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Immunology

Illegitimate recombination generates a class switch from C_μ to C_δ in an IgD-secreting plasmacytoma

(immunoglobulin genes/C_μ *deletion*/DNA sequence)

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ABSTRACT **We present here the sequence characterization of a $C_\mu \rightarrow C_\delta$ immunoglobulin (Ig) heavy chain class switch. In the murine IgD-secreting plasmacytoma TEPC 1017, deletion of most of the μ switch recombination region (S_μ) and the entire C_μ gene occurred in the absence of switch region sequences 5′ to C_δ. This unique rearrangement resulted from an illegitimate recombination of sequences with only patchy homology to each other. The infrequent and variable nature of illegitimate recombination may explain the low frequency of IgD-secreting plasma cells in normal mouse tissues.**

The chromosomal rearrangements that occur in the genomes of both prokaryotic and eukaryotic organisms can be grouped into two categories. General (or homologous) recombination takes place between homologous chromosomes during meiosis in eukaryotes, utilizing extensive base pairing to provide new combinations of genes without alteration of their order. Nonhomologous recombination, on the other hand, does not demand extensive sequence homology and can alter the order of genes present on the germline chromosome. This latter category includes recombinational events that occur between sites of short and often imperfect sequence homology, such as the site-specific rearrangements of variable (*V*) and constant (*C*) region genes that accompany the developmental expression of Ig heavy (H) and light (L) chains in B cells. During early B-cell differentiation, germline V_H, D_H, and J_H segments undergo recombination (reviewed in ref. 1) to form a functional V_H region gene. Initially, this V_H gene is expressed with the nearest C_H gene, C_μ, to form μ-heavy-chain mRNA of membrane IgM (2–4). As the B cell matures, C_δ, the adjacent C_H gene downstream, is cotranscribed with C_μ without further DNA recombination (5, 6). This results in joint expression of IgM and IgD on the membrane, apparently through alternative processing of the RNA (5–8).

Upon stimulation with antigen or mitogen, B cells lose IgD from the cell surface and differentiate into IgM-secreting plasma cells (9). Some of the cells further differentiate to secrete IgG, IgE, or IgA by expression of downstream C_H genes C_γ, C_ε, or C_α (reviewed in refs. 10, 11). This phenomenon, referred to as the class switch, occurs by nonhomologous recombination between repetitive switch site (*S*) sequences located in front of C_μ and in front of the downstream C_H genes (reviewed in ref. 11). *S* eliminates C_μ and any intervening C_H genes so that the C_H gene to be transcribed is positioned closest to the functional *V* region.

Although IgD is an abundant cell surface isotype on most mature B cells (12), it is secreted only in minute quantities during any stage of normal differentiation in mice (13). It is not known if the rare secretion of IgD in normal cells is mediated by nonhomologous recombination deleting C_μ in a class switch from IgM-producing to IgD-producing cells or by RNA processing as in the case of membrane δ expression. A typical class switch is unlikely since we (14) and others (15, 16) have recently shown that there is no *S* region between C_μ and C_δ in murine DNA. RNA processing is also unlikely since the few IgD-secreting tumor cells characterized (17–19) have deleted the C_μ gene (5, 8, 20).

To determine the recombination mechanism utilized for secretion of IgD in one of these tumors, we have cloned the productively rearranged C_δ allele and determined the nucleotide sequence spanning the recombination joint in the plasmacytoma TEPC 1017. Our results show that recombination of unique DNA sequences that lack special sites or obvious homology has produced a functional *VDJ*–C_δ transcriptional unit and eliminated the C_μ gene. The "illegitimate" nature of this unusual immunoglobulin class switching event may explain the low frequency of IgD-secreting cells in normal mice.

MATERIALS AND METHODS

TEPC 1017 tumors (17) were propagated in BALB/c mice. DNA was extracted, blotted, and hybridized as described (21–23). An *Mbo* I partial library of TEPC 1017 tumor DNA was constructed in Charon (Ch) 30 (24). Clones were screened with a δ cDNA clone, pδ54J (25), as described (26, 27). The sequence (28) spanning the deletion in TEPC 1017 was determined from a 3.2-kilobase-pair (kbp) *Hin*dIII subclone of genomic phage Ch30-573.1. The BALB/c germline sequences were obtained from published sequences (14) and from genomic clones and subclones of the regions involved in the TEPC 1017 deletion (7, 14, 29). A cDNA clone, VDJ3–C_δ, was obtained by rescreening the same TEPC 1017 library (25) from which pδ54J was derived. The *Pst* I fragments of this cDNA were subcloned into pUC8 (30) and sequenced by labeling at vector linker sites. Computer algorithms (31, 32) were utilized to search for sequence homologies.

RESULTS

The 3′-Deletion End Point in TEPC 1017 Is Close to C_δ. To map the 3′ side of the C_μ deletion in the IgD-secreting plasmacytoma TEPC 1017, Southern blots prepared from digested tumor and normal mouse liver DNA were hybridized with probe C, a δ cDNA produced from δ mRNA. The probe C structure is indicated by hatched boxes above or below the maps in Fig. 1. (Lines joining the hatched boxes represent sequences spliced out of precursor RNA in the

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Abbreviations: H, heavy; L, light; V, variable; C, constant; D, diversity; J, joining; USIR, unique-sequence inverted repeat; *S*, switch recombination; kbp, kilobase pair(s); bp, base pair(s).

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formation of mature δ mRNA.) Bold numbers give the sizes in kbp of rearranged bands in the TEPC 1017 tumor DNA. As indicated by the interpretive maps in Fig. 1 *A* and *B*, no deletion or rearrangement occurred in the TEPC 1017 C_δ and δ_s regions between the respective *Bgl* II sites, confirming previous findings (34). However the 3.9-kbp germline *Hin*dIII fragment that spans the C_δ exons was rearranged to 3.2 kbp in TEPC 1017 DNA as shown in the Southern blot using probe C. Therefore, rearrangement of TEPC 1017 DNA occurred between the *Hin*dIII and *Bgl* II sites 5′ to C_δ (Fig. 1*A*). The 3.9-kbp *Hin*dIII band in the TEPC 1017 lane identifies an unrearranged germline chromosome in this near tetraploid tumor (unpublished data).

The presence in TEPC 1017 DNA of an 11.5-kbp rearranged *Eco*RI fragment (Fig. 1*B*) that hybridized to the δ cDNA probe (data not shown) indicated that the *Eco*RI site 5′ to the germline *Bgl* II site (above) was deleted and that the rearrangement had occurred within 670 bp of the $C_\delta 1$ exon.

The 5′-Deletion End Point in TEPC 1017 Eliminates C_μ and Most of S_μ from the Rearranged Allele. To identify the 5′ boundary of the rearrangement, we probed *Eco*RI–*Bgl* II-digested TEPC 1017 and mouse liver DNA with probe B (shown above the maps in Fig. 1 *A* and *B*). The 6.2-kbp *Eco*RI–*Bgl* II germline fragment, which includes S_μ and the $C_\mu 1$ exon, was rearranged to 1.8 kbp in TEPC 1017 DNA, as shown in the Southern blot using probe B. Therefore, from these data and from the size of the rearranged *Eco*RI fragment spanning C_δ (11.5 kbp in Fig. 1*B*), we concluded that the deletion in the rearranged chromosomes of TEPC 1017 eliminates most of the S_μ and all of the C_μ region.

The Expressed TEPC 1017 V_H Allele is Rearranged to J_H3. To examine the *V* and the J_H regions of TEPC 1017, a fragment containing J_H4 and its flanking region (probe A in Fig. 1 *A* and *B*) was employed as a probe of *Eco*RI-digested liver and TEPC 1017 tumor DNAs. In addition to the 6.4-kbp germline band (Fig. 1*A*), probe A hybridized to a single 4.3-kbp *Eco*RI fragment in 1017 DNA (Fig. 1*B*). Additional mapping in the joining (*J*) region showed that the only rearrangement is to J_H3 (data not shown). To confirm that the observed genomic TEPC 1017 rearrangement represents the expressed gene, we isolated and sequenced an overlapping cDNA (Fig. 2) from the same TEPC 1017 library (25) from which we obtained the δ cDNA used as probe C. The sequence of Fig. 2 predicts the existence of unique *Nco* I and *Pvu* II restriction sites in the leader and *V* region segments of the cDNA. This provided a strategy to determine whether sequences rearranged 5′ to J_H3 in TEPC 1017 genomic DNA corresponded to the *V* region expressed in the mRNA (*VDJ3*–

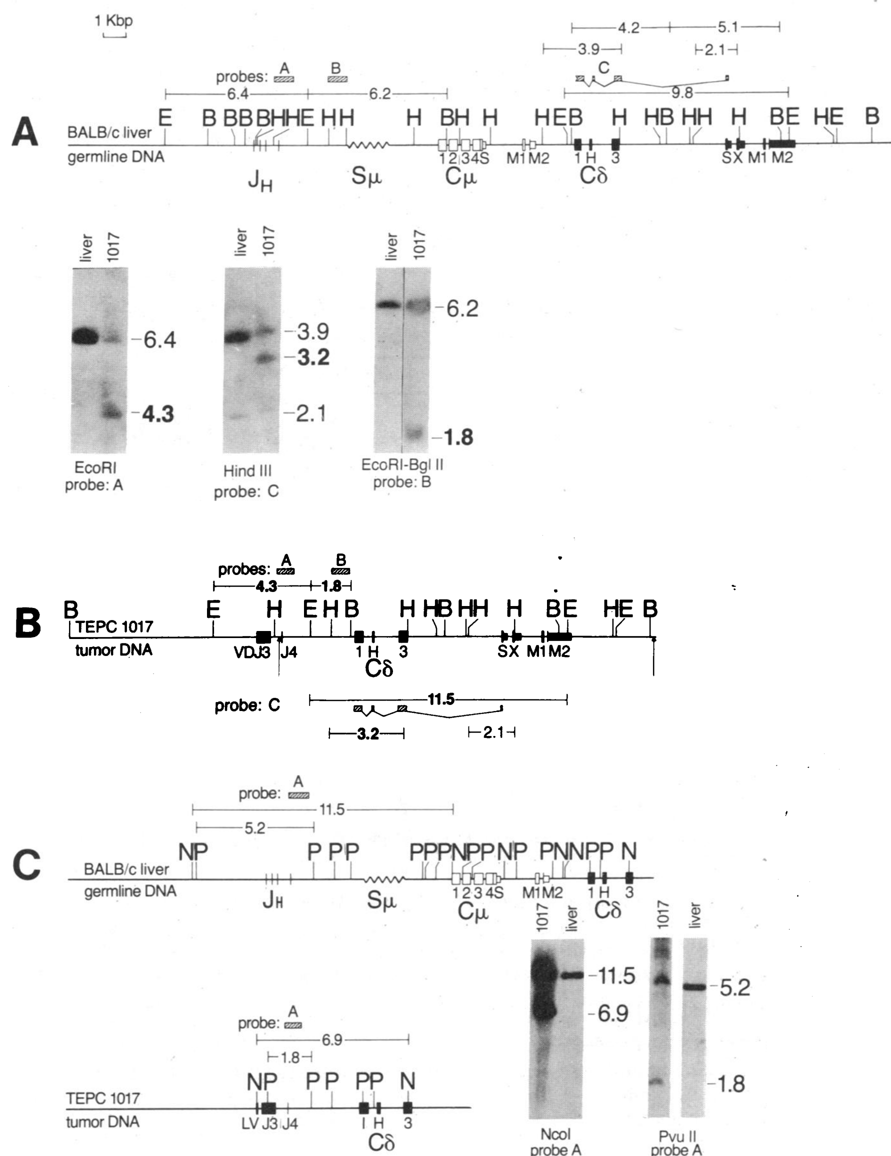

FIG. 1. Comparison of the DNA of TEPC 1017 IgD-secreting plasmacytoma with germline DNA in the C_μ–C_δ region. High molecular weight DNAs (≈25 μg each) from BALB/c liver and TEPC 1017 tumors were digested with the restriction enzymes *Bgl* II (B), *Eco*RI (E), *Hin*dIII (H), *Nco* I (N), and *Pvu* II (P), electrophoresed in 1% agarose gels, and transferred to nitrocellulose paper (21). The blots were hybridized with labeled DNA fragment probe A, B, or C (structures indicated as hatched boxes above or below the interpretive maps): *A*, a 700-base-pair (bp) *Hin*dIII–*Xba* I fragment containing the BALB/c J4 exon and its flanking regions; *B*, an 800-bp *Hin*dIII–*Bgl* II fragment from the TEPC 1017 clone Ch30-573.1 spanning the deletion site; and *C*, a 900-bp *Pst* I fragment prepared from pδ54J cDNA containing $C_\delta 1$, $C_\delta H$, $C_\delta 3$, and δ_s (25). Selected autoradiographs are presented also. Sizes (kbp) of hybridizing bands are indicated above or below the maps and to the side of each autoradiograph. C_H exons are denoted on the maps by solid (C_δ) or open (C_μ) boxes; secreted (*S*), membrane (*M1*, *M2*), *V*, diversity (*D*), and J_H exons are shown similarly. The 3′-untranslated regions are denoted by the narrow boxes. The δ exon with unknown function (X) has been described (33). The μ switch recombination region (S_μ) is shown as ∿∿.

C_δ). As shown in the germline map in Fig. 1*C*, probe A is included within an 11.5-kbp *Nco* I fragment and within a 5.2-kbp *Pvu* II fragment. If the *V* region of *VDJ3–C*$_\delta$ were recombined with J_H3, the predicted lengths of these fragments would be 6.9 and 1.8 kbp, respectively, in TEPC 1017 genomic DNA. The sizes of hybridizing bands on Southern blots (Fig. 1*C*) were in agreement with the predicted values. These results argue that all non-germline chromosomes in TEPC 1017 DNA are identically rearranged to produce the expressed H chain.

δ Expression in TEPC 1017 Is Mediated Via Illegitimate Recombination. To determine the mechanism utilized to delete C_μ, we isolated a genomic clone from a TEPC 1017 library. As shown by arrows in the Fig. 1*B* map, Ch30–573.1 extends 14.6 kbp from the *Mbo* I site between J_H3 and J_H4 and includes all C_δ, δ_s, and δ_m gene segments. Extensive analysis (not shown) confirmed that the map of the clone agreed with that of the TEPC 1017 genomic DNA.

The molecular details of the TEPC 1017 recombination are summarized in Fig. 3. In addition to S_μ, several regions of simple-sequence repetitive DNA occur in the μ–δ locus (14) (denoted 1–6 in Fig. 3; see legend for details). Although repetitive regions are often the site of DNA deletions (39), repeats 1–3 are included well within the deletion and repeats 4–6 are 3′ to it. The germline sequences spanning the recombination joint are indicated in Fig. 3 by underlining. The germline sequences that are deleted are not underlined. The joint is ≈300 bp 5′ of the S_μ region, as defined by a high density of the consensus pentamer unit (G-A-G-C-T)$_{3-17}$ (G-G-G-G-T) (40). The 5′-deletion end points previously determined for other plasmacytomas (see legend to Fig. 3) also map 5′ to the high concentration of consensus pentamers.

The 3′ end point of the TEPC 1017 deletion is within a region of unique DNA sequence 255 bp 5′ to the $C_\delta 1$ exon (Fig. 3). A computer search of the surrounding region for S_μ pentamers, for variants that are repeated in other switch sites (e.g., G-A-G-Y-T, G-G-G-Y-T, where Y=pyrimidine), and for sequences (38) identified near the point of recombination in some tumors (e.g., T-G-G-G, T-G-A-G, and Y-A-G-G-T-G-G) revealed only a single occurrence of G-G-G-G-T within several hundred base pairs. Analysis (31, 32) of several kilobase pairs of flanking sequences revealed no long stretches of base homologies. The two 500-bp segments immediately surrounding the joint gave a patchy homology (39%), which is only slightly higher than for the comparison of two unrelated sequences within the J_H region. Therefore, we concluded that an illegitimate recombination between unique sequences has led, by virtue of C_μ elimination, to a DNA configuration that provides efficient expression of C_δ.

DISCUSSION

Unusual Rearrangement of the C_δ Region in TEPC 1017. We have examined the molecular events that accompany the secretion of IgD in the mouse plasmacytoma TEPC 1017, one of four IgD-secreting tumors that have been reported (17–19). All carry deletions of C_μ, but TEPC 1017 is the only one to be examined at the sequence level. The rearrangement in TEPC 1017 is unusual in that it occurred so close to the body of the C_δ gene (255 bp upstream), in contrast to switch deletion sites in other plasmacytomas, which are commonly 1 or 2 kbp from the first C_H exon (15). However, the most unusual feature of the TEPC 1017 rearrangement is that it occurred in the absence of a typical *S* region 5′ to the C_δ gene. All studies of other productive *S* in plasmacytomas and hybridoma cells have shown that deletion of DNA sequences 5′ to the expressed C_H gene (either γ, α, or ε) involves repetitive *S* regions (reviewed in refs. 10, 15). The deletion end points in TEPC 1033 (unpublished results) and in the IgD-secreting hybridoma B1-8.1 (5) differ from TEPC 1017 but achieve the same result: elimination of C_μ.

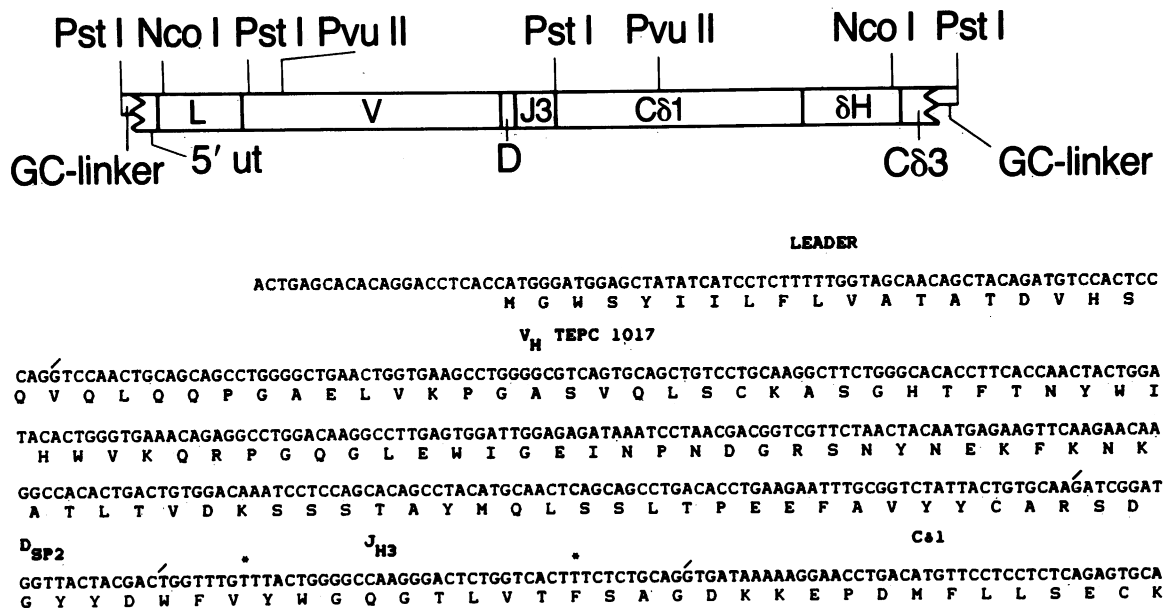

FIG. 2. Physical map and cDNA sequence of the expressed δ chain mRNA of TEPC 1017. Plasmid pVDJ3-C$_\delta$, cloned by G-C tailing into the *Pst* I site of pBR322 (25), contains the complete leader (*L*), *V*, *D*, J_H, $C_\delta 1$, and $_\delta H$ segments. It terminates (indicated by vertical jagged lines) at the 5′ end at position -21 of the 5′-untranslated region (5′ ut) and at the 3′ end 20 bases into $C_\delta 3$. The 5′-most *Nco* I and *Pvu* II sites (indicated in the map) were critical for confirming the expressed genomic equivalent (see text for details). The coding strand of the cDNA sequence is displayed below the map. Predicted amino acids are indicated in a single-letter code directly below the first base of the corresponding codon: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr. The TEPC 1017 V_H belongs to the mouse V_H subgroup II (35). The TEPC 1017 *D* segment (G-A-T-G-G-T-T-A-C-T-A-C) appears to be a somatic mutant of the *Sp2* family (36). The TEPC 1017 J_H3 sequence differs from the germline (29) at two positions (*), which predict amino acid changes of Ala → Val and Val → Phe in 5′ → 3′ order. The nucleotide sequence of the remaining C_δ region (not presented here) agrees with the published sequences for genomic (14) and δ cDNA (37) and confirms the 5′ RNA splice site for $C_\delta 1$ (37).

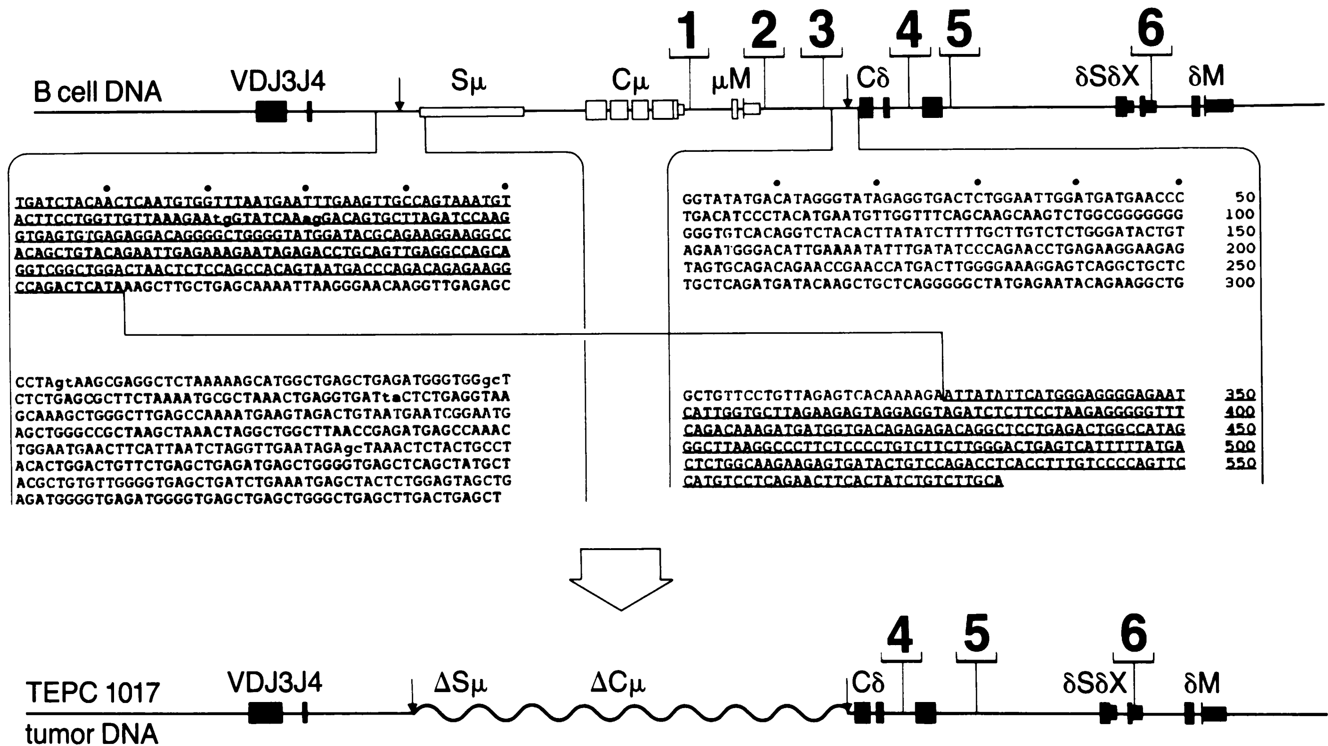

FIG. 3. Deletion of C_μ and rearrangement of C_δ accompanying a C_μ to C_δ switch in the IgD-secreting plasmacytoma TEPC 1017. The upper map shows the presumed DNA structure of an IgM-secreting B-cell precursor. The lower map shows the rearranged TEPC 1017 tumor DNA. The wavy line (∿) indicates the area of the deletion (ΔS_μ, ΔC_μ). Both ends of the deletion are designated with vertical arrows above the maps. Exons are denoted as described in the legend to Fig. 1. The locations of repeated sequences (14) are shown above the maps as 1–6: repeat 1, $(C\text{-}A)_{33}$; repeat 2, $(G\text{-}G\text{-}G\text{-}A\text{-}G\text{-}A)_{12}(G\text{-}A)_{28}$; repeat 3, the unique-sequence inverted repeat (USIR); repeat 4, $(C\text{-}T)_{30}(C\text{-}A)_{30}$; repeat 5, $(G\text{-}A\text{-}A\text{-}A\text{-}A)_{11}$; and repeat 6, $(G\text{-}A)_{16}$. Enclosed within the vertical lines between the maps are germline sequences spanning both sides of the TEPC 1017 deletion. The complete sequence analysis of the J_H–C_μ–C_δ 26-kbp region will be published elsewhere. The sequence retained in TEPC 1017 is underlined. The 5′ end of the TEPC 1017 deletion is at position 261 in the DNA sequences to the left, and the 3′ end of the deletion is at position 326 to the right. We have drawn the deletion sites between the first and second A in the sequences, but several possibilities exist for recombination in the stretch of As present in both regions. The 5′-deletion end points of other plasmacytomas are identified between the lowercase letters in the DNA sequence to the left: MOPC 141-γ2b (position 71); TEPC 15-α (position 80); M167-α and MOPC 603-α (position 305); 53-569-ε (position 348); MC101-γ1 (position 388); MPC 11-γ2b (position 534); IF-2-γ_1 (position 627); and J558-α (position 634–637) (reviewed in ref. 38). The body of the S_μ region is 3′ to the sequences shown to the left here (see upper map). The $C_\delta I$ exon (not shown) begins at position 582 in the sequences to the right.

Illegitimate Recombination in TEPC 1017. Most examples of illegitimate recombination in eukaryotic cells involve the insertion or deletion of mobile genetic elements, as in yeast (41) and *Drosophila* (42), or the stable integration of viral DNA (such as simian virus 40) into nonhomologous sites on the host chromosome (43, 44). Illegitimate recombination has been demonstrated also in the nonspecific end-to-end joining of simian virus 40 and pBR322 sequences by monkey kidney cells (45, 46). In all cases, there is little or no sequence homology involved in the recombination. The illegitimate recombination leading to IgD secretion in TEPC 1017 shares these features but, in contrast, may be generated by unequal exchange as seen in other C_H genes. Conventional class switches, on the other hand, differ from the TEPC 1017 deletion in that heteroduplex formation results from alignment of homologous consensus pentamers on both the 5′ and 3′ sides of the breakpoint, even though the site of recombination *per se* may be upstream.

In considering a mechanism for the illegitimate recombination in TEPC 1017, we were struck by the proximity of the 3′-deletion end point to the large USIR (repeat 3 in Fig. 3). Formation of an almost perfectly paired 162-bp stem of the USIR appears to be energetically favored, judging from sequence calculations (14) and from direct denaturation electron microscopic analysis (7). A potential role for this structure in heteroduplex formation was suggested by computer-generated sequence alignments. Arbitrarily defined 500-bp sequence blocks that span the 5′ and 3′ sides of the USIR stem gave the highest percent homology (47% and 37%, respectively) when aligned with blocks that spanned the 5′- and 3′-deletion end points (data not shown). The alignments required sizable gaps, but the resulting best-fit pairing would bring the deletion end sites to within 20 bp of each other. Others have postulated that inverted repeats constitute special sites of cleavage that lead to initiation of strand exchange in prokaryotes (47, 48).

Copious Secretion of C_H Gene Products May Require Shortened Transcription Units. Each Ig class has specific membrane and secreted forms that differ only in the carboxyl-terminal portion of the H chains. A separate exon that encodes the secreted terminus is present as an extension of the last C_H domain in all C_H genes except δ. The δ gene is unique in that the δ_s exon is separated from the body of the C_δ gene by an intervening sequence of 4.5 kbp.

In all Ig-secreting cells (plasmacytomas and hybridomas) investigated, deletion of intervening C_H regions occurs. Thus, the most strongly expressed mRNA (that for the secreted form) is defined by the first transcriptional stop sequence in the DNA following the promoter. Expression of secreted IgD from a DNA configuration in which C_μ was present would require a minimal transcriptional unit of ≈26 kilobases, almost three times that calculated for the RNA precursor of other Ig classes in plasmacytomas (average, ≈10 kbp). In contrast, a deletion of C_μ as observed in TEPC 1017 would result in a RNA precursor of only 11 kilobases. Transcriptional control of this pattern of expression may be exerted at

the level of termination (49). We cannot rule out, however, that factors independent of the deletion of C_μ (e.g., secondary structure, methylation, etc.) might up-regulate the expression of secreted δ chains in TEPC 1017 nor can we exclude the nonspecific effects of the transformed state.

Secretion of IgD by Normal Murine B Cells. Our analysis of TEPC 1017 may provide an answer for the mode of IgD secretion by tumors but it raises a more fundamental question concerning the mechanism utilized in the normal immune response. Even though IgD-secreting plasma-cell subsets have been identified in the spleen (50), levels of $_\delta s$ chain mRNA (5, 34) or protein (34) in spleen are barely detectable. We previously hypothesized (33, 49), based on the presence of a $_\delta s$ precursor mRNA in plasma cell-depleted splenic B cells (49), that the low levels of $_\delta s$ message are transcribed from the (VDJ–C_μ–C_δ) unrearranged DNA template. However, lymph nodes, which contain significantly more secreted IgD (D. Yuan, personal communication), may be the primary source of IgD in the serum. This tissue would represent the most logical source to search for an analogue of the unique illegitimate recombination utilized by TEPC 1017.

Finally, the dramatic difference seen in the structures of IgD in mouse (37) and man (51) suggests a major evolutionary divergence of C_δ in the two species. This divergence may also be reflected in the noncoding regions between C_μ and C_δ. Recent studies in our laboratories (unpublished results) indicate that the human C_δ gene is preceded by a class switch-like repetitive sequence that may allow expression of δ by means of nonhomologous recombination. This more efficient mechanism for C_μ deletion may account for the significantly higher serum IgD levels and for the increased occurrence of IgD-secreting myelomas in humans.

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