

CHROMOSOMAL LOCATION OF HUMAN KAPPA  
AND LAMBDA IMMUNOGLOBULIN LIGHT CHAIN  
CONSTANT REGION GENES

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There is a large body of information relating to the molecular organization of immunoglobulin (Ig) gene clusters and the somatic rearrangements involved in the formation of active Ig genes in antibody-producing cells (1). Recombinant DNA technology has permitted the short-range mapping of variable region genes, J regions, constant region genes, and switch regions between classes of heavy chain constant region genes in antibody-producing cells and germ line cells. Both light and heavy chain gene clusters occupy large segments of DNA, and study of the long range organization of these genes might require other techniques.

In contrast to the detailed studies of human Ig genes at the molecular level, there is only very limited information concerning the chromosomal location of these genes. Previous studies have depended on expression of Ig genes in somatic cell hybrids, and Ig heavy chain genes were assigned to human chromosome 14 (2) by this method. Nevertheless, there are serious limitations to this approach. This method is limited by the fact that Ig expression involves somatic rearrangement of the genes on only one of a pair of homologous chromosomes. Thus, hybrid cell clones that do not express the gene may retain the homologue carrying the excluded allele and, therefore, will be noninformative in this type of analysis.

The availability of cloned Ig gene probes has greatly simplified analysis of the chromosomal location of these genes. Somatic cell hybrids segregating mouse chromosomes have been analyzed with mouse Ig nucleic acid probes. This has permitted the recent assignment of murine kappa (3), lambda (4), and heavy chain (5) constant and variable region genes to mouse chromosomes 6, 16, and 12, respectively.

In this study, we analyzed 24 independent human fibroblast/rodent somatic cell hybrids with nucleic acid probes prepared from cloned human light chain constant region genes. Our results indicate that the kappa and lambda constant region genes are located on human chromosomes 2 and 22, respectively. Our preliminary results have been reported previously (6). In agreement with our results, Malcolm et al. (7) have recently assigned a kappa variable region gene to human chromosome 2 by *in situ* hybridization, and Erikson et al. (8) have assigned the lambda gene to human chromosome 22 by analysis of gene expression in somatic cell hybrids. Hence, these three studies provide confirmed chromosome assignments for both human kappa and lambda Ig genes.

## Materials and Methods

**Cell Cultures.** Human fibroblast lines used in cell fusions were diploid WI 38 (ATCC CCL75), an *hprt*<sup>-</sup> SV40 transformed WI 18 line VA2 (9), and a *tk*<sup>-</sup> HeLa derivative AV3 (ATCC CCL 21). Rodent cell lines used were murine *hprt*<sup>-</sup> L-A9 (10), mouse *tk*<sup>-</sup> L-B82 (10) and LMTKC1 1D (11), and an *hprt*<sup>-</sup> derivative of the hamster CHV79 line. Cells were grown in monolayer cultures in modified Eagle's minimum essential medium containing 5 or 10% fetal bovine serum. Eagle's spinner medium was used for suspension cultures.

**Cell Hybridization.** Human and rodent cells were cocultivated (1:1 mixture) in plastic petri dishes (6- or 10-cm Diam) for 24 h before induction of cell fusion with 52.5% polyethylene glycol 1000 (12). Selective hypoxanthine-aminopterin-thymidine medium was applied 24 or 48 h after fusion, and independent colonies were cloned after ~2 wk. Hybrid cell lines were usually expanded in nonselective medium and analyzed 1-6 mo after isolation.

**Isoenzyme Analyses.** Washed cell pellets were simultaneously prepared for DNA isolation and isoenzyme analyses from hybrid cell lines that had been expanded to  $2-10 \times 10^8$  cells. These pellets were stored frozen at  $-80^\circ\text{C}$  until used. The human chromosomes present in each hybrid cell line were determined from starch gel electrophoretic analyses (13, 14) of isoenzyme markers that have been previously assigned to each of the human chromosomes (15). The isoenzyme markers used were enolase 1 (ENO-1; EC4.2.1.11), peptidase C (PepC; EC3.4.11.-), phosphoglucosylase 1 (PGM-1; EC2.7.5.1), and phosphoglucosylase dehydrogenase (6-PGD; EC1.1.1.44) (chromosome 1); soluble malate dehydrogenase (MDH-S; EC1.1.1.37), and acid phosphatase-1 (ACP-1; EC3.1.3.2) (chromosome 2p); soluble isocitrate dehydrogenase (IDH-S; EC1.1.1.42) (chromosome 2q); aminoacylase-1 (ACY-1; EC3.5.1.14) and sometimes glutathione peroxidase 1 (GPX-1; EC1.11.1.9) (chromosome 3); peptidase S (PepS; EC3.4.11.-) and phosphoglucosylase 2 (PGM-2; EC2.7.5.1) (chromosome 4); hexosaminidase B (Hex B; EC3.2.1.30) (chromosome 5); soluble malic enzyme (ME-1; EC1.1.1.40), phosphoglucosylase 3 (PGM-3; EC2.7.5.1), and mitochondrial superoxide dismutase (SOD2; EC1.15.1.1) (chromosome 6); beta glucuronidase (GUS; EC3.2.1.31) and uridine phosphorylase (UP; EC2.4.2.3) (chromosome 7); glutathione reductase (GSR; EC1.6.4.2) (chromosome 8); adenylate kinase-1 (AK-1; EC2.7.4.3) and soluble aconitase (ACON-S; EC4.2.1.3) (chromosome 9); soluble glutamate oxaloacetate transaminase (GOT-S; EC2.6.1.1) and adenosine kinase (ADK; EC2.7.1.20) (chromosome 10); lactate dehydrogenase A (LDH-A; EC1.1.1.27) and esterase-A4 (EsA4; EC3.1.1.1) (chromosome 11); lactate dehydrogenase B (LDH-B; EC1.1.1.27) and peptidase B (Pep B; EC3.4.11.-) (chromosome 12); esterase D (EsD; EC3.1.1.1) (chromosome 13); purine nucleoside phosphorylase (NP; EC2.4.2.1) (chromosome 14); mannose phosphate isomerase (MPI; EC5.3.1.8), pyruvate kinase-3 (PK-3; EC2.7.1.40), and hexosaminidase-A (Hex A; EC3.2.1.30) (chromosome 15); adenine phosphoribosyltransferase (APRT; EC2.4.2.7) and NADH diaphorase-4 (DIA-4; EC1.6.2.2) (chromosome 16); galactokinase (GALK; EC2.7.1.6) (chromosome 17); peptidase A (Pep A; EC3.4.11.-) (chromosome 18); glucosephosphate isomerase (GPI; EC5.3.1.9) and peptidase D (PepD; EC3.4.13.9) (chromosome 19); adenosine deaminase (ADA; EC3.5.4.4) (chromosome 20); soluble superoxide dismutase (SOD-1; EC1.15.1.1) (chromosome 21); mitochondrial aconitase (ACON-M; EC4.2.1.3) and NADH diaphorase-1 (DIA-1; EC1.6.2.2) (chromosome 22); hypoxanthine phosphoribosyltransferase (HPRT; EC2.4.2.8) and glucose-6-phosphate dehydrogenase (G6PD; EC1.1.1.49) (X chromosome).

The presence of the short arm of human chromosome 6 (chromosome 6p) was also determined by blot hybridization with a human HLA nucleic acid probe. The human/mouse hybrid cell lines were tested for sensitivity to diphtheria toxin, a marker located on human chromosome 5. The human chromosome content of some hybrids was confirmed by karyotypic analysis of samples of these same cell populations using alkaline giemsa (16) or giemsa banding (17) procedures.

**DNA Isolation and Restriction Endonuclease Digestion.** DNA samples were prepared from cell lines according to the procedure of Polsky et al. (18). Control DNA samples were similarly prepared from mouse and Chinese hamster livers and human placenta. Enzymatic digestions were carried out with EcoRI and BamHI according to standard procedures.

**In Situ Hybridization Analysis.** Digested DNA samples were size fractionated by 1% agarose gel electrophoresis and transferred to nitrocellulose (19) or DBM-derivatized paper (20).

Hybridization was carried out in 40% formamide-1  $\times$  SSC (0.15 M NaCl, 0.015 M sodium citrate) at 40°C in the presence of cloned, nick translated (21),  $^{32}$ P-labeled DNA probes prepared as follows: kappa probe, a 2.5 Kb EcoRI fragment containing the human kappa constant region coding and flanking sequences (22); and lambda probe, a 0.7 Kb EcoRI fragment containing coding and flanking sequences of the human  $\lambda$  MCG gene (23, 24) and extending from an artificial EcoRI site to a naturally occurring EcoRI site 3' to the MCG gene (unpublished results). Filters were washed at 52°C in 0.1  $\times$  SSC before development of autoradiograms.

### Results

Six of the human/rodent hybrid cell lines contained the human lambda constant region gene cluster, as demonstrated by hybridization with the  $C_\lambda$  probe (Fig. 1). The cluster of six lambda constant region genes usually was present on three EcoRI fragments (8, 14, and 16 Kb). This is the most common arrangement of the human  $C_\lambda$  genes, but polymorphism of restriction sites flanking the lambda constant genes has been reported (24). The most common polymorphism has been replacement of the 8 Kb EcoRI fragment with an 18 Kb fragment, and this pattern was observed in the human VA2 parental line (not shown) and the two VA/mouse hybrid lines (21 and 25) that contained the human  $C_\lambda$  cluster. The identification of the six hybrid cell lines containing the human lambda-constant genes was completely confirmed by hybridization of the BamHI fragments with the  $C_\lambda$  probe (not shown). The hybridization conditions resulted in weak cross-species hybridization to hamster and mouse DNA, but this posed no problem in the analyses. The most prominent rodent band

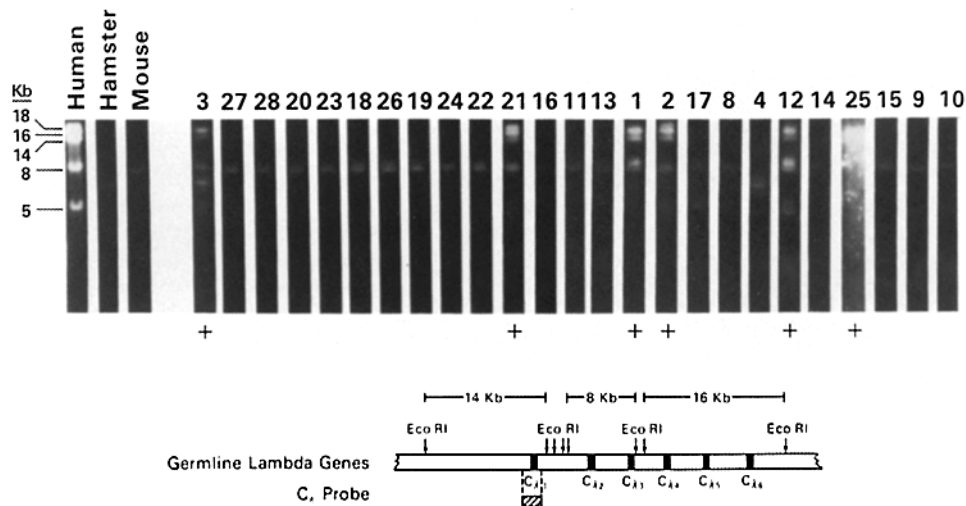


FIG. 1. *In situ* hybridization of the  $^{32}$ P-labeled human  $C_\lambda$  probe with DNA fragments. DNA isolated from control and somatic hybrid cell lines was digested with EcoRI, fractionated by agarose gel electrophoresis (30  $\mu$ g/lane), transferred to nitrocellulose, hybridized with a human  $C_\lambda$  probe, and visualized by autoradiography. Lanes hybridizing with  $C_\lambda$  (+) and hybrid cell line numbers are indicated. Line 4 represents hamster cells containing no human chromosomes. The origin of the probe is shown (hatched box). The 8, 14, 16, and 18 Kb EcoRI fragments containing human  $C_\lambda$  genes are seen in the human control lane. This particular DNA is heterozygous for the 8/18 Kb restriction fragment length polymorphism (24). In addition, a less strongly hybridizing 5 Kb fragment is seen (human lane). It represents a  $C_\lambda$  pseudogene (unpublished) and it is also detected in some of the hybrid lines. The most prominent cross-hybridizing rodent band was an 8.6 Kb restriction fragment containing mouse  $C_{\lambda 1}$  (25).

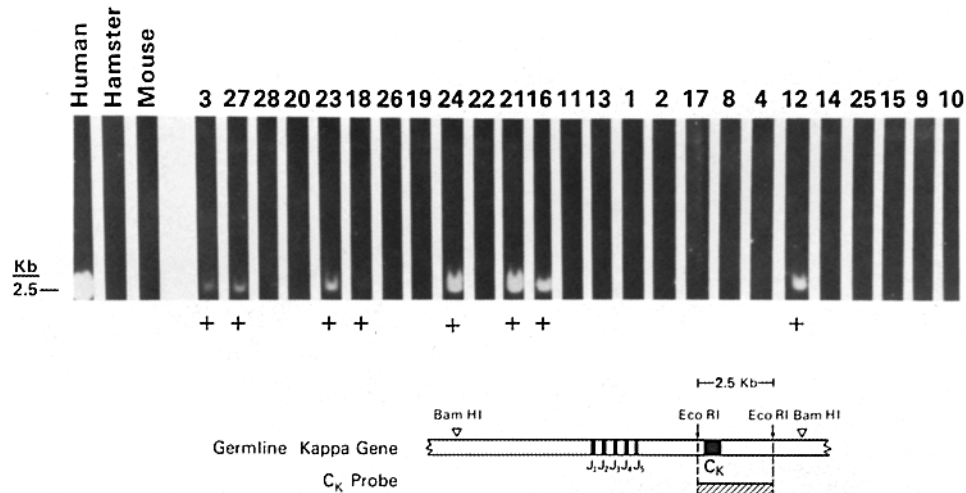


FIG. 2. Hybridization of size fractionated DNA fragments with a  $^{32}\text{P}$ -labeled human  $\text{C}_\kappa$  probe after transfer to nitrocellulose. DNA digestion (EcoRI), electrophoresis, transfer, and hybridization were identical to that described in Fig. 1. The hybrid cell line is shown above each lane, and those hybridizing with  $\text{C}_\kappa$  are indicated (+). The  $\text{C}_\kappa$  probe is depicted below the lanes (hatched box).

was an 8 Kbp EcoRI fragment (25), present in each of the human/mouse somatic cell hybrids. In addition, there was faint hybridization of the  $\text{C}_\lambda$  probe with a 5 Kbp EcoRI human  $\lambda$  pseudogene band (unpublished results) in some hybrid cell lines. The hybridization intensity varied considerably among the hybrid cell lines containing the  $\text{C}_\lambda$  gene cluster. This probably reflects different fractions of the cell population containing the  $\text{C}_\lambda$  genes in the six hybrid cell lines, because the same amount of DNA was loaded in each lane. This interpretation was supported by results of isoenzyme analyses and by subcloning the hybrid cell lines and determining the fraction of the clones that retained the  $\text{C}_\lambda$  gene cluster.

A different group of hybrid cell lines hybridized with  $\text{C}_\kappa$ , indicating that the kappa and lambda constant-region genes are located on different human chromosomes. The 2.5 Kbp EcoRI  $\text{C}_\kappa$ -containing fragment was found in eight hybrid cell lines (Fig. 2). Hybridization of the human  $\text{C}_\kappa$  probe with a 12 Kbp BamHI fragment was observed in these same cell lines (not shown). The intensity of hybridization again varied considerably between different hybrid cell lines, suggesting that only part of some cell populations retained the kappa constant gene.

Isoenzyme analyses were performed on all hybrid cell lines to determine the specific human chromosomes present in each line (Fig. 3). Each human chromosome was present in several different hybrid cell lines. The total human chromosome content of these lines varied from 1 to 19 different chromosomes. Whenever possible, several different isoenzyme markers were used to establish the presence or absence of each specific human chromosome. Usually syntenic markers were all detected when a specific human chromosome was present. Consistent discrepancies for detection of syntenic markers in any line are indicated (Fig. 3). None of the human chromosome 6 long-arm (6q) markers (ME-1, PGM-3, and SOD-2) were found in hybrid line 1, but the human HLA short-arm (6p) marker was demonstrated by filter hybridization of an EcoRI digest with a human HLA probe. Karyotypic analysis of this line

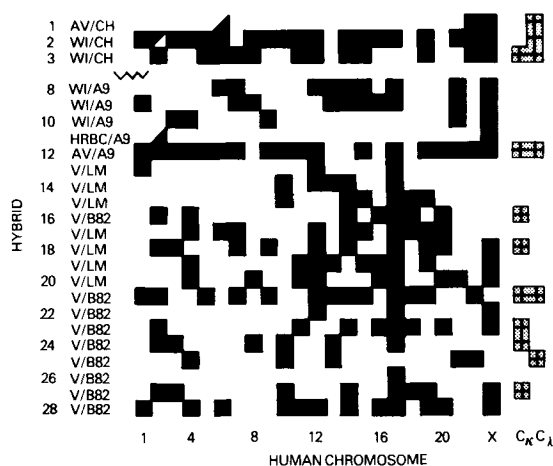


FIG. 3. Distribution of specific human chromosomes in each human/rodent hybrid cell line. Individual hybrid cell lines are represented on the ordinate, and specific human chromosomes are represented on the abscissa. Solid boxes indicate the presence of a particular human chromosome in a hybrid line, and open boxes indicate absence of the chromosome. The symbol  $\blacktriangle$  and  $\blacktriangledown$  indicate the presence of markers for only the short arm or long arm of the chromosome, respectively. The parental origin of the hybrid lines is presented in the second column; the designations are AV = AV3, WI = WI 38, V = VA2, CH = *hprt*<sup>-</sup> CHV79, and LM = LMTKC1 1D. The presence of C $\kappa$  and C $\lambda$  sequences in hybrid cell lines is shown in the last 2 columns.

revealed a deletion of the long arm of chromosome 6 beyond 6q 13 (D. E. Moore and W. McBride, unpublished results). The human chromosome 2 long-arm (IDH-S) and short-arm (MDH-S and ACP-1) markers segregated concordantly in all cell lines except hybrid lines 2 and 11, which exhibited only 2q and 2p markers, respectively. Karyotypic analysis of hybrid line 11 demonstrated that the only human chromosomes were the X chromosome and a human chromosome fragment fused to the centromere of a mouse chromosome.

The results (Fig. 3) indicate that the lambda constant gene cluster is located on human chromosome 22. There were no discordancies between the segregation of C $\lambda$  and chromosome 22, whereas C $\lambda$  segregated discordantly ( $\geq 21\%$ ) with all other human chromosomes except chromosome 5. This latter chromosome segregated discordantly with C $\lambda$  in two primary hybrids (8%) and in many subclones (Fig. 4). The assignment of C $\lambda$  to chromosome 22 is further strengthened by the fact that both karyotypic and isoenzyme analyses demonstrate that the only human chromosomes present in one C $\lambda$ -containing cell line (hybrid 1) are 22, 6p, and the X chromosome. Analysis of subclones of hybrid lines 1, 2, 3, and 12 provided conclusive evidence for our chromosomal assignment of C $\lambda$  (Fig. 4). Chromosome 22 segregated concordantly with C $\lambda$  in all 34 subclones of these four hybrid cell lines. In contrast, there was discordant segregation of chromosome 5 and C $\lambda$  in 19 of the 34 subclones.

The kappa constant gene segregated concordantly with the intact human chromosome 2 in all cases and discordantly ( $\geq 25\%$ ) with all other human chromosomes (Fig. 3). Neither the hybrid cell line containing the isozyme markers for chromosome 2p (hybrid 11) nor the markers for 2q (hybrid 2) contained C $\kappa$ . Concordant segregation of human chromosome 2 and C $\kappa$  was also observed in all 25 subclones that were analyzed (Fig. 4). There was a reasonably good correlation between the relative

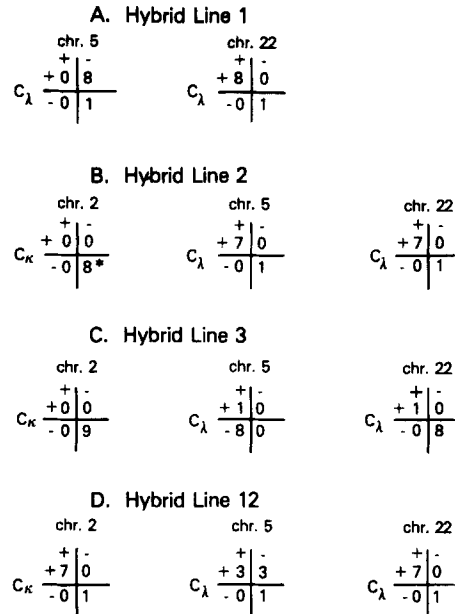


FIG. 4. Segregation of C<sub>κ</sub> and C<sub>λ</sub> with human chromosomes 2, 5, and 22 in subclones of four hybrid cell lines. Subclones of each line were analyzed for the presence of specific chromosomes (isozyme determinations) and the retention of C<sub>κ</sub> and C<sub>λ</sub> genes (blot hybridization of size fractionated DNA restriction digests). Either retention or loss of both a chromosome and the appropriate light chain constant region Ig genes (minus 45° slope) indicates concordancy, whereas independent segregation of a chromosome and C<sub>κ</sub> or C<sub>λ</sub> (positive 45° slope) represents discordancy. Asterisk indicates six of eight subclones of hybrid line 2 retained the chromosome 2q marker (IDH-S), but the chromosome 2p marker (MDH-S) was not present.

intensity of filter hybridization of C<sub>κ</sub> and C<sub>λ</sub> with different primary cell lines and the fraction of cells containing chromosomes 2 and 22, respectively, as estimated by relative intensities in the isozyme assays.

### Discussion

The chromosomal location of human kappa and lambda constant genes has been determined by analysis of a group of human/rodent somatic cell hybrids with nucleic acid probes prepared from cloned human kappa and lambda constant region genes. The analytical procedure involved digestion of DNA from hybrid cells with various restriction endonucleases, size fractionation of DNA fragments by electrophoresis, transfer to nitrocellulose or DBM paper, and hybridization with radioactively labeled C<sub>κ</sub> and C<sub>λ</sub> probes. The results permit assignment of C<sub>κ</sub> to human chromosome 2 and C<sub>λ</sub> to chromosome 22.

There is a very interesting relationship between human lymphoproliferative malignancies and the light chain Ig gene-bearing chromosomes 2 (C<sub>κ</sub>) and 22 (C<sub>λ</sub>). Translocation of material from some other chromosome to the end of number 14 has been reported in many human lymphomas (26), and this chromosome contains the heavy chain genes (2, 27-29). The 14q+ chromosome in Burkitt's Lymphoma has been shown to result from a t[8; 14] translocation (30). In those cases not involving this particular chromosomal rearrangement, two alternate translocations have been

reported. The three translocations that have been observed in both endemic and non-African Burkitt's Lymphoma are t[8; 14], t[2; 8], and t[8; 22], and these same translocations have also been described in other B cell malignancies (31). Bernheim et al. (31) noted that the common anomaly present in Burkitt's Lymphoma actually involves chromosome 8. One assignment to this chromosome is the gene for the large, external, transformation-sensitive protein, fibronectin (32). It is highly significant that Ig genes are located on each of the other three chromosomes involved in these translocations with number 8. The specific break point on chromosome 8 is identical in all these B cell malignancies, and the break points on chromosomes 2, 14, and 22 are also the same in all these reported cases. Similar types of translocations have been observed previously in murine lymphomas. A consistent translocation of the distal part of chromosome 15 to either chromosome 6 or 12 was reported in mouse plasmacytomas (33). The murine kappa (chromosome 6) and heavy chain (chromosome 12) genes are located on these chromosomes. It was postulated that these specific translocations might be related to the V-J type gene rearrangements and IgC<sub>H</sub> switching that occurs in B cells during Ig gene differentiation. An interesting translocation involving human chromosome 22 in a non-B cell neoplasm is the 9:22 balanced translocation that is observed in >90% of the patients with chronic myeloid leukemia (26). The Ig gene probes will be useful in studying translocations involving these chromosomes, and they might provide clues concerning the mechanisms involved in these neoplastic transformations.

Assignment of Ig genes to specific human chromosomes also provides a method for mapping other human genes that are interesting clinically but that are not expressed in somatic cell hybrids. Family studies (34) have previously demonstrated a loose linkage between several markers, including the Kidd and Colton blood group loci and the constant region of kappa. Because C <sub>$\kappa$</sub>  is located on human chromosome 2, these other important loci can now also be assigned to this chromosome.

Gene mapping by recombinant DNA techniques has been limited to rather short intervals in the range of 100 Kb. It has not been possible to determine the distance between constant and variable region Ig genes of the same class by this procedure. It was reported that constant and variable region Ig genes are syntenic in the murine genome (3-5). The recent study by Malcolm et al. (7) combined with our results indicates that the human C <sub>$\kappa$</sub>  and V <sub>$\kappa$</sub>  genes are also syntenic. Analysis of somatic cell hybrids with nucleic acid probes should permit long-range ordering of these genes and provide information concerning their evolution and arrangement. For example, examination of our somatic cell hybrid panel with a human lambda pseudogene probe indicates that it is not syntenic with the C <sub>$\lambda$</sub>  gene cluster (G. Hollis, P. Hieter, P. Leder, D. Swan, and W. McBride, unpublished results). Similar procedures can be used to determine whether J regions, IgC<sub>H</sub> switch regions, and flanking sequences are unique to Ig genes or present on additional chromosomes as well.

The occurrence of occasional chromosomal breaks and translocations in somatic cell hybrids (Fig. 3) has been observed by others (35). Consistent breaks or translocations in the human parental line would create more serious problems. The probability of potential erroneous assignments as a result of these problems has been substantially reduced in this study by using several different parental cell lines and by further analyzing the segregation of C <sub>$\kappa$</sub>  and C <sub>$\lambda$</sub>  in many subclones of multiple independent hybrid lines. Pronounced differences in the sensitivity for detecting a

gene by nucleic acid hybridization and identifying the presence of a chromosome by isoenzyme assays could also pose difficulties. We estimate that the presence of one  $C_{\kappa}$ - or  $C_{\lambda}$ -bearing human chromosome per ten hybrid cells could be detected by the nucleic acid hybridization procedure. The sensitivity of the isoenzyme assays for human chromosome 2 markers was probably similar, and those for chromosome 22 were somewhat less sensitive.

Our chromosomal assignments for human  $C_{\kappa}$  and  $C_{\lambda}$  genes are supported by simultaneous work in other laboratories using different methods. Erikson et al. (8) observed association of lambda gene expression with human chromosome 22 in hybrids, and Malcolm et al. (7) mapped  $V_{\kappa}$  to chromosome 2 by *in situ* hybridization. Malcolm et al. (7) also reported regional localization of  $V_{\kappa}$  to the proximal quarter of the short arm of chromosome 2. Two of our hybrid lines are potentially informative for regional localization of  $C_{\kappa}$  and currently permit some speculation. Line 11 (Fig. 3) expresses human MDH-S and ACP-1 but not IDH-S, whereas the reverse pattern of expression was observed in line 2. Efforts to precisely identify the chromosome 2 fragment in line 11 by Giemsa banding have not been successful. The length of this translocated portion of chromosome 2 has been determined in Giemsa 11-stained metaphases using the human X chromosome as an internal standard. These results indicate that its length is only one-half the entire length of the chromosome 2 short arm. The location of the MDH-S and ACP-1 markers is band 2p23, near the distal end of the short arm. These results suggest that  $C_{\kappa}$  may be located either on the proximal one-half of the short arm of chromosome 2 or the long arm, excluding the immediate IDH-S region. The more probable location of  $C_{\kappa}$  is the proximal one-half of 2p if  $V_{\kappa}$  is located on the short arm. The formation of active Ig genes involves V-J recombination with deletion of at least a portion of the intervening region (36, 37). Thus, location of  $V_{\kappa}$  and  $C_{\kappa}$  on different arms of chromosome 2 could result in deletion of the centromere, which appears improbable. Analysis of hybrids containing well-characterized translocations or deletions involving human chromosomes 2 and 22, or *in situ* hybridization, is required to provide definitive regional localization of these genes.

### Summary

The chromosomal location of human constant region light chain immunoglobulin (Ig) genes has been determined by analyzing a group of human fibroblast/rodent somatic cell hybrids with nucleic acid probes prepared from cloned human kappa and lambda constant region genes. Human chromosomes in each cell line were identified by isoenzyme analysis. The DNA from hybrid cells was digested with restriction endonucleases, size fractionated by gel electrophoresis, transferred to nitrocellulose or DBM paper, and hybridized with  $^{32}\text{P}$ -labeled nucleic acid probes. The  $C_{\kappa}$  gene was assigned to human chromosome 2 and the  $C_{\lambda}$  genes to chromosome 22, based upon analysis of these hybrid cell lines, and these assignments were confirmed by analysis of subclones. A group of previously unassigned loci can be mapped to chromosome 2 by virtue of their close linkage to  $C_{\kappa}$ . The  $\lambda$  and  $\kappa$  light chain and heavy chain Ig genes have now been assigned to all three human chromosomes that are involved in translocations with chromosome 8 in human B cell neoplasms. These techniques and probes provide a means to study the detailed arrangement of human Ig genes and their pseudogenes.



The hybrid cell line A9/HRBC2 (hybrid line 11) was kindly provided by Dr. Marcello Siniscalco (38). Bromodeoxyuridine and 6-thioguanine resistant mutants of AV3 and VA2, respectively, were isolated by Dr. A. Gazdar and generously furnished to us. We thank Dr. Janet Rowley for helpful discussions. We are grateful to Dr. Jon Seidman for providing a  $^{32}\text{P}$ -labeled (nick-translation) probe prepared from a cloned human HLA fragment, pHLA-1 (39), and to Ms. Debi Keithley for DNA isolation and gel electrophoresis of DNA restriction fragments, Ms. D. E. Moore for karyological analyses, Mr. A. Kerr for tissue culture assistance, and Ms. G. Taff for preparation of the manuscript.

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