Volume 12 Number 10 1984 Nucleic Acids Research

Immunoglobulin gene 'remnant' DNA – implications for antibody gene recombination

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Received 13 January 1984; Revised and Accepted 18 April 1984

ABSTRACT

Many immunoglobulin (Ig)-producing cells retain the DNA that separates Ig variable (V) and constant (C) region genes in the germline. This "remnant" DNA must be moved during the recombination process that joins V and C genes via a joining (J) segment. We have analyzed remnant DNAs in several Ig-producing cell lines. The nucleotide sequences of kappa (κ) light chain remnant DNAs indicate close relationships to V-J joining. We find fused Vκ and Jκ recognition sequences in five remnant DNAs, suggesting reciprocal relationships to the fused Vκ and Jκ segments produced by V-J joining. However, of sixteen plasmacytoma remnant DNAs analyzed, all involve only recombination with Jκ1. Thus, in most cell lines, remnant DNAs are not directly reciprocal to recombined κ-genes. On the other hand, our analyses of some myelomas do indicate indirect relationships between remnant DNAs and κ-genes. Our results suggest that multiple steps of DNA recombination occur during Ig-gene rearrangement. Because remnant DNA joining sites do not exhibit the flexibility that has been observed in Ig-gene V-J joining, our findings also suggest that the joining mechanism may involve endonuclease, exonuclease and ligase activities.

INTRODUCTION

Antibody genes require recombination of variable (V) and constant (C) region gene segments to produce a functional transcription unit. This recombination involves the joining of one of many V genes to a C gene via one of several J (for joining) segments (for a review see ref. 1). Whereas a number of possible models for the recombinational mechanism involved in the juxtaposition and joining of V and J segments can be formulated, early evidence favored a simple "excision-deletion" model, in which the DNA between V and J segments was simply excised and lost from the genome (2,3). More recently, in conflict with this excision-deletion model, several reports have indicated that the DNA initially separating V and J genes in the germline is not always absent from the genomes of antibody producing cells (4-8). Studies have shown that this "remnant" DNA, unexpectedly remaining in the genome when a simple excision-deletion model would predict its absence, has a structural

organization that suggests a by-product relationship to the V-J joining event (4,7). Despite this apparent by-product relationship to antibody gene recombination, no direct correlations have been found between the remnant DNAs and the V-J joined antibody genes in three antibody-producing cell lines studied to date (4,7). This lack of direct correlation, together with the observation that remnant DNAs are found in only 50% of myelomas (4-6), has led to proposals that V-J recombination occurs via interchromatid rather than intrachromatid joining (6,7). Since in interchromatid joining, the products of V-J recombination (remnant DNA and V-J joined antibody genes) would automatically separate by segregation, this model is consistent with the apparent lack of correlation between remnant DNAs and recombined antibody genes in any given cell.

We have analyzed the remnant DNAs present in several B-cell lines. Our results indicate that, whereas remnant DNAs appear to be related to the antibody gene recombination process, the relationship is complex. Our results indicate that antibody gene DNA recombination may occur in several steps during B-cell ontogeny and thus obscure the reciprocal relationship between remnant DNAs and recombined kappa genes. In addition, our results suggest that the enzymatic functions in Ig gene joining involve at least 1) an endonuclease that cuts precisely next to V, D and J recognition sequences, 2) an exonuclease that acts only on the V, D or J exon termini generated by the endonuclease and 3) a ligase that joins the termini.

MATERIALS AND METHODS

Cell Lines

The following B-cell lines were analyzed in this study: the kappa-producing myelomas, MOPC21, MOPC41, MOPC321, J606, MOPC167, and MOPC511 (Litton); the lambda-producing myelomas, RPC-20, S178 and M315 (Litton); the kappa-producing B-cell lymphoma, WEHI279 (9); the lambda-producing B-cell lymphomas, CH1 and CH2 (10); and the Abelson virus transformed B-cell lines; ABE8 (9) and BM18-4 (11). In addition, the kappa-producing hybridomas, HPC16 (12), 100.6F9.1, 101.3C2.1, 101.3G8.4, 101.6G6.2 and 137.5G6 (13) were also analyzed.

Methods

Preparation of genomic and phage DNAs, cloning of genomic DNAs in phage vectors, gel electrophoresis, Southern filter hybridization, preparation of ^{32}P nick-translated hybridization probes, autoradiography, and DNA sequencing techniques have been described previously (14,15).

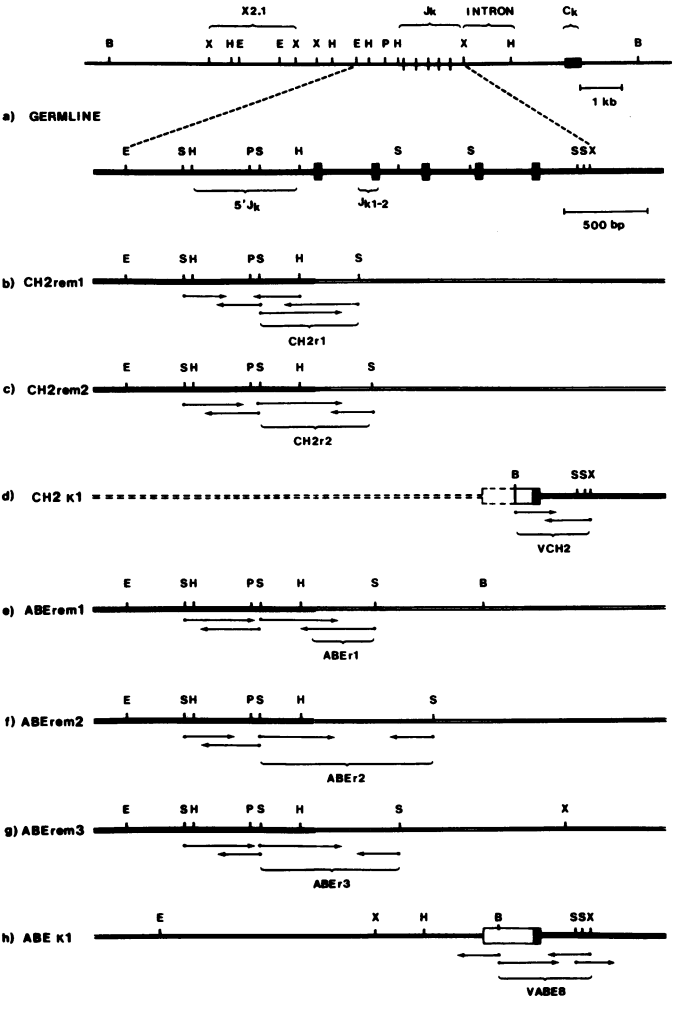

Fig. 1 - Restriction maps of kappa DNA and remnant DNAs. Germline restriction maps of the Balb/c Jκ-Cκ locus are shown in (a) to indicate the hybridization probes used to analyze genomic DNAs and for comparison with recombined kappa DNA segments isolated from tumor DNAs (b-h). Restriction maps of recombined remnant DNA (b,c,e,f,g) and recombined kappa gene (d,h) clones from the cell lines, CH2 and ABE8, are also shown. DNA sequence analyses of the clones are indicated by arrows. Segments in the maps corresponding to Jκ-Cκ associated DNA are indicated by solid bars whereas segments corresponding to Vκ associated DNA are indicated by open bars. Hybridization probes isolated from each clone are indicated by brackets. The dashed lines 5' of VCH2 indicate that the isolated clone ended at the indicated BamH1 restriction site. Restriction enzymes are abbreviated as follows: BamHI (B), EcoRI (E), XbaI (X), HindIII (H), PvuII (P) and Sau3A (S). Not all Sau3A restriction sites have been mapped in the various clones.

RESULTS

Remnant DNAs Are Found in Many Antibody Producing Cells

To investigate the organization of kappa gene segments, the DNA probes shown in Fig. 1 were isolated. These probes, together with Southern blot analyses, were used to discern the context of kappa light chain C-region genes in various B-cell lymphomas, myelomas and hybridomas (e.g. Fig. 2). Analyses of twenty cell lines are summarized in Table I. As expected, all kappa-producing cells exhibit recombined Cκ genes. In addition, all lambda-producing cells also exhibit recombined Cκ genes or have deleted the Cκ locus, consistent with previously published observations (16-18). In addition to recombined Cκ genes, roughly half of the cell lines exhibit "remnant" DNAs. Remnant DNAs characteristically hybridize to sequences 5' of the Jκ genes but do not hybridize to Cκ exon sequences (Figs. 1 and 2). As many as three separate remnant DNA fragments are found in a single cell line. Remnant DNAs are not found in Balb/c kidney DNA or in the DNAs from a panel of nine thymoma cell lines (data not shown). Apparently, remnant DNAs are uniquely, and frequently, found in cells which have undergone V-J recombination. These results are consistent with other reports that have described the detection of remnant DNAs (4-8).

It is of interest to note that several of the hybridomas that we have analyzed exhibit remnant DNAs even though the myeloma partners in these fusion cells show no remnant DNAs (Table I). Thus, in these hybridomas, the normal B-cell fusion partner apparently contributed a remnant DNA segment as well as an active antibody gene. This indicates that remnant DNAs are found in normal B-cells and are not an artifact of the tumor state. Experiments using quantitative Southern blot hybridization have also suggested that remnant DNAs are found in normal B-cells (6).

Nucleotide Sequence Structures of Remnant DNAs Suggest a By-Product Relationship to V-J Joining

To further characterize the structure of remnant DNAs, several recombinant phage containing remnant DNA sequences were isolated. Five distinct remnant DNAs were obtained from two cell lines; three of these were from the B-cell lymphoma, ABE8, and two were from the B-cell lymphoma, CH2. Southern blot analysis indicated that the recombination sites in these remnant DNAs were located close to the Jκ region. The relevant portions of each remnant DNA were sequenced as indicated in Fig. 1. Nucleotide sequences of the regions surrounding remnant DNA recombination sites are shown in Fig. 3.

The nucleotide sequences of these five remnant DNA fragments exhibit a

TABLE I - Kappa DNAs in Myelomas, Lymphomas and Hybridomas

Cell Line	Size[a]	Type[b]
MOPC41	13.0 11.0 8.1	nfCκ fCκ R
MOPC21	8.0 7.5 5.8	R nfCκ fCκ
MOPC167	5.2	fCκ
MOPC511	5.2	fCκ
MOPC321	13.0 11.5 8.0 4.8	G aCκ aCκ fCκ
J606	24 7.4 5.0	R rCκ rCκ
S178	8.2 7.8 6.3 6.0	R nfCκ aCκ nfCκ
MOPC315	8.2	RS[c]
RPC-20	14.0 8.0	R R
WEHI279	13.0 13.0 8.0	G fCκ R
CH1	5.5 5.0	nfCκ nfCκ
CH2	24 9.5 6.5	R R RS[c]
ABE8	15.0 10.0 8.0 4.8	R R R nfCκ
BM18-4	15.0 13.0 10.5 9.0 8.3 7.0	R G rCκ R[e] rCκ rCκ
HPC16[d]	8.0 6.0 5.2	R nfCκ fCκ
101.3C2.1[d]	13.0 12.0	G rCκ
101.3G8.4[d]	13.0 7.8 5.2	G R fCκ
101.6G6.2[d]	25 13.0 7.5	R G rCκ
100.6F9.1[d]	13.0 13.0 7.6	G rCκ R
137.5G6[d]	13.0 5.2	G fCκ

a. Sizes of BamHI restriction fragments are given in kilobases (kb).

b. The type of kappa DNA represented in each restriction fragment is indicated. The assignment of types was based on the patterns of hybridization with the various probes indicated in Fig. 1. Germline kappa segments (G) hybridize with all the probes shown in Fig. 1. Remnant DNAs (R) hybridize with the X2.1, 5'Jκ and Jκ probes but not with the Jκ1-2, intron or Cκ probes. Recombined kappa genes (rCκ) hybridize with Cκ, intron and Jκ probes but not with the 5'Jκ or X2.1 probes. In some cell lines it was possible to assign recombined kappa genes as functional (fCκ) or non-functional (nfCκ) based on previous work. Aberrant kappa genes (aCκ) hybridize with Cκ and intron probes but not with Jκ, 5'Jκ or X2.1 probes. These genes most likely are similar to the recombined kappa gene in MPC11, described in ref. 34, where a Vκ gene has recombined into the intron that separates Jκ and Cκ.

c. RS segments represent unusual kappa recombination events that are found in the CH2 and MOPC315 cell lines. These segments hybridize with Jκ and intron probes but not with X2.1, 5'Jκ or Cκ probes. These DNA segments are described in detail elsewhere (35).

d. In hybridomas, only the kappa DNA segments from the normal B-cell partner are listed. Segments from the myeloma parent are omitted.

e. Among all remnant DNAs tested, only this BM18-4 remnant DNA hybridizes with the Jκ1-2 probe shown in Fig. 1.

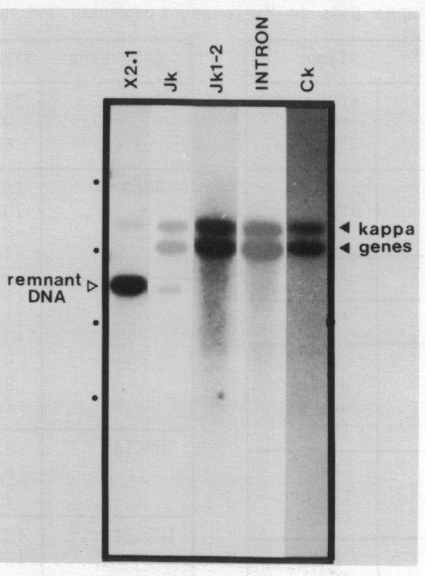

Fig. 2 - Southern blot analyses of MOPC41 DNA. BamHI digested MOPC41 DNA was hybridized with the probes indicated in each lane (see Fig. 1 for designations of probes). The segments of DNAs that hybridized with the probes were assigned as "remnant DNA" or "kappa genes" as indicated based on their patterns of hybridization with the various probes (see legend to Table I). The weak hybridization signal seen at 13kb in the X2.1 lane most likely represents a small amount of germline Jκ-Cκ loci present in host cells infiltrating the in vivo passaged MOPC41 tumor. Relative positions of λ HindIII restriction fragments (23, 9.8, 6.6, 4.5 kb) used as molecular size markers are indicated by dots.

notable feature which indicates their possible origin. All show identical sequences reading into the Jκ region up to the last base of the V-J joining recognition sequence for Jκ1. As shown in Fig. 3, the recognition site for Jκ1 is one example of the conserved sequences found 5' of all J (or D) segments involved in light and heavy chain antibody gene recombination in mice and humans (19-20). These sequences are characterized by strong homology to a consensus site which comprises a nanomer having the sequence, GGTTTTTGT separated by a spacer region from a heptamer having the sequence, CACTGTG. Similarly, a conserved sequence having the consensus form of CACAGTC-spacer-ACAAAAACC is found 3' of all V (or D) segments in mice and humans. In the kappa light chain system, the spacer lengths for J and V segments are 23 and 12 nucleotides, respectively. The palindromic and conserved nature of V and J recognition sequences suggests that they represent sites of binding for recombination enzymes involved in V-J joining (19-20).

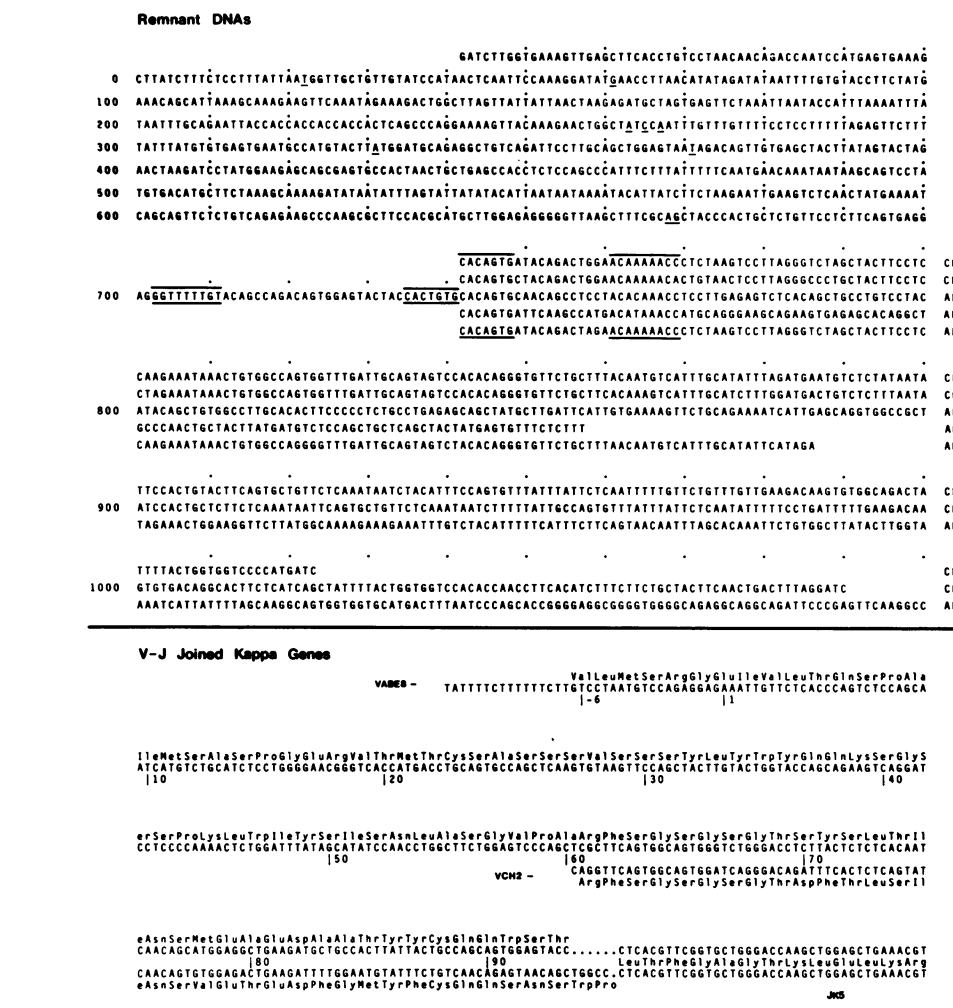

Fig. 3 - DNA sequences of CH2 and ABE8 remnant DNAs and recombined kappa genes. The top panel shows sequences for five remnant DNAs. The nucleotide numbering is as described in ref. 24. Positions 0-741 correspond to sequences 5' of Jκ1. The underlined nucleotides differ from the previously published germline sequence (24) but are the same in all five remnant DNAs and, thus, probably reflect errors in the previous sequence. The nucleotides delineated by bars represent Jκ and Vκ recognition sequences (see text). The lower panel shows the sequences of V-J joined genes in CH2 and ABE8. Both genes involve recombination at Jκ5. Amino acids are numbered as in ref. 27. The V region sequences were aligned using the invariant residues found in all Balb/c mouse kappa light chains (27). The dotted lines represent nucleotides that were probably lost during V-J joining.

Immediately contiguous to the Jκ recognition sites in the five remnant DNAs shown in Fig. 3, nucleotide sequences which are highly homologous to Vκ recongition sites are found. In each case, the characteristic CACAGTG heptamer and ACAAAAACC nanomer sequences are observed and are separated by 12 nucleotide spacer regions. Because J and V recognition sites represent the end boundaries of the DNA that separates V and J sequences in the germline and which must be removed during V-J joining, the remnant DNAs in Fig. 3 exhibit structures that suggest a reciprocal relationship to V-J recombined antibody genes. Similar structures have been found for remnant DNAs in three other myelomas (4,7). Coupled with the observation that remnant DNAs are commonly and exclusively found in cells which have undergone antibody gene recombination, these particular remnant DNA structures strongly indicate that they represent by-products of V-J recombination.

Recombination Sites in Remnant DNAs Are Precise

Several studies on recombined antibody genes have indicated that the precise location of V-J joining can vary (21-23). There is a degree of flexibility in the nucleotide positions of both V and J gene segments at which recombinational fusion can occur. This flexibility can augment the potential antibody repertoire by altering amino acid codons at the V-J recombination site but also results in non-functional recombined antibody genes due to shifts in codon reading frames (21-23).

In contrast to the flexibility observed in antibody gene V-J recombination sites, all five remnant DNA segments shown in Fig. 3 exhibit precisely identical sites of recombination. In each case, the Jκ1 recognition site is immediately contiguous to a Vκ recognition site with no additional nucleotides either inserted or removed between. This is particularly noteworthy since a single nucleotide separates the Jκ1 recognition site from the Jκ1 coding sequence in the germline (24) and as many as four nucleotides are found between the recognition sites and coding sequences of various germline Vκ genes (e.g. 7,25-26). Thus, any hypothetical antibody gene that might be a reciprocal product of the five remnant DNA recombinations shown in Fig. 3 would produce a protein having an additional amino acid inserted within the third hypervariable region relative to most kappa chains. Such additional amino acids have been observed in some antibodies but are not common among known kappa chain protein sequences (27).

Remnant DNAs Found in Plasmacytomas Involve Only Recombination at Jκ1

Since all five sequenced remnant DNAs (Fig. 3) exhibited only recombination at the Jκ1 recognition site, it was of interest to determine whether any

remnant DNAs involve recombination at other Jκ sites. A probe which contained sequences between Jκ1 and Jκ2 (Fig. 1) was isolated and hybridized to Southern blots of DNAs from cell lines that were known to contain remnant DNAs. Any plasmacytoma remnant DNAs similar to those depicted in Fig. 3 but recombined at Jκ2, Jκ3, Jκ4 or Jκ5 instead of Jκ1 should hybridize with this Jκ1-2 probe. As indicated in Table I, the Jκ1-2 probe did not hybridize to any of the remnant DNAs that were detected previously (with the exception of one remnant DNA in BM18-4; see below) although it did hybridize, as expected, to germline Jκ regions and to antibody genes which were known to be recombined at Jκ1 (the MOPC41 functional and non-functional kappa genes [28,29]; Fig.2). Thus, of sixteen remnant DNAs found in plasmacytomas or hybridomas, all apparently involved recombination at the Jκ1 recognition site. No remnant DNAs directly reciprocal to antibody genes recombined at Jκ2, Jκ3, Jκ4, or Jκ5, were found.

Relationships Between Vκ Gene Segments in Remnant DNAs and Recombined Antibody Genes.

Because Jκ segments found in remnant DNAs and recombined antibody genes in individual cell lines were not directly reciprocal, it was of interest to determine whether any relationships could be discerned between the Vκ segments in these recombined DNAs. In the cell line, CH2, hydridization probes corresponding to Vκ segments (either the Vκ gene itself or its immediate 3' flanking region) were isolated from clones representing the two remnant DNAs and one V-J joined kappa gene found in the CH2 genome (Fig. 1). These probes were then hybridized to Southern blots of germline DNA to determine whether the recombined V-regions came from the same or different V-gene families. As seen in Fig. 4, although the two V-gene segments found in CH2 remnant DNAs appear to be members of the same family (as also indicated by their nucleotide sequence; see Fig. 3), neither of the remnant DNA Vκ segments is related to the V-gene found in the CH2 recombined kappa gene. Intriguingly, however, the remnant DNA V-segments found in the CH2 cell line are both closely related (100% and 90% homology) to a remnant DNA V-segment previously reported for the myeloma, T (compare Fig. 3 with Fig. 2 in ref 7). Furthermore, the single recombined Vκ gene in CH2 is closely related (88% homology) to a non-functional recombined Vκ gene also found in the myeloma T (compare Fig. 3 with Fig. 4 in ref. 30). Thus, in these two distinct cell lines, the presence of similar remnant DNAs appears to be correlated with the presence of similar V-J joined kappa genes.

Relationships between remnant DNA and kappa gene Vκ segments were also investigated in the B-lymphoma cell line, ABE8. Hybridization probes for ABE8

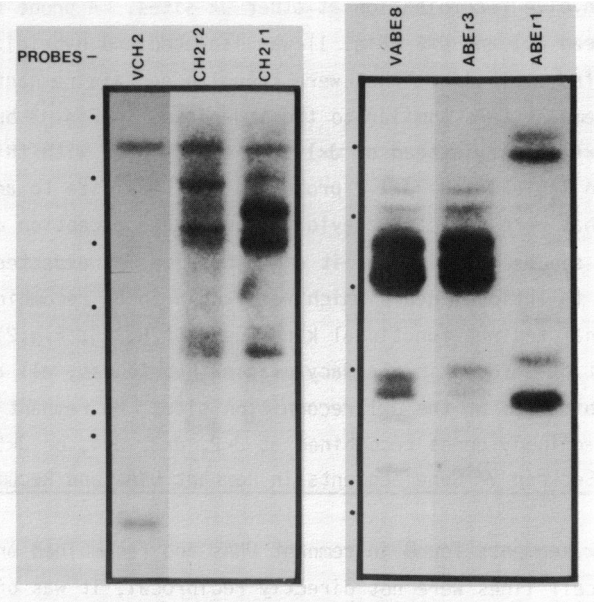

Fig. 4 - Comparison of Vκ families giving rise to remnant DNAs and recombined kappa genes in CH2 and ABE8. Southern blots of Eco RI digested Balb/c kidney DNA were hybridized to the various probes indicated in each lane. The probes are shown in Fig. 1. Dots indicate the relative positions of λ Hind III restriction fragments (23, 9.8, 6.6, 4.5, 2.5 and 2.2 kb) used as molecular size markers. All the probes except ABEr1 contain some Jκ-associated sequences (see Fig. 1) and, thus, hybridize to germline 13kb Jκ-Cκ BamHI fragments as well as Vκ fragments.

Vκ segments were isolated as indicated in Fig. 1. As shown in Fig. 4, Southern blot analyses demonstrated a close relationship between the V region segments present in one ABE8 remnant DNA and in the single recombined ABE8 kappa gene. Both V regions apparently derive from the same germline Vκ family although slight differences in relative band intensities indicate that the two probes do not represent the identical germline Vκ gene. Two other remnant DNAs in ABE8 derive from entirely distinct Vκ families (Figs. 3 and 4) and, thus are apparently not related to the single recombined kappa gene in ABE8.

The presence or absence of Vκ genes in the CH2 and ABE8 cell lines was also investigated. As shown in Fig. 5, when probes for the remnant DNA or kappa gene V regions in CH2 were hybridized to germline and CH2 DNAs, complete deletions of certain germline genes (arrows in Fig. 5.) in the CH2 genome were observed. No similar deletions were found when CH2 was analyzed with six other Vκ probes (see legend to Fig. 5). Thus, in CH2 cells, only a few Vκ genes, near points of kappa DNA recombination, have been recombined or totally

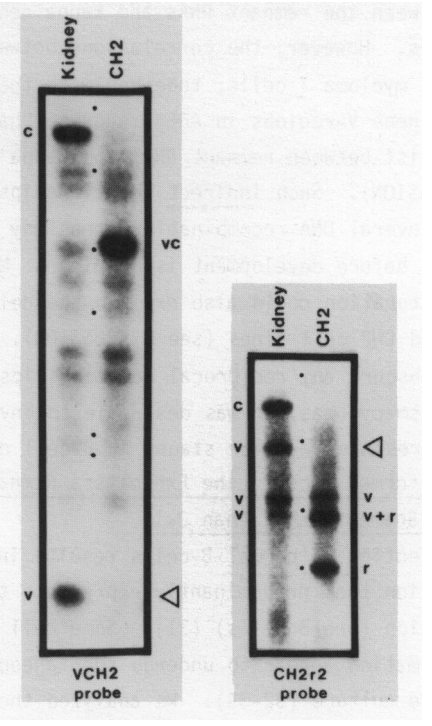

Fig. 5 - Deletions of Vκ genes in CH2. Southern blots of Eco RI digested Balb/c kidney and CH2 DNAs were hybridized with the probes indicated (see Fig. 1). Kappa germline variable (v) and constant (c) region DNA segments are indicated, as are V-J joined kappa genes (vc) and remnant DNAs (r). Assignments for the various types of gene segments were based on the Southern blots shown, together with others hybridized with probes specific for Cκ and remnant sequences. The blot hybridized with the VCH2 probe was washed at lower stringency than normal (1 x SSC rather than 0.1 x SSC) to allow visualization of more distantly related V-genes. Complete deletions of certain Vκ genes in the CH2 cell line are indicated by arrows. Dots indicate the positions of λ Hind III markers as in Fig. 4. Comparisons of six other distinct V gene families in kidney and CH2 cells were also done with VM11, VM21, VA20, Vκ167 (5), VABE8 and ABEr1 (Fig. 1) probes. No Vκ gene deletions were seen in the CH2 genome using these Vκ probes. In addition, no Vκ gene deletions were seen in the ABE8 genome using any of the Vκ probes.

deleted. We have previously reported similar observations with the myeloma, MOPC21 (5). On the other hand, no deletions of any Vκ genes were found in Southern blot analyses of the ABE8 genome (5 and legend to Fig. 5).

The data from experiments using Vκ-region hybridization probes, albeit complex, are consistent with the picture that emerges by merely comparing Jκ-region usage in remnant DNAs and kappa genes. It is obvious that no direct

relationsips exist between the remnant DNAs and kappa genes present in most plasmacytoma cell lines. However, the correlations between remnant DNAs and kappa genes in CH2 and myeloma T cells, together with the closely related remnant DNA and kappa gene V-regions in ABE8, suggest that indirect relationships might exist between remnant DNAs and kappa genes in B-cell lines (see Fig. 7 and DISCUSSION). Such indirect relationships would be consistent with the notion that several DNA recombination steps may occur within a differentiating B-cell before development is complete. Multiple recombination steps during B-cell maturation could also explain the deletion of certain Vκ genes in the MOPC21 and CH2 cell lines (see DISCUSSION). Because multiple recombinations would obscure any reciprocal relationships between remnant DNAs and kappa genes in plasmacytomas, it was desirable to investigate remant DNAs in cell lines that represented earlier stages in B-cell development.

An Abelson Virus Transformed B-cell Line Exhibits a Remnant DNA that Involves Recombination at a Jκ Segment Other than Jκ1.

Abelson virus infection of normal B-cells results in the transformation of a B-cell subpopulation that predominantly represents those cells at early stages of differentiation (pre-B cells) (31). Some cell lines established by Abelson virus transformation appear to undergo spontaneous antibody gene recombination in tissue culture (32-33). We analyzed the kappa chain genes in one Abelson line, BM18-4, to determine whether the characteristics of remnant DNAs and antibody genes found in plasmacytomas would be similar to those found in an "early" B-cell.

Fig. 6 shows results from Southern blot hybridization of BM18-4 DNA with various kappa probes. Analyses with Cκ or intron region probes (Figure 6, lane C) show a major germline kappa constant region band with several minor bands also present. This hybridization pattern is similar to those seen in several other Abelson cell lines (8,32) and reflects recombinational activity of kappa light chain genes in the BM18-4 cell line (D. Persiani and E.S., unpublished). Similarly, analyses with remnant DNA probes also show a major germline band and minor recombined species (Figure 6, lane A). These data are consistent with the results observed in plasmacytomas and suppport the notion that the presence of remnant DNAs in B-cells are linked to the presence of recombined antibody genes.

Significantly, however, remnant DNAs in the Abelson cell line, BM18-4, differ in one respect from the remnant DNAs observed in plasmacytomas and B-cell lymphomas. As shown in Fig. 6 (lane B), one remnant DNA in BM18-4 cells hybridizes to the Jκ1-2 probe and, thus, appears to reflect recombination at a

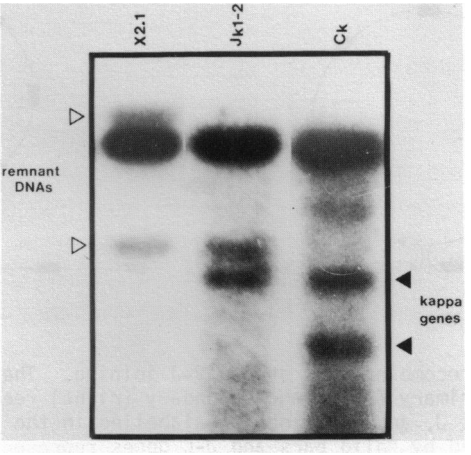

Fig. 6 - Remnant DNAs and kappa genes in the BM18-4 cell line. Southern blots of BamHI digested BM18-4 DNA were hybridized with the probes indicated. The blots indicate that, in BM18-4, one remnant DNA and one recombined kappa gene hybridize with the Jκ1-2 probe. In each lane a major hybridization band at 13kb (corresponding to the germline Cκ gene) is seen. Sizes of other minor bands are: lane A, 15 and 9.0 kb; lane B, 9.0 and 8.3 kb; lane C, 10.5, 8.3 and 7.0 kb. The major remnant DNAs (open arrows) and recombined kappa genes (solid arrows) in the BM18-4 cell line are indicated.

Jκ segment other than Jκ1. Apparently, the exclusive association of remnant DNAs with recombination at Jκ1 observed in cells representing mature B-cells is not found in cells that represent "early" B-cells. It is also interesting that, in BM18-4, two predominant recombined kappa genes and two predominant remnant DNAs are observed (Fig. 6). From the hybridization data with the Jκ1-2 probe we can deduce that one kappa gene is apparently recombined at Jκ1 whereas the other kappa gene is recombined at a Jκ region other than Jκ1. Similarly, one remnant DNA is recombined at Jκ1 whereas the other is recombined at a Jκ region other than Jκ1. These results suggest that kappa genes and remnant DNAs in the BM18-4 cell line may be directly reciprocal, contrasting sharply with the non-reciprocal behavior found in plasmacytomas. Further characterization of the kappa genes and remnant DNAs in BM18-4 will be needed, however to substantiate this possibility.

DISCUSSION

We have found that the DNA that initially separates kappa V and C genes in the germline is retained in many antibody producing cells that have undergone Ig gene recombination. These retained "remnant" DNAs exhibit structures,

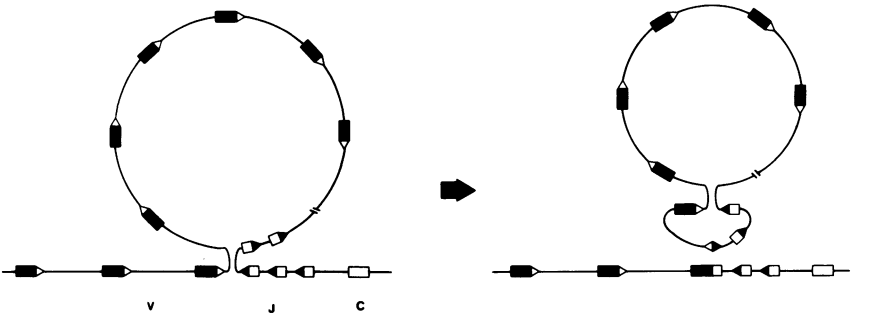

Fig. 7 - Secondary recombinations during V-J joining. The diagram pictures schematically primary (left) and secondary (right) recombinations in the kappa locus. V, J, and C regions are labelled in the left panel with V genes represented by solid bars and J-C genes represented by open bars. Recognition sequences associated with V genes (open triangles) and J genes (solid triangles) are also indicated. Relative sizes of the various DNA segments have been distorted for the purpose of diagrammatic clarity. An excision mechanism is depicted in this figure; similar model can be constructed assuming other recombination mechanisms (see text).

with directly-linked, recombined V and J recognition sequences, that strongly suggest a close relationship to recombined antibody genes. In plasmacytomas, however, remnant DNAs are not directly reciprocal to recombined antibody genes. Instead, our data indicate that secondary recombinational events are interposed between the first kappa gene recombinations that occur in a differentiating B-cell and the final remnant DNA and recombined antibody gene products found in a mature B-cell (represented by plasmacytomas and B-cell lymphomas). This notion is supported by our initial characterization of a cell line that represents an "early" B-cell. In this cell line, our results suggest that remnant DNAs and recombined kappa genes may indeed be reciprocal.

Several mechanisms for antibody gene recombination have have been postulated (see below). For the purposes of discussion, one model depicting the events that give rise to remnant DNAs and recombined kappa genes in B-cells is shown in Fig. 7. In the figure, two sequential recombination events are illustrated. In the first recombination event, DNA between antibody V and J segments is looped out and excised. This event generates an episome which can subsequently undergo a second recombination step. The experimental data that we have obtained from several cell lines are consistent with this model. Secondary recombinations such as shown in Fig. 7 can explain why remnant DNAs in differentiated B-cells (represented by plasmacytomas and most lymphomas)

are not directly reciprocal to recombined kappa genes. In some instances, such as in the ABE8 cell line, primary and secondary recombinations may involve Vκ genes that closely neighbor on the chromosome. This could result in remnant DNAs and kappa genes that involve V genes from the same Vκ gene family. In other cases, primary and secondary recombinations might involve widely separated V genes. Under these circumstances, no relationships would be easily detectable between remnant DNA and kappa gene V segments. The situations in the CH2 and myeloma T cell lines appear to fit this pattern. It is interesting that, in CH2 and myeloma T, similarities in recombined kappa genes seem to correlate with similarities in remnant DNAs. This could imply that the relationship between primary and secondary recombinations may not be entirely random.

The secondary recombination event depicted in Fig. 7 should result in two circular episome segments and, thus, two remnant DNAs. However, it is likely that episome DNAs require chromosomal reintegration to be retained during long-term cellular proliferation. Our finding of only Jκ1 associated remnant DNAs in plasmacytomas might suggest that sequences necessary for reintegration are only found on the larger episome in Fig. 7 and that the smaller episome is invariably lost from the genome during cell passage. The loss of a small number of Vκ genes from the genomes of CH2 and MOPC21 cells is consistent with this notion because some Vκ genes may also be present on the smaller episome that is lost during cellular proliferation. Furthermore, in those instances when the larger episome in Fig. 7 does not reintegrate it might also be lost during cell passage; this could account for the absence of any remnant DNAs in roughly one-half of all plasmacytomas (5,6) and the extensive deletions of Vκ genes seen in some cell lines (3,5).

Three general models for antibody gene recombination have been postulated. These models invoke either (A) DNA excision/reintegration (4,5), (B) interchromatid exchange (6,7) or (C) DNA inversion (8,29) to account for V-J joining. Our results are not consistent with simple versions of any of these models. On the other hand, ad hoc assumptions, such as we describe above for an excision/reintegration model, can reconcile our data with any of the three models. Clearly, the complexity introduced by secondary recombinations in individual B-cells makes analysis of remnant DNAs in most plasmacytomas difficult. The availability of Abelson-virus transformed cell lines actually undergoing recombination will greatly facilitate study of the V-J joining process and may allow elucidation of the recombinational mechanism (or mechanisms) involved.

The detailed sequence structures that we have determined for five remnant DNAs exhibit directly-joined Ig gene recombination recognition sequences. All five remnant DNAs show recombinations at precisely the identical nucleotides. In contrast, the joining sites of recombined kappa genes exhibit a great deal of flexibility (21-23). These two observations could suggest that the antibody gene recombinational mechanism may involve both exonuclease and endonuclease activities. The precise joining sites observed in remnant DNAs may indicate that the V-J joining process initiates with endonucleolytic cuts precisely contiguous to the recognition sequences that have been postulated to be involved in Ig gene recombination. Such a mechanism would be quite similar to the mode of action found for many bacterial type II restriction endonucleases (reviewed in ref. 33). On the other hand, the joining site flexibility found in antibody genes suggests that, prior to ligation, an exonuclease may processively remove nucleotides from the DNA ends of V, D or J segments that are generated by endonucleolytic scission. Because remnant DNAs have thus far been found to be precisely joined, it would appear that such an exonuclease acts specifically on the DNA ends actually involved in making up the third hypervariable region of a recombined antibody gene. This notion is consistent with the apparent importance of variability in Ig gene joining in modulating the exact nature of the antigen binding site in the antibody protein.

It is somewhat surprising to find that, in plasmacytomas, remnant DNAs appear to be invariably associated with recombination at Jκ1. Even assuming multiple recombination events within an individual cell, one might expect that some remnant DNAs would be associated with Jκ segments other than Jκ1 in the same manner that many antibody genes (even those that are non-functional) are associated with Jκ2, Jκ4, etc. It is generally assumed that a specific molecular event (such as the insertion of IgM molecules into the membrane surface of a B-cell) in some manner terminates the antibody gene recombination process. If this termination process acted only on kappa DNA regions closely-linked to the Cκ gene, then secondary recombinations of kappa remnant DNAs might continue until the DNA sequence signals for recombination were exhausted. Such a model could explain the exclusive association of remnant DNAs with Jκ1 recombination. Clearly, however, further exploration of the developmental controls on Ig gene recombination will be needed to probe this possibility.

ACKNOWLEDGEMENTS

We are grateful to M. Potter, G. Haughton, N. Warner, C. Sibley, N. Rosenberg, C. Berek and J.L. Claflin for generously providing cell lines and thank Jim Miller for many stimulating discussions. This work was supported by NSF (PCM 78-13205) and NIH (CA/AI 25754 and DE 02600) grants to U.S. and NSF (PCM 83-02110) grant to E.S.

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