

DOUBLE ISOTYPE PRODUCTION BY A NEOPLASTIC B CELL LINE

II. Allelically Excluded Production of μ and $\gamma 1$ Heavy Chains Without C_H Gene Rearrangement

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Ig class switching is a process in which a single clone of B lymphocytes synthesizes IgM antibody, and subsequently synthesizes another isotype other than IgD with the same specificity for antigen. Thus, the same heavy chain variable region gene (V_H) formerly associated with the C_μ gene is subsequently associated with one of several constant region genes (C_H) 3' to C_δ . (reviewed in reference 1). Expression of C_H genes downstream to C_δ (e.g., C_γ , C_ϵ , C_α) in transformed cell lines involves deletion of DNA 5' to the expressed C_H gene (1, 2). Studies of the structure of Ig genes in myelomas or hybridomas (reviewed in references 3 and 4) have led to the conclusion that the isotype switch is mediated by DNA recombination between tandemly repeated switch (S)¹ sequences located 5' to each C_H gene, with the exception of C_δ production (5, 6). Cloned cell lines, including BCL₁.B₁ (7), simultaneously express both μ and δ genes in the same unrearranged DNA context as in μ -producing cells, and therefore, must produce these isotypes by alternative RNA processing and/or termination of a 26-kb RNA transcript (7-11).

Mitogen- or antigen-activated cells are reported to express IgM in combination with isotypes other than δ (12-18). Southern blot analyses have raised the issue of whether these normal cells use downstream C_H genes, either singularly or in combination, in the same manner as plasmacytomas and hybridomas. Yaoita et al. (17) presented evidence that C_μ is not deleted in surface IgM⁺IgE⁺ (sIgM⁺sIgE⁺) spleen cells from SJA/9 mice infected with parasites. Their finding that all C_H genes were retained in the germline context suggested that the expression of IgM and IgE is mediated by the splicing of an 180-kb RNA

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¹ Abbreviations used in this paper: s, switch region; sIg, surface Ig; UT, untranslated region.

transcript. Because of the induction of avid Fc receptors for IgE after infection of SJA/9 mice with such parasites, there is a strong possibility that the majority of the $\sim 10\%$ sIgE⁺ cells were not synthesizing IgE, but were absorbing it from the culture medium. This hypothesis is strongly supported by recent data of Katona et al. (19). Studies by Perlmutter and Gilbert (18) also support the long-transcript model. Using a combination of cell sorting, Southern blotting, and sandwich RNA hybridization, they concluded that sIgG1⁺ splenic cells have long RNA transcripts generated from unrearranged DNA that contain both γ and μ sequences. However, as in the case of the report by Yaoita et al., their interpretations depend upon cell homogeneity. For example, they claimed to have successfully sorted 1–3% sIgG1⁺ cells to 99% purity, yet no data were shown to estimate the level of contaminating sIgM⁺ cells in their sIgG1⁺ population.

The mechanism of double isotype production could give insights into the genetic regulation of Ig synthesis in differentiating B cells. To analyze such mechanisms requires a constitutively expressing clonal analogue. In our accompanying paper, we described a switch variant derived from the BCL₁.B₁ in vitro line in which each cell expresses both surface IgM and surface IgG1 and also secretes both isotypes. The IgM and IgG1 share the same idiotype and use the same VDJ rearrangement. In this paper, we present evidence, based on the C_H gene context of several independently isolated IgM/IgG1-producing clones (collectively termed BCL₁.B₂), that both μ and $\gamma 1$ heavy chains are transcribed in germline DNA configuration from a single copy of chromosome 12.

Materials and Methods

Cell Lines and Subcloning. The derivation of the two BCL₁.B₁ subclones, BCL₁.2.62 (IgM producer) and BCL₁.2.58 (IgM/IgG1 producer), is described in the accompanying paper. BCL₁.2.54 is a sister subclone of BCL₁.2.58. For DNA analysis, we have employed additional independently derived IgM/IgG1 subclones of the parental BCL₁.B₁ line. The subclone BCL₁.13.92 was 1 of the 12 BCL₁.B₂ subclones obtained at a frequency of 1.35×10^{-2} (12/888) by depositing one cell per well using a FACS III. The medium used for subcloning is the same as described in the accompanying paper. The subclone BCL₁.6.1 is a subclone of BCL₁.2.58, derived in the same way. The subclones BCL₁.15.9 and BCL₁.15.19 were two of the seven BCL₁.B₂ subclones derived similarly at a frequency of 5×10^{-3} (7/1429), except that no WEHI 274 SN was added to the medium. As in the case of BCL₁.2.58, all subclones except BCL₁.6.1 secrete more IgM than IgG1 and preliminary data of surface immunofluorescence indicate that all subclones express sIgM and sIgG. BCL₁.6.1 secretes more IgG1 than IgM (Y.-W. Chen et al., unpublished data).

Southern Hybridization Analysis. Procedures for genomic DNA isolation and blotting were described in the preceding paper. All washings were done in $3 \times$ SSC plus 0.1% SDS, three times at room temperature, followed by washing in $0.1 \times$ SSC, 0.1% SDS at 42°C for 1 h, with the exception of the p ϵ - (see probes) probed filter, which was washed at 65°C. Restriction endonucleases were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN), New England Biolabs (Beverly, MA), and Bethesda Research Laboratories (Gaithersburg, MD) and used according to the manufacturer's instructions. Electrophoresis was carried out as described in the accompanying paper (in 1% agarose at 40 V for 18 h).

Probes. Genomic or cDNA probes were prepared by gel isolation (20) of restriction fragments from previously characterized recombinant clones, except for p $\gamma 1$ and p ϵ where the whole plasmids were used. To insure that no repetitive sequences were present in the probes, particularly in fragments derived from introns, DNAs were digested with various

enzymes and subjected to Southern analysis with ^{32}P -labeled mouse genomic DNA as a probe. Only nonhybridizing fragments were employed for further experiments.

Relative positions of the probes are shown in Figs. 2, A–R and 6, S–U. All DNAs were labeled by nick translation (21) to a specific activity of $\sim 10^8$ cpm/ μg . Probe A: A 700-bp Pvu II/Pst I fragment of p μ 3741 (cDNA clone) (22) containing 18 bp of C μ 2, the entire C μ 3 and half of the C μ 4 region of μ chain mRNA. Probe B: A mixture of 1.95-kbp and 1.25-kbp Hha I/Bam HI fragments from pB2 (genomic clone) (P. Fell, personal communication) containing the C μ 1, C μ 2, C μ 3, and C μ 4 exons of C μ . Probe C: A 900-bp Pst I/Pst I fragment of p δ 54J (cDNA clone) (23) containing C δ 1, δ H, C δ 3 and the secreted carboxyl terminus (δ s) of δ chain mRNA. Probe D: A 1.3-kbp Bam HI/Bam HI fragment of pCp13 (genomic clone) (24) containing the carboxyl terminal membrane (M) exons δ M1 and δ M2 of the C δ gene. Probe E: A 1.2-kbp Eco RI/Eco RI fragment of γ 3-13 (genomic clone) (25) containing C δ -C γ 3 intronic sequences. Probe F: A 1.0-kbp Eco RI/Eco RI fragment of γ 3-13 (25), located downstream of probe E. Probe G: A 2.0-kbp Hind III/Hind III fragment of pH311 (subcloned from genomic phage γ 3-25) (25) containing 5' flanking region of the C γ 3 gene. Probe H: A 1.0-kbp Xba I/Hind III fragment of pJW7 (genomic clone) (26) containing the 5' flanking region of the C γ 3 gene. Probe I: A 473-bp Bste II/Sph I fragment of pDRI (genomic clone) (26) containing most of C μ 3 and 3' untranslated region (UT) of the C γ 3 gene. Probe J: A 1.4-kbp Xba I/Hind III fragment of pJW7 (genomic clone) (26) containing C γ 3, M2 segment and 3' UT. Probe K: A 1.0-kbp Sac I/Hind III fragment of γ 1-6 (genomic clone) (25) containing the flanking region 5' to S γ 1 (switch region of C γ 1 gene). Probe L: A 1.7-kbp Xba I/Eco RI fragment of γ 1 (genomic clone) (27) containing the 5' flanking region of the C γ 1 gene (3' to the S γ 1 region). Probe M: γ 1 (27); pBR322 plasmid containing a 6.6-kbp Eco RI insert of the entire C γ 1 gene and its 5' and 3' flanking regions. Probe N: A 2.1-kbp Xba I/Sac I fragment of γ 1 (genomic clone) (27) containing the C γ 1 structural gene. Probe O: A 1.2-kbp Sac I/Eco RI fragment of γ 1 (genomic clone) (27) containing the 3' flanking region of the C γ 1 gene. Probe P: A 1.0-kbp Bam HI/Kpn I fragment of p γ 2b(11)⁷ (cDNA clone) (28) containing the entire coding sequence of γ 2b chain message. Probe Q: p ϵ (29); pBR322 plasmid containing a 4.4-kbp Bam HI insert of the C ϵ structural gene and its 5' and 3' flanking regions. Probe R: A 900-bp Bste II/Bste II fragment of p α (J558)¹³ (cDNA clone) (30) containing C α 1, C α 2, and most of C α 3 of α chain mRNA. Probe S: A 2.0-kbp Bam HI/Eco RI fragment of pJ μ 3J μ 4 (subcloned from genomic clone CH4A-142.7) (31) containing J μ 3, J μ 4 and ~ 1.5 kb of 3' flanking sequence. Probe T: A 674-bp Eco RI/Hind III fragment located just 3' to probe S subcloned from genomic clone CH-28.289.1 (27; P. W. Tucker, unpublished data). Probe U: A 760-bp Hind III/Hind III fragment located just 3' to probe T (subcloned from genomic clone CH-28.289.1) (P. W. Tucker, unpublished data). Probe V: 1,774-bp Sph I/Bam HI fragment of pDRI (26) containing the entire C γ 3 coding region and 3' UT of the secreted terminus.

Karyotyping. Cells from the BCL $_1$ B $_1$ and BCL $_1$.2.58 lines were grown to exponential phase and cells were harvested for karyotypic G-banding analysis (32, 33).

Densitometry. For band quantification, autoradiographs were scanned with a densitometer (model GS 300, Hoefer Instruments), and densities were calculated using a Bio-Rad model 33E2A integrator.

Results

BCL $_1$.B $_1$ and BCL $_1$.2.58 Cells Carry Two Copies of a Translocated Chromosome 12. Two reports (34, 35) of the karyotype of the in vivo BCL $_1$ cell line were in conflict with respect to the copy number and constitution of the IgH-bearing chromosome 12 (36). The single J μ rearrangement observed in the Eco RI blots of the in vitro lines (Fig. 5 of companion report) was consistent with either a single copy of VDJ or multiple copies of the same VDJ. Resolution of this question, imperative for evaluation of the Southern blotting data presented

below, required a careful cytogenetic evaluation of the in vitro-adapted parental and variant lines.

Chromosome analysis of BCL₁B₁ and one of the IgM/IgG1-producing subclones, BCL₁.2.58, was performed by Giemsa banding techniques. The modal chromosome number in BCL₁.B₁ was 62 (with 55–63 chromosomes in 52 cells analyzed). The modal chromosome number in BCL₁.2.58 was 63 (57–66 chromosomes in 65 cells analyzed). We karyotyped 25 cells from BCL₁.2.58 and 19 cells from BCL₁.B₁. We found that the in vitro BCL₁.B₁ line has two copies of chromosome 12 per cell. These two chromosomes appear to be identical, and both have an unusual translocation to chromosome 16 [t(12;16)], which carries the λ light chain gene (37) (Fig. 1*b*). Likewise, in BCL₁.2.58, 24 out of 25 cells carry two t(12;16) chromosomes (Fig. 1*c*). One of 25 cells has only one t(12;16), probably due to a random loss of this chromosome during the preparation of the slides. It appears that at the microscopic level both t(12;16) chromosomes identified in BCL₁.2.58 are indistinguishable from those of BCL₁.B₁. One normal chromosome 16 was found in every cell karyotyped.

The breakpoint on chromosome 12 is in the distal region (12F2), telomeric to the V_H region cluster (Fig. 1*a*) (36, 38). The breakpoint on chromosome 16 is at a region proximal (16B3) to the centromere, based on the ideogram proposed by Nesbitt and Franke (39). Presumably, this would translocate λ genes to chromosome 12, although their definitive band location on chromosome 16 has not been established. Therefore, heavy chain expression in BCL₁.B₁ and BCL₁.B₂ is derived from either one or two copies of an apparently identical translocated chromosome 12, but the breakpoint of the translocation is 5' to the rearranged V_H gene(s). Light chain expression (λ_3) could conceivably be generated from either the translocated or normal chromosome 16.

Strategy for Determining the J_H-C_H Gene Context of BCL₁.B₁ and BCL₁.B₂. In the accompanying paper we have shown that all BCL₁.2.58 cells synthesize both IgM and IgG1, and both isotypes carry the same serologically defined idiotype. To determine whether the expression of μ and γ 1 genes is allelically excluded and, if so, the DNA context of the C_H locus, we have carried out an extensive overlapping Southern blotting analysis of BCL₁.B₁, BCL₁.2.58 and several other independently derived IgM/IgG1-producing subclones (Materials and Methods). Fig. 2 summarizes the probes and restriction digestions employed with respect to the composite germline C_H map of Shimizu et al. (25). Considerable care was taken to insure the absence of repetitive sequences.

All C μ Alleles Are Present in Germline Configuration. As shown in Fig. 2 and lane 1 of Fig. 3, Bam HI cleaves within C μ , generating a germline 9.4-kbp 5' fragment that spans the S μ region, and a 10.5-kbp 3' fragment that extends into the C δ locus. Only these two germline bands are present in BCL₁.B₁ (lane 2), BCL₁.2.62, an IgM-secreting subclone (lane 3), BCL₁.2.58 (lane 7), and in four other BCL₁.B₂ subclones (lanes 4–6, 8). As expected from the published maps of the C μ -C δ region, rehybridization of this blot to C δ (probe C) detected the 10.5-kbp fragment, insuring that no rearrangement had taken place between C μ and C δ or within C δ (data not shown). Similarly, Eco RI digests probed for C μ gave only the 12.5-kbp germline fragment in all DNAs. From these results, in

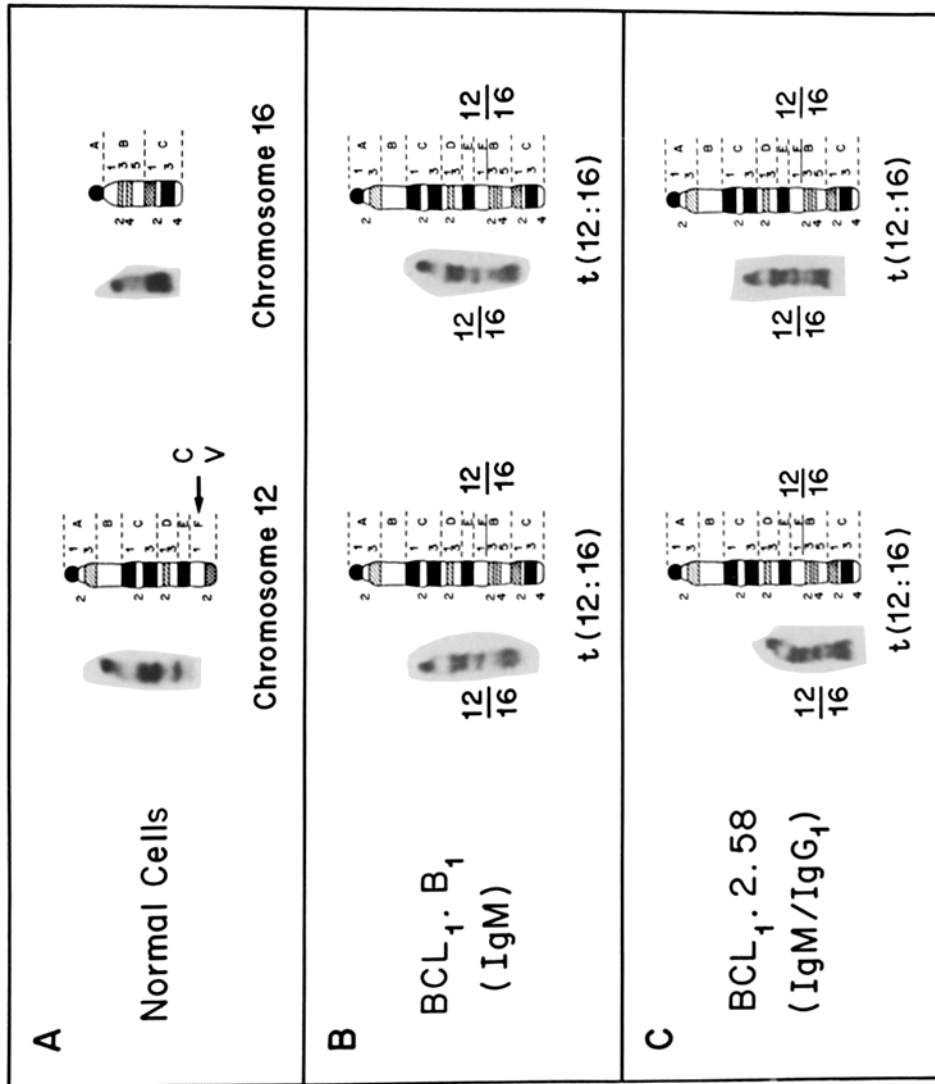


FIGURE 1. Translocation of the IgH-bearing chromosome 12 to the Igλ-bearing chromosome 16 in both alleles of BCL₁B₁ and BCL₁-2.58 cell lines. (A) Representative normal chromosomes 12 and 16. Indicated on the schematic ideograms (39) are the mapped positions of heavy chain C and V loci (38). The λ genes on chromosome 16 have not been precisely mapped. (B) Representative pair of t(12;16) alleles from the IgM-producing parental line. The translocation breakpoint (12F2;16B3) is denoted by a solid horizontal line on the ideogram. (C) Representative t(12;16)s from an IgM/IgG₁ variant cell line.

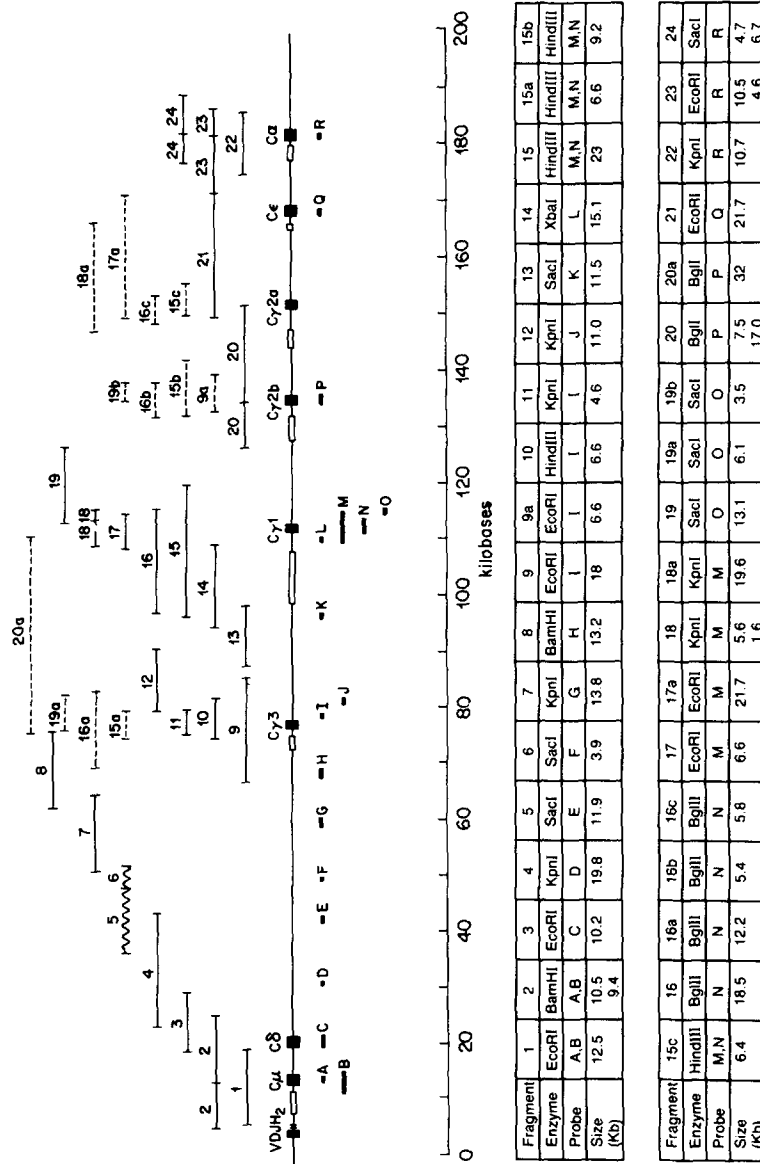


FIGURE 2. Summary of genomic Southern hybridization analyses of the IgH allele responsible for both IgM and IgG1 production. The map in the center is to the scale according to Shimizu et al. (25). Structural genes are shown as closed boxes and corresponding switch recombination regions are shown as open boxes. Above the map, solid lines (1-24) depict the location and length of the restriction fragment (or fragments, as indicated by redundant numbers) that hybridize to the probes whose size and position are indicated below the map as narrow boxes (A-R). Dashed lines above the map represent fragment sizes inferred by crosshybridization. The wavy line represents a segment of repetitive DNA that, in addition to the expected fragments (fragments 5 and 6), yielded many other bands that migrated identically in all DNAs. Numerous attempts to confirm this 7.9-kbp repetitive region employing alternative digestions and probes were unsuccessful. The table below the map correlates fragment location with its size and the enzyme(s) and probe(s) used in a particular experiment. Most of the data were generated from the complete panel of BCL₁-B₂ subclones described in Materials and Methods.

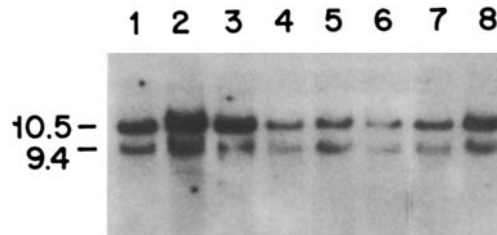


FIGURE 3. Analysis of $C\mu$ gene context. High molecular weight DNAs were digested with Bam HI (see Figs. 2 and 6 for relative location of sites), and were fractionated and blotted as described in Materials and Methods, then were hybridized with $C\mu$ (probe B). Lane 1, BALB/c liver DNA; lane 2, BCL₁.B₁; lane 3, BCL₁.2.62; lane 4, BCL₁.15.19; lane 5, BCL₁.15.9; lane 6, BCL₁.13.92; lane 7, BCL₁.2.58; lane 8, BCL₁.2.54. kb markers are shown in margin.

conjunction with data from Kpn I digestions presented below, we conclude that no rearrangement involving $S\mu$, $C\mu$, or $C\delta$ alleles contribute to double production of μ and $\gamma 1$ in BCL₁.B₂.

All C_H Genes 3' to $C\mu$, including $C\gamma_1$, are in Germline Configuration. The lack of $C\mu$ rearrangement suggested that γ_1 expression in BCL₁.B₂ subclones does not occur by prototypic class-switch recombination. To resolve the issue directly, we carried out an exhaustive set of C_H probings, which overlapped the entire region (~100 kbp) between $C\delta$ and $C\gamma_1$ as well as the DNA downstream to $C\gamma_1$. The complete analysis was performed on Balb/c liver, BCL₁.B₁, BCL₁.2.62 and BCL₁.2.58, and partial analyses were carried out on additional BCL₁.B₂ subclones. The data are summarized in Fig. 2, with the most crucial blots for the region between $C\gamma_3$ and $C\gamma_1$ presented in Fig. 4. Digestion with Hind III generates a diagnostic fragment (fragment 15 in Fig. 2) which spans the $\gamma 1$ switch region and generally detects rearrangement of this locus. This is shown in Fig. 4a by the two rearranged bands (15 and 16 kbp) in the control digestion of an IgG1 secreting hybridoma (lane 1). In contrast, we detect only the germline band of $C\gamma_1$ (23 kbp) and the more faintly crosshybridizing germline fragments of $C\gamma_{2b}$, $C\gamma_3$ and $C\gamma_{2a}$ (9.2, 6.6, and 6.4 kbp, respectively) in liver (lane 2), BCL₁ IgM producers (lanes 4 and 5) and BCL₁.B₂ (lanes 3, 6–8). Likewise, with the more upstream probes L, K and J (Fig. 4, b–d), the BCL₁ and BCL₁.B₂ patterns are identical to those of the liver.

Although no rearrangement occurred between γ_3 and γ_1 , it still remained possible that expression of γ_1 might be affected by a recombination between δ and γ_3 . For example, a deletion within this region would appreciably reduce the length of a putative J_H - $C\mu$ - $C\gamma$ transcription unit. As summarized in Fig. 2, we detected no rearrangements in BCL₁.B₁ and BCL₁.B₂ relative to liver within this ~55 kbp area with the exception of only 7.9 kbp of repetitive DNA (between fragment 4 and 7) that was difficult to confirm. Similarly, in the BCL₁.B₂ cells, downstream genes from $C\gamma 1$ to $C\alpha$ remain in germline context. Taken together, these results strongly argue that DNA rearrangement of C_H genes is not responsible for expression of γ_1 in BCL₁.B₂ cells.

Deletion of the Majority of the C_H Locus Has Occurred on the Nonproductive Chromosome 12 of BCL₁.B₂. As noted above, the $C\mu$ and $C\delta$ genes of both BCL₁.B₁ and BCL₁.B₂ are unrearranged and are linked on a Bam HI fragment

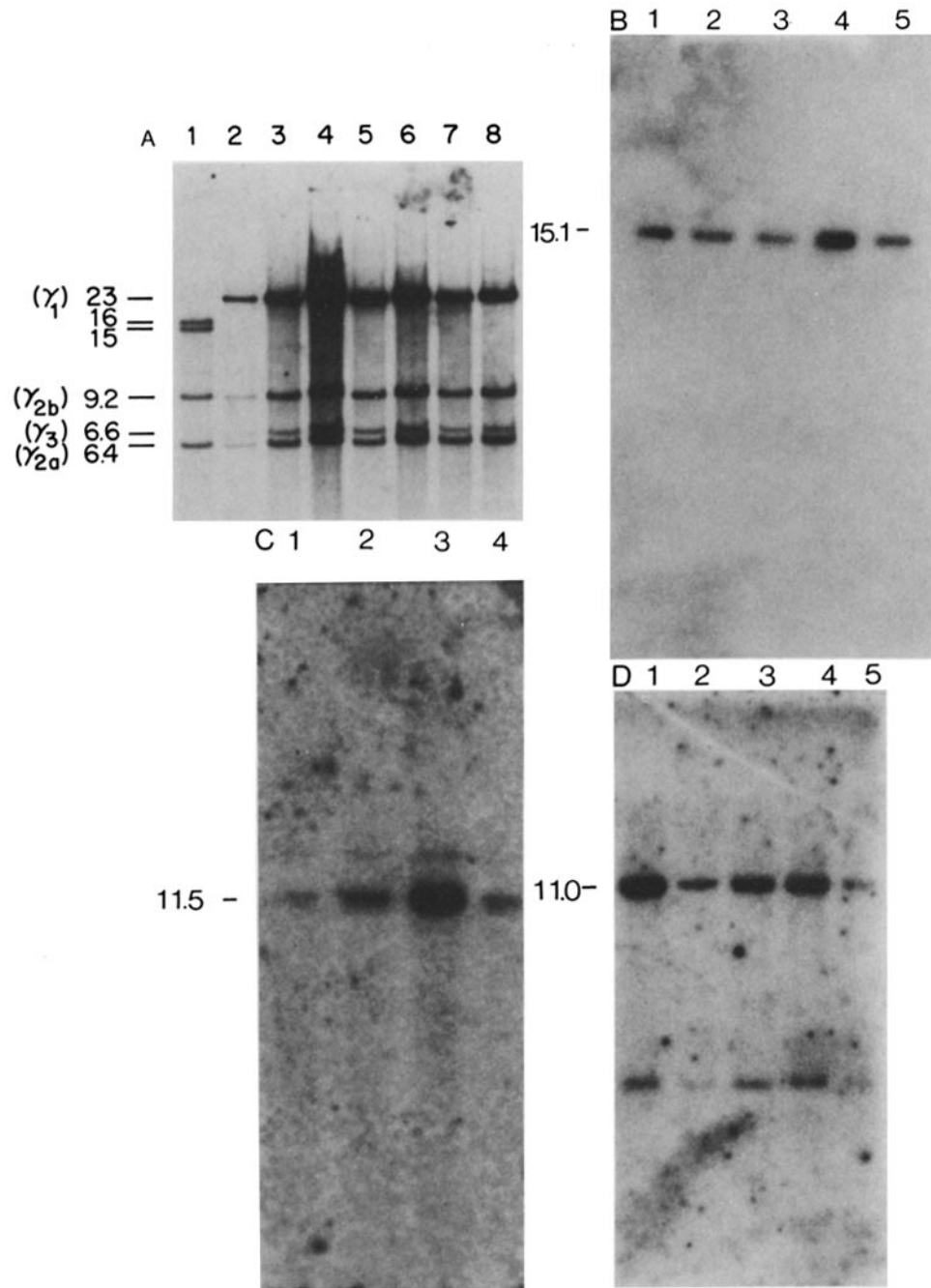


FIGURE 4. C_{γ} genes and the region between $C_{\gamma 3}$ and $C_{\gamma 1}$ are unrearranged in BCL₁.B₂ double producers. (A) Hybridization of a Hind III digest (Fig. 2, fragment 15) to $C_{\gamma 1}$ probe M. Lane 1, 9B12 (IgG1-secreting hybridoma); lane 2, BALB/c liver; lane 3, BCL₁.2.58; lane 4, BCL₁.B₁; lane 5, BCL₁.2.62; lane 6, BCL₁.2.54; lane 7, BCL₁.13.92; lane 8, BCL₁.15.9. (B) Hybridization of an Xba I digestion (Fig. 2, fragment 14) to $C_{\gamma 3}$ - $C_{\gamma 1}$ intronic probe L. Lane 1, BCL₁.2.62; lane 2, BCL₁.B₁; lane 3, BCL₁.2.58; lane 4, BCL₁.6.1; lane 5, Balb/c liver DNA. (C) Hybridization of a Sac I digestion (fragment 13, Fig. 2) to $C_{\gamma 3}$ - $C_{\gamma 1}$ intronic probe K. Lane 1, BCL₁.B₁; lane 2, BCL₁.2.58; lane 3, BCL₁.6.1; lane 4, Balb/c liver DNA. (D) Hybridization of a Kpn I digestion (fragment 12, Fig. 2) to $C_{\gamma 3}$ 3' probe J. Lanes are as in B. The additional bands seen in Fig. 4C (13.1 kbp) and 4d (5.6 kbp) are residual hybridization from the previous probings of the same filters. kb markers are shown for A-D.

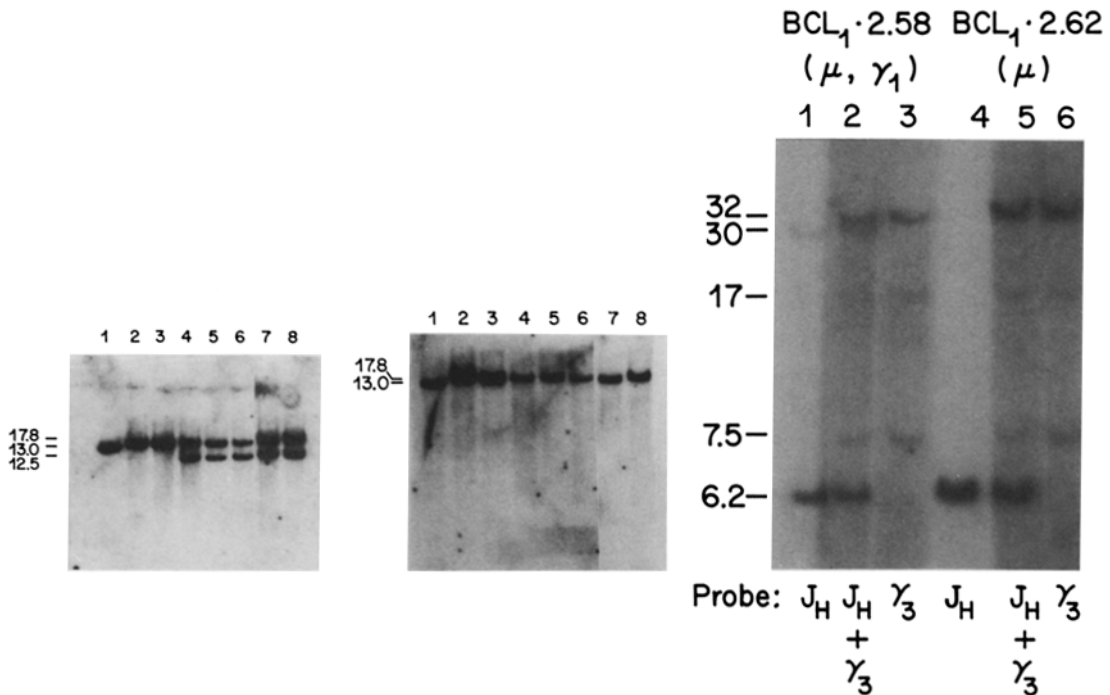


FIGURE 5. BCL₁.B₂ clones carry two different alleles of chromosome 12. Southern blots of Kpn I-digested DNAs (see Fig. 6 for positions of sites) were hybridized in *A* to probe S (J_H³-J_H⁴) and in *B* to probe B (C_μ). Lane 1, BALB/c liver; lane 2, BCL₁.B₁; lane 3, BCL₁.2.62; lane 4, BCL₁.15.19; lane 5, BCL₁.15.9; lane 6, BCL₁.13.92; lane 7, BCL₁.2.58; lane 8, BCL₁.2.54. (*C*) J_H and C_{γ1} genes are not linked in μ only (lanes 4-6, BCL₁.2.62) and μγ1- (lanes 1-3, BCL₁.2.58) producing subclones. DNAs were digested with Bgl I and were hybridized to J_H (probe S) (lanes 1 and 4), C_{γ3} (probe V) (lanes 3 and 6), and a mixture of probes S and V (lanes 2 and 5). In Fig. 5c, lanes 2, 3, 5, and 6, the 17 kbp and 7.5 kbp fragments were C_{γ2b} gene crosshybridizations with probe W. The 6.2 kbp fragment is the Bgl I fragment containing VDJ sequences located on the H⁺ allele (see Fig. 6). kb markers are shown.

(Fig. 3). Both lines contain two copies of the t(12;16) chromosome. But a single, apparently identical variable region rearrangement is observed when Eco RI blots of BCL₁.2.58, BCL₁.B₁ and BCL₁.2.62 DNA are probed with a J_H region fragment (probe S and Fig. 6 of accompanying paper). In addition, a single VDJ_{H2}-containing band was obtained in Xba I, Hind III, Bam HI and Bgl I digests of all the BCL₁.B₂ subclones probed with J_H (accompanying paper and data not shown). These data suggested that each BCL₁.B₂ allele possessed an identically rearranged VDJ. However, Kpn I digests revealed a rearrangement of one of the two BCL₁.B₂ alleles with respect to BCL₁.B₁. As shown in Fig. 5a, in addition to the rearranged 17.8-kbp Kpn I fragment found in BCL₁.B₁ (lane 2) and BCL₁.2.62 (lane 3), we detected a 12.5-kbp fragment in BCL₁.2.58 (lane 7). The identically rearranged band is present in the other BCL₁.B₂ subclones (lanes 4-6, 8), suggesting that all BCL₁.B₂ lines, although independently cloned, were derived from a common parental variant. When a similar Kpn I blot was probed with C_μ (Fig. 5b), only a single hybridizing band, corresponding to the

17.8-kbp fragment from the unrearranged allele of BCL₁.B₁, was detected in BCL₁.B₂. To map the rearrangement more precisely, we performed similar Kpn I blots with probes (T and U) that extend directly from the Eco RI site in the J_H-C_μ intron. In all cases, only the 17.8-kbp fragment hybridized (data not shown). Since there is only a single Eco RI band in J_H3J_H4 blots, the rearrangement had to occur just 3' (<50 bp) to the Eco RI site in the J_H-C_μ intron. Otherwise, the 12.5-kbp band would have been detected by probe T or U in BCL₁.B₂.

Hybridization of the Kpn I digests to probe M had shown that the 12.5-kbp fragment did not contain C_{γ1} (Fig. 2, fragment 18). However, there are a number of Kpn I sites between C_{γ3} and C_{γ1} in germline DNA (25), and there is a slight possibility of fragment comigration in the analysis of Fig. 4. Therefore, we digested BCL₁.B₁ and BCL₁.B₂ DNAs with Bgl I, an enzyme that generates a fragment that spans the entire C_{γ3}-S_{γ1} germline region (Fig. 2, fragment 20a), and cuts at a single site between J_H and C_μ, ~480 bp downstream of the Eco RI site of the rearrangement junction (P. W. Tucker, unpublished results). As seen in Fig. 5c, hybridization either separately or jointly with J_H (probe S) and C_{γ3} (probe V) gave different sized bands (30 and 32 kbp, respectively). These data prove that J_H and C_{γ1} are not linked on either allele of the IgM/IgG1 producing variants.

Simultaneous Expression of μ and γ1 in BCL₁.B₂ is Allelically Excluded. The above results, taken with the analyses of the C_H genes, suggest that the observed rearrangement on one allele of BCL₁.B₂ is unusual. It deletes, at a minimum, part of J_H-C_μ intron, all C_μ and, by direct inference of the C_μ-C_δ linkage (Fig. 3), C_δ. However, the germline patterns (Figs. 2 and 4) and the analysis of Fig. 5c show unequivocally that this rearrangement is not accomplished by recombination into downstream C_H sequences. Therefore, the 3' side of the rearrangement in BCL₁.B₂ appears to be unrelated to Ig C_H DNA, yet it is not large enough to be detected cytogenetically.

A proposed map of the two IgH alleles in BCL₁.B₂ is shown in Fig. 6. The model predicts that downstream C_H genes on the productive chromosome (i.e., on the H⁺ allele) exist in single copy in BCL₁.B₂ but in double copy in BCL₁.B₁, BCL₁.2.62, or liver. On the other hand, J_H genes should exist in double copy in parental, variant, and germline DNA. To test this hypothesis, we hybridized Eco RI digests (Fig. 7a) of the appropriate DNAs to a mixture of J_H (probe S) and C_{γ3} (probe V), and Hind III digests (Fig. 7b) to a mixture of J_H (probe S) and C_{γ1} (probe M). The hybridization intensities were quantified by densitometry and the ratio of J_H/C_{γ3} (4 kbp:18 kbp, Fig. 7a) and J_H/C_{γ1} (2.55 kbp:23 kbp, Fig. 7b) were determined. As shown in Table I, the J_H/C_{γ3} and J_H/C_{γ1} ratios in BCL₁.B₁, BCL₁.2.62 are approximately twice those in the IgM/IgG1-producing variants, BCL₁.2.58 and BCL₁.13.92. We conclude that allelic exclusion is operative in BCL₁.B₂ in generating both μ and γ₁ chains from a single rearranged VDJ.

Discussion

Two major conclusions can be drawn from the results of our studies: (a) IgG1 is expressed in the BCL₁.B₂ clones in the absence of DNA rearrangement of the constant region genes; and (b) the heavy chains of both IgM and IgG1 are

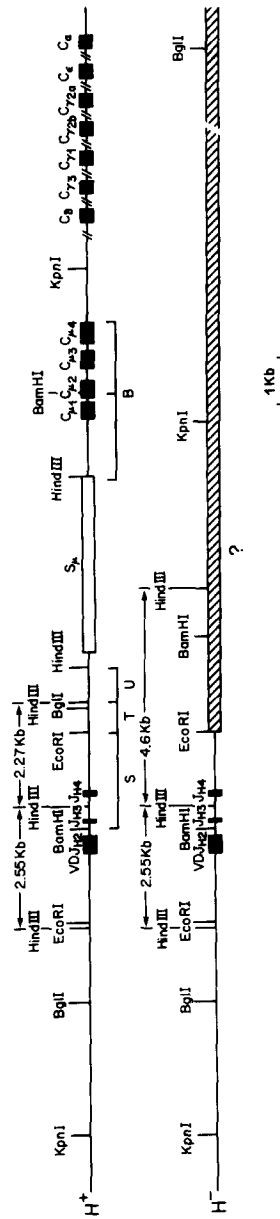


FIGURE 6. Schematic representation of the productive (H⁺) and nonproductive (H⁻) heavy chain alleles of BCL-1, B₂ double producers. Location and lengths of probes S, T, U, and B are indicated. The hatched DNA to the right of the H⁻ Eco RI site is of undetermined (?) origin.

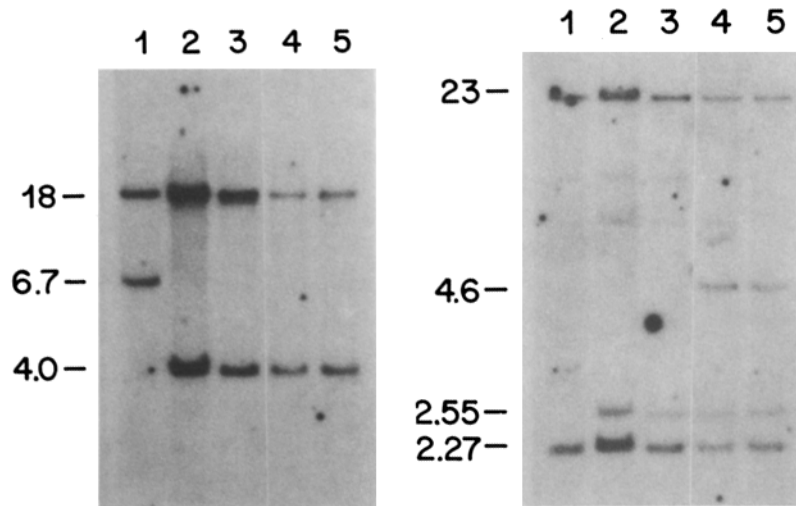


FIGURE 7. Quantitative analysis of J_H, C_{γ3}, and C_{γ1} gene context. (A) Southern blots of Eco RI digests were hybridized separately or with a mixture of J_H and C_{γ3} probes. (B) Hybridization of Hind III digests to J_H and C_{γ1}. Lane 1, BALB/c liver; lane 2, BCL₁.2.62; lane 3, BCL₁.B1; lane 4, BCL₁.13.92; lane 5, BCL₁.2.58. Densitometry of the appropriate bands is summarized in Table I. kb markers are shown for A and B.

TABLE I
Allelic Exclusion Is Operative in BCL₁.B₂ Double Producers

Cells	IgH	J _H /C _{γ3} *	J _H /C _{γ1} *
BCL ₁ .2.58	μγ1	1.25	0.80
BCL ₁ .13.92	μγ1	1.48	0.77
BCL ₁ .2.62	μ	0.74	0.44
BCL ₁ .B1	μ	0.70	0.49
BALB/c liver	—	0.71	—

* Densitometry was performed on the 4 kbp and 18 kbp bands of Fig. 7a and the 2.55 kbp and 23 kbp bands of Fig. 7b. Ratios represent the average of two J_H/C_{γ3} and three J_H/C_{γ1} determinations.

expressed from a single rearranged VDJ segment on the same chromosome 12. Therefore, we conclude that simultaneous production of IgM/IgG1 in BCL₁.B₂, as in the case of IgM and IgD in BCL₁.B₁, is controlled exclusively at the RNA level.

Our findings provide the first example of allelically excluded, double isotype expression in a neoplastic B cell clone. A similar conclusion was drawn to explain dual μ and γ2b production in a derivative of a μ-producing Abelson murine leukemia-transformed cell line (40). Contrary to the initial report, it is now clear (41, 42) that γ2b synthesis in this line is accompanied by deletion of sequences between J_H and C_{γ2b}, and thus cannot be explained by differential RNA processing. Our data also support the observations of Yaoita et al. (17) and Perlmutter and Gilbert (18), who used purified populations of normal lymphocytes. The caveats associated with these studies (19) are overcome here since we have shown that virtually all cells in BCL₁.2.58 synthesize, express, and secrete IgM and

IgG1. Furthermore, the clonal BCL₁.B₂ lines have provided the opportunity for a more extensive analysis of the DNA context. In addition to establishing germline configuration of sequences anticipated to undergo rearrangement during switch recombination (e.g., S_μ, C_μ, and S_{γ1}), we have also shown that the remainder of the C_H locus is unrearranged on the productive allele. This observation argues against the hypothesis that C_H genes might be translocated, via nonclassical switch sequences and deletion of C_δ, to a position downstream to C_μ, possibly replacing δ with another isotype (7, 11).

Although we have provided strong evidence for use of a single VDJ, it resides on a chromosome 12 that has undergone an unusual translocation, distal to the Ig locus, with chromosome 16. At the microscopic level, the t(12;16) appears to be identically duplicated in both the BCL₁.B₁ and BCL₁.B₂ cell lines. However, our blotting data define the nonproductive t(12;16) allele (H⁻ in Fig. 6) by virtue of its loss of Ig-related sequences 3' to the Eco RI site within the J_H-C_μ intron. These findings have important implications in considering both the derivation of the *in vivo* BCL₁ leukemia, its subsequent *in vitro* adaptation, and the eventual mechanism for allelic exclusion in BCL₁.B₂. The *in vivo* BCL₁ leukemia cells appear to have a stable karyotype, with the exception of chromosome 12. Two early reports (34, 35) are in agreement with our data (Y.-W. Chen and G. V. Dev, unpublished results), with respect to the modal chromosome number (35 chromosomes in reference 34 and our data, and 36 chromosomes in reference 35). However, Schroeder et al. (34) reported a single normal chromosome 12 and a translocated chromosome 12 to an unidentified recipient in early passages of the line. One year later, Voss et al. (35) found no evidence for normal copies of chromosome 12, but identified three translocated alleles. We observed a single copy of the same t(12;16) in the *in vivo* line (Y.-W. Chen and G. V. Dev, unpublished results) that is duplicated in the *in vitro* BCL₁.B₁ and BCL₁.B₂ lines, both of which have a modal chromosome number of ~60. We suggest that the precursor to the original leukemic cell carried a normal chromosome 12 as nonproductive and retained it during early passages, at which time the productive chromosome 12 underwent translocation(s). Adaptation to cell culture resulted in a duplication of most of the karyotype, including t(12;16), and may have required a second more subtle event (e.g., somatic mutation), which was undetectable at the cytogenetic or DNA rearrangement level, to effect allelic exclusion in BCL₁.B₁. Alternatively, both of the t(12;16) alleles remain active in BCL₁. If the former is true, then the third event, the rearrangement 3' to VDJ on the H⁻ allele of BCL₁.B₂, is not required for allelic exclusion, but may instead reflect some *trans*-acting regulatory requirement for switching the productive allele from μ/δ to μ/γ1. The significance of this DNA rearrangement is underscored by the fact that all independent BCL₁.B₂ variants maintain it.

Aside from its unusual karyotype, do the BCL₁.B₂ variants have a counterpart amongst normal B cells? Based on its surface Ig phenotype, its low level of constitutive IgM secretion (reviewed in reference 43), and its heavy chain gene transcription profile (44), the parental BCL₁.B₁ line appears to represent an immature B cell. It has been employed as a model for numerous functional studies by virtue of its ability to undergo further differentiation when cultured

with LPS (45), anti-Ig (46), or T cell-derived lymphokines (47). The fact that all BCL₁.B₂ variants isolated for IgG secretion secrete both IgM and IgG1, as well as express them on their surface, may reflect their derivation from a single precursor. It may also denote a basic difference between these cells and the nonsecreting memory cell populations analyzed by others (17, 18) at the molecular level. Regardless of whether these differences in mode of expression are real or are a consequence of the transformed state, the BCL₁.B₂ phenotype shows that high expression of the secreted and the membrane form of γ mRNA can be generated from C_H genes in germline configuration.

Finally, with regard to mechanisms, the only plausible explanation for the present data is that μ and γ 1 mRNA are expressed with a common VDJ gene by alternative RNA processing. This could be accomplished by a discontinuous mechanism in which the RNA polymerase translocates from the template to transcribe discrete segments of DNA, as proposed for trypanosome variable antigen (48) and certain viral genes (49, 50). Alternatively, there could be a continuous transcription mechanism (i.e., long transcript model), such as that apparently used by the drosophila bithorax locus (51), which has been invoked by others (17, 18) to account for their results in normal B cells. In the latter scenario, the γ 1 chains could then be translated from mRNA derived by processing a primary transcript that includes sequences from both C μ and C γ 1 genes. The μ mRNA may be derived either from an identical transcript of ~120 kb or from one that terminates in the intervening sequence between C μ and C δ . Based on nuclear transcription studies in BCL₁.B₁ cells (44) and other IgM/IgD double producers (52, 53), we favor the latter alternative. This would require differential regulation of transcriptional termination, perhaps at two points. First, the majority of the RNA polymerases would unload 3' to C μ , since in BCL₁.B₂ cells, μ RNA abundance is greater than γ 1 RNA abundance (accompanying report and data not shown). Second, a small proportion of the polymerases would read through the μ termination region and exit the template downstream of the C_H gene to be expressed (C γ 1 in BCL₁.B₂). A choice in the second polymerase unloading event may dictate (and simplify) not only the subsequent RNA splicing decision, but may also bear on the acquisition of isotype commitment; i.e., once a cell expresses an isotype other than IgM and IgD, it is then committed and restricted to secretion of that isotype upon stimulation (54). The best clonal example of this model is the I.29 B cell lymphoma, which undergoes spontaneous and/or inducible switch recombination from IgM to IgA (55). The C α gene is preferentially open, as defined by hypomethylation, in the IgM-synthesizing cells that are committed to switch to this isotype (56). Similarly, in the transition of BCL₁ to BCL₁.B₂, C γ 1 appears to be exclusively selected. Perhaps transcription through, and equally important, termination beyond a C_H gene (C γ 1 in BCL₁.B₂) is critical in inducing an active chromatin structure for subsequent expression of that same C_H gene. The resulting prediction is that BCL₁.B₂ cells, on receiving the appropriate signal, would undergo exclusive switch recombination to C γ 1. This cell line, therefore, provides us with the opportunity of testing the mechanisms of double isotype production and its consequences.

Summary

In our accompanying paper, we described a switch variant (BCL₁.2.58) that expresses membrane and secreted forms of IgM and IgG1. Both IgM and IgG1 share the same idiotype and use the same VDJ rearrangement. Here, a detailed Southern blot analysis of the entire constant region of the Ig heavy chain (Ig C_H) locus of parental (BCL₁.B₁) and variants (BCL₁.B₂) DNA showed no detectable rearrangement. Similar analysis of the J_H-C_μ region led to the conclusion that two heavy chain alleles present in the IgM/IgG1-producing variants carried the same VDJ rearrangement but differed in their 3' flanking regions. One chromosome 12 did not carry any Ig C_H genes, whereas, the other chromosome 12 carried one copy of C_H genes. In BCL₁.B₁, however, each of the chromosome 12 alleles carried a full copy of C_H genes. Karyotypic analysis confirmed the presence of two translocated t(12;16) chromosomes in both BCL₁.2.58 and BCL₁.B₁ cells, with a break 5' to the V_H locus at the distal region (12F2) of chromosome 12, and at the proximal region below the centromere (16B3) of chromosome 16. We conclude that double production of IgM and IgG1 in BCL₁.B₂ is accomplished by transcription of the corresponding C_H genes in germline configuration using a single VDJ on the same chromosome 12.

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Note added in proof: Results of sandwich hybridization and nuclear run-on transcription are consistent with the long transcript model for dual μ and $\gamma 1$ expression in BCL₁.B₂.

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