Expression of IgD may use both DNA rearrangement and RNA splicing mechanisms

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ABSTRACT From a library of mouse sperm DNA, we have isolated two overlapping clones which contain the Cg gene. One of these clones also contains the Cδ gene. The Cg gene is separated from the Cg membrane exons by approximately 2 kilobases (kb) of DNA. The Cδ gene was identified by (a) hybridization to poly(A)+RNA prepared from the IgD-producing rat plasma cell tumor IR731, and (b) homology of a translated nucleotide sequence to the amino acid sequence of the human δ chain. The Cδ gene spans 8 kb of DNA in the germ line. Plasmid subclones of the Cg gene were used as probes in Southern and RNA blot experiments. RNA blot analysis of poly(A)+RNA from IR731 and a μ*δ* B-cell hybridoma revealed 1.6- and 2.7-kb δ mRNA species with different 3' ends, which presumably encode the secreted and membrane-bound forms, respectively, of the δ chain. Southern blot analysis of DNA from two μ*δ* lymphomas revealed that the Cδ gene is in the germ-line configuration in each case. Restriction map analysis of Cg and Cδ genomic clones isolated from a library of normal μ*δ* B-cell DNA also gave no evidence for DNA rearrangement in the region between the Cg and Cδ genes. Taken together, these data suggest that IgD expression in μ*δ* B cells does not involve a VH-to-Cg DNA switch rearrangement. We propose that simultaneous expression of Cg and Cg in a single VH gene is mediated by two alternative routes of RNA processing of a primary nuclear transcript which contains the VH, Cg, and Cδ genes. In contrast, analogous experiments with myeloma IR731 DNA revealed that the Cg gene has been deleted from the myeloma DNA and that the Cg gene has undergone DNA rearrangement, presumably including a switch recombination of the VH gene from the Cg to the Cg gene. These results indicate that two alternative mechanisms may be used in the expression of IgD molecules—RNA splicing in B cells and DNA rearrangement in plasma cells.

An immunoglobulin heavy chain is composed of a variable (Vh) region and one of five classes of constant (Ch) region: Cμ (IgM), Cδ (IgD), Cε (IgG), Cα (IgA), and Cγ (IgE). In mice, the five CH classes are encoded by eight distinct genes: Cμ, Cδ, Cε, Cα, Cγ1, Cγ2, Cγ3, and Cγ4. Early in its development, a lymphocyte (B cell) bears only IgM on its surface; later, IgD molecules are often expressed together with IgM molecules (for review, see ref. 1). Upon interaction with antigen, a B cell proliferates and differentiates, ultimately becoming a plasma cell. The class of immunoglobulin produced by its progeny may change, from IgM (and IgD) to IgA, IgG, or IgE.

The molecular mechanisms by which these genes are expressed during B-cell development have been partially characterized. The VH gene is joined to the CH gene by assembly of three gene segments: V, D, and J (2, 3). Presumably, upon recognition of antigen, the VH gene, along with some Cγ5′-flanking sequence, is joined to another CH region in a phenomenon called the heavy-chain switch (4–6). Honjo and coworkers (7, 8) have suggested that the intervening DNA, containing the Cγ gene and perhaps other CH genes, is deleted.

Both IgM and IgD molecules are present on the surface of the B cell. Experiments utilizing allotypic markers have shown that their expression conforms to the rule of allelic exclusion: both heavy chains on the surface of an individual cell are encoded by the same chromosome (9). Moreover, considerable evidence suggests that these two cell-surface molecules bear identical VH regions (10–13). These data are difficult to reconcile with the Cg gene deletion model because they imply that a VH gene can be expressed with the Cg gene without concomitant deletion of the Cg gene. A number of mechanisms have been suggested to account for these observations, including the "copy-insertion" mechanism (14) in which a copy of the VH gene is joined to the Cg gene while the original remains joined to the Cg gene, and differential RNA processing of a single transcript containing VH, Cg, and Cg genes (15).

This paper reports experiments carried out to test these hypotheses. The results support RNA splicing as the mechanism by which the Cg and Cg genes are expressed simultaneously in B cells and also suggest that VH-to-Cg DNA switch recombination may occur in plasma cells that express only IgD.

MATERIALS AND METHODS

Rat IgD Myeloma. The rat myeloma IR731 (16), a plasmacytoma, was passaged subcutaneously in Lou/M/Wsl N rats (NIH). Total cell poly(A)+RNA was prepared from IR731 by a method similar to that of Chirgwin et al. (17), followed by oligo(dT)-cellulose chromatography. This RNA was hydrolyzed with base to an estimated average size of ~500 nucleotides and labeled with 32P by using [γ-32P]ATP and polynucleotide kinase for use as a probe. Cytoplasmic poly(A)+RNA for RNA blots was prepared from IR731 as described (18).

BALB/c μ*δ* Lymphomas. These lymphomas, the generous gifts of R. Asofsky and K. Jin Kim, were passaged subcutaneously in BALB/c mice (19). The presence of cell-surface IgM and IgD molecules was verified by immunofluorescence using anti-IgM (Cappel) and monoclonal anti-IgD (Becton Dickinson) reagents. GCL-2.1 cells were from W. Raschke. DNA from these and other tissues was prepared by the method of Blin and Stafford (20).

Germ-Line CH Clones. The germ-line clone ChSpμ7 has been described (4, 21). The clone ChSp37 was isolated from the same library of mouse sperm DNA as ChSpμ7. The positions of CH sequences in these clones were determined by hybridization of [5′-32P]poly(A)+RNA from IR731 to blots of restriction digests of these clones. Subclones of the hybridizing region

Abbreviations: VH, heavy chain variable; CH, heavy chain constant; kb, kilobase(s).
regions were generated by ligation of restriction fragments into the corresponding site or sites of pBR322 and were used as probes in Southern (22) and RNA blot experiments. The cDNA clone p104Eµ/12 (21) was used as a probe for C\textsubscript{\textdelta} sequences. The J\textsubscript{\textdelta} probe, containing the J\textsubscript{\textdelta} gene cluster and 3'-flanking sequence, was prepared by M. Steinmetz.

**BALB/c \mu \delta Normal B-Cell DNA Library.** IgM-positive cells were isolated from BALB/c spleens by using a fluorescence-activated cell sorter. Ninety-three to 97% of the purified cell population stained positively for surface IgM, and 99% was positive for surface IgD. A library of 12 × 10\textsuperscript{6} recombinant phage was constructed by ligation of EcoRI partial digests of \mu \delta spleen cell DNA to phage vector Charon 4A (23), followed by in vitro packaging (24).

All manipulations of microorganisms containing recombinant DNA were carried out under P2/EK2 or P2/EK1 conditions prior to January 1980, after which P1/EK2 and P1/EK1 conditions were used.

DNA sequence analysis was as described (25).

**RESULTS AND DISCUSSION**

**Characterization of Genomic Clones.** [5',-32P]Poly(A)\textsuperscript{-}RNA prepared from IR731 was used to screen a number of genomic clones known to contain mouse immunoglobulin C\textsubscript{\textH} region genes or their flanking sequences. Two BALB/c sperm DNA clones, ChSp47 and ChSp37, hybridized to this probe. These clones were subjected to restriction map analysis (Fig. 1). The [5',-32P]poly(A)\textsuperscript{-}RNA probe hybridized to three discrete regions of the cloned DNA.

**Identification of the C\textsubscript{\textdelta} Gene.** Restriction fragments hybridizing to IR731 poly(A)\textsuperscript{-}RNA were subcloned into the plasmid vector pBR322 (Fig. 1). A DNA sequence determined near the 3' end of the p824 clone (Fig. 2, part B) appeared to encode the first third of an immunoglobulin domain, with a cysteine residue and several conserved amino acids in the appropriate positions. This amino acid sequence is translated from the only open reading frame and is associated with a possible downstream splicing site. This sequence displays striking homology to the protein sequence of the C\textsubscript{\textdelta} domain of human IgD (26): 18 of 33 residues, including a stretch of 10 surrounding the cysteine residue are identical, and an additional 10 are either conservative substitutions or can be attributed to a single-base difference in the genetic code. Thus, this sequence codes for the C\textsubscript{\textdelta} domain of mouse IgD.

**C\textsubscript{\textdelta} Gene Encodes Two mRNAs with Alternative 3' Ends.** The germ-line DNA subcloned in p82 contains the C\textsubscript{\textdelta} domain of the \delta gene and, as argued above, probably additional C\textsubscript{i} domains as well. Labeled total cellular poly(A)\textsuperscript{-}RNA from IR731 also hybridized with sequences 3-7 kb to the C\textsubscript{\textdelta} encoding sequence (Fig. 1). These regions were subcloned as p832 and p88. Both p832 and p88 contain only single-copy sequences and do not cross hybridize with each other or with p82. That this DNA also contains \delta gene sequences was demonstrated by RNA blots with IR731 mRNA and nick-translated p832 and p88 as probes. Hybridization of C\textsubscript{\textdelta} probes (p82) to IR731 poly(A)\textsuperscript{-}RNA revealed a major \delta mRNA species of 1.6 kb and a minor species of 2.7 kb (Fig. 3). In addition, the 1.6-kb species hybridized with p832, and the 2.7-kb mRNA hybridized with p88. These results suggest that p832 and p88 contain separate, noncontiguous gene segments which represent alternative 3' ends for the 1.6- and 2.7-kb mRNAs. At present we do not know whether each gene sequence is composed of one or more exons. Nonetheless, these results indicate that C\textsubscript{\textdelta} gene sequences that are contiguous in \delta mRNA occupy about 8 kb of germ-line DNA.
We have also observed two corresponding species in a mouse μ-δ' cell line (GCL-2.1) (Fig. 3).

Because the 1.6-kb mRNA is the major species in IR731 myeloma cells which secrete IgD, we propose that p832 encodes a δ terminus for δ chain secretion. The p88 sequence in the 2.7-kb mRNA may encode a δ m terminus for membrane-bound δ chains. We propose that these alternative 3' sequences are spliced to the Cγ3 domain to generate either δ or δ m mRNA. This arrangement is different from that of μ and μ m RNA, in which the μ m terminus encoding sequence is contiguous with the C4 domain (15, 18).

μ-δ' B-Cell DNA Contains Rearranged Cμ and Germ-Line Cγ Genes. The lymphoma lines L10A and K46 are B-cell tumors and express both IgM and IgD molecules on the cell surface (19). We carried out Southern blot experiments with DNA prepared from these tumor cells, using BALB/c embryo DNA as a control. Fig. 4A shows a Southern blot of a Kpn I digest of mouse embryo and K46 DNA with the Cμ cDNA clone as probe. In embryo DNA, this Cμ probe hybridizes to a fragment 13 kb long. This fragment contains both the Cμ gene and the JH gene cluster (27). In K46 DNA, the Cμ gene appears on an ∼20-kb restriction fragment, which indicates that a DNA rearrangement has occurred, presumably the V-J joining event (27) (similar results were obtained with L10A DNA). Note that a faint band is present in K46 DNA which corresponds to the germ-line Cμ gene. Both K46 and L10A are polyclonal (28), and this band could represent an unrearranged chromosome. Alternatively, it could arise from DNA derived from contaminating host tissue.

The corresponding data for the Cγ gene are shown in Fig. 4B for K46, embryo, and L10A DNAs. The probe used in this experiment was a 0.68-kb BamHI/Xba I restriction fragment from p82 which contains the Cγ3 domain and 340 nucleotides of pBR322. This Cγ probe hybridizes to identical bands in both embryo and lymphoma DNA restriction digests. No other hybridization could be detected. Furthermore, the bands observed in Fig. 4B are identical in size to those observed in ChSpa7 and ChSp37, which demonstrates that these latter clones contain no detectable cloning artifacts.

Analogous experiments were carried out with p88 and p832 as probes. In each case, hybridization to germ-line and lymphoma DNA restriction digests gave similar results (data not shown), indicating that no rearrangement of the Cγ gene or its flanking sequences occurs in μ-δ' lymphoma DNA.

We also have obtained similar results by using the μ-δ' normal B-cell library. When 1.75 × 10⁶ and 3.2 × 10⁶ recombinant phages were screened with the Cμ and Cγ probes, respectively, a total of 4 Cμ clones and 11 Cγ clones were isolated. Six of the 11 Cμ clones also contained the Jμ locus, and 5 of these showed rearrangements consistent with Vμ-D-Jμ joining. Restriction map analysis of the Cμ and Cγ clones revealed that they all contain only germ-line DNA within the Cμ gene, between Cμ and the downstream EcoRI site (Fig. 1), and in the Cγ gene and 3'-flanking sequence. The possibility that we simply failed to clone or detect a rearranged Cγ gene cannot be ruled out; however, these results are consistent with those described above and suggest that, insofar as can be detected by gel electrophoresis, the Cμ gene in μ-δ' B cell and lymphoma DNA is unrearranged.

Thus, we conclude that the simultaneous expression of IgM and IgD molecules on the B lymphocyte surface does not involve rearrangement of the Cγ gene or, by implication, a Vγ-Cγ DNA switch recombination.

IR731 Myeloma DNA Contains a Rearranged Cγ Gene and Has Deleted the Cμ Gene. Using the μ cDNA clone and the restriction fragment encoding the Cγ3 domain as probes, we also examined the disposition of the Cμ and Cγ genes in IR731 myeloma DNA. DNA from Lou/M/Wsl N rat liver served as a control (Fig. 5). Both probes cross hybridized with the rat genes on a Southern blot of Lou/M/Wsl N liver DNA. Results obtained with the Cμ probe indicate that the Cμ gene has been largely deleted from the myeloma DNA (Fig. 5A). The corresponding experiment with the Cγ3 probe and three restriction endonucleases revealed that the Cγ gene had undergone DNA rearrangement.

These data indicate that the Cγ gene in this IgD-producing myeloma has been rearranged, unlike that in mouse μ-δ' B cells. Several explanations for these contrasting results should be considered. First, IgD expression in the rat may be fundamentally different from that in the mouse, although we believe that there exists no other evidence to indicate that this might be the case. Second, these observations could be an artifact of the myeloma condition. For example, the significance of "abortive" rearrangements observed in mouse plasmacytomas (29) is not understood. Finally, these results could conceivably be attributed to a minor substrain difference between Lou/M/Wsl N, the tumor host, and Lou/C/Wsl (16), in which IR731 appeared.

![Figure 4](image-url)
To address the latter two questions, we carried out Southern blot experiments using p88, p832, a γ3 cDNA clone from myeloma J606, and a plasmid subclone containing the mouse JH gene cluster and its 3' flanking sequence. The p88 and p832 clones gave similar results with Lou/M/Wsl N liver and Lou/C/Wsl myeloma (Fig. 5C) DNA: in each case, both hybridized to a single 5.7-kb EcoRI fragment. The γ3 cDNA probe also hybridized to 9.2-, 14-, and 16-kb EcoRI fragments in both DNA samples. Thus, the Cγ genes and the counterparts of p832 and p88 in the rat genome display no polymorphism in the two substrains. These results suggest that DNA rearrangement rather than restriction enzyme site polymorphism is responsible for the observed difference in hybridization of the Cγ probe to Lou/M/Wsl N liver and IR731 DNA and that this rearrangement has occurred on the 5' side of p832 and p88.

Although the Cγ gene had been deleted from IR731 DNA, results obtained with the Jγ probe (Fig. 5C) indicate that the JH genes and 3'-flanking sequence are still present and have been rearranged, as would be expected for a V-D-JH joining event. This observation suggests that a Vγ-Cγ switch recombination has indeed occurred in IR731 DNA.

**Two Alternative Mechanisms for the Expression of IgD.** Based on the results described above, we propose the existence of two different molecular mechanisms for the expression of the Cγ gene. First, in μ' δ' B cells, these results suggest regulation at the level of RNA processing, probably involving multiple sites for splicing and poly(A) addition. This type of control has been observed in late adenovirus mRNA processing (30–32) and has been implicated in the synthesis of membrane-bound and secreted IgM (μm and μ) mRNA from a single transcript (15). This mechanism presupposes the existence of a poly(A) addition site 3' to the Cγ gene. Extension of a transcript beyond the poly(A) addition sites of μ, μm, mRNA would then generate the precursor of δ mRNA. Wall et al. (33) pointed out that, according to this model, the preferential utilization of the μ, poly(A) site which would accompany the initiation of μ synthesis would halt production of membrane-bound IgM and IgD molecules. Results have been obtained which agree with this prediction (34–36). The Cγ gene thus could be another example in eukaryotes in which developmentally regulated RNA splicing generates alternative protein forms. We believe that this control mechanism will be a general one in eukaryotic gene expression.

The second mechanism, apparently used by the rat IgD myeloma IR731, involves deletion of Cγ and rearrangement of Cγ, presumably via a Vγ-Cγ switch recombination. We infer the presence of one or more switch sites (37), probably in the region between Cγ and the IgM M exons. At present, the mechanism of this particular switching event remains unknown. We predict that, generally, IgD-secreting cells also will exhibit rearrangement of the Cγ gene. A schematic representation of these mechanisms is given in Fig. 6.

**Cγ Gene Linkage Family and Cγ Gene Expression.** The data in this paper and those from other laboratories (38–40), allow construction of a linkage map of the immunoglobulin heavy chain gene family (Fig. 6, top line). Because the separation between the Cγ and Cγ genes is substantially smaller than that for other Cγ genes, we believe that the Cγ-Cγ system is likely to be the only pair of Cγ genes that uses an RNA processing mechanism as proposed above for their expression. Expression of other heavy chain genes (C, C, C, C) most likely will occur only by the Cγ switching mechanism involving DNA rearrangement.

![Diagram](image)

**Fig. 6.** Proposed mechanisms for expression of IgD in B cells and plasma cells. The top line represents the current germ-line linkage map (38–40) of the immunoglobulin Cγ locus. Known distances are in kb. The putative δ exon is indicated by ?.
Note. After this manuscript was submitted for review, two articles (41, 42) describing detailed structural studies of the mouse C\(_2\) gene appeared. Our data on the structure of the C\(_2\) gene largely agree with the data presented in them.

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