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Genetics

$C\mu$ gene rearrangement of mouse immunoglobulin genes in normal B cells occurs on both the expressed and nonexpressed chromosomes

(allelic exclusion/B lymphocytes/V-J rearrangement/restriction fragment length polymorphism)

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ABSTRACT **We have examined the organization of heavy-chain immunoglobulin genes on both the expressed and nonexpressed chromosomes of normal B lymphocytes from allotype heterozygous (BALB/c × C57BL/J)F_1 mice. The $C\mu$ genes of BALB/c mice are on 12.4-kilobase *Eco*RI and 13.1-kilobase *Kpn* I restriction fragments, whereas those of C57BL/J mice are on 13.6-kilobase *Eco*RI and 14.3-kilobase *Kpn* I restriction fragments, allowing the examination of rearrangements on each chromosome independently. B lymphocytes from spleen and Peyer's patches expressing both IgD and IgM of the BALB/c allotype were isolated with a fluorescence-activated cell sorter. *Eco*RI and *Kpn* I restriction digests were hybridized with a $C\mu$ gene-containing probe. The $C\mu$ gene is present on both chromosomes. DNA rearrangements occur on both the expressed and nonexpressed chromosome within the 3.6-kilobase *Kpn* I/*Eco*RI restriction fragment containing the joining (J_H) gene locus. We conclude that allelic exclusion of heavy-chain immunoglobulin gene expression is not mediated by J_H-region DNA rearrangement of the expressed chromosome only. In contrast, analysis of the $C\kappa$ gene region from the same sorted B-cell DNA reveals a substantial quantity of germline context DNA. We also demonstrate that the deletions observed on the *Eco*RI fragment containing the $C\mu$ gene in myeloma cells and in $C\mu$ gene-containing recombinant DNAs do not usually occur in normally differentiating B lymphocytes and are likely to be confined to myeloma tumor cells.**

An unusual feature of immunoglobulin gene expression is the phenomenon of allelic exclusion. Only one member of an allelic pair of immunoglobulin heavy chains is produced by a B lymphocyte (1). Each immunoglobulin heavy chain is encoded by at least four known gene segments: variable (V_H), diversity (D_H), joining (J_H), and constant (C_H) regions (2–4). Expression of a complete immunoglobulin chain requires the combining of V_H, D_H, J_H, and C_H gene segments (3, 4). Allelic exclusion is most simply explained by rearrangement of only one chromosome, allowing expression of that allele, whereas the alternate allele remains in the germ-line configuration.

Studies of light-chain gene organization in κ-producing myeloma and hybridoma cell lines indicate that, although in some cases there can be unrearranged κ genes (5–7), several cloned cell lines have no discernible $C\kappa$ genes in the germ-line configuration (7, 8). Examination of the $C\kappa$ gene region in a relatively pure population of normal, κ-bearing splenic B lymphocytes indicates that approximately half of the κ-containing chromosomes remained in the germ-line context (9). This suggests a mechanism of DNA rearrangement coinciding with light-chain gene expression.

The role of heavy-chain gene rearrangements in allelic exclusion is unclear. Heavy-chain μ genes from several BALB/c IgM- and IgG-secreting myelomas contain a variety of DNA rearrangements in the $C\mu$ gene region that do not involve J_H gene segments (10, 11–13). It is possible that these rearrangements may reflect continuous propagation of these neoplastic cells rather than physiological events that occur during normal B-lymphocyte differentiation.

The experiments described in this report examine the role of immunoglobulin heavy-chain gene rearrangements in normal B lymphocytes exhibiting allelic exclusion. Genetically defined restriction fragment length polymorphisms distinguish the two $C\mu$ gene alleles, *Igh-6a* and *Igh-6b*. Probing for the $C\mu$ gene in allotypically excluded B cells yields unambiguous evidence that immunoglobulin gene rearrangements occur near or in the J_H gene region on both the expressed and nonexpressed chromosomes.

MATERIALS AND METHODS

Purification of Sperm Cells. Sperm were isolated essentially as described by Joho *et al.* (14).

Purification of *Igh-5a*-Bearing B Lymphocytes. Spleens or Peyer's patches from 6- to 12-week-old (BALB/c × C57BL/J)F_1 mice were gently teased into medium 199/phosphate-buffered saline, 1:1 (vol/vol), 5% (vol/vol) fetal calf serum, and 5 mM NaN_3. Cells were washed once in medium and resuspended in 1 ml of fetal calf serum. Then 10 ml of ACK solution (0.15 M NH_4Cl/10 mM $KHCO_3$) was added to spleen suspensions for 3 min on ice to lyse erythrocytes. Lymphocytes were collected by centrifugation and subsequently stained with nitroiodophenyl$_7$ (NIP_7)-conjugated H10.4-22, a monoclonal antibody against *Igh-5a* (δ^a chain) (15), and fluoresceinated rabbit anti-NIP antiserum (unpublished data). All antisera were deaggregated at 100,000 × *g* for 10 min before use. Briefly, 1 μg of NIP_7-H10.4-22 was incubated per 10^6 cells at 10^8 cells per ml of medium for 15 min on ice, followed by one wash through fetal calf serum. Fluoresceinated rabbit anti-NIP was used under the same conditions. Cells were resuspended to a final concentration of 10^7 cells per ml in medium and sorted on the fluorescence-activated cell sorter (FACS) III (Becton Dickinson). Light-scattering criteria were used to sort only viable cells (16).

DNA Preparations. Sperm were lysed in 200 mM Tris, pH 8/100 mM EDTA/1% Sarkosyl/15 mM 2-mercaptoethanol/proteinase K (100 μg/ml) and incubated at 50°C for 2 hr. DNA was purified by CsCl/ethidium bromide density gradient equilibrium centrifugation. Ethidium bromide was removed by extraction with butanol, and the DNA was exhaustively dialyzed against 10 mM Tris, pH 8/1 mM EDTA. Sorted cell populations were collected by centrifugation. DNA was prepared in a manner similar to that of sperm DNA, except that 2-mercaptoethanol was omitted during cell lysis.

Hybridization Probes. pμ27.3 contains a 10.5-kilobase (kb) *Eco*RI fragment derived from λChμ27, a $C\mu$ gene-containing

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Abbreviations: kb, kilobase; NIP, nitroiodophenyl; FACS, fluorescence-activated cell sorter; V_H, variable region of the heavy chain; D_H, diversity region of the heavy chain; C_H, constant region of the heavy chain; J_H, joining region of the heavy chain.

BALB/c germ-line clone (19), the generous gift of Philip Early and Leroy Hood (California Institute of Technology). A 2-kb deletion occurred during λ cloning within the genomic 12.4-kb *Eco*RI fragment containing the *Cμ* gene (17). The pμ27.3 subclone contains only sequences derived from this 12.4-kb genomic fragment. pCκ1, the generous gift of Howard Gershenfeld, contains a 5.8-kb germ-line restriction fragment containing all *Jκ* gene segments and the *Cκ* gene. Both the *Cμ* and *Cκ* gene-containing restriction fragments were contained in pBR322.

Agarose Gel Electrophoresis, Transfer to Solid Supports, and Nucleic Acid Hybridization. Five micrograms of DNA was digested with either *Eco*RI or *Kpn* I, precipitated with ethanol, resuspended in 10 mM Tris, pH 8.0/1 mM EDTA, and electrophoresed through 0.5% agarose for 12–20 hr at 0.5 V/cm. Preparation of the gel and transfer to diazobenzyloxymethyl-paper was essentially as described by Wahl *et al.* (18), except that the gel was UV-irradiated rather than treated with acid. After transfer, the diazobenzyloxymethyl-paper was washed in 360 mM NaCl/20 mM sodium phosphate, pH 7.0/2 mM EDTA, treated with 0.1 M NaOH for 5 min at room temperature, and washed again in the same buffer. The DNA-paper was incubated in prehybridization solution, containing 50% (vol/vol) formamide, 900 mM NaCl/50 mM sodium phosphate, pH 7.0/5 mM EDTA, 200 μg of denatured salmon sperm DNA per ml, and (at 5 times the normal concentrations) 0.1% bovine serum albumin/Ficoll/polyvinylpyrrolidone (19) in a heat-sealable plastic bag at 42°C for 1 hr. Plasmid DNAs were ^{32}P-labeled by nick-translation according to Rigby *et al.* (20), with minor modifications. Approximately 20×10^6 dpm of DNA probe ($1–3 \times 10^8$ dpm/μg) was annealed with a 12 × 14 cm sheet of DNA-paper. Hybridization solution (containing 50% formamide, 90 mM NaCl/50 mM sodium phosphate, pH 7.0/5 mM EDTA, 200 μg of denatured salmon sperm DNA per ml, 0.1% bovine serum albumin/Ficoll/polyvinylpyrrolidone, and 0.2% $NaDodSO_4$) containing probe was incubated with the filter for 48 hr at 42°C on a rocking platform. After hybridization, DNA-paper samples were washed in 50% formamide, 90 mM NaCl/50 mM sodium phosphate, pH 7.0/5 mM EDTA, and 0.2% $NaDodSO_4$ at 42°C. DNA-paper samples were autoradiographed with either Kodak XR or DuPont Cronex 4 x-ray film and a DuPont Lightning Plus intensifying screen at −70°C.

RESULTS

Isolation of B Cells Expressing Both *Igh-5a* and *Igh-6a*. B cells from spleen or Peyer's patches were isolated by virtue of their reactivity with an anti-*Igh-5a* (anti-δ allotype) hybridoma antibody. We confirmed that more than 97% of B cells expressing δ chains simultaneously express μ chains (data not shown; refs. 21 and 22). In addition, in allotypic heterozygotic mice, the coexpressed μ and δ chains on a B-cell surface are the same allotype (22).

Spleen cells or Peyer's patch cells were stained with H10-4.22, an anti-*Igh-5a* monoclonal antibody, and analyzed on the FACS III. *Igh-5a*-positive cells comprised 30.8% of spleen cells (≈50% of the B cells) and 33.9% of Peyer's patch cells (≈50% of the B cells). The fluorescence criteria applied for sorting *Igh-5a*-positive cells were chosen to exclude weakly positive cells to reduce contamination by non-*Igh-5a*-positive cells. Only 26.7% of cells in spleen (Fig. 1*a*) and 27.4% of cells in Peyer's patches (Fig. 1*c*) were actually collected. In Fig. 1 *b* and *d*, reanalysis of the collected fractions shows that the sorted *Igh-5a*-positive cells are 97.9% *Igh-5a*-positive from spleen and 95.4% *Igh-5a*-positive from Peyer's patches. The same criteria were used for determining the frequency of *Igh-5a*-positive cells in both the original and the collected populations.

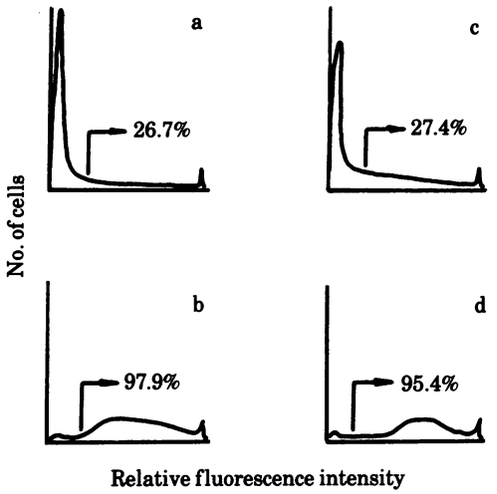

FIG. 1. FACS III analysis of spleen and Peyer's patch cells stained with NIP_7-H10.4-22 monoclonal antibody (anti-δ allotype) before and after cell sorting. (*a*) Analysis of spleen cells before sorting. Percentage indicated is the fraction of the cell population with fluorescence greater than or equal to that used for sorting. (*b*) Analysis of sorted spleen cells. Percentage indicated is the purity of the collected population. (*c*) Analysis of Peyer's patch cells before sorting. Percentage indicated is the fraction of the cell population with fluorescence greater than or equal to that used for sorting. (*d*) Analysis of sorted Peyer's patch cell. Percentage indicated is the purity of the collected population.

Restriction Fragment Length Differences Correlate with Allotype. Distinguishing rearrangements of the expressed and nonexpressed immunoglobulin heavy-chain genes requires a means of identifying each chromosome. We thought it likely that two different distantly related mouse strains might have sequence divergence in the region of immunoglobulin DNA. DNA insertions, deletions, and point mutations that occurred in or near *Cμ* genes could result in restriction fragment length polymorphisms. It would then be possible to identify the *Cμ* gene on each chromosome in a F_1 mouse. The BALB/c (*Igh*^{a} haplotype) and C57BL/J (*Igh*^{b} haplotype) strains are derived from mice initially collected in two widely separated areas of the world (23). Sperm DNA from these two strains was digested with various restriction enzymes, electrophoresed, and transferred to diazotized paper. These blots were annealed with pμ27.3, the genomic *Eco*RI fragment containing the *Cμ* gene. With several restriction enzymes, different sized fragments were observed with these two strains (data not shown). In Fig. 2, a map of the relevant restriction sites of the BALB/c and the C57BL/J genomes is presented. All four known BALB/c J_H genes are located on the 3.6-kb *Kpn* I/*Eco*RI fragment 5′ to the *Cμ* gene (see Fig. 2 and refs. 4 and 24). The 6.2-kb *Eco*RI fragment that contains the J_H gene segments of BALB/c is identical by limited restriction enzyme mapping to that of C57BL/J (data not shown). Thus, it is likely that the J_H gene segments of C57BL/J are located in the corresponding genomic region. The 12.4-kb *Eco*RI fragment and the 13.1-kb *Kpn* I fragment containing the *Cμ* gene correlates with the *a* allotype of BALB/c mice (Fig. 3, lanes a and f). Likewise, the 13.6-kb *Eco*RI fragment and the 14.3-kb *Kpn* I fragment correlates with the *Cμ* gene of the *b* allotype (Fig. 3, lanes b and g). (BALB/c × C57BL/J)F_1 mice, *Igh-6a,Igh-6b* heterozygotes, have both the 12.4-kb and the 13.6-kb *Eco*RI fragments containing the *Cμ* gene (Fig. 3, lanes c and h).

Rearrangement of Heavy-Chain Immunoglobulin Genes Occurs on Both Chromosomes. A *Kpn* I restriction digest of allotypically excluded B-cell DNA from (BALB/c × C57BL/J)F_1

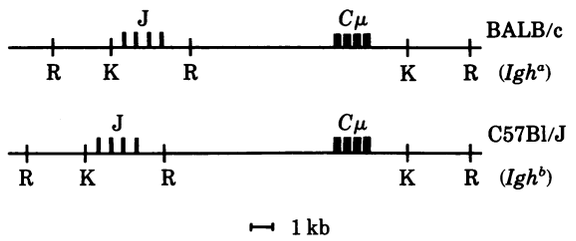

FIG. 2. Map of *Kpn* I (K) and *Eco*RI (R) restriction sites flanking the *Cμ* germ-line gene of BALB/c (*Igh*^{a}) and C57BL/J (*Igh*^{b}) strains. J_H gene segments and the *Cμ* gene are indicated by black boxes. The *Kpn* I fragment containing the *Cμ* gene is 13.1 kb in BALB/c and 14.3 kb in C57BL/J. The *Eco*RI fragment containing the *Cμ* gene is 12.4 kb in BALB/c and 13.6 kb in C57BL/J.

mice hybridized with pμ27.3 indicates that rearrangement occurs on both the BALB/c (expressed) chromosome and the C57BL/J (nonexpressed) chromosome (Fig. 3, lanes i and j). A rearrangement of a V_H gene segment or non-immunoglobulin DNA to the J_H gene region would drastically reduce or eliminate the intensity of the *Cμ* gene-containing *Kpn* I band. The disappearance of this band would be consistent with a large number of different rearrangements that have generated many new, different *Kpn* I fragment lengths. In Fig. 3, lane i, *Kpn* I-digested DNA from *Igh-5a,Igh-6a*-expressing spleen contains no detectable band. The faint band at 4.8 kb is the *Kpn* I fragment located 3′ to the *Cμ* gene that contains sequences present in the pμ27.3 hybridization probe. A second FACS sort of *Igh-5a,Igh-6a*-bearing spleen cells also did not reveal any detectable *Cμ* germ-line context hybridization bands of *Kpn* I-digested DNA, even after overexposure of the autoradiogram (data not shown). *Kpn* I-digested DNA from Peyer's patch *Igh-5a,Igh-6a*-expressing B cells hybridized with the pμ27.3 probe shows faint bands at the germ-line lengths of 13.1 kb and 14.3 kb and also

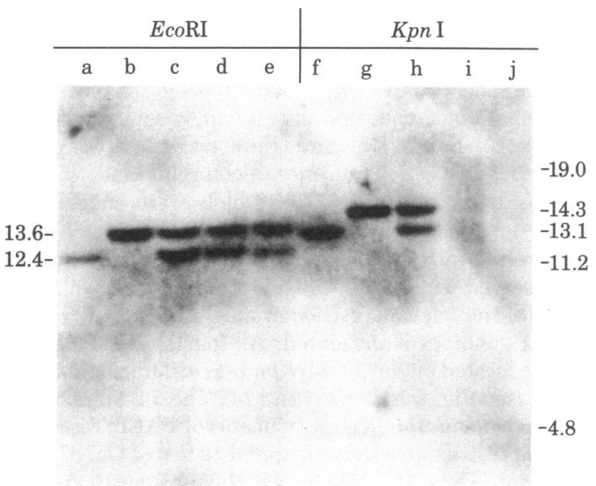

FIG. 3. Hybridization of ^{32}P-labeled pμ27.3 to a restriction spectrum of sperm and FACS-sorted *Igh-5a,Igh-6a* bearing B-cell DNAs. DNA was digested with either *Eco*RI (lanes a–e) or *Kpn* I (lanes f–j), electrophoresed through agarose, and transferred to diazobenzyloxymethyl-paper. The DNA-paper was annealed with ^{32}P-labeled pμ27.3, a plasmid containing the *Eco*RI fragment containing the *Cμ* gene from BALB/c. The DNA-paper filter was washed and autoradiographed. Lanes a and f, BALB/c sperm DNA; lanes b and g, C57BL/J sperm DNA; lanes c and h, (BALB/c × C57BL/J)F_1 sperm DNA; lanes d and i, DNA from (BALB/c × C57BL/J)F_1 splenic B cells expressing *Igh-5a,Igh-6a* (IgD, IgM of the BALB/c allotype); lanes e and j, DNA from (BALB/c × C57BL/J)F_1 Peyer's patch B cells expressing *Igh-5a,Igh-6a*. The numbers are kb.

at 11.2 kb and 19.0 kb, in addition to the expected hybridization to the 4.8-kb *Kpn* I fragment (Fig. 3, lane j). We consider it likely that the 13.1-kb and 14.3-kb *Kpn* I bands reflect a small percentage of contaminating non-B cells. Peyer's patch cell suspensions, unlike spleen cell suspensions, typically contain 25–50% nonviable cells, many of which are nonspecifically stained with H10.4-22. Some nonspecifically stained cells may have been selected despite stringent light-scattering criteria for viability. The germ-line 14.3-kb band (of the nonexpressed haplotype) could result if DNA rearrangements do not always occur on the nonexpressed chromosome in Peyer's patch B cells. We consider this unlikely because bands are present at both the expressed and nonexpressed germ-line positions. The faint bands at 19.0 kb and 11.2 kb may indicate common $V_H(D_H)J_H$ rearrangements selected for in Peyer's patches.

Disappearance of the *Cμ* gene *Kpn* I bands from B-cell DNA could reflect either rearrangements or loss of the corresponding *Cμ* gene region. Because the 12.4-kb and 13.6-kb *Eco*RI fragments containing the *Cμ* gene are entirely 3′ to the J_H region (Fig. 2), DNA rearrangements occurring within or 5′ to the J_H region should not affect *Eco*RI fragment lengths. Therefore, we examined the DNA fragments generated by *Eco*RI digestion. As can be seen in Fig. 3, lanes d and e, both spleen and Peyer's patch B cells have few, if any, rearrangements of or deletions in the *Eco*RI fragment of either chromosome. No significant reduction of *Cμ* gene signal strength is apparent and no new hybridizing bands are revealed.

Some *Cκ* Genes Are Not Rearranged. More than 95% of the splenic and Peyer's patch B cells express κ (data not shown). Because at least 95% of the allotypically excluded B lymphocytes used in this study should also express κ, we could test for the presence of unrearranged (germ-line context) *Cκ* genes. Hybridization of a *Jκ* and *Cκ* gene-containing probe (pCκ1) to

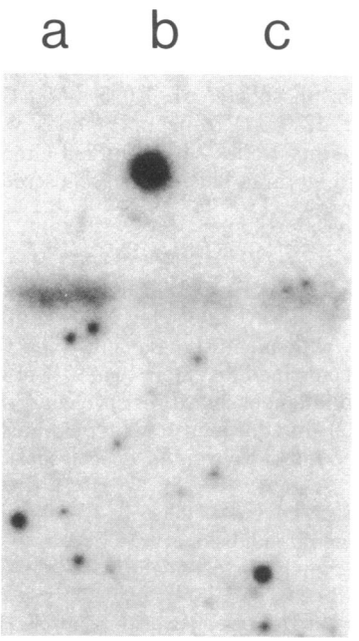

FIG. 4. Hybridization of ^{32}P-labeled pCκ1 to *Eco*RI restriction fragments of sperm and FACS-sorted *Igh-5a,Igh-6a*-bearing B-cell DNAs. DNA was digested with *Eco*RI, electrophoresed through agarose, and transferred to diazotized paper. The DNA-paper was annealed with ^{32}P-labeled pCκ1, a plasmid containing the BALB/c *Jκ* gene segments and the *Cκ* gene, washed, and autoradiographed. Lane a, BALB/c × C57BL/J)F_1 sperm DNA; lane b, DNA from (BALB/c × C57BL/J)F_1 splenic B cells expressing *Igh-5a,Igh-6a*; lane c, DNA from (BALB/c × C57BL/J)F_1 Peyer's patch B cells expressing *Igh-5a,Igh-6a*.

an *Eco*RI digest will reveal a band for germ-line context DNA of intensity corresponding to the fraction of nonrearranged sequences (9). Hybridization of pCκ1 to an *Eco*RI restriction digest of DNA from *Igh-5a,Igh-6a*-bearing spleen cells and Peyer's patch cells (Fig. 4, lanes b and c) reveals a substantial fraction of germ-line Cκ gene sequences. Clearly, a large percentage of B cells must have only one rearranged chromosome, unless frequent rearrangements occur that preserve the germ-line Jκ-Cκ *Eco*RI fragment length.

DISCUSSION

Normal B Cells Offer Unique Advantages for Study of Heavy-Chain Gene Rearrangements. We have shown that normal IgD,IgM-bearing B lymphocytes contain rearrangements of heavy-chain immunoglobulin genes on both chromosomes. Previous investigations using myeloma cells to study the role of DNA rearrangements in mediating allelic exclusion have led to conflicting data. A variety of rearrangements, not likely to involve J_H gene segments, including deletions, have frequently been discovered on the *Eco*RI fragment containing the Cμ gene from myeloma cells (10, 11, 13). These rearrangements may have occurred on one or more of the nonexpressed chromosomes during the continuous propagation of these transformed cells. Deletions occurring 5′ to the Cμ gene have been found in isolated Cμ gene recombinant DNA clones (refs. 13, 25, and 26 and unpublished observations). In this study, however, the *Eco*RI digest of both spleen and Peyer's patch B-cell DNA shows that the Cμ gene is present on germ-line-length restriction fragments on both chromosomes (Fig. 3, lanes d and e). Because no new Cμ gene-containing bands are apparent and there is no noticeable reduction of band intensity, it is unlikely that rearrangements occur between the Cμ gene and the *Eco*RI site 5′ to Cμ in these normal, allelically excluded B cells. We conclude that deletions and other rearrangements in the region of the Cμ gene occur infrequently, if at all, in normal B cells. Such rearrangements in myeloma cells probably do not represent a physiological process unless they occur during the transition of B cells to plasma cells.

Cμ Genes of Normal IgD,IgM-Bearing B Cells Appear To Be Greater Than 90% Rearranged. To study immunoglobulin DNA rearrangements in allelically excluded normal IgD,IgM-bearing B cells, we have exploited *Eco*RI and *Kpn* I restriction fragment length polymorphisms which correlate with the Cμ allotypes of BALB/c (*Igh*^{a}) and C57BL/J (*Igh*^{b}). A *Kpn* I restriction digest of allotypically excluded IgD,IgM-bearing B-cell DNA annealed with ^{32}P-labeled pμ27.3 reveals extensive DNA rearrangements in the region of J_H gene segments on both the expressed and nonexpressed chromosomes (Fig. 3, lanes d, e, i, and j). These DNA rearrangements occur within the 3.6-kb *Kpn* I/*Eco*RI germ-line fragment that contains J_H gene segments. (Fig. 2). We consider it likely that less than 10% germ-line context Cμ genes are present in the B-cell populations (Fig. 3, lanes a–c). The expressed chromosome (BALB/c-derived) rearrangements must be productive $V_H(D_H)J_H$ joining, because the cell population analyzed was selected on the basis of immunoglobulin expression. The nature of the alteration of the nonexpressed chromosome (C57BL/J-derived) has not been elucidated (Fig. 5). It may be a viable, yet unexpressed, $V_H(D_H)J_H$ rearrangement with the same or different $V_H(D_H)J_H$ rearrangement as on the expressed chromosome or it may be a nonviable rearrangement.

The *Kpn* I restriction digest of DNA from Peyer's patch B cells reveals two faint germ-line bands containing the Cμ gene (Fig. 3, lane j), probably resulting from contamination by non-B cells. The 19.0-kb and 11.2-kb faint bands may represent frequently occurring rearrangements of a limited number of $V_H(D_H)J_H$ joinings or common alterations of the nonexpressed chromosome.

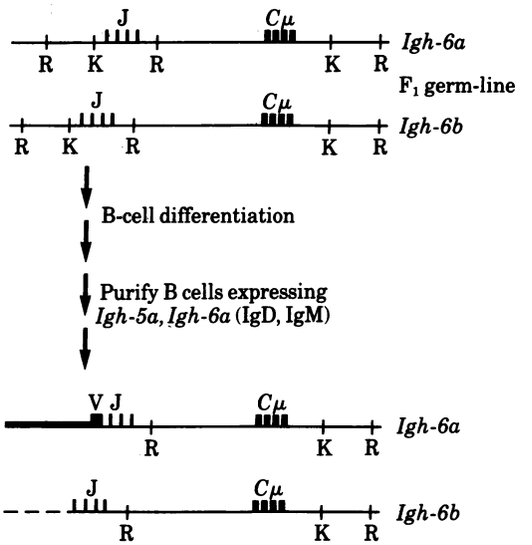

FIG. 5. Cμ gene rearrangements in normal B lymphocytes: An interpretation of the data of Fig. 3. A restriction map of the germ-line Cμ gene region from *Igh*^{a}/*Igh*^{b} heterozygotes [(BALB/c × C57BL/J)F_1 mice] is presented. Allotypically excluded B cells expressing the BALB/c allotype have one chromosome *Igh*^{a} (BALB/c-derived) with a viable, productive $V_H(D_H)J_H$ rearrangement. One example of a rearranged *Igh*^{a} (BALB/c-derived) chromosome is illustrated. The thick line to the left of the V_H gene segment sequence indicates sequences found 5′ to the germ-line V_H gene (4, 21). The nonexpressed chromosome *Igh*^{b} (C57BL/J-derived) has a rearrangement in the 3.6-kb *Kpn* I/*Eco*RI fragment. An example of a possible rearrangement is illustrated. The dashed line indicates that the derivation of the translocated sequence is unknown. K, *Kpn* I site; R, *Eco*RI site.

Role of Immunoglobulin Gene Rearrangements in Allelic Exclusion. Heavy-chain allelic exclusion in normal B cells is clearly not mediated by DNA rearrangements in the J_H region of the expressed chromosome only. κ light-chain allelic exclusion, however, may be correlated with DNA rearrangements. In two studies of normal κ-bearing splenic B cells (Fig. 4; ref. 9), a significant fraction of the Cκ genes remains in germ-line context. It is possible that different rearrangement processes are involved in heavy-chain and light-chain gene expression. For example, there is evidence for temporal regulation of expression of immunoglobulin genes: putative pre-B cells in bone marrow and fetal liver express μ-chain proteins before they express light chains (27). An analysis of Cμ and Cκ immunoglobulin gene rearrangements in differentiating pre-B cells might further clarify the relationships of immunoglobulin gene rearrangements to allelic exclusion.

There are two likely explanations of heavy-chain allelic exclusion: (*i*) The Cμ gene rearrangement on the nonexpressed chromosome is a nonproductive rearrangement. A $V_H(D_H)J_H$ rearrangement resulting in codon reading frameshifts, $V_H(D_H)$ translocation not immediately adjacent to a J_H gene segment, or non-V_H DNA translocated to a J_H sequence would result in a nonfunctional polypeptide if transcription and translation occurred. (*ii*) Both chromosomes may contain potentially viable $V_H(D_H)J_H$ rearrangements of the same or different $V_H(D_H)J_H$ rearrangements, but an active regulatory process limits the ultimate expression to one chromosome. An analysis of the rearrangements of the nonexpressed chromosome should distinguish between these possibilities.

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