ACTIVE λ AND κ ANTIBODY GENE REARRANGEMENT IN ABELSON MURINE LEUKEMIA VIRUS–TRANSFORMED PRE-B CELL LINES

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Transformation of murine adult bone marrow cells by Abelson Murine Leukemia Virus (A-MuLV)[1] can result in the generation of permanent cell lines (Abelson lines) representing early stages of B lymphocyte differentiation (1). Because it is difficult to obtain large numbers of B cell precursors capable of Ig gene recombination from normal cell populations, Abelson cell lines provide an accessible model system in which to study the development and regulation of immunoglobulin (Ig) gene rearrangement. Many A-MuLV transformants exhibit characteristics of the pre-B cell stage of B cell development, i.e., rearranged and expressed heavy (H), but not light (L), chain genes (2–6). Active Ig H-chain gene rearrangement has been found to occur in certain A-MuLV-transformed pre-B cell lines during maintenance in tissue culture (3). In addition, some Abelson lines that have rearranged H-chain genes proceed to recombine κ light chain genes (7–10) in culture, and some have been observed to spontaneously undergo deletion of κ constant region (Cκ) genes (7, and this report).

We have previously described a class of recombination events that are associated with Cκ deletion (11, 12). These recombinations involve a segment of DNA, designated as RS (for recombining sequence), which joins directly to either a κ variable region (Vκ) gene segment or to the Jκ-Cκ intron (11, 12). RS DNA lies at least 15 kb downstream of the Cκ exon on chromosome 6; thus either type of RS recombination results in Cκ deletion (12). RS recombination events are mediated by recognition sequences that are similar to those involved in V-J joining (12). RS recombinations are found frequently in hybridomas producing λ light chains, but not in κ-producing hybridomas (11), suggesting RS recombination may be linked to λ gene rearrangement in developing B cells. In addition, a recombining DNA element (kde) that appears to be analogous to RS and that is involved with Cκ deletion in human B cells has also been identified (13). RS DNA and kde segments share extensive nucleotide sequence homology, indicating that these elements have been evolutionarily conserved (13a). These results

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[1] *Abbreviations used in this paper:* A-MuLV, Abelson murine leukemia virus; RS, recombining sequence.

suggest that RS recombinations may have an important role in normal murine B cell development.

Mature B cells express either κ light chains or λ light chains but not both. No examples of A-MuLV-transformed pre-B cell lines capable of undergoing λ gene rearrangements have been reported to date. Previous studies have suggested the hypothesis that κ gene recombinations precede λ gene recombinations in normal maturing B cells because λ-producing myelomas and lymphomas almost always exhibit recombined κ genes, whereas κ-producing tumor cells almost always exhibit λ genes in a germline (nonrearranged) context (14–17). The absence of λ gene rearrangement among those Abelson lines capable of active κ gene recombination is consistent with this hypothesis (7, 8). In this report, we characterize the first Abelson cell line capable of active λ gene recombination in culture and compare this to an Abelson line that only recombines κ genes. Our studies suggest that maturing pre-B cells pass through two developmental stages, first recombining κ genes and then recombining both κ and λ genes. We also show that RS recombinations appear to occur at the appropriate developmental timepoint to act as signals for the initiation of λ gene rearrangement.

Materials and Methods

Cell Lines. The BM18-4 cell line was obtained from Naomi Rosenberg (Tufts University, Boston, MA) and the ABC-1 cell line was obtained from Susanna Lewis and David Baltimore (Massachusetts Institute of Technology, Cambridge, MA). The lines were maintained in tissue culture in RPMI 1640 (Gibco, Grand Island, NY) medium with 50 μM 2-ME supplemented with 10% heat-inactivated FCS. BM18-4 originated as a single-focus lymphoid colony derived by in vitro A-MuLV infection of adult bone marrow cells from a BALB/c mouse (18). The BM18-4 cell line has previously been shown to contain low levels of terminal deoxynucleotidyl transferase and is positive for the cell surface antigens Lyb-2, Lyt-4, and SC-1 (18). No Ig protein has been found in BM18-4 (18). The ABC-1 cell line also originated from adult BALB/c mouse bone marrow cells transformed in vitro by A-MuLV (19). Previous reports have indicated that no ABC-1 cells express cell surface Ig, although low levels of cytoplasmic μ chain have been detected within some cells of the ABC-1 population (20, 21). In addition, reported analyses of the AT1 subline, which was derived from ABC-1, indicated that a small proportion of AT1 cells exhibited positive staining for cytoplasmic λ light chain protein (21). ABC-1 cells possess low but detectable levels of terminal deoxynucleotidyl transferase (20), but do not express the surface markers Thy-1.2, Ia, TL 1, 2, 3, 4, or peanut agglutinin (20).

Subcloning. BM18-4 and ABC-1 cells were subcloned by limiting dilution, 0.2 cells/well in 96-well microtiter plates. Growth of individual clones was evident 10–14 d after subcloning. 20% (or less) of the wells in a plate exhibited growth, indicating that individual subclone populations were most likely initially derived from a single cell. DNA was prepared from cells 2.5 to 3 wk after the initial subcloning, at which time cells were also frozen and stored in liquid nitrogen.

DNA Probes. Cloned DNAs used as hybridization probes were pES202, a 400 bp fragment of the Cκ exon cloned into pBR322 (22); pHBCκ, a 3.5 kb Hind III/Bam HI Cκ-containing piece that was cloned into pBR322 (7); pJκ, a 2.8 kb Hind III fragment that includes all five Jκ genes cloned into pBR322 (22); pDP2-5, a 2.1 kb embryonic Xba I fragment located ~4 kb upstream of Jκ1 cloned into pUC13; pJ11, a 2.2 kb Bam HI/Eco RI fragment that contains JH gene segments JH3 and JH4 in addition to ~1 kb of intron sequence 3′ of JH4 cloned into pBR322 (23); pDhFL16.1 5′, a 700 bp Pst I fragment that contains sequences ~1 kb upstream of the germline DHFL16.1 cloned into pUC12 (provided by Peter Brodeur, Tufts University, Boston, MA); pλ1V+C, a full length cDNA cloned into pBR322 derived from MOPC 104E (24); pVλ, a 230 bp fragment from the

5′ untranslated region to amino acid 57 in Vλ1 cloned into pBR322 (25); Vλ1 fragment, a 1 kb Xba I/Hind III fragment containing the germline Vλ1 gene segment that was isolated from pLT2 (26); and rs0.8, an 800 bp Sau 3A fragment specific for RS DNA sequences which was cloned into pBR322 (11, 12). pES202, pHBCκ and pJκ were used to identify κ chain rearrangements; pλ1V+C, pVλ, and the Xba I/Hind III Vλ1 fragment were used to identify λ chain rearrangements; and rs0.8 was used to identify RS DNA rearrangements. pDP2-5 was used to detect κ remnant DNAs.

Agarose Gel Electrophoresis and Transfer to Nitrocellulose. DNA was prepared from BALB/c kidney and cultured A-MuLV cell lines as described previously (27). Restriction enzyme–digested genomic DNAs were loaded onto 0.8% agarose gels (20 μg/lane), electrophoresed (buffer: 40 mM Tris-HCl, pH 7.8, 20 mM sodium acetate, 2 mM EDTA) and transferred to nitrocellulose (Sartorius, Hayward, CA) by the method of Southern (28). All hybridization probes were nick-translated to a specific activity of $\sim 10^8$ cpm/μg. Both pretreatments and hybridizations of nitrocellulose blots were at 65°C in 0.45 M NaCl, 0.045 M sodium citrate, pH 7, 0.2% polyvinylpyrrolidone, 0.2% BSA, 0.2% Ficoll, 0.1% SDS, 50 μg/ml salmon sperm DNA. Hybridization was for 48 h, followed by washing at 65°C in 0.15 M NaCl, 0.0015 M sodium citrate, pH 7. Filters were exposed to x-ray film at −70°C.

Isolation of BM18-4.20.2.19 JH-containing Clone. The Bam HI/Eco RI JH containing fragment of pJ11 (23) was used to detect and isolate a recombined JH clone from an EMBL4 (29) library of Eco RI–digested DNA from the cell line BM18-4.20.2.19.

Western Blot Analysis. $\sim 5 \times 10^6$ cells were lysed in a phospholysis buffer containing 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 0.1 M NaCl and 0.01 M sodium phosphate. Lysates were reduced in the presence of mercaptoethanol, fractionated by electrophoresis through a 10% SDS-polyacrylamide gel, and electrotransferred to nitrocellulose. The blot was reacted with ^{125}I-labeled affinity-purified rabbit anti–mouse IgM at 4°C overnight in 1% casein-PBS, washed three times in PBS, and exposed to x-ray film at −70°C.

Results

Ig Gene Recombination in the BM18-4 Cell Line. The V and C regions of H and L immunoglobulin chains are encoded in the germline by multiple gene segments that must be brought together by somatic recombination to form a functional gene (30). Previous analyses of the BM18-4 line had suggested that κ gene recombination might be occurring within this cell population (22). We performed subclone analyses to ascertain the status of κ gene rearrangement within BM18-4. Southern blot analyses of primary, secondary, and tertiary BM18-4 subclone DNAs using either Cκ or Jκ probes are shown in Fig. 1. In these analyses, germline Cκ alleles give rise to a 13 kb Bam HI hybridization band, whereas V-J joined κ genes will generally result in hybridization bands that are smaller or larger than 13 kb depending on the Vκ segment involved in the joining. As is evident in Fig. 1, many BM18-4 subclones display germline Cκ genes as well as submolar or molar recombined κ genes. Submolar κ genes, generally indicated by weaker hybridization intensities, are likely to be present in only a portion of the cell population analyzed, and indicate heterogeneity within a subclone. Because most, if not all, subclones were derived from single cells, the diverse κ gene rearrangements observed even after sequential subcloning indicate that new V-J joined κ genes are continually arising during the growth of the BM18-4 line. Because a randomly selected group of BM18-4 subclones exhibited identical rearranged JH segments and identical A-MuLV integration sites in Southern blot analyses (data not shown), it appears that the BM18-4 line resulted from the

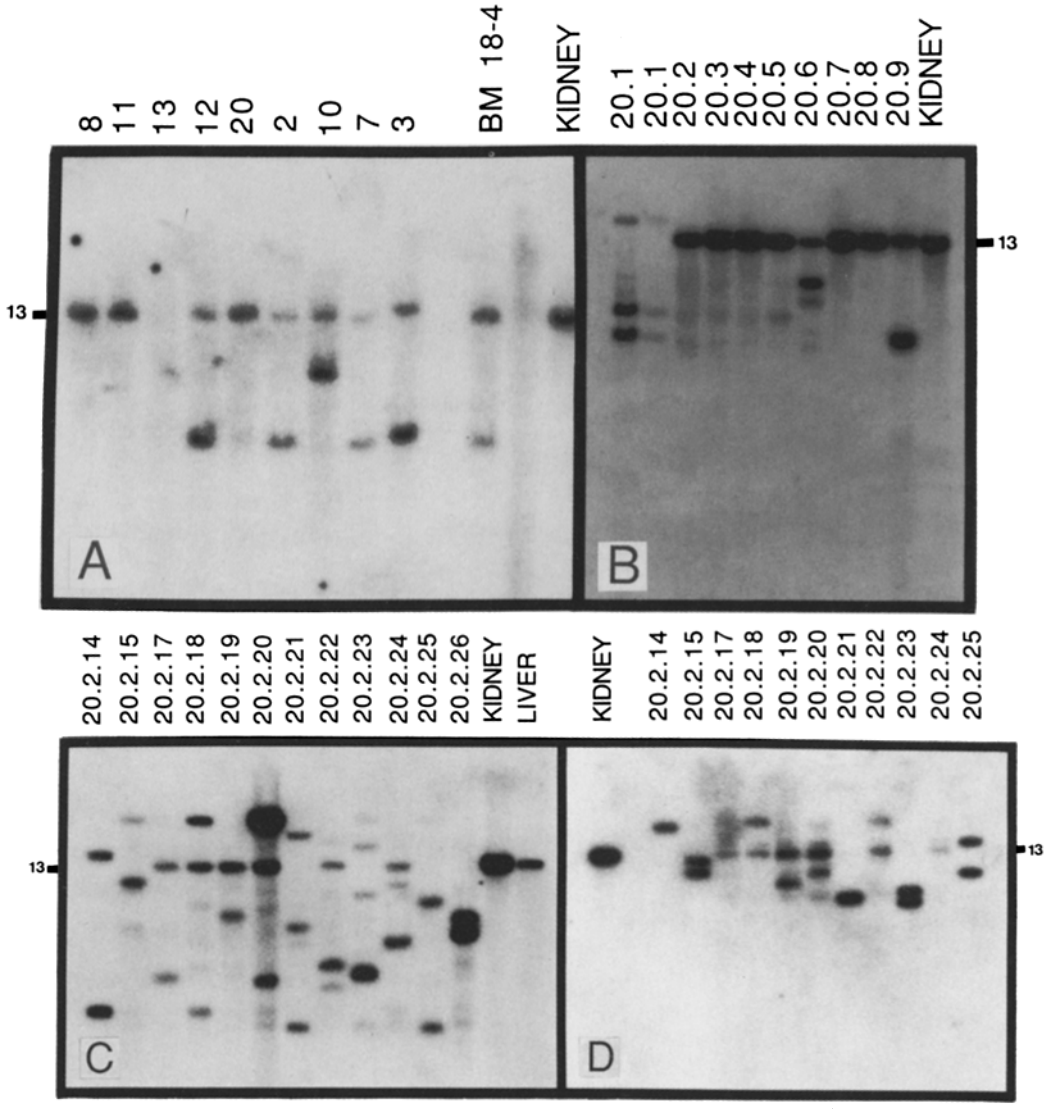

FIGURE 1. κ gene recombination in BM18-4 sublines. Southern blot analyses of genomic DNAs hybridized with κ region probes. Total genomic DNAs (~20 μg) from BALB/c kidney and BM18-4 subclones were digested with Bam HI and probed with a Cκ probe in primary BM18-4 subclones (*A*) or with a Jκ region probe for subsequent subclone analysis of BM18-4.20 (*B*) and BM18-4.20.2 (*C*). Bam HI–digested genomic DNAs of BM18-4.20.2 subclones were also probed with pDP2-5 (a 5′ of Jκ1 probe) and reveal the presence of remnant DNAs within BM18-4 sublines (*D*). Bam HI–digested BALB/c kidney DNA lanes show one 13 kb hybridization band, which includes the Jκ-Cκ region.

transformation of a single pre-B cell that has retained the capacity for active and spontaneous κ gene recombination during propagation in tissue culture.

Several reports have shown that the DNA sequences that separate Vκ and Jκ segments in the germline are not always lost from the cell after Vκ → Jκ joining (7, 22, 31–34). These retained DNAs, designated as remnant or reciprocal

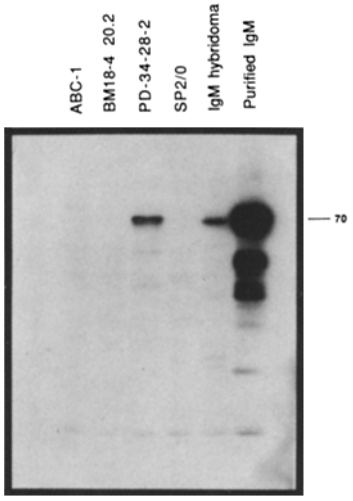

FIGURE 2. Western blot analysis of μ-chain protein from A-MuLV-transformed cell lines BM18-4, ABC-1, and PD-34-28-2. ~5 × 10^6 cells were lysed, lysates were reduced in the presence of 2-ME, and fractionated by electrophoresis through a 10% SDS-polyacrylamide gel and electrotransferred to nitrocellulose paper. The blot was reacted with ^{125}I-labeled affinity-purified rabbit anti-mouse IgM. The IgM-producing hybridoma dE2 and purified IgM from dE2 were used as μ^+ controls and the SP2/0 cell line was used as a μ^- control. Most sublines of the A-MuLV line PD have been shown to express cytoplasmic μ chain (7); the PD subline PD-34-28-2 was therefore also included as a control in this analysis.

DNAs, have been proposed to be a by-product of κ gene rearrangement (7, 22, 31–34). Analyses of BM18-4 subclones show that recombined remnant DNA segments are found in many BM18-4 cells (Fig. 1*D*). This finding supports the hypothesis that remnant DNAs are a product of V-J joining and are created concomitantly with recombined κ genes.

We have also screened the BM18-4 line for recombination of λ light chain genes. Despite the fact that we can detect molar or submolar recombined κ genes in almost all BM18-4 subclones, we have not observed any evidence of λ gene recombination in analyses of 23 distinct BM18-4 subclones (not shown).

Previous reports indicate that BM18-4 does not express any heavy or light chain proteins (18). By Southern blot analyses using JH and Cμ probes, we found that the single recombined JH segment in BM18-4 is linked to the Cμ gene segment and has not undergone any class-switch recombinations (not shown). We cloned this recombined JH segment and, after hybridization with a 5′ of D probe, found that it represented a DJ join with no attached V_H segment. This incomplete μ gene is apparently nonfunctional because Western blot analyses (Fig. 2) of cytoplasmic proteins in BM18-4 do not show any mature μ chains or any Dμ chains (35).

Ig Gene Recombination in the ABC-1 Cell Line. Because a subline of ABC-1 had been reported to produce λ light chains (21), we analyzed the parental line and several primary subclones to determine the status of κ and λ gene recombination in ABC-1. As shown in Fig. 3*A*, recombined κ genes are found in the parental ABC-1 line and in the primary ABC-1 subclones. The parental line exhibits a predominant germline Jκ-Cκ component together with a variety of submolar recombined bands, whereas individual ABC-1 primary subclones exhibit a variety of distinct recombined κ genes. Thus, with regard to κ genes, the ABC-1 line appears quite similar to the BM18-4 line described above. However, in contrast to the BM18-4 line, ABC-1 cells also exhibit evidence of λ gene recombination (Fig. 3*B*). Southern blot analyses of the parental ABC-1 population using a λ1V+C probe show Eco RI hybridization bands of 7.4 kb and 2.8 kb that

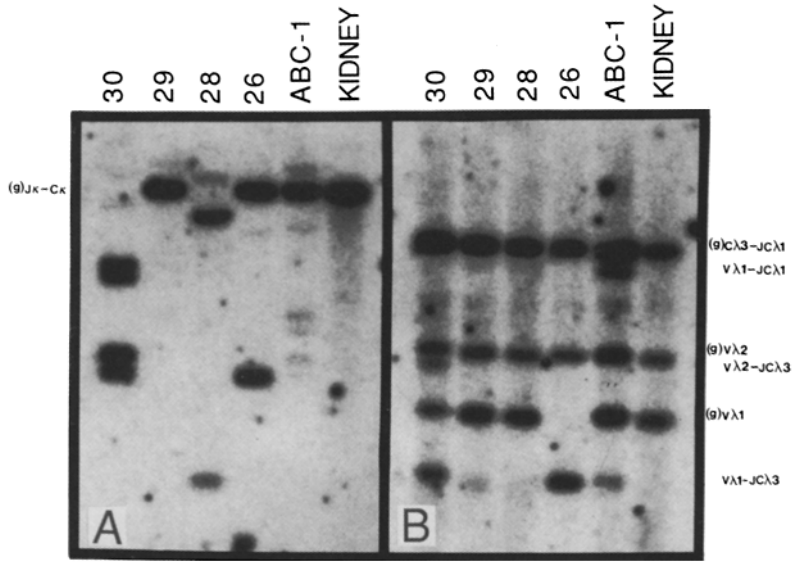

FIGURE 3. κ and λ gene recombinations in primary sublines of ABC-1. Southern blot of genomic DNAs probed with κ gene–specific (*A*) or λ gene–specific (*B*) probes. In *A*, genomic DNAs have been digested with Bam HI and hybridized to a Jκ probe. In *B*, Eco RI–digested DNAs (~20 μg) from BALB/c kidney and ABC-1 subclones were probed with the λ gene–specific probe, pλ1V+C. The sizes (in kb) of predicted germline or recombined λ gene segments in BALB/c mice are listed below. 8.6, 4.8, and 3.5 kb bands correspond to germline Cλ3-Jλ1Cλ1, Vλ2, and Vλ1 gene segments, respectively. The rearranged bands at 7.4, 4.4, and 2.8 kb correspond to the recombined λ genes Vλ1-Jλ1Cλ1, Vλ2-Jλ3Cλ3, and Vλ1-Jλ3Cλ3, respectively. Band assignments have been confirmed using Vλ1, Cλ1, and Cλ2 subregion–specific probes (data not shown).

correspond to Vλ1 → Jλ1 and Vλ1 → Jλ3 recombinations, respectively, as well as bands corresponding to germline λ gene segments. The heterogeneity of the ABC-1 parental population is apparent when the primary subclones are examined. Many of the ABC-1 subclones exhibit both recombined κ and λ genes, and distinct combinations of recombined κ and λ genes are evident among each of the primary subclones. The ABC-1 line appears to be derived from a single transformed progenitor cell because all subclones exhibit a common rearranged JH hybridization band and all subclones share the same A-MuLV proviral integration site as revealed by a A-MuLV v-*abl* hybridization probe (not shown). These results suggest that some ABC-1 cells have undergone κ and λ gene recombination during establishment and maintenance of the ABC-1 cell line in tissue culture.

We were interested in determining whether any ABC-1 cells within the parental population had retained the capacity for continuing λ and/or κ gene recombination. Our analysis of the λ genes in several ABC-1 primary subclones indicated that Vλ1 → Jλ1 and Vλ1 → Jλ3 recombined bands were present in submolar concentrations (e.g., see ABC-1.28 in Fig. 3*B*). Because these subclones were derived from single cells, this observation suggested that recombination of λ genes had occurred during the subsequent outgrowth of the subclone before preparation of DNA for Southern blot analysis. We performed secondary subcloning of several ABC-1 primary subclones to determine whether the apparently

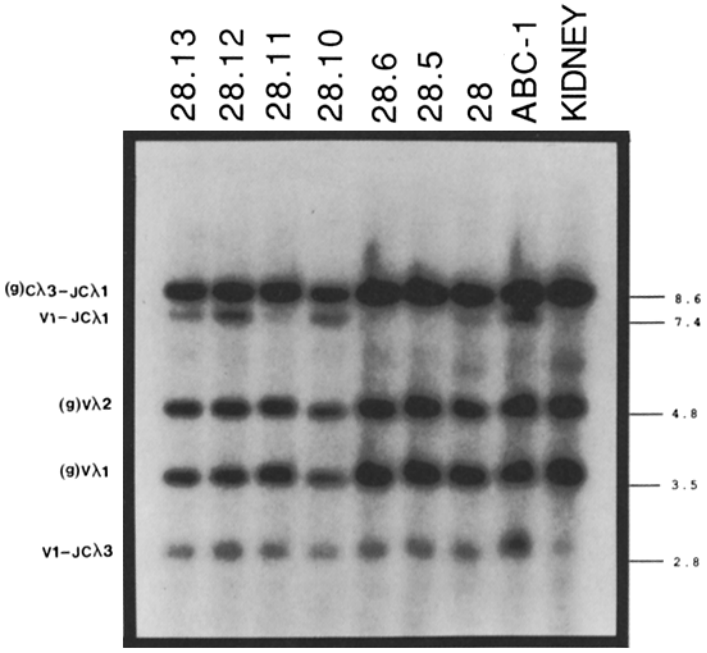

FIGURE 4. λ gene rearrangements are evident in secondary subclones of ABC-1.28. Eco RI–digested genomic DNAs from kidney, ABC-1, ABC-1.28, and its subclones have been probed with pλ1V+C. Band assignments are as in Fig. 3*B*.

submolar recombined λ hybridization bands were, indeed, indicative of heterogeneity within the primary subclone populations and whether recombination of λ genes in ABC-1 cells would continue after further subdivision. Southern blot analyses of secondary subclones derived from ABC-1.28 are shown in Fig. 4. Some ABC-1.28 subclones are found to have their Jλ1 genes entirely in a germline configuration, clearly indicating that the recombined λ1 genes observed in the progenitor ABC-1.28 population were, in fact, not present in all ABC-1.28 cells. In addition, several secondary subclones display recombined λ genes and, among these, some clearly have submolar levels of recombined genes. Further analyses of two other primary subclones, ABC-1.29 and ABC-1.30, and of tertiary subclones derived from selected secondary ABC-1.28 subclones gave analogous results (not shown). From this data, it is apparent that the ABC-1 cell line retains the capacity for continuing recombination of λ genes during passage in tissue culture.

As described above, our parental ABC-1 population showed many submolar recombined κ genes when analyzed using a Jκ probe; this suggested that some ABC-1 cells might be capable of κ gene recombination in culture. To determine whether a single ABC-1 cell had the capacity to rearrange both κ genes and λ genes, we investigated the κ gene organization in one of the ABC-1 subclones that we had found to be capable of λ gene recombination. We chose to analyze ABC-1.29 because this subclone exhibited a germline κ allele that should provide a suitable substrate for any recombinational activity. ABC-1.29 has an RS recombination on one κ chromosome (see below) so that there is only one Jκ

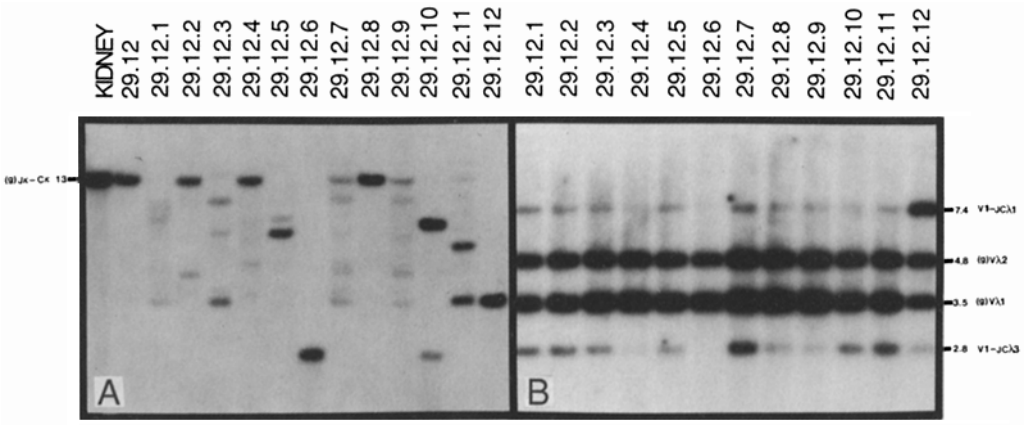

FIGURE 5. κ and λ gene organization in tertiary subclones derived from ABC-1.29. Subclones of ABC-1.29.12 were digested with Bam HI and probed with the Cκ-region probe pHBCK (*A*) or digested with Eco RI and probed with the Vλ1 fragment (*B*). Hybridization signal at 13 kb corresponds to the germline Jκ-Cκ locus (*A*). λ gene band assignments are as in Fig. 3*B*.

allele present in these cells. Southern blot analyses of 16 secondary subclones derived from ABC-1.29 showed that two subclones had faint submolar κ hybridization bands indicative of κ gene recombination (not shown). One of these secondary subclones, ABC-1.29.12, was further divided by tertiary subcloning; Fig. 5 shows the κ and λ gene organizations in these tertiary subclones. The clear evidence of both κ and λ gene recombination in all of the ABC-1.29.12 tertiary subclones demonstrates that both of these light chain gene families can recombine in some ABC-1 cells.

We have also analyzed ABC-1 cells for κ and λ remnant DNAs. As in BM18-4, ABC-1 subclones often display κ remnant DNAs (not shown). However, using a hybridization probe that contains 200 bp of sequences flanking the 3′ end of the germline Vλ1 gene, we have not detected evidence of λ remnant DNA segments among eight ABC-1 subclones having recombined λ genes (not shown).

The active recombination of λ genes in the ABC-1 cell line provides the first opportunity to directly compare the gene rearrangements for different λ isotypes. In subclone analyses of ABC-1 cells, the predominant λ gene recombinations that are observed are of the Vλ1 → Jλ3 or Vλ1 → Jλ1 type. Although precise comparisons of recombination frequencies are not possible because Southern blot analyses must be performed on relatively large numbers of cells, simply counting the number of subclones with Vλ1 → Jλ3 or Vλ1 → Jλ1 recombinations indicates that the rearrangement of Vλ1 to Jλ1 occurs at about the same rate as rearrangement of Vλ1 to Jλ3 (e.g., Figs. 3, 4, and 5). On the other hand, we have not detected any Vλ2 → Jλ1, Vλ1 → Jλ2, Vλ2 → Jλ2, or Vλ2 → Jλ4 recombinations among primary and secondary ABC-1 subclones. The lack of Vλ2 → Jλ2 recombinations was surprising because λ2 proteins have been reported to be present on almost as many mature B cells as λ1 proteins (36, 37). Tertiary subclone analysis of ABC-1.28.13 did reveal one Vλ2 → Jλ2 recombination (Fig. 6), indicating that this recombinational event can occur within ABC-1 cells. Clearly, however, Vλ2 → Jλ2 recombination occurs much less frequently

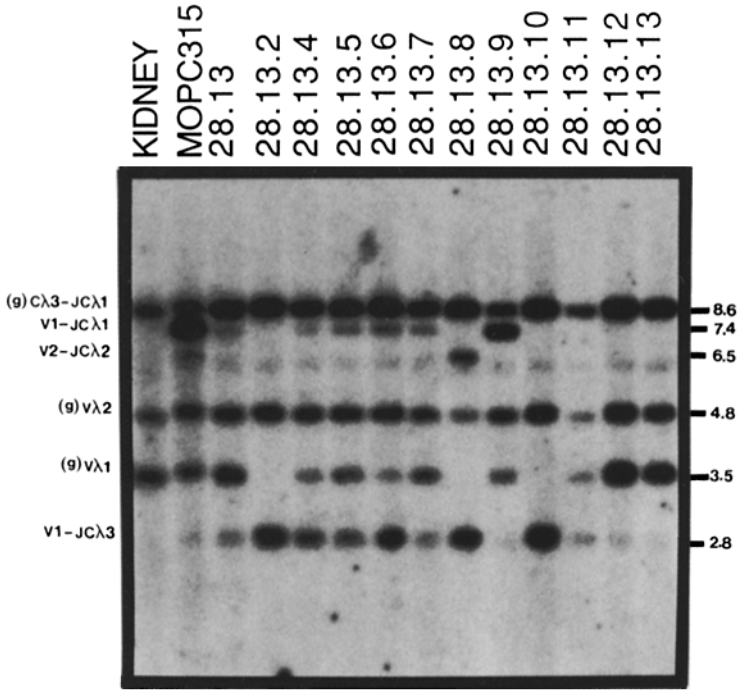

FIGURE 6. λ gene recombinations are evident in tertiary subclones derived from ABC-1.28. Eco RI–digested genomic DNAs from BALB/c kidney, MOPC315, and tertiary subclones derived from ABC-1.28.13 were probed with pλ1V+C. The 6.5 kb band found in the λ2-producing myeloma MOPC315, and ABC-1.28.13.8 corresponds to a Vλ2-Jλ2Cλ2 rearrangement. The weak hybridization signal seen at ~6.0 kb in some lanes represents incomplete digestion with Eco RI (48). Hybridization of this blot to the subregion-specific probe pVλ eliminates hybridization to the partial digestion product at 6.0 kb and clearly also reveals the recombined 6.5 kb fragment in both MOPC315 and ABC-1.28.13.8, indicative of Vλ2-Jλ2Cλ2 (data not shown).

in ABC-1 cells than Vλ1 → Jλ3 or Vλ1 → Jλ1 recombination. Among the ABC-1.28.13 subclones shown in Fig. 6, several apparently exhibit Vλ1 → Jλ3 rearrangements on both alleles, as shown by a lack of germline Vλ1 genes. In these subclones, germline Vλ2 segments (Fig. 6) and germline Jλ2Cλ2-Jλ4Cλ4 segments (not shown) are still present, suggesting that Vλ2, Jλ2Cλ2, and Jλ4Cλ4 are located upstream of Vλ1 or downstream of Jλ3Cλ3-Jλ1Cλ1 in the germline chromosome (see Discussion).

In one ABC-1 subclone, ABC-1.30, a hybridization band indicative of a Vλ2 → Jλ3 rearrangement, is observed (Fig. 3*B*). The 4.4 kb fragment observed in Eco RI–digested ABC-1.30 DNA is diagnostic of a Vλ2 → Jλ3 rearrangement (38, 39); this band assignment was also confirmed using Vλ and Cλ subregion-specific probes (not shown). The Vλ2 → Jλ3 gene recombination has been reported in one λ-producing hybridoma, but is apparently rare (39). Most ABC-1.30 cells exhibit both Vλ2 → Jλ3 and Vλ1 → Jλ3 recombined alleles because all nine subclones derived from ABC-1.30 show the same diagnostic hybridization bands as observed in the parental population (Fig. 7). However, in one subclone (ABC-1.30.7 in Fig. 7), an additional Vλ1 → Jλ1 hybridization band is found;

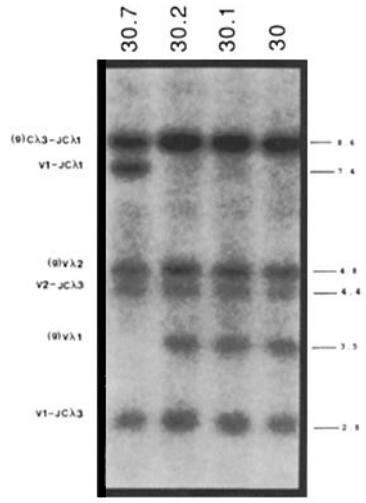

FIGURE 7. λ gene organization of secondary subclones derived from ABC-1.30. Southern blot hybridization of Eco RI–digested genomic DNAs probed with pλ1V+C. Rearranged bands at 7.4 and 2.8 kb represent recombined Vλ1-Jλ1Cλ1 and Vλ1-Jλ3Cλ3 λ gene segments. The Vλ2-Jλ3Cλ3 rearrangement at 4.4 kb is found among all nine secondary subclones of ABC-1.30 (additional data not shown). Restriction enzyme digestion of genomic DNA with Eco RI separates Jλ3 from the rest of the germline Cλ3-Jλ1Cλ1 cluster onto two different restriction fragments (48). Therefore, Vλ2 → Jλ3 and Vλ1 → Jλ3 recombinations do not alter the 8.6 kb Eco RI Cλ3-Jλ1Cλ1 fragment.

this suggests that most ABC-1.30 cells are aneuploid, having three copies of the λ chromosomal locus (see Fig. 7 legend). Significantly, a germline Vλ1 allele found in other ABC-1.30 secondary subclones (Fig. 7 and other data not shown) is deleted in ABC-1.30.7. This finding implies that germline Vλ1 gene segments have been deleted from the recombined Vλ2 → Jλ3 chromosomes found in most ABC-1.30 cells and that, therefore, the Vλ1 gene is located between Vλ2 and Jλ3 in the germ line (see Discussion).

The presence of cytoplasmic μ chain in a minor proportion of ABC-1 cells has previously been indicated by immunofluorescence studies (21). Our Southern blot analyses of 14 randomly selected ABC-1 subclones showed only a single recombined JH region, which is linked to the Cμ gene (not shown). By using the pDhFL16.1 5′ probe, we find that this recombined JH allele appears to represent a DJ join (not shown). In Western blot analyses of cytoplasmic proteins, we have not detected any mature μ chains or Dμ chains in ABC-1 cells (Fig. 2).

RS DNA Recombination in BM18-4 and ABC-1 Cells. We have previously described a class of κ chromosome rearrangements that are often found in λ-producing B cells (11, 12). These rearrangements involve a segment of DNA that is located downstream of the Cκ exon and that we have designated as RS (for recombining sequence) DNA. Because RS DNA recombinations are directed by Ig gene recognition sequences (12), we wished to determine whether RS rearrangements occurred in the same B cell developmental stage as antibody gene rearrangement.

In the BM18-4 parental line, and in most BM18-4 subclones, recombined RS DNAs are not detected (Fig. 8*A*). However, in a few BM18-4 subclones, such as BM18-4.13 (Fig. 8*A*), recombined RS sequences are found. The frequencies of RS and V-J recombinations in the BM18-4 cell line cannot be precisely established by Southern blot analyses. However, among 21 BM18-4 subclones that show Vκ → Jκ joining (as indicated by submolar or equimolar recombined Jκ regions) only two show evidence of RS recombination. Thus, it appears that RS recombination occurs at the same developmental stage as κ gene recombination but at a lower frequency. In addition, the BM18-4.13 subclone has two distinct RS

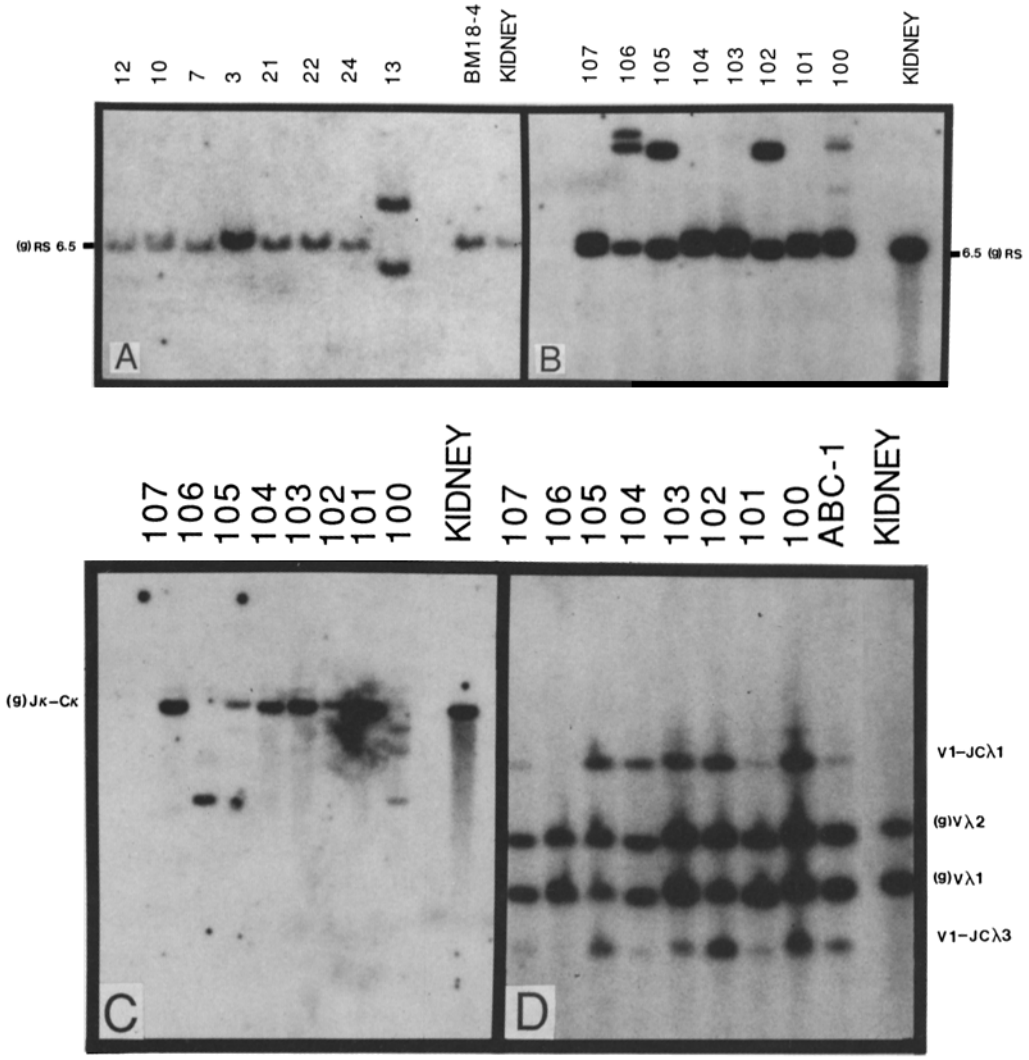

FIGURE 8. RS recombination in A-MuLV pre-B cell lines BM18-4 and ABC-1. Eco RI–digested genomic DNAs from BM18-4 subclones (*A*) or primary ABC-1.100 series subclones (*B*) were probed with an RS sequence–specific probe. A germline RS DNA band is detected at 6.5 kb as indicated. Shorter autoradiographic exposures of *B* show that the broad hybridization signal at ~6.5 kb in subclones ABC-1.100, .101, .103, .104, and .107 can be resolved into a germline RS segment of 6.5 kb and an equimolar recombined RS segment of 6.8 kb. Genomic DNAs of the same ABC-1 subclones in *B* were also digested with Bam HI and probed with a Jκ-region probe (*C*) or digested with Eco RI and probed with the Vλ1 fragment (*D*).

recombinations (Fig. 8*A*) and also has completely lost the Cκ region (Fig. 1*A*) and all of the Jκ region (not shown). These results indicate that RS recombinations are associated with Cκ deletion, as we have previously suggested (11, 12).

We have proposed that RS DNA recombination might provide a developmental signal to initiate the recombination of λ genes (11, 12). Because some ABC-1 cells can recombine λ genes in culture, we were interested in whether RS recombinations are evident in ABC-1 cells. In contrast to BM18-4 cells, many

ABC-1 subclones display recombined RS segments (Fig. 8*B*). Among 21 primary ABC-1 subclones that exhibit recombined λ genes, 20 display recombined RS sequences. In most cases, individual ABC-1 subclones exhibit only one major recombined RS DNA segment (Fig. 8*B*). In each of the three ABC-1 subclones (ABC-1.28, ABC-1.29, ABC-1.30) that we have extensively characterized by subclone analyses and ascertained to be capable of active λ gene recombination, we find two equimolar RS DNA hybridization bands, one germline and one recombined (not shown). Thus, in the ABC-1 cell line, λ recombination is clearly associated with the presence of recombined RS sequences. In addition, it appears that RS recombinations can occur in ABC-1 cells. In subclone analyses of ABC-1.30 we have observed the loss of a Cκ allele in two of nine members in a subclone series (not shown). In these two members, the loss of Cκ is associated with the appearance of a recombined RS segment not found in other subclones within the series (not shown), suggesting that RS recombination has caused the deletion of a Cκ allele.

In ABC-1 subclones that have recombined λ genes, the Jκ-Cκ alleles present can be either germline, rearranged, or deleted (Figs. 8*C* and 3*A*). However, because RS recombinations involve rearrangement of the κ locus, all ABC-1 subclones exhibiting recombined λ genes that we have analyzed also have one or more κ chromosomes rearranged (Fig. 8, *B–D*).

Discussion

Light Chain Gene Assembly in BM18-4 and ABC-1. The results of these studies show that the two Abelson cell lines, BM18-4 and ABC-1, are capable of L-chain gene recombination during growth in tissue culture. The BM18-4 line exhibits κ gene recombination but no λ gene recombination, whereas the ABC-1 line exhibits both κ and λ gene recombination. The ABC-1 cell line is the first Abelson line reported to be capable of λ gene rearrangement in culture and represents a unique opportunity to characterize this type of pre-B cell.

Four other A-MuLV-transformed cell lines, PD, 300-19, 18-81, and TX1-4 have been shown to undergo κ gene recombination in culture (7–10). From reported characterizations, it appears that all of these cell lines produce H-chain proteins. Although one κ-recombining subline of PD (PD31) was originally reported to have lost μ-chain expression (7), more recent work indicates that an "IgM crossreactive fusion protein" may be produced by PD31 (40). We, however, have found no evidence for any expression of H-like chains in the BM18-4 line, nor has any Ig ever been previously detected on the surface or in the cytoplasm of BM18-4 cells by immunofluorescence (18). We also find no evidence of H-like chains in our ABC-1 subclones. It has been proposed that the production of a functional H-chain protein might act as a signal to induce the recombination of L-chain genes in pre-B cells (14, 15, 41). Because some cells in the ABC-1 line have been reported to produce μ chains (20, 21) and because the BM18-4 line may have expressed H chains at some time either before or after viral transformation, our results do not address this point. After the initial induction of L-chain gene recombination, however, our results do indicate that a pre-B cell can retain the capacity for active L-chain gene recombination even without any H-

chain expression. This suggests that the developmental progression of a pre-B cell from H-chain to L-chain gene recombination may be irreversible.

Many BM18-4 and ABC-1 subclones that exhibit recombined κ genes are also found to retain recombined κ remnant or reciprocal DNA segments (7, 22, 31–34). These κ reciprocal fragments appear to be generated by gene inversion during the Vκ → Jκ joining process (42, 43). The lack of λ remnant DNA segments among ABC-1 cells, even though these cells are undergoing λ gene rearrangement, suggests that inversions do not occur during V-J joining in the small murine λ gene family.

λ *Gene Recombinations in ABC-1.* Among ABC-1 subclones, we have observed λ gene recombination events corresponding to the three murine λ isotypes, λ1, λ2, and λ3. However, the frequencies of the λ isotype recombinations that we find among ABC-1 cells are quite distinct from the λ isotype ratios that are expressed in mice. In normal mouse sera the isotype ratios of λ1:λ2:λ3 are 8:1:1, respectively (44, 45). Within mouse splenic B cell populations, cytoplasmic λ1:λ2:λ3 ratios are about 3:2:1 (36, 37), and are likely to reflect the numerical ratios of B cells producing various λ isotypes, because approximately half of the λ-producing B cells in either mouse fetal liver or adult spleen produce λ1, whereas the other half produce either λ2 or λ3 (37). In the ABC-1 line, however, λ1 and λ3 recombinations appear to occur at roughly equivalent rates, whereas λ2 recombinations occur much less frequently. It is not clear why these rates of recombination in ABC-1 are different from the λ isotype frequencies among B cells. Perhaps, even in the fetal liver, there are antigenic selection or T cell regulatory effects that act to expand certain B cell clones. Alternatively, the rates of λ1, λ2, and λ3 recombination that we observe may not directly correlate with the rates of producing functional λ1, λ2, and λ3 genes. For instance, it has been suggested that defects found in mouse λ3 recognition sequences might result in a higher proportion of nonfunctional λ3 gene recombinations (46). Thus, λ3 recombinations could occur at the same rate as λ1 recombinations in pre-B cells but λ3 recombinations might produce functional genes less frequently.

It is also possible that the ratios of λ isotype recombination in the ABC-1 cell line may not be representative of the frequencies of λ-gene recombination in all normal maturing pre-B cells. Some pre-B cells may predominantly recombine λ2 genes, whereas others predominantly rearrange λ1 and λ3 genes. The absence of certain λ gene rearrangements among ABC-1 subclones could be due to a low-level (or absence) of recombination or to selection effects on cell viability. As one example, the absence of Vλ2 → Jλ4 recombinations in ABC-1 could be due to a 2-bp deletion found within the Jλ4 recognition sequence (46, 47) that might abrogate recombinational activity.

The various λ gene recombinations observed in ABC-1 subclones suggest that the germline arrangement of λ genes in the mouse is: 5′ . . . Vλ2 . . . Jλ2Cλ2-Jλ4Cλ4 . . . Vλ1 . . . Jλ3Cλ3-Jλ1Cλ1 . . . 3′. Previous reports have shown that the four murine Jλ-Cλ gene segments are organized into two clusters, Jλ3Cλ3-Jλ1Cλ1 and Jλ2Cλ2-Jλ4Cλ4, located on chromosome 16 (25, 46–49). Because we detect no λ remnant DNAs among ABC-1 cells, we assume that Vλ → Jλ recombinations in the mouse occur by an excision-deletion mechanism resulting in the loss of the DNA that separates the Vλ and Jλ segments before joining. We

find that Vλ2 and Jλ2Cλ2-Jλ4Cλ4 segments are still present in ABC-1 subclones having Vλ1 → Jλ3 recombinations on both alleles suggesting that Vλ2 and Jλ2Cλ2-Jλ4Cλ4 are either upstream of Vλ1 or downstream of Jλ3Cλ3-Jλ1Cλ1. In addition, we find that the Vλ2 → Jλ3 recombination in ABC-1.30 apparently deletes the Vλ1 gene segment suggesting that the Vλ2 segment is upstream of Vλ1. Taken together, these results lead to the gene order described above. This order has also been proposed based on analyses of the recombined λ genes present in a panel of λ-producing hybridomas (39) and, thus, is supported by data from two different classes of cell lines.

Developmental Regulation of Light Chain Gene Recombination. Studies of the Abelson lines, BM18-4 (this report), PD (7), and 300-19 (8), clearly show that κ gene recombination can proceed with no concomitant rearrangement of λ genes in Abelson lines and support the proposal that light chain Ig gene recombination is a developmentally regulated process in maturing mouse B cells. Our results with the λ-recombining ABC-1 cell line carry this notion further. The κ alleles among ABC-1 cells are frequently not germline; instead, the κ genes are often recombined. If RS recombinations are included as rearrangements of κ chromosomes, then all of the ABC-1 subclones that we have found to exhibit recombined λ genes also have one or more κ alleles rearranged. Furthermore, several ABC-1 subclones that have only germline λ genes still exhibit recombined κ genes. These results support a model in which pre-B cells first undergo κ gene recombination (with no concomitant λ gene rearrangement) and then, if no productive κ genes are formed, subsequently initiate λ gene recombination (14–17). Because we find that ABC-1 cells are capable of both κ and λ gene recombination, it appears that the initiation of λ gene assembly is not necessarily accompanied by a cessation of κ gene rearrangement. This result is incorporated into a modified model of pre-B cell differentiation that we discuss below.

RS Recombination Occurs Within the Same Developmental Time Frame as Light Chain Gene Recombination and May Be Linked to the Onset of λ Gene Recombination. We have previously reported that RS recombinations are frequently observed in λ-producing B cells but not in κ-producing B cells (11), and that RS recombinations are linked with Cκ deletions (11, 12). We have also suggested that RS recombination might play a role in the switch from κ recombination to λ recombination in maturing B cells (11, 12). Because RS recombinations are mediated by Ig gene recognition sequences, we predicted that the recombinases involved in V-J and V-D-J joining might also be responsible for RS rearrangement (12). In the BM18-4 line, where most cells are apparently in the process of Vκ → Jκ joining, we now find that some cells actively recombine RS sequences. This observation clearly indicates that RS rearrangements occur in the same developmental time-frame as κ gene recombination and supports the notion that the same enzymes are mediating both the V-J joining events and the RS recombinations in these pre-B cell lines. In addition, the κ-recombining PD cell line displays RS recombination (not shown) in those few subclones that have undergone Cκ deletions (7). Significantly, in both BM18-4 and PD, the frequency of RS recombination is lower than the frequency of Vκ → Jκ joining (see below).

Because of the observed correlation of RS recombination with λ-production in mature B cells (11), we were particularly interested in the status of RS

sequences in the ABC-1 cell line, which is capable of active λ gene rearrangement. The finding that the majority of ABC-1 cells contain recombined RS segments suggests that RS recombination may precede λ recombination in most pre-B cells. Indeed, our results indicate that at least 95% of ABC-1 cells that show evidence of λ recombination also have one or more recombined RS alleles. These data augment the earlier studies of λ-producing hybridomas and accentuate the linkage between RS recombination and λ gene rearrangement.

Taken together, our results with the PD, BM18-4, and ABC-1 cell lines suggest that RS rearrangement occurs mainly toward the end of the κ-recombining phase of pre-B cell development, before the initiation of λ recombination. Although RS recombinations are found in some BM18-4 and PD subclones, we have not yet found any evidence of λ gene recombinations among these two cell lines. This observation, however, does not rule out a role for recombined RS sequences in the initiation of λ gene rearrangement; many Abelson lines are not capable of fully differentiating during passage in tissue culture. For instance, a number of Abelson lines make functional μ heavy chains and yet do not progress to the stage of L-chain gene recombination (2, 3, 6).

Although no causal role for RS recombination in the initiation of λ gene rearrangement has yet been demonstrated, the association of these two events is so striking that we wish to propose a model for light chain gene assembly that incorporates our observations and provides a framework for further investigation (Fig. 9). Because many λ-recombining ABC-1 cells and many λ-producing B cells appear to have only one RS recombination (11, and this report), we will assume that RS recombination acts in a positive manner to induce λ-gene rearrangement. We suggest that the processes that initiate Vκ → Jκ joining in a pre-B cell concurrently activate RS recombination. Our findings of RS recombination in the κ-recombining BM18-4 and PD cell lines support this notion. Because RS recombinations occur less frequently than Vκ → Jκ joinings in BM18-4 and PD, we propose that, in most pre-B cells, the first κ gene recombinational event is likely to be a V → J join. If this join forms a functional κ gene, then a functional κ protein is made; this event appears to end Ig gene recombination (14, 15). If, however, the V → J join results in a nonfunctional κ gene, no κ protein is made and recombination continues. Eventually, on a probabilistic basis, a pre-B cell that has undergone only nonfunctional Vκ → Jκ joins will undergo an RS recombination; we propose that this event might activate a *trans*-acting factor that induces λ gene rearrangement. Because RS recombinations can involve a sequence located in the Jκ-Cκ intron, all pre-B cells are capable of RS rearrangement no matter how many V → J joins have occurred in the cell previously. Some pre-B cells might undergo an RS recombination before any Vκ → Jκ joins have occurred; our hypothesis would suggest that such cells would then progress immediately to λ recombination. The cell line, ABC-1.29 (which undergoes λ recombination), could represent such an example; this cell has a germline κ configuration on one allele and an RS recombination on the other. Our results with the ABC-1 cell line suggest that pre-B cells that are undergoing λ-gene recombination also continue to rearrange κ genes and RS sequences. This implies that some κ-producing B cells might arise from λ-recombining precursors and, thus, could exhibit recombined λ genes (Fig. 9). We would expect this type of κ-

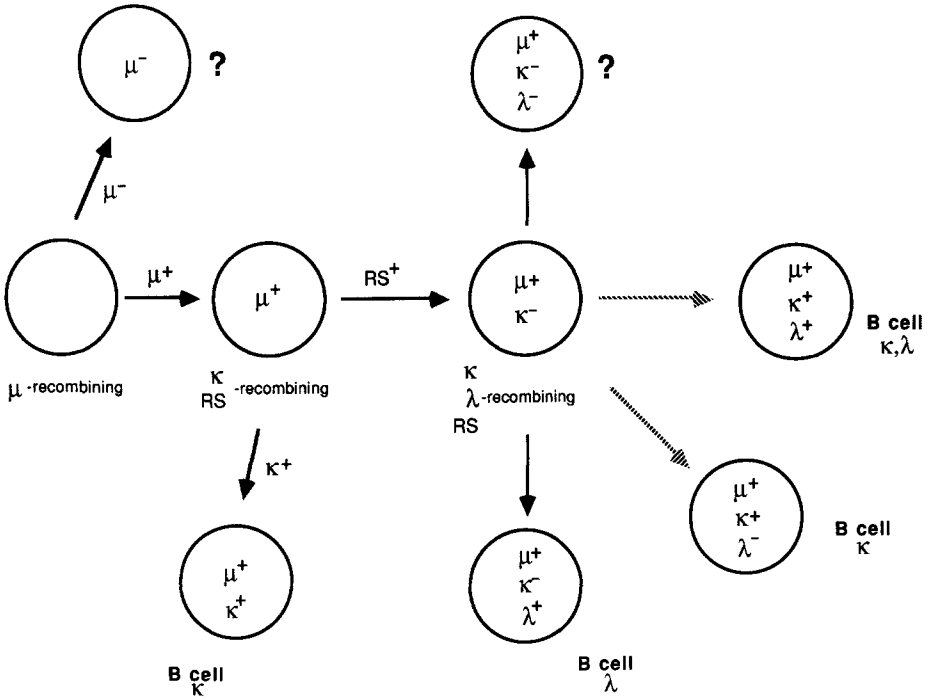

FIGURE 9. A proposed model for pre-B cell differentiation. The status of rearranged Ig genes at each stage of maturation is indicated. Plus (+) superscripts indicate functional rearranged genes, whereas minus (−) superscripts indicate nonfunctional recombined alleles. The cells undergoing active Ig gene recombination are designated and the types of gene recombination that are occurring in each precursor type are listed.

producing B cell to be rare, however, due to the Cκ deletions (associated with RS recombination) occurring in the precursor cell. A small number of κ-producing tumor cells that exhibit recombined λ genes have been reported (15, 50, 51). We have found a nonfunctional recombined λ1 gene in the κ-producing cell line, P3, and a functional recombined λ1 gene in the κ-producing myeloma, MOPC511 (J. Durdik and E. Selsing, unpublished data). In accord with the model that we have described above, both P3 and MOPC511 also exhibit recombined RS sequences (11 and unpublished results).

Recently, λ gene rearrangements have been reported in a Ly-1$^+$ tumor cell, NFS-5 (52). Because NFS-5 has a functional κ gene present and also undergoes a type of H-chain gene recombination (52, 53), it is not apparent how this particular cell relates to the model of B-cell differentiation that we have proposed. Further studies of RS recombination, as well as H, κ, and λ gene recombination in Ly-1$^+$ and Ly-1$^-$ pre-B cells may resolve this question.

Although the model we have outlined above is consistent with the data that we have obtained, it must remain speculative until a mechanism that links RS recombination with the initiation of λ gene recombination is found. Our characterization of clonal cell lines that can, in tissue culture, undergo κ and λ gene recombination will greatly facilitate further study of the molecular details of RS recombination and pre-B cell differentiation.

Summary

The two Abelson murine leukemia virus (A-MuLV)-transformed cell lines, BM18-4 and ABC-1, undergo immunoglobulin L-chain gene recombination during passage in tissue culture. BM18-4 cells are capable of κ gene recombination, whereas ABC-1 cells are capable of both κ and λ gene recombination. The expression of H chains is apparently not necessary for continuing L chain gene recombination in either of these cells, although H-chain expression may have been involved in the initiation of L-chain gene recombination. All ABC-1 cells that have λ gene rearrangements also display recombined κ alleles, supporting the hypothesis that κ and λ gene recombination are initiated in an ordered, developmentally regulated manner in maturing B cells. However, analyses of the ABC-1 line indicate that pre-B cells that have initiated λ gene recombination do not terminate κ gene rearrangement.

The λ gene recombinations that occur in the ABC-1 cell line indicate that the germline order of λ gene segments is: 5′ . . . Vλ2 . . . Jλ2Cλ2-Jλ4Cλ4 . . . Vλ1 . . . Jλ3Cλ3-Jλ1Cλ1 . . . 3′. In addition, the frequencies of λ1, λ2, and λ3 gene recombinations among ABC-1 cells are quite different than the frequencies of B cells producing λ1, λ2, and λ3 L-chains in the mouse.

RS DNA recombinations also occur in the BM18-4 and ABC-1 cell lines, supporting the notion that Ig gene recombinases are involved in RS rearrangement. Recombined RS segments are infrequent among BM18-4 cells but common among ABC-1 cells, suggesting that RS recombinational events often occur in maturing pre-B cells just before initiation of λ gene rearrangements. This developmental timing is consistent with the hypothesis that RS recombination may be involved in the initiation of λ gene assembly.

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