# CHROMOSOMAL ORGANIZATION OF THE HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I GENE FAMILY

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The human MHC, the HLA complex, is a genetic region located on the short arm of chromosome 6 (1). HLA class I genes encode a 44-kD polypeptide that associates with the non-MHC-encoded 12-kD polypeptide, $\beta_2$-microglobulin (reviewed in reference 2). The most extensively characterized members of the HLA class I gene family are the genes encoding the major transplantation antigens, HLA-A, HLA-B, and HLA-C. A genetic map of 6p based on recombination fractions places *HLA-B* 0.2 cM proximal of *HLA-C*, and *HLA-A* 0.8 cM distal to *HLA-C*.

Southern blot analysis has shown that the HLA class I gene family contains many more hybridizing DNA segments than can be accounted for by *HLA-A*, *-B*, and *-C* genes (3). To precisely define the extent of the HLA class I gene family, we have undertaken the isolation and characterization of genomic clones for each of the HLA class I genes present in the genome of the human B-lymphoblastoid cell line (B-LCL)[1] 721. Three of these clones have been shown to contain the non-A, -B, and -C class I genes *HLA-E* (4), HLA-5.4 (Geraghty, D. E., H. T. Orr, and B. H. Koller, submitted for publication), and HLA-6.0 (5). A panel of HLA loss mutants was used to position the *HLA-E* gene between *HLA-C* and *HLA-A* (4).

In this report we describe the subcloning of locus-specific DNA markers from each of the non-HLA-A, -B, and -C genomic clones that detect restriction fragment-length polymorphisms in genomic DNA. These markers are used to analyze irradiation-induced HLA loss mutants derived from B-LCL 721 (6–8) and a large, HLA-typed pedigree (9). By a combination of deletion analysis and linkage analysis, a map of the HLA class I gene family has been obtained.

## Materials and Methods

*Cell Lines.* $\gamma$ ray-induced HLA loss mutants (Table I) were derived after $\gamma$ irradiation of the human B-LCL 721, as previously described (6–8). These lines were typed at the HLA-A, -B, and -DR loci and at the glyoxylase-1 (GLO-I) locus by serological and electrophoretic methods, respectively. EBV-transformed lymphoblastoid cell lines were established from 95 members of a large pedigree (9). Each member of this pedigree was typed for HLA-A and HLA-B antigens by the University of Minnesota Histocompatibility Laboratory (Minneapolis, MN).

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[1] *Abbreviations used in this paper:* B-LCL, B lymphoblastoid cell line; GLO-I, glyoxylase-1.

*DNA Probes.* A cosmid library was constructed from B-LCL 721 genomic DNA by ligating partial Sau 3A fragments of 35–40 kb into the Bam HI site of pHC79. After in vitro packaging and bacterial transfection, $10^6$ colonies were screened at low stringency with an HLA-B cDNA clone (10). The 89 class I-positive clones were digested with Hind III and Southern blots were probed with the HLA-B cDNA at low stringency. Cosmids containing the same size class I-positive DNA fragments and corresponding in size to class I Hind III fragments detected on a Southern blot of B-LCL 721 DNA were grouped together. The cosmid Southern blots were also probed at high stringency with *HLA-A*-, *HLA-B*-, and *HLA-C*-specific probes (see below) to identify clones containing these genes. From each cosmid group a representative cosmid was selected and a locus-specific DNA probe subcloned. Clones corresponding to three HLA class I genomic Hind III segments were not represented in the cosmid library. These three HLA class I sequences were, therefore, cloned from λ partial genomic libraries (4, 5).

Table II lists each of the HLA DNA probes used, the HLA class I subregion from which each was subcloned, and the polymorphic restriction fragments detected by each probe in B-LCL 721 genomic DNA. Isolation of the HLA-A and HLA-B probes have been described previously (11). The HLA-C probe was subcloned from DNA flanking the *HLA-C* gene (4). At the HLA-5.4p/16.0p subregion, two class I sequences are located within contiguous Hind III-generated restriction fragments of 5.4 and 16.0 kb. Probe 26.1.27 is from DNA 18 kb 5′ of the HLA-5.4p/16.0p subregion and probe 26.1.4 is from DNA immediately 5′ of the HLA-16.0p gene. The HLA-7.5p/9.0p/HLA-6.0 subregion consists of two class I-containing sequences within contiguous Hind III-generated restriction fragments of 7.5 and 9.0 kb, and a third class I gene, HLA-6.0 (5), located ~30 kb 3′ of the 9.0-kb class I sequence. Probe 11.2.1 is from DNA located 5 kb 3′ to HLA-9.0p, and probe 23.2d is from DNA 20 kb 5′ to HLA-7.5p. 12.3.6 is a subclone from a cosmid containing the HLA-9.2p segment. Probe 5.4SH is a Hind III-Sst I fragment subcloned from the 5′ flanking region of the novel HLA class I gene HLA-5.4 and B1.1.EH.11 is from a region 25 kb 5′ of the *HLA-E* gene (4). Probe 26.3 is a Xba I-Hind III fragment subcloned from the HLA-3.0p subregion.

p7H4 is a DNA clone isolated from a genomic library constructed with DNA from a human-hamster hybrid cell line containing human chromosome 6p (12). p7H4 was localized by pedigree analysis to the terminus of 6p, 45 cM from HLA (10). This probe detects polymorphic Eco RV restriction fragments of 9.1 and 11.5 kb in B-LCL 721 DNA.

*DNA Analysis.* Isolation of high molecular weight genomic DNA from B-LCLs, agarose gel electrophoresis, Southern blotting of DNA onto nylon membranes, and probe hybridization and wash conditions were as previously described (13).

Pedigree linkage analyses were performed using the LINKAGE computer program package (14). Recombination frequency between marker loci was estimated by determining lod scores and location scores.

## Results

*Strategy for the Construction of an HLA Class I Chromosomal Map Using HLA Deletion Mutants of B-LCL 721.* Single-copy DNA segments were randomly subcloned from genomic clones of each HLA class I subregion. Each subclone was tested for its ability to detect heterozygosity for a restriction enzyme site polymorphism in B-LCL 721 genomic DNA by probing a Southern blot of digested DNA from two complementary HLA haplotype loss mutant B-LCLs, i.e., DNA from an HLA-A1:GLO2 deletion mutant cell line (e.g., .127) and DNA from an HLA-A2:GLO1 deletion mutant cell line (e.g., .45.1). Once a polymorphism was confirmed, the corresponding probe was then used to analyze digested DNA from various HLA deletion mutant cell lines (Table I). In addition to the HLA haplotype loss mutant cell lines, cell lines screened with each probe included HLA-A2 loss mutant cell lines, HLA-B8 loss mutant cell lines, and an HLA-A homozygous loss mutant cell line (Table I).

*Restriction Fragment-length Polymorphisms Detected by DNA Probes from HLA Class I*

TABLE I

*Loss of Markers from Chromosome 6p in Mutant Cell Lines Derived from Human B-LCL 721*

| Cell line | Chromosome 6p markers | | | | | |
|---|---|---|---|---|---|---|
| | GLO-I | HLA-DR | HLA-B | HLA-C | HLA-A | 7H4 |
| | | | | *kb* | | *kb* |
| Parental B-LCL | | | | | | |
| 721 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| HLA-A1:B8 haplotype loss | | | | | | |
| .13 | 2 | - | - | - | - | 9.1 |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| .19 | 2 | - | - | ND | - | 9.1 |
| | 1 | 1 | 5 | ND | 2 | 11.5 |
| .31 | 2 | - | - | - | - | 9.1 |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| .34 | 2 | - | - | - | - | 9.1 |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| .40 | 2 | - | - | - | - | 9.1 |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| .45.1 | - | - | - | - | - | - |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| HLA-A2:B5 haplotype loss | | | | | | |
| .77.1 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | - | - | - | - | - | - |
| .111 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | - | - | - | - | 11.5 |
| .116 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | - | - | - | - | 11.5 |
| .117 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | - | - | - | - | 11.5 |
| .118 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | - | - | - | - | 11.5 |
| .127 | 2 | 3 | 8 | ND | 1 | 9.1 |
| HLA-A2 loss | | | | | | |
| .64 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | 1 | 5 | 14 | - | 11.5 |
| .74 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | 1 | 5 | 14 | - | - |
| .87 | 2 | 3 | 8 | ND | 1 | 9.1 |
| | 1 | 1 | 5 | ND | - | - |
| .108 | 2 | 3 | 8 | 22 | 1 | 9.1 |
| | 1 | 1 | 5 | 14 | - | 11.5 |
| HLA-B8 loss | | | | | | |
| .5 | 2 | 3 | - | ND | 1 | 9.1 |
| | 1 | 1 | 5 | ND | 2 | 11.5 |
| .12 | 2 | 3 | - | - | 1 | 9.1 |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| .18 | 2 | 3 | - | - | 1 | ND |
| | 1 | 1 | 5 | 14 | 2 | ND |
| .25 | 2 | 3 | - | - | 1 | 9.1 |
| | 1 | 1 | 5 | 14 | 2 | 11.5 |
| HLA-A1:B8, A2 loss | | | | | | |
| .144 | - | - | - | ND | - | - |
| | 1 | 1 | 5 | ND | - | 11.5 |

Glyoxylase I (GLOI) and HLA-DR, HLA-A, and HLA-B were determined by electrophoretic and serological procedures, respectively. Loss of HLA-A, HLA-B, HLA-C, and 7H4 DNA was determined by Southern blotting with gene-specific probes (see Table II). Parental cell line B-LCL 721 is described in reference 6. Mutant cell lines were selected as described in references 6–8. Many mutants have been analyzed prevously by karyotyping (6, 7, and unpublished results) and/or by Southern blotting and HLA locus-specific probes (3, 23–25).

*Subregions.* Hybridization patterns obtained from DNA from the B-LCL mutants with two of the HLA locus probes, 11.2.1 and 26.1.27, are shown in Fig. 1, *A* and *B*, respectively. Probes detecting class I-associated restriction fragment-length polymorphisms in B-LCL 721 and the data obtained upon probing DNA from B-LCL 721 and $\gamma$ irradiation-induced HLA loss mutants derived from B-LCL 721 are summarized in Table II.

*The HLA-B Subregion.* Taq I-generated polymorphic restriction fragments of 8.0 and 3.2 kb are detected by the *HLA-B*-specific probe 1.1a. Both fragments are detected in DNA from the parental B-LCL 721. DNA from the complementary HLA haplotype deletion mutants examined does not contain the 8.0-kb band (HLA-A1:B8 loss mutants) or the 3.2-kb band (HLA-A2:B5 loss mutants). In DNA from the two HLA-B8 loss mutants, .12 and .25, the 8.0-kb fragment is absent. As expected from serological studies, HLA-B5-associated DNA is not deleted in HLA-A2 loss mutants.

*The HLA-C Subregion.* The *HLA-C* subregion-specific probe, 114.5.32, detects Bam HI-generated restriction fragments of 22.0 and 14.0 kb in DNA from B-LCL 721. One fragment, of 22.0 kb, is absent in DNA from the HLA-A1:B8 loss mutants tested and the other band, of 14.0 kb, is not present in DNA from the HLA-A2:B5 deletion mutants. The 22.0-kb fragment is also absent in the three HLA-B8 loss mutants examined. Thus, the deletion of DNA in these three cell lines includes both the *HLA-B* and *HLA-C* loci from the short arm of one chromosome 6.

*The HLA-A Subregion.* Two RFLPs in B-LCL 721 DNA are detectable with the *HLA-A* probe 2a.1, Msp I-generated fragments of 1.9 and 2.3 kb, and Hind III-generated fragments of 4.7 and 5.1 kb. Analysis of DNA from the complementary HLA haplotype loss mutant cell lines indicates that the 1.9-kb Msp I and the 4.7-kb Hind

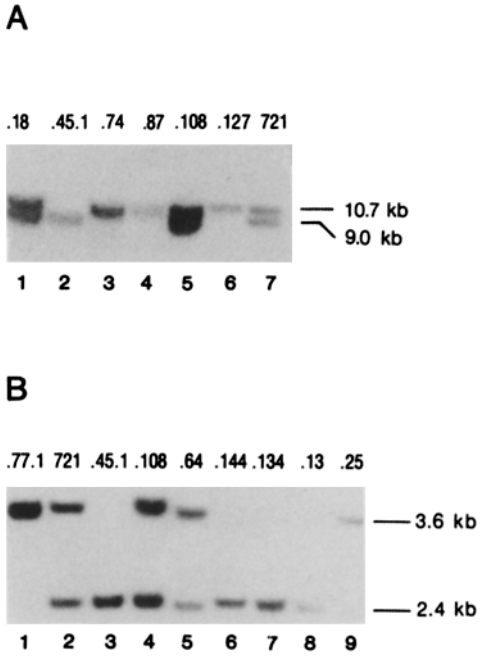


FIGURE 1. Hybridization patterns obtained after probing DNA from HLA loss B-LCLs with two of the HLA locus-specific probes. (*A*) Genomic DNA was digested with the restriction enzyme Msp I and the Southern blot was probed with 11.2.1. (*B*) Genomic DNA was digested with the restriction enzyme Kpn I and the blot was probed with 26.1.27. Above each lane is indicated the B-LCL from which the genomic DNA was obtained. See Table I for the HLA loci absent from B-LCL.

TABLE II

*Analysis of HLA Loss Mutants with DNA Probes from HLA Class I Subregions*

| Subregion | Probe | RFLP in B-LCL 721 | A1:B8 loss cell lines | | | | | | A2:B5 loss cell lines | | | | | | A2 loss cell lines | | | | B8 loss cell lines | | | | A1:B8, A2 loss cell line |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | .13 | .19 | .31 | .34 | .40 | .45.1 | .77.1 | .111 | .116 | .117 | .118 | .127 | .64 | .74 | .87 | .108 | .5 | .12 | .18 | .25 | .144 |
| | | *kb* | | | | | | | | | | | | | | | | | | | | | |
| HLA-B | 1.1a | T 8.0 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | – | – | – | – | ND |
| | | T 3.2 | + | + | + | + | + | + | – | – | – | – | – | – | + | + | + | + | + | + | + | + | |
| HLA-C | 114.5.32 | B 22.0 | – | ND | – | – | – | – | + | + | + | + | + | ND | + | + | + | + | ND | – | – | – | ND |
| | | B 14.0 | + | | + | + | + | + | – | – | – | – | – | | + | + | + | + | | + | + | + | |
| HLA-E | B1.1.EH.11 | P 4.2 | – | ND | – | – | – | | + | + | + | + | + | ND | + | + | + | + | ND | – | – | – | ND |
| | | P 3.8 | + | | + | + | + | + | – | – | – | – | – | | + | – | – | + | | + | + | + | |
| HLA-A | 2a.1 | M 1.9 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | ND | + | – |
| | | H 2.3 | + | + | + | + | + | + | – | – | – | – | – | + | – | – | – | – | + | + | | + | – |
| | | H 4.7 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – |
| | | H 5.1 | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | + | + | + | + | – |
| 5.4p/16.0p | 26.1.27 | K 3.6 | – | – | – | – | – | – | + | + | + | ND | ND | ND | ND | + | + | + | + | ND | + | ND | – |
| | | K 2.4 | + | + | + | + | + | + | – | – | – | | | | | – | – | + | + | | + | | + |
| | 26.1.4 | M 3.5 | – | – | – | – | – | + | ND | + | + | + | + | + | + | + | + | + | + | + | + | + | – |
| | | M 3.0 | + | + | + | + | + | + | | – | – | – | – | – | – | – | – | – | + | + | + | + | – |
| 7.5p/9.0p/ HLA-6.0 | 11.2.1 | M 10.7 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – |
| | | M 9.0 | + | + | + | + | + | + | – | – | – | – | – | – | + | – | – | + | + | + | + | + | + |
| | 23.2d | X 8.8 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – |
| | | X 5.7 | + | + | + | + | + | + | – | – | – | – | – | – | + | – | – | + | + | + | + | + | + |
| 9.2p | 12.3.6 | A 3.4 | – | – | – | – | – | – | + | + | + | + | ND | ND | + | + | + | + | + | + | + | + | – |
| | | A 3.95 | + | + | + | + | + | + | – | – | – | – | | | + | – | – | + | + | + | + | + | + |
| HLA-5.4 | 5.4SH | A 1.5 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – |
| | | A 1.9 | + | + | + | + | + | + | – | – | – | – | – | – | + | – | – | + | + | + | + | + | + |
| | | X 1.8 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – |
| | | X 1.3 | + | + | + | + | + | + | – | – | – | – | – | – | + | – | – | + | + | + | + | + | + |
| 3.0p | 26.3 | Pv 2.4 | – | – | – | – | – | ND | ND | + | + | + | + | ND | + | + | + | + | + | + | + | + | – |
| | | Pv 1.75 | + | + | + | + | + | | | – | – | – | – | | + | – | – | + | + | + | + | + | + |

T, Taq I; B, Bam HI; P, Pst I; M, Msp I; H, Hind III; K, Kpn I; X, Xba I; A, Ava II; Pv, Pvu II.

III fragments are associated with the HLA-A1 gene, and the 2.3-kb Msp I and 5.1-kb Hind III fragments are associated with the HLA-A2 gene. Absence of the 2.3-kb Msp I fragment and the 5.1-kb Hind III fragment in DNA from the HLA-A2 loss mutants and the absence of all bands detected by 2a.1 in DNA from the *HLA-A* homozygous loss mutant .144 are consistent with these assignments.

*The HLA-E Subregion.* *HLA-E* is a recently described HLA class I gene (4). An HLA-E locus-specific probe, B1.1.EH.11, was subcloned from the DNA 25 kb 5′ of the *HLA-E* gene. Previous analysis of DNA from HLA loss mutants with this probe placed the *HLA-E* locus between *HLA-C* and *HLA-A*. The earlier data obtained with the HLA-E-specific probe and the results of probing DNA from additional HLA mutants with this probe are given in Table II. The location of the HLA-E subregion between HLA-C and HLA-A is supported by this additional data. One allele of *HLA-E* DNA is absent in all three of the HLA-B8 loss mutants and the other allele is gone in two of the four HLA-A2 loss mutants examined. The region of overlap between the deletions in the HLA-B8 loss mutants and the HLA-A2 loss mutants (Table I), i.e., the location of the HLA-E subregion, is between HLA-C and HLA-A.

*The HLA-5.4p/16.0p Subregion.* This HLA class I subregion is defined by the contiguous Hind III restriction fragments of 5.4 and 16.0 kb. Within each of these restriction fragments is an HLA class I sequence that by DNA sequencing is likely to be a class I pseudogene (D. Geraghty and B. Koller, unpublished data). Two single-copy DNA segments, 26.1.27 and 26.1.4, separated by 18 kb of DNA, were subcloned from a cosmid insert containing the 5.4p and 16.0p sequences. The restriction fragment-length polymorphisms detected in B-LCL 721 DNA and the hybridization patterns obtained with these probes with the HLA loss mutants are presented in Table II. DNA detectable by the 26.1.4 probe is located within the deletion of all four of the HLA-A2 loss mutants and within the homozygous deletion in mutant .144 (Table II). DNA detected by probe 26.1.27 is also absent in three of the four HLA-A2 loss mutants. However, 26.1.27 DNA remains in the HLA-A2 loss mutant .108 and one copy of 26.1.27 DNA remains in the HLA-A homozygous loss mutant .144 (Table II). These data localize the 5.4p/16.0p subregion to the HLA-A region. Furthermore, the differential loss of 26.1.4 vs. 26.1.27 DNA in mutants .108 and .144 indicates that one $\gamma$ irradiation-induced breakpoint in these two cell lines lies within the 5.4p/16.0p subregion.

*The HLA-9.2p Subregion.* This HLA subregion consists of a single class I sequence located within a 9.2-kb Hind III restriction fragment. DNA sequence analysis shows that the 9.2-kb fragment contains an HLA class I pseudogene (B. Koller and D. Geraghty, unpublished data). The single copy probe, 12.3.6, from the 9.2p subregion, detects an Ava II polymorphism in B-LCL 721 DNA (Table II). Presence of 9.2p DNA in DNA from the HLA-A2 loss mutants .64 and .108 while 5.4p/16.0p DNA is absent in these mutants indicates that the HLA-9.2p subregion is located physically further from HLA-A than is the HLA-5.4p/16.0p subregion and/or these two subregions are positioned on opposite sides of the HLA-A gene.

*The HLA-7.5p/9.0p/HLA-6.0 Subregion and the HLA-5.4 and 3.0p Fragments.* These three HLA class I subregions are defined by class I-containing restriction fragments of 7.5, 9.0, and 6.0 kb, 5.4 kb, and 3.0 kb, respectively. The HLA-6.0 (6) and HLA-5.4 genes are intact class I genes. The HLA-5.4 gene has been designated *HLA-F*. Both encode an HLA class I H chain with a shortened cytoplasmic segment. HLA-6.0

maps to the 7.5p/9.0p subregion, since a cosmid insert containing the 7.5p/9.0p genes also contained the 5′ end of the HLA-6.0 gene (15). RFLPs detected in B-LCL 721 DNA by single-copy probes from these subregions are presented in Table II. Analysis of DNA from the HLA loss mutants shows that 7.5p/9.0p/HLA-6.0, HLA-5.4, and 3.0p are located within a region flanking the HLA-A region. DNA from these subregions is absent in two of the four HLA-A2 loss mutants and is present in all of the HLA-B8 loss mutants examined.

*The 5.9p and 8.0p2 Subregions.* Polymorphic DNA markers could not be identified for these two HLA class I subregions. Nevertheless, the 5.9p fragment was localized within the MHC. This fragment contains an HLA class I pseudogene within a Hind III restriction fragment of 5.9 kb. A single-copy probe isolated from the 5.9-kb subregion fails to hybridize to DNA from the homozygous HLA-A loss mutant .144 (Table II). Thus, this result places the 5.9p subregion withing the homozygous deletion in cell line .144. At this time it is not possible to position the HLA-8.0p2 fragment within the class I gene map. Single-copy DNA segments subcloned from this class I subregion did not detect any RFLPs in B-LCL 721 genomic DNA (data not shown). Moreover, HLA-8.0p2 DNA remains in the HLA-A homozygous loss mutant .144.

*HLA Class I Gene Order Determined by Genetic Linkage Analysis.* Although results obtained with the HLA deletion mutant B-LCLs indicate that the HLA-6.0 and HLA-5.4 genes are located distal to *HLA-C*, these two genes could not be positioned relative to *HLA-A*. Therefore, 95 members from a large, HLA-typed pedigree were screened with the DNA markers 5.4SH, 23.2d, and 23.3.6 from the HLA-5.4, HLA-6.0, and HLA-9.2p loci, respectively, and genetic linkage analyses were performed.

Table III presents results of two-point linkage analysis using these DNA markers and HLA-A. Significant linkage was found between HLA-A and 5.4SH, between HLA-A and 23.2d, between HLA-A and 12.3.6, and between 5.45H and 12.3.6, i.e., maximum lod scores $\geq 3.0$. Two informative recombinations between HLA and 5.4SH were found. Fig. 2 presents the data for one of these informative recombinations.

TABLE III
*Two-point Linkage Results*

| | LOD score | | | | | |
|---|---|---|---|---|---|---|
| $\theta$ | HLA-A 5.4SH | HLA-A 23.2d | HLA-A 12.3.6 | 5.4SH 23.2d | 5.4SH 12.3.6 | 23.2d 12.3.6 |
| .00 | $-\infty$ | $-\infty$ | $-\infty$ | 2.12 | $-\infty$ | 1.47 |
| .05 | 6.54 | 4.26 | 2.91 | 1.93 | 3.38 | 1.28 |
| .10 | 6.76 | 4.41 | 3.08 | 1.68 | 3.00 | 1.08 |
| .15 | 6.17 | 4.07 | 2.77 | 1.40 | 2.51 | 0.88 |
| .20 | 5.26 | 3.55 | 2.30 | 1.11 | 1.99 | 0.68 |
| .25 | 4.20 | 2.95 | 1.78 | 0.82 | 1.50 | 0.51 |
| .30 | 3.09 | 2.31 | 1.25 | 0.55 | 1.05 | 0.34 |
| .35 | 1.99 | 1.66 | 0.75 | 0.32 | 0.65 | 0.22 |
| .40 | 1.01 | 1.03 | 0.35 | 0.14 | 0.32 | 0.11 |
| .45 | 0.29 | 0.46 | 0.09 | 0.03 | 0.09 | 0.04 |
| .50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MAX LOD | 6.82 | 4.44 | 3.11 | 2.12 | 3.42 | 1.47 |
| $\theta$ | .08 | .08 | .08 | .00 | .03 | .001 |

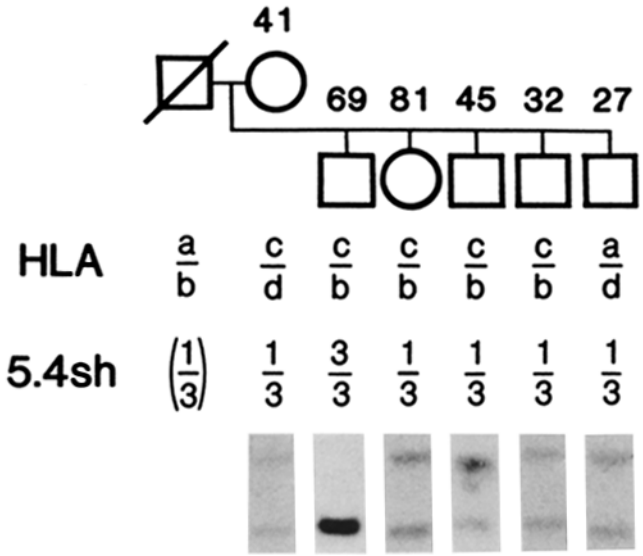


FIGURE 2. An individual having a recombination between HLA and the DNA marker 5.4SH. A nuclear family from the large HLA-typed pedigree (9) is depicted. HLA-A and HLA-B data are presented as haplotypes. The Southern blot data for marker 5.4SH upon digestion with the restriction enzyme Xba I is depicted showing the fragments corresponding to allele 1 (*upper band*) and to allele 3 (*lower band*).

Individual 69 shares both HLA haplotypes with three of his sibs, individuals 81, 45, and 32. However, individual 69 is homozygous for 5.4SH, while 81, 45, and 32 are heterozygous for 5.4SH. Thus, individual 69's HLA-5.4SH genotype is the result of a recombination between HLA and 5.4SH. Importantly, *HLA-A* and *HLA-B* segregated together from 5.4SH in individual 69.

To determine gene order and genetic distances between loci, three-point and four-point linkage analyses were performed (14). The results are shown in Table IV. In both analyses 5.4SH and 23.2 mapped to the same genetic locus, i.e., no recombinants separating these two markers were detected. Three-point linkage analysis (Table IV) indicated a most favored gene order (order 1) with 5.4SH/23.2d 8 cM distal to *HLA-A*. The second most favored order (order 3) places 5.4SH/23.2d 11 cM proximal of *HLA-B*. The least favored order (order 2) places 5.4SH/23.2d between *HLA-A* and *HLA-B*. Relative odds for the first two orders vs. the third were $1.8 \times 10^{10}$ and $1.8 \times 10^{9}$, respectively. A 10-fold difference in relative odds is not considered to be statistically significant. However, results with the HLA deletion mutant LCLs show that 5.4SH and 23.2d could not be located proximal to *HLA-B*,

TABLE IV
*Multi-point Linkage Results*

| Order | Three-point Linkage Results | Relative Odds |
|---|---|---|
| 1 | HLA-B-(0.01)-HLA-A-(0.08)-5.45H/23.2d | $1.8 \times 10^{10}$ |
| 2 | HLA-B-(0.01)-5.45H/23.2d-(0.01)-HLA-A | 1 |
| 3 | 5.45H/23.2d-(0.11)HLA-B-(0.01)-HLA-A | $1.8 \times 10^{9}$ |
| | Four-point Linkage Results | |
| 4 | HLA-B-(0.01)-HLA-A-(0.08)-5.45H/23.2d-(0.02)-12.3.6 | $1.76 \times 10^{11}$ |
| 5 | HLA-B-(0.02)-5.4H/23.2d-(0.01)-HLA-A-(0.11)-12.3.6 | 1 |

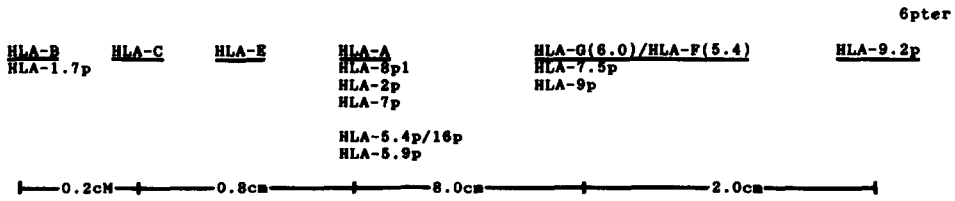


FIGURE 3. A chromosomal map of the HLA class I gene family. The position of HLA-E was determined previously using the HLA loss B-LCL cell lines (4). HLA class I loci linked either within the same cosmid insert or by overlapping cosmids to the HLA-A, HLA-B, or HLA-G (6.0)/HLA-F (5.4) genes are indicated immediately below each locus (17). The HLA-5.4p/16p locus is placed at HLA-A based on analysis of deletion B-LCLs as is the HLA-5.9p locus (see Table I and text). The genetic distances between each marker locus are indicated in cM.

since mutants were found in which both *HLA-A* and 5.4SH/23.2d DNA were deleted while HLA-B DNA was retained, e.g., mutants .74 and .87 in Table II. These data eliminate the proximal order (order 3) as a possibility, leaving only the order with 5.4SH/23.2d distal to *HLA-A* (order 1), favored by relative odds of $1.8 \times 10^{10}$ to 1 over the remaining order with 5.4SH/23.2d between *HLA-B* and *HLA-A*.

Four-point linkage analysis with HLA-A, HLA-B, 5.4SH/23.2d, and 12.3.6 also places the 5.4SH/23.2d locus distal to *HLA-A*. In addition, the 12.3.6 locus is located distal to the 5.4SH/23.2 locus. The most favored four-point order, order 4 in Table IV, places 5.4SH/23.2d 8 cM distal to *HLA-A* and 12.3.6 2 cM distal to 5.4SH/23.2d. This order has relative odds of $1.76 \times 10^{11}$ over the next most favored four-point order (order 5).

*Construction of an HLA Class I Chromosomal Map.* Data from analysis of genomic clones (15), HLA deletion B-LCLs, and multi-point linkage analyses of a large HLA-typed pedigree were combined to generate a chromosomal map of the HLA class I gene region that contains 16 of the 17 class I segments isolated from B-LCL 721. The map (Fig. 3) consists of six genetic loci, HLA-B, HLA-E, HLA-A, HLA-F (5.4)/HLA-6.0, and HLA-9.2p. This region is bounded by the HLA-B locus at the proximal end and by the HLA-9.2p locus at the distal end and spans a genetic distance of 11 cM. Additional class I segments are positioned at some of these loci by virtue of their physical linkage to the marker gene or by analysis of the HLA deletion B-LCLs. Thus, the HLA-1.7p segment is placed at the HLA-B locus, since the two are located within the same cosmid insert (15). In a similar manner, the HLA-8p1, HLA-2p, and HLA-7p segments are placed at the HLA-A locus (15). The HLA-5.4p and -16p segments are also positioned at the HLA-A locus, since in every HLA deletion where B-LCL deletes HLA-A DNA, HLA-5.4p and -16p DNA are also deleted (Table II). The HLA-7.5p and HLA-9p segments are placed at the HLA-6.0/HLA-F (5.4) locus by overlapping cosmid clones (15). Finally with the placement of the HLA-6.0 gene on the genetic map, we propose that this gene be designated HLA-G.

## Discussion

RFLPs have been identified for 16 of the 17 HLA class I segments present within the genome of human B-LCL 721. Analysis of mutant .19 DNA with the HLA locus-specific probes shows that each of the non-HLA-A, -B, and -C loci are located within the 6p deletion present in mutant .19 (Table II). Southern blot analysis of mutant

.19 indicates that the deletion of DNA extends from a breakpoint between HLA-DPI and DR 1 (16) to a position proximal to the marker 7H4 (12). Therefore, the entire HLA class I gene family in B-LCL 721 is located within this region on chromosome 6. This is consistent with the earlier description of a single predominant site of hybridization for a HLA class I probe by chromosomal in situ hybridization (17). The one class I fragment that might not be located in this region of the short arm of chromosome 6 is HLA-8p2, for which a polymorphic DNA marker has yet to be obtained.

Placement of the non-A, -B, -C class I genes *HLA-E*, *HLA-F* (5.4), and *HLA-G* (6.0) distal to *HLA-C* supports earlier predictions on the organization of the HLA complex based on evolutionary considerations (18). If one assumes that the non-A, -B, and -C genes are related to the murine Qa and Tla genes, then the organization of the HLA class I genes shown in Fig. 3 is similar to the supposed organization of H-2 class I genes before the translocation of the H2-K locus from the vicinity of Qa to its current position centromeric to the I region (19).

One implication of the HLA map depicted in Fig. 3 is that the general organization of mammalian MHC class I gene family evolved before speciation. Yet, the human class I gene family occupies a substantially larger genetic region than does the murine class I gene family, i.e., 11 cM between HLA-B and HLA-9.2p vs. 1.6 cM from H-2D to Tla (20). Estimates, based on pulse field gel electrophoresis linkage, of the physical size of the HLA class I region are slightly less than 2,000 kb (21, 22). This is somewhat larger than the 1,000-kb distance estimated to separate H-2D from Tla in the mouse genome (20). Thus, while some of the increase in genetic size of the HLA class I region over the H-2 class I region can be accounted for by additional DNA, the vast majority seems to be due to a relative high recombination frequency distal to HLA-A in human.

An interesting point concerns whether such recombinational hotspots occur distal to HLA-A in all HLA-haplotypes. Thus, it will be important to examine other large HLA-typed pedigrees with the appropriate DNA markers. Variation between HLA haplotypes in the presence of recombinational hotspots distal to HLA-A may provide an explanation for the association of certain diseases with some HLA haplotypes and not with other haplotypes.

## Summary

17 HLA class I genes have been isolated from the genome of B-lymphoblastoid cell line 721. Sequence analysis and transfection studies indicate that three genes, in addition to those encoding the HLA-A, -B, and -C antigens can direct the synthesis of a class I $\alpha$ protein (4, 5, 21). Using gene-specific DNA probes to analyze the presence of restriction fragment-length polymorphisms within a large pedigree and in panel of HLA deletion mutant cell lines, we show here that two of these genes, designated *HLA-G* and *HLA-F*, are located on the short arm of chromosome 6 telomeric to the HLA-A locus. The third expressed non-A, -B, and -C class I gene, *HLA-E*, is located between *HLA-A* and *HLA-C* (4). In addition, the remaining 11 class I pseudogenes and gene fragments are localized relative to established markers on chromosome 6p.

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