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Immunology

Somatic rearrangements forming active immunoglobulin μ genes in B and T lymphoid cell lines

(recombinant phage/rearranged genes/deletions/allelic exclusion/heavy chain class switch)

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ABSTRACT We have cloned an active gene for an immunoglobulin μ heavy (H) chain, bearing the variable (V_H), joining (J_H), and constant (C_μ) sequences expressed in the IgM-secreting mouse plasmacytoma HPC-76. The μ gene was formed by somatic recombination between a V_H gene and one of several J_H genes, which are located about 7.7 kilobase pairs from the C_μ gene in embryo DNA. The J_H-C_μ intervening sequence has suffered a deletion of about 2.7 kilobase pairs in HPC-76. Because the deletion encompasses sequences required to switch an expressed V_H-J_H gene from C_μ to another C_H gene, it may represent a mechanism for "freezing" a lymphocyte clone at the stage of IgM expression. For the second (inactive) C_μ allele in HPC-76, the entire joining and switch regions have been deleted; functional inactivation of one allele may thus represent one mechanism by which a lymphocyte clone restricts expression to a single allele (allelic exclusion). Probes generated from the cloned μ gene allowed examination of the J_H locus in B, Abelson "pre-B," and T lymphoma cell lines and a myeloid line, all of which contain RNA species bearing C_μ sequences. The B and pre-B lines exhibited recombination within both alleles of the J_H locus, suggesting that both alleles may be expressed in some cells. The absence of the J_H genes 5′ to the recombination sites favors a deletion mechanism for V_H-J_H joining. Recombination within the J_H locus was also detected in two out of four T lymphoma lines, but not in the myeloid line. This indicates that the mechanism by which B cells generate immunoglobulin diversity is operational in some T cells. Lines that synthesize μ RNA without J_H rearrangement may have activated the C_μ gene directly or have undergone recombination at a more distant locus.

An immunoglobulin (Ig) chain, whether light (κ or λ) or heavy (μ, α, γ, etc.), is the product of several distinct germline genes: a variable region (V) gene encoding most of the V amino acid sequence, a joining region (J) gene for the remaining V sequence, and a constant region (C) gene (1–10). The J gene is associated with the C gene but separated from it by an intervening sequence (2–8). Yet another element, the D_H gene, may encode a few amino acids preceding the J region of certain heavy (H) chains (5, 11). A functional Ig gene is formed by somatic recombination between V, (D), and J genes (2–7), most likely by deletion of all DNA between these elements (4, 10). After transcription, RNA corresponding to the J-C intervening sequence is removed by splicing and the resulting Ig mRNA bears contiguous V, (D), J, and C sequences (12). The progeny of a bone-marrow-derived lymphocyte (B cell) secrete only one light (L) and one heavy chain, each being derived from a single allele ("allelic exclusion") (13). However, the multiplicity of V genes (9, 10, 14–17) and J genes (3–6, 11) ensures that the B-cell population expresses a diverse array of Ig sequences.

μ heavy chains play a pivotal role in the immune response. They are the Ig chains expressed earliest during B-lymphoid differentiation, being detectable in the cytoplasm of "pre-B" cells, which do not yet express light chains or membrane Ig (18). A B lymphocyte can subsequently switch from synthesis of μ to another heavy chain type (for example, γ or α) while maintaining the same V_H sequence and, thus, the same antigen specificity (ref. 13; see also refs. 7 and 10). Recent evidence suggests that switching is achieved by a shift of the active V_H-(D_H)-J_H gene associated with C_μ to another C_H gene (7, 19), again by deletion (10, 20–23).

Although the antigen receptor on B cells is membrane Ig, the molecular basis for the antigen specificity of thymus-derived lymphocytes (T cells) is not yet established. In recent studies we showed that RNA bearing C_μ sequences is expressed in certain T lymphoma cell lines (24), in mouse thymocytes (25), and, surprisingly, in myeloid lines (24). This result is consistent with the controversial proposition that Ig heavy chains are components of the antigen receptor on T cells (26).

We wished to investigate rearrangements involving the J_H-C_μ locus in cells of the B lineage and to determine whether similar events occur in T cells. To this end, we have cloned a V_H-J_H-C_μ gene from an IgM-secreting plasmacytoma, the first active μ gene to be described, and we show that it was formed by somatic recombination. We have used fragments of the cloned μ gene as probes to examine the J_H locus in B and pre-B lymphoma, T lymphoma, and myeloid cell lines expressing μ RNA. The B and pre-B lines have undergone recombination within the J_H locus, presumably reflecting V_H-J_H joining; recombination occurred for both alleles, an observation that must be reconciled with allelic exclusion. Significantly, two T-cell lines have also undergone recombination within the J_H locus. This finding argues that the mechanism used by B cells to generate Ig diversity is operational in some T cells.

MATERIALS AND METHODS

Isolation of a Recombinant Phage Bearing V_H and J_H Sequences. Recombinant phage (8) bearing *Eco*RI fragments of DNA from plasmacytoma HPC-76 were screened by hybridization (27) with a ^{32}P-labeled (28) J_{H76}-specific probe (fragment b in figure 1 of ref. 8). DNA from the phage Ch-H76μ119 so identified yielded a 7.3-kilobase pair (kb) *Eco*RI fragment which hybridized to both the J_{H76} probe and a V_{H76}-specific probe (fragment a in figure 1 of ref. 8). Work was conducted in a P3 facility with EK2 host-vector systems.

Analysis of J_H and C_μ Genes Within Digests of Genomic DNA by Southern Blotting. Restriction endonuclease digests were fractionated by electrophoresis on agarose, blotted onto nitrocellulose filters (29), hybridized with ^{32}P-labeled probes, and autoradiographed as described (10). Cell lines STRij-4-2.2 (ST4), WEHI-22.1 (W22), STRij-1.3 (ST1), S49.1 (S49), WEHI-265.1 (W265), ABLS-8.1 (A8), AVRij-1.3 (A1), and WEHI-231.1 (W231) are described elsewhere (24).

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Abbreviations: V, variable; C, constant; J, joining region; L, light chain; H, heavy chain; kb, kilobase pair(s).

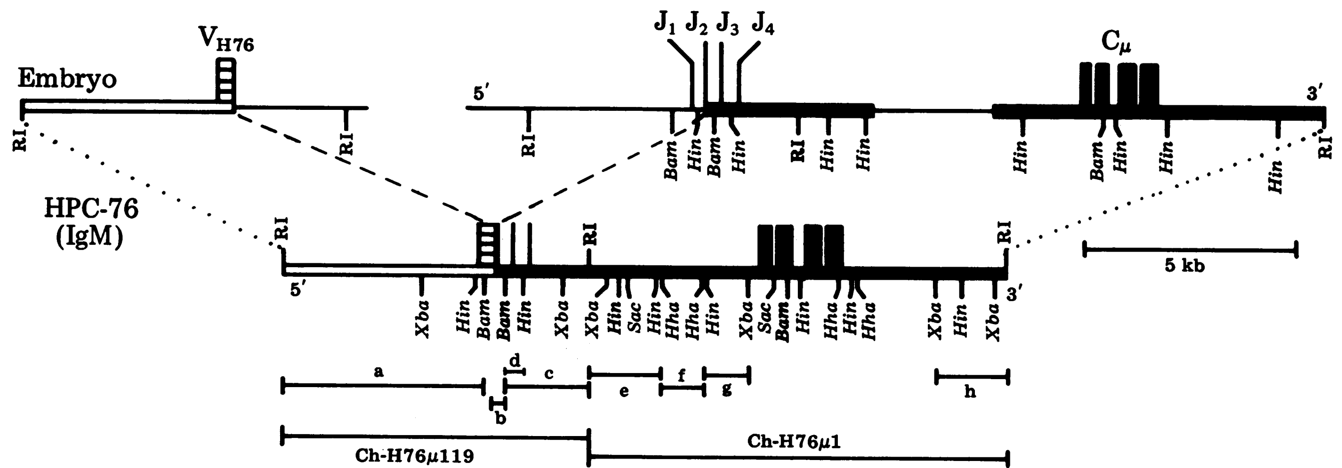

FIG. 1. Formation of the functional HPC-76 μ gene by somatic recombination between the embryonic V_{H76} gene and the second J_H gene. The four known J_H sequences are: J_1 (J_{H107}), J_2 (J_{H315}), J_3 (J_{HA4}), and J_4 (J_{H173}) (refs. 5 and 6; O. Bernard and N. Gough, personal communication). Restriction sites were determined by analysis of cloned *Eco*RI fragments from phages μ119 and μ1 or by Southern blot analysis of embryo DNA. The region between the J_H and C_μ genes in embryo DNA indicated by a narrow line has been deleted from HPC-76 DNA (see text). Fragments used as probes were derived as follows: *a* and *c*, *Bam*HI digestion of subcloned 7.3-kb *Eco*RI fragment from μ119; *b*, *Hae* III digestion of subcloned 0.5-kb *Bam*HI fragment from μ119; *d*, *Hin*dIII digestion of subcloned fragment *c*; *e* and *f*, *Hha* I digestion of subcloned 9.9-kb *Eco*RI fragment from μ1; *g*, *Xba* I digestion of subcloned 2.3-kb *Hin*dIII fragment from μ1; *h*, *Xba* I digestion of subcloned 5.2-kb *Bam*HI/*Eco*RI fragment from μ1.

RESULTS

Structure of a Functional μ Gene. We have cloned the V_H-J_H gene expressed in the IgM-secreting plasmacytoma HPC-76. DNA from the V_H-J_H clone Ch-H76μ119 hybridized to both J_{H76}- and V_{H76}-specific probes, whereas DNA from clones bearing homologous (9) unrearranged V_H genes hybridized only to the V_{H76} probe. We have previously described (8) a clone from HPC-76 DNA bearing a C_μ gene (Ch-H76μ1). The two cloned *Eco*RI fragments abut in the HPC-76 genome, as shown in Fig. 1. This was proved by demonstrating that a 3′-terminal segment of the VJ clone (probe *c* in Fig. 1) and a 5′-terminal segment of the C_μ clone (probe *e*) both hybridized to fragments of HPC-76 DNA spanning the common *Eco*RI site. Thus, in Southern blot (29) experiments, both probes hybridized to a 7.4-kb *Bam*HI fragment of HPC-76 DNA (Fig. 2*A*, tracks a and c) and to a 2.3-kb *Hin*dIII fragment (not shown), as predicted by the *Bam*HI and *Hin*dIII sites shown in boldface on the clone restriction maps (Fig. 1). A comparison (6) of sequences from μ119 and μ1 with that of HPC-76 μ mRNA has established that the clones encode the complete HPC-76 μ sequence and thus represent the functional μ gene.

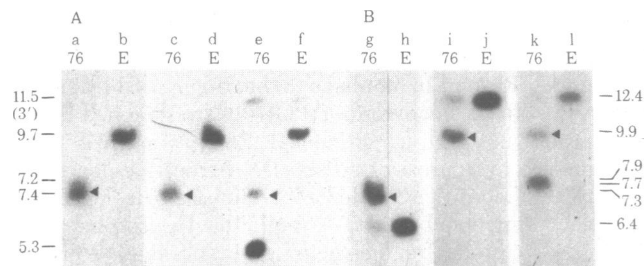

FIG. 2. Comparison of the context of J_H and C_μ genes in DNA from BALB/c embryo (E) and HPC-76 (76) by Southern blot analysis. (*A*) *Bam*HI digests; (*B*) *Eco*RI digests. The probes were: tracks a, b, g, and h, fragment *c* of Fig. 1; tracks c, d, i, and j, fragment *e* of Fig. 1; tracks e, f, k, and l, fragment from the 5′ end of the C_μ gene (fragment *c* in figure 1 of ref. 8), which includes a short sequence 3′ to the *Bam*HI site within the C_μ gene and thus also weakly labels the 3′ 11.5-kb *Bam*HI fragment (tracks e and f). *Bam*HI fragment sizes (in kb) are refinements of those in ref. 10. HPC-76 DNA used in *B* contains detectable levels of germline sequences due to contamination with nonlymphoid DNA (see ref. 10). Arrowheads indicate fragments derived from the active HPC-76 μ gene. The top and bottom of the autoradiograph, which contained no bands, have been cut off.

As shown in Fig. 1, the active HPC-76 μ gene was formed by recombination between a germline V_{H76} gene and a distantly located J_H gene. The recombined J_H gene is the second (6) of the four clustered J_H genes near the C_μ gene (refs. 5 and 6; O. Bernard and N. Gough, personal communication). Evidence for the recombination event is that neither the J_2 gene nor the V_{H76} gene remains in the germline sequence arrangement (i.e., context) in HPC-76 DNA. Thus, Southern blot analysis showed that the J_H genes, detected by probe *c*, are located in a 7.3-kb *Eco*RI fragment of HPC-76 DNA (Fig. 2, track g) rather than in a 6.4-kb fragment as in embryo DNA (track h) and that the V_{H76} sequence, detected by probe *a*, is located in a 7.3-kb *Eco*RI fragment rather than in a 7.7-kb *Eco*RI fragment (not shown). V_{H76} rearrangement is specific to HPC-76, because seven other plasmacytomas retained the V_{H76} gene in germline context.

HPC-76 Bears a Deletion Between the Active J_H and C_μ Genes. The location of the C_μ gene within μ1 (8) and of the J_H genes within μ119 (6) establishes that the J_4 gene is separated from the C_μ gene by a 5.0-kb intervening sequence in HPC-76 DNA (Fig. 1). However, these genes are $\approx$8.3 kb apart in the germline (Fig. 1) because the *Bam*HI site between J_2 and J_3 lies $\approx$9.7 kb from that within the C_μ gene, as determined by the size of the embryo *Bam*HI fragment revealed by probe *c* (Fig. 2, track b), probe *e* (track d), and a 5′ C_μ-specific probe (track f). This suggests that HPC-76 has suffered a deletion of $\approx$3.3 kb within this intervening sequence (Fig. 1). To determine the position of the deletion, we compared the sizes of the relevant *Hin*dIII fragments in embryo DNA with those expected from the clone maps (Fig. 3). The 2.5-, 1.0-, and 2.4-kb embryo *Hin*dIII fragments revealed by probes *c*, *e*, and *g* correspond well with the 2.3-, 0.9-, and 2.3-kb fragments expected from the clone maps, but probes *e* and *f* revealed an $\approx$4-kb embryo fragment rather than the 1.1-kb fragment found in μ1. Hence, the deletion occurred within the $\approx$4-kb embryo segment. We determined the position of the deletion more precisely by comparing the restriction map of μ1 with that reported (19) for the germline C_μ gene. A germline *Sac* site (asterisk in Fig. 3) is absent from HPC-76 DNA but the *Hha* site about 200 base pairs upstream is present, so the left boundary of the deletion must lie between these two sites (Fig. 3). The deletion is not a

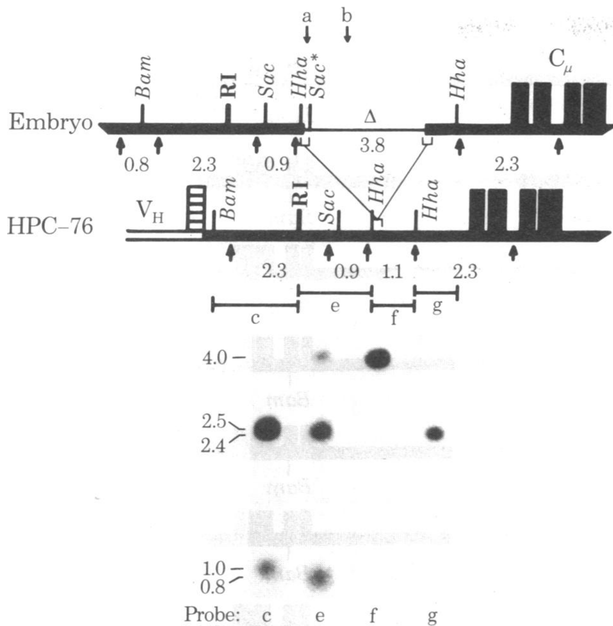

FIG. 3. A deletion within the sequence flanking the active HPC-76 C_μ gene. Southern blot analysis was performed on *Hin*dIII digests of embryo DNA with the indicated probes. In the restriction maps of embryo and HPC-76 DNAs, *Hin*dIII sites are indicated as arrows below the line; *Hin*dIII fragment sizes (in kb) are derived from analysis of μ1 and μ119 from refs. 5 and 19 and from the *Hha* I and *Sac* I sites in embryo from ref. 19. The region of embryo DNA indicated by a narrow line represents the sequence deleted from HPC-76 DNA. Arrows a and b indicate recombination sites inferred for switching expression from C_μ to $C_{\gamma 1}$ (19) and C_α (7), respectively.

cloning artifact because the C_μ-bearing *Eco*RI fragments of μ1 and HPC-76 DNA are indistinguishable in size (8, 10).

Inactivation of the Other C_μ Allele in HPC-76. We suspected that the other, presumably inactive, C_μ allele, which occurs in three copies per tetraploid cell (8, 10), also contained a deletion because it is found in smaller *Eco*RI (7.9 kb; track k, Fig. 2) and *Bam*HI (5.3 kb; track e) fragments. Significantly, neither of those fragments is labeled by the J_3/J_4 probe *c* (Fig. 2, tracks a and g) nor by probe *e* (tracks c and i). In addition to the fragment associated with the active gene, the J_3/J_4 probe faintly labeled a 7.7-kb *Eco*RI fragment (track g) and a 7.2-kb *Bam*HI fragment (track a), but their intensity is not sufficient to account for even one copy of the J_H region. Other experiments (not shown) revealed that the 7.9-kb *Eco*RI fragment bearing the inactive C_μ gene was labeled by probes *g* and *h* but not by probe *f*, indicating that the deletion extends to within about 2 kb of the C_μ gene. As shown in Fig. 5, we conclude that recombination at this allele deleted all J_H genes and most of the J_H-C_μ intervening sequence, thereby inactivating the C_μ gene.

Recombination Within the J_H Locus in T and B Cells. Probes generated from μ119 have enabled us to examine whether the J_H locus has undergone recombination in B, Abelson pre-B, and T lymphoma cell lines and a myeloid line, all of which are nearly diploid. Fig. 4*A* shows *Eco*RI digests of DNA from different lines scored with the J_3/J_4 probe (fragment *c*); Fig. 4*B* shows *Bam*HI digests scored with a J_2-specific probe (fragment *b*). Fig. 5 depicts the structure of J_H loci inferred from these results and those reported previously (24).

In ST4 T lymphoma cells, which contain μ RNAs (24), rearrangement has occurred at both alleles of the J_H locus; the J_3/J_4 probe hybridizes to *Eco*RI fragments of 12.5 and 5.3 kb (Fig. 4, track b), in contrast with the 6.4-kb embryo fragment

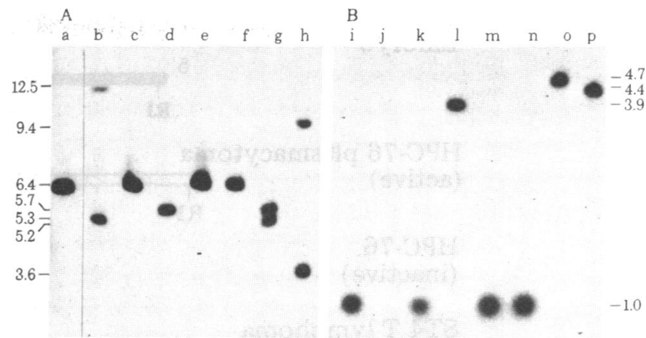

FIG. 4. Detection of J_H genes in pre-B and T lymphoma and myeloid cell lines. Southern blot analysis was performed on DNA from embryo (tracks a and i), from T lymphoma lines ST4 (tracks b and j), W22 (tracks c and k), ST1 (tracks d and l), and S49 (tracks e and m), from myeloid line W265 (tracks f and n), and from Abelson pre-B lines A8 (tracks g and o) and A1 (tracks h and p). (*A*) *Eco*RI digests hybridized with a probe for the J_3 and J_4 genes (fragment *c* in Fig. 1); (*B*) *Bam*HI digests hybridized with a J_2 gene probe (fragment *b*). The blank top and bottom of each autoradiograph have been cut off.

(track a). Moreover, this probe reveals two *Bam*HI fragments of 11.7 and 14.5 kb (not shown), which are indistinguishable in size from those detected (24) by a probe specific for the 5′ end of the C_μ gene. Because no alterations were detected in either the *Eco*RI fragment bearing the C_μ gene (not shown) or the *Bam* fragment lying 3′ to the C_μ gene (24), both rearrangements in ST4 must eliminate the embryonic *Bam*HI site between the J_2 and J_3 genes but not the *Eco*RI site to its right (Fig. 5), suggesting that recombination has occurred with J_3 or J_4. Because a J_3-specific probe (fragment *d*, Fig. 1) hybridized to only a single fragment in *Bam*HI and in *Eco*RI digests (the 11.7-kb and 5.3-kb fragments, respectively; not shown), the J_3 gene must have been deleted from one chromosome. Hence, recombination appears to have occurred with J_3 on one allele and J_4 on the other, as indicated in Fig. 5. If so, the J_2 gene and its flanking sequence should have been deleted from both alleles, and, indeed, no fragment of ST4 DNA was labeled by the J_2 probe (Fig. 4*B*, track j).

ST1 T lymphoma cells, which do not contain detectable levels of μ RNA (24), do contain rearranged J_H genes. The J_3/J_4 probe hybridized to an *Eco*RI fragment of 5.6 kb (Fig. 4, track d) and no sequences remained in the embryonic location. In *Bam*HI digests, the fragment detected by the J_3/J_4 probe was the same size as in embryo DNA (not shown), but that detected by the J_2 probe was different (compare tracks i and l in Fig. 4*B*). Hence, the germline *Bam*HI site 1 kb 5′ to the site between J_2 and J_3 has been removed. Recombination thus probably occurred at the J_1 or J_2 gene (Fig. 5). Because only one type of rearrangement was detected in ST1, similar events may have occurred at both alleles or one allele may have been deleted.

In pre-B lines A1 and A8 and the B line W231, which contain μ RNA (24), both alleles of the J_H locus are rearranged. In each line, the J_3/J_4 probe hybridized to two new *Eco*RI fragments (e.g., compare tracks g and h with track a in Fig. 4*A*); the J_3 probe labeled both of the A1 and A8 fragments but only one W231 fragment (not shown). For each line, *Bam*HI digestion showed that only one allele retained the *Bam*HI site between J_2 and J_3 (not shown). We infer that one allele in each line recombined at J_1 or J_2 and the other recombined at J_3 for A1 and A8 and J_4 for W231 (Fig. 5). This inference is supported by the detection of only a single *Bam*HI fragment by the J_2 probe (e.g., tracks o and p in Fig. 4*B*), the J_2 gene presumably having been deleted from one chromosome.

The myeloid line W265 and the T lymphoma cell lines W22

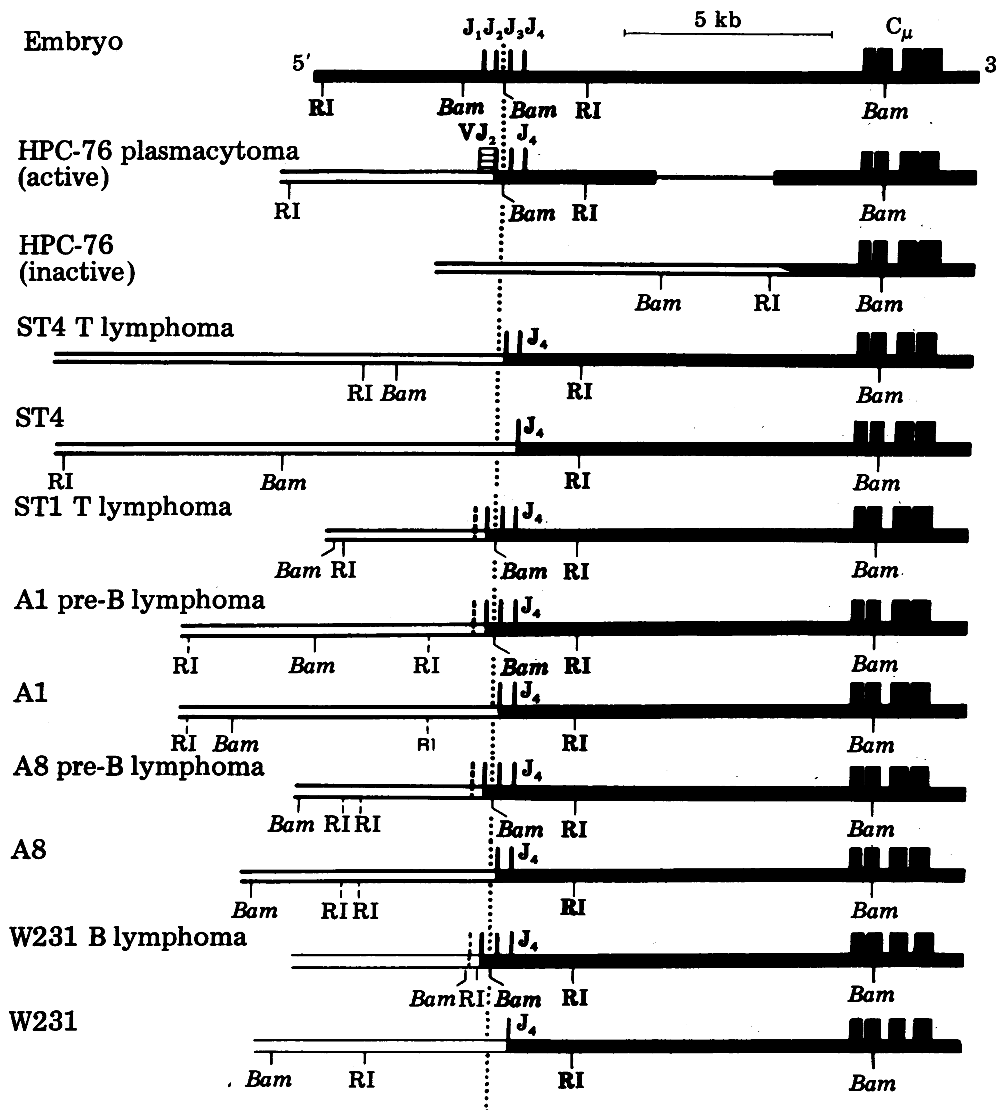

FIG. 5. Somatic rearrangements in B and T cells. *Bam*HI and *Eco*RI sites are shown near alleles of the C_μ gene in the germline (BALB/c embryo), the IgM-secreting plasmacytoma HPC-76, T-lymphoma lines ST4 and ST1, Abelson pre-B lines A1 and A8, and B lymphoma line W231. All the DNAs yield an 11.5-kb *Bam*HI fragment hybridizing to a 3′ C_μ probe so that this region 3′ to C_μ is retained in germline context, as are those sequences and sites 5′ to C_μ which are indicated in bold face. When it is not clear which *Eco* RI site belongs to a particular allele, both sites are indicated by broken lines. When it is not clear which of two J_H genes has recombined with a new region of DNA (shown open), the bold line is extended to the J_H gene closest to C_μ and the other J_H gene is shown as a broken line. The region deleted from the active HPC-76 allele (see text) is shown as a thin line.

and S49 do not appear to contain rearranged J_H genes; no fragment differing from its embryo counterpart was detected in *Eco*RI or *Bam*HI digests (Fig. 4) or, for W265 and W22, in a *Xba* I digest (not shown). W265 and W22 contain μ RNA, whereas S49 does not (24).

DISCUSSION

Analysis of the cloned HPC-76 μ gene shows that formation of a functional μ gene, probably a prerequisite for synthesis of any Ig heavy chain, occurs by somatic recombination between a specific V_H and a J_H gene (Fig. 1). The absence of the J_H genes 5′ to recombination sites in B and Abelson pre-B lymphoma lines supports our proposal (10) that V_H-J_H joining occurs by deletion of intervening DNA, as appears to be the case for $V_\lambda C_\lambda$ joining (4) and for C_H switching (10, 20–23).

Deletion as a Mechanism for "Freezing" C_H Expression. Although the J_H locus lies about 7.7 kb from the C_μ gene in the germline, a deletion has removed about 2.7 kb of this intervening sequence from the functional allele in HPC-76. The deletion does not affect processing of μ precursor RNA; HPC-76 contains properly spliced mRNA and synthesizes μ chains. Significantly, the deletion encompasses sequences concerned with switching an active V_H-J_H gene from C_μ to other C_H genes. Arrows a and b in Fig. 3 mark switch sites for recombination with $C_{\gamma 1}$ (19) and C_α (7) genes. A deletion removing all switch sites would prevent C_H switching and could thus represent a mechanism for "freezing" a lymphocyte clone at the stage of IgM expression.

Relationship of Rearrangement to Allelic Exclusion. Rearrangement of Ig genes in many plasmacytomas is not restricted to one allele (8, 10, 15, 30, 31). We have shown here that rearrangement of the second C_μ allele in HPC-76 has removed the entire joining and switch region (Fig. 5). Because neither V_H-to-J_H translocation nor C_H switching can occur without this region, the entire C_H locus on that chromosome has presumably been inactivated. Thus, aberrant rearrangement leading to

functional inactivation of one allele is one attractive mechanism for allelic exclusion.

In contrast, both alleles of the J_H-C_μ locus in the pre-B lines A1 and A8 and the B lymphoma line W231 (Fig. 5) appear to have undergone V-J recombination. If so, allelic exclusion must not operate in all cells or must operate at some later step in gene expression. The observation (32) that about half the C_κ genes in a B-cell population remain in germline context indicates that both C_κ alleles do not rearrange in the majority of normal B cells, but it does not exclude rearrangement of both in a minority (even 20–30%). The results with the B lymphomas argue strongly against models (32) in which only one allele is capable of V-J recombination.

Our results favor the notion (33) that recombination occurs with a certain frequency, independently for each allele. An inefficient joining mechanism, combined with a significant frequency of aberrant rearrangements, would then account for allelic exclusion in most, but not all, B cells.

Some T Cells Undergo Recombination Within the J_H Locus. Rearrangement within the J_H locus was also found in T lymphoma cell lines ST4 and ST1, again involving both alleles (Fig. 5). Recombination occurred at, or very near, a known J_H gene, and the portion of the J_H locus 5′ to the recombination site was deleted. Restriction mapping established that, for each line, different DNA sequences had recombined with the J_H region (Fig. 5). This marked similarity to events in B cells suggests that the DNA recombined at the J_H locus bears a V_H or an analogous gene. Although ST4 contains as much μ RNA as pre-B lines, none was detectable in ST1 (24), perhaps because it represents a developmental stage prior or subsequent to μ expression. These observations, in conjunction with our demonstration of μ RNA species in normal thymocytes (25), provide compelling evidence that the Ig heavy chain locus is active in T cells. Although other explanations are obviously possible (24), the most attractive hypothesis is that a polypeptide encoded at this locus constitutes a component of the T-cell antigen receptor. However, μ polypeptides cannot be detected serologically in T lymphoma lines or in some pre-B lines that contain μ RNA (I. D. Walker and A. W. Harris, personal communication).

One T lymphoma (W22) and a myeloid (W265) cell line, both known to express μ RNA of the same sizes as ST4 (24) and thymocytes (25), apparently retain the C_μ and four known J_H genes in germline context. This also appears to be the case for several other T and myeloid lines expressing μ RNA. Any rearrangements up to 3.8 kb beyond the known J_H cluster should have been detectable (Fig. 5). It is conceivable that only a small fraction of the cells in these cloned lines undergo rearrangement and synthesize μ RNA. However, it seems more likely that some T cells and certain cells of the granulocyte–macrophage lineage activate the C_μ gene in a new manner, either independently of rearrangement or by recombination at a more distant J_H (or D_H) locus.

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