences 5' to the deletions appeared normal (see Fig. 3). However, since the switch region contains the highly repetitive five-nucleotide motif (GAGCT, GGGCT . . . etc.), deletions and duplications might not be obvious. The RIV gene, unlike OMM, contained abnormalities in the region 5' to the V/J deletion. Similarly, the mu heavy chain disease gene, BW, contained a frame shift resulting in stop codons in the V region. The mu protein was still synthesized, albeit in an aberrant form, because abnormal splicing removed the entire V region with its contained termination signals (see above). The errors in the V regions of RIV and BW may or may not be associated with the deletion event. It is possible that some HCD proteins represent rearrangement of germline V regions that contain abnormalities, and can only be expressed as proteins if their heptamer-nanomer sequences are intact and if the abnormal V sequences do not appear in the spliced mRNA.

The examination of structural genes coding for heavy chain disease proteins suggests that regions normally associated with DNA rearrangements, presumably having their chromatin in a more open configuration, are more prone to events that generate deletions. Thus it may be more the open nature of the active chromosome than the specifics of the processes regulating switching and rearrangement that predisposes Ig heavy chain genes to develop the deletions that are expressed in HCD proteins.

Acknowledgments

We thank Jeffrey Ravetch for his help and advice in the initial electrophoretic separation of OMM DNA. Computer programs used in this work were provided by the Bionet National Computer Resource for Molecular Biology (National Institutes of Health grant P41 RR-01685-05).

These studies were supported by Merit Review funds from the Veterans Administration and National Institutes of Health grant AM-14031.

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