Isolation of HLA locus-specific DNA probes from the 3'-untranslated region

(HLA-A and -B/HLA loss mutants/high-stringency hybridization)

Beverly H. Koller*, Beth Sidwell*, Robert DeMars*, and Harry T. Orr*

*Department of Laboratory Medicine/Pathology, University of Minnesota, Minneapolis, MN 55455; and **Laboratory of Genetics and Department of Human Oncology, University of Wisconsin, Madison, WI 53706

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ABSTRACT When human class I cDNA clones containing coding sequences are used to probe genomic DNA, 15-20 fragments, each containing a complete class I gene or pseudogene, are seen. Identification of which genomic DNA segments encode the HLA-A and -B antigens has to date required transfection of mouse L cells with cloned class I genes or analysis of HLA loss mutants. In this report we show that under high-stringency conditions, probes constructed from the 3'-untranslated region can be used to specify the segments of DNA that encode the HLA-A and -B antigens in the human lymphoblastoid cell line LCL 721. Examination of DNA from unrelated individuals indicates that these probes are locus specific and will permit identification of HLA-A and -B genes in the population.

The definition of the HLA system arose from the discovery of leukocyte agglutinins in the sera of polytransfused individuals (1) and multiparous women (2, 3). Two antigenic groups were initially described; the group Four, or HLA-B, and LA, or HLA-A. As more sera were described, it was found that the antigens fell into two series, HLA-A and -B, each of which behaved as if controlled by a set of alleles at two closely linked loci (4). Today, >20 HLA-A and >40 HLA-B alleles have been described (5). As well as providing a major barrier to transplantation, the HLA antigens are responsible for restriction of T-cell-mediated cytotoxicity to virus-infected target cells (reviewed in ref. 6).

Both HLA-A and -B antigens are integral membrane glycoproteins of about 44,000 daltons expressed on the cell surface of most tissues in noncovalent association with β2-microglobulin (reviewed in ref. 7). Protein sequence analysis of products of the HLA-A and -B loci shows overall homology of 80% (8). However, few of the differences appear to be locus specific. Comparison of the protein sequences of HLA-A2 and -B7 shows that most of the differences between these two proteins are found clustered in three areas of their NH2-terminal regions. Between amino acid residues 75 and 80, homology drops to 30% and from residues 105–116 and 177–194, 50% homology is seen. However, these areas are also poorly conserved between the HLA-A2 and HLA-A28 heavy chains (9). Moreover, residues that distinguish HLA-B7 from HLA-A2 are found in another -A allele, HLA-A28. Thus, since positions that differ from HLA-A and -B also vary between alleles of a single locus, the limited sequences available have not been useful in identifying residues unique to HLA-A or -B alleles (9, 10). Monoclonal antibodies reacting with the protein products of only one allele of each locus have been isolated (e.g., ref. 11). Monoclonal antibodies that react with the protein products of a number of HLA alleles, but not necessarily of the same locus, have also been described (12). Thus, although monoclonal antibodies to HLA class I antigens have been described, none uniquely defines the alleles according to locus.

Two mechanisms have been invoked to explain the apparent lack of locus-specific sequences. The first suggests that divergence in the population is limited by functional constraints placed on the antigens. Thus, at any given position only a limited spectrum of amino acids will result in a functional molecule. The second suggests that much of the polymorphism seen in class I genes is the result of gene conversion. Gene conversion could result in a movement of sequences from one class I locus to another, resulting in both polymorphism and loss of locus-specific sequences that may have evolved since gene duplication.

The high level of sequence conservation in HLA class I proteins extends to the DNA level. Over 15 different fragments generated by restriction endonucleases hybridize with cDNA probes for class I coding sequences (13–15). Hybridization of these fragments with probes from both the 5' and 3' regions of the class I cDNA clones indicates that they contain an entire gene. The large number of class I genes that hybridize these cDNA probes has made identification of the HLA-A and -B genes difficult. Two approaches have been used. Class I genomic clones have been transfected into mouse L cells and assayed for their ability to direct synthesis of HLA-A and -B surface antigens (16, 17). An alternate approach has been to analyze a series of HLA loss mutants derived from lymphoblastoid cell line (LCL) 721 (18). Correlation of loss of restriction enzyme-generated fragments of genomic DNA hybridizing with class I cDNA probes, with loss of reactivity with specific HLA sera, has resulted in the identification of the HLA-A1, -A2, and -B8 genes in this cell line (19).

In this report, we describe probes isolated from the 3'-untranslated region of HLA-A and -B genes that, under high-stringency conditions, hybridize only to one locus in LCL 721, demonstrating that locus-specific sequences can be identified for members of the class I multigene family. Hybridization of these probes with genomic DNA from individuals with different HLA-A and -B types suggests that the locus specificity of the 3'-untranslated region is not restricted to the HLA-A and -B alleles found in LCL 721. Thus, these probes can be used to identify all HLA-A and -B genes.

MATERIALS AND METHODS

Cell Lines. LCL 721 (HLA-A1, -B8, -A2, -B5) is an Epstein-Barr virus-transformed human B cell. HLA loss mutants derived from LCL 721 after γ-irradiation (300 rad; 1 rad = 0.01 gray) and selection with complement and alloantisera (18, 20) or monoclonal antibodies against specific HLA antigens (15, 19).

Southern Blot Analysis. Genomic DNA was isolated from LCL 721 and its mutants as described (19). Restriction en-
zymes were purchased from Bethesda Research Laboratories, Boehringer Mannheim, and New England BioLabs and used according to supplier specifications. Gel electrophoresis was performed as described (17) and blotting was according to Southern (21). Filter prehybridization and hybridization were as described (19). Filters were washed initially at room temperature in 0.15 M sodium chloride/0.015 M sodium citrate, pH 7/0.5% NaDodSO₄, followed by washes in either 15 mM sodium chloride/1.5 mM sodium citrate, pH 7, at 42°C (low stringency) or 15 mM sodium chloride/1.5 mM sodium citrate, pH 7, at 65°C (high stringency).

Probes. An HLA-B locus-specific probe, pHLA-1.1 (Fig. 1b), was prepared by digesting cDNA clone pHLA-1 (22) with restriction endonucleases Pvu II and Pst I. The DNA digest was subjected to electrophoresis in a 5% acrylamide gel. A 358-base-pair (bp) fragment was isolated from the acrylamide gel according to Maxam and Gilbert (23). This fragment contains only 3'-untranslated sequences and does not include the poly(A) tail or poly(A) addition site. An HLA-A locus-specific probe, pHLA-2a.1, was prepared from the HLA-A2 genomic clone pHLA-2a (Fig. 1a). A 490-bp Pvu II-Msp I fragment of pHLA-2a was subcloned into the Acc I and Smal I sites of pUC9 (24). By sequence analysis, this subclone contains most of the HLA-A2 3'-untranslated region and 72 bp of 3'-flanking DNA. The 490-bp insert was isolated as described above (23). Probes were radiolabeled by nick-translation (25) of 0.5 µg of insert DNA.

RESULTS AND DISCUSSION

HLA-A Locus Probe. The HLA-A locus probe pHLA-2a.1 was prepared from the genomic clone pHLA-2a, which by analysis of HLA mutants (19), sequence analysis, and transfection studies (unpublished data), has been shown to encode the HLA-A2 antigen. Sequence analysis and comparison to pHLA-1 indicated that the 490-bp subclone of pHLA-2a (Fig. 1a) contains all of the HLA-A2 3'-untranslated region and 72 bp of 3'-flanking DNA. When this subclone was used to probe HindIII-digested genomic DNA from LCL 721 at low stringency (15 mM sodium chloride/1.5 mM sodium citrate, pH 7, at 42°C), a pattern very similar to that seen with an HLA-B7 cDNA clone (15) was obtained (Fig. 2, lane a). Only two of the bands visualized with the HLA-B7 cDNA probe failed to hybridize with this HLA-A2 subclone. When the stringency was increased (15 mM sodium chloride/1.5 mM sodium citrate, pH 7, at 65°C), a markedly different result was obtained. Although the HLA-B7 cDNA probe continued to hybridize to all but a few of the HindIII-generated fragments at high stringency (unpublished data), only two bands continued to bind to the pHLA-2a.1 subclone (Fig. 2, lane e). One of these, the 5.1-kilobase (kb) fragment, correlated with the expression of the HLA-A2 antigen (19). The second, a 4.7-kb band, corresponded by both an analysis of mutants and population studies to HLA-A1 (19). To further verify the specificity of the HLA-A probe, DNA was prepared from HLA loss mutants derived from LCL 721. If pHLA-2a.1 is locus specific, hybridization of this probe to these mutant lines should correlate with their expression of HLA-A genes. When HindIII-digested DNA from 721.72, an HLA-A2, -B5, -DR1 loss mutant (26), was examined, only the 4.7-kb band seen in the parental line 721 was detected (Fig. 2, lane h). Conversely, when we examined HindIII-digested DNA from the mutant 721.45.1, from which the HLA-A1, -B8, -DR3 haplotype had been deleted (18), the band at 4.7 kb was no longer seen while the 5.1-kb band remained (Fig. 2, lane h). To ensure that hybridization was HLA-A locus specific, the HLA-A null cell line 721.144 was examined. This line was obtained after γ-irradiation of the HLA-A1, -B8, -DR3 loss mutant 721.45.1, followed by immunoselection for cells no longer expressing HLA-A2 (ref. 15; unpublished data). Both the 4.7- and 5.1-kb fragments were absent from HindIII-digested DNA from line 721.144 (Fig. 2, lane f). Thus, the hybridization data obtained from LCL 721 and the HLA loss mutants show that at high stringency the 3'-untranslated region of HLA-A2 can be used as an HLA-A locus-specific probe for LCL 721.

HLA-B Locus Probe. pHLA-1 (22) is a cDNA clone obtained from the cell line LKT (HLA-A1, -B8 homozygous). Sequence homology between pHLA-1 and an HLA-B7 cDNA clone suggests that pHLA-1 originates from an HLA-B locus mRNA (27). When pHLA-1 was digested with the restriction enzyme Pvu II, two fragments were obtained. The 150-bp fragment contains the sequence encoding the cytoplasmic region and a small portion of the transmembrane region. The larger 358-bp fragment consists entirely of the 3'-
untranslated region. When the latter fragment, pHLA-1.1, was used to probe at high stringency DNA from LCL 721 digested with Bgl II, only two bands were seen (Fig. 3, lane a), one at 7.0 kb and another at 5.8 kb. Examination of haplotype loss mutants correlated the 7.0-kb band with the HLA-A1, -B8 haplotype and the 5.8-kb band with the HLA-A2, -B5 haplotype—that is, HLA-A1, -B8 loss mutants no longer contained a 7.0-kb band while retaining the 5.8-kb band (Fig. 3, lanes d and e). In HLA-A2, -B5 loss mutants, the 5.8-kb band was no longer detected, while the 7.0-kb band remained (Fig. 3, lanes h and i). To determine which locus on the HLA-A1, -B8 haplotype hybridized the probe, Bgl II-digested DNAs from five HLA-B8 loss mutants—721.5, 721.16, 721.10, 721.18, and 721.25—were examined (Fig. 3, lanes b, c, j–l) and four were shown to have lost the 7.0-kb band while retaining the 5.8-kb fragment. Mutant 721.16 (Fig. 3, lane c) retained both Bgl II-generated fragments. Earlier hybridization studies using an HLA-B7 cDNA clone as a probe indicated that HLA-B8 single-loss mutants can be divided into two groups (19), those whose loss of expression of the HLA-B8 gene can be correlated with loss of DNA hybridizing with the HLA-B7 cDNA probe and those in which no loss of class I DNA is apparent. The HLA-B8 loss mutant 721.16 falls into this later category (19).

As indicated above, the 5.8-kb Bgl II-generated DNA band correlated with the HLA-A2, -B5 haplotype in LCL 721 and therefore was present in the HLA-A1, -B8, -DR3 loss mutant 721.45.1 (Fig. 3, lane e). When mutant 721.45.1 was mutagenized further with γ-rays to create the HLA-A null line 721.144, the 5.8-kb Bgl II-generated band was retained (Fig. 3, lane f). As line 721.144 has lost the HLA-A2 structural gene (17), the 5.8-kb fragment that hybridizes with pHLA-1.1 cannot originate from the HLA-A2 gene. Therefore, the 5.8-kb Bgl II-generated DNA fragment contains the HLA-B5 gene or a gene closely associated with it. The strong evidence that pHHLA-1 is a clone of the HLA-B8 gene makes it likely that the 5.8-kb band does contain the HLA-B5 gene. Therefore, at high stringency a probe obtained from the 3′ untranslated region of an HLA-B cDNA clone, pHLA-1, hybridizes to class I genes of LCL 721 in a locus-specific manner.

Mutant 721.53 is phenotypically HLA-B null (28). Since previous studies failed to establish a correlation between the HLA-B5 allele and a restriction fragment hybridizing to the HLA-B7 cDNA probe, it was not possible to determine whether loss of HLA-B5 expression in mutant 721.53 was due to a deletion of the HLA-B5 gene (20). Although our interpretation is limited because the fragment to which the probe from the 3′-untranslated region of pHLA-1 hybridized may not contain the complete gene, the presence of the 5.8-kb band in mutant 721.53 (Fig. 3, lane g) suggests that the loss of HLA-B5 expression in mutant 721.53 may not be due to a γ-ray-induced deletion of the HLA-B5 gene.

As studies thus far have been restricted to the LCL 721, it is possible that pHLA-1.1 and pHLA-2a.1 hybridize only to the genes of a limited array of HLA-A and -B alleles. To address this question, pHLA-2a.1 was used to probe genomic DNA from 23 unrelated individuals (Fig. 4, lanes b–m and p–z). Two hybridization patterns were observed. In some individuals, two bands were seen. In others, only one band was visualized; however, this band has an intensity double that of a single copy gene. These data, and similar studies with pHLA-1.1 (unpublished data), support the contention that probes from the 3′-untranslated region can be used to identify HLA-A and -B genes.

The HLA-A and -B genes are part of a multigene family with >15 members (13–15). The high level of sequence conservation within the class I family has made it difficult to use class I cDNA probes containing coding information to identify and study the products of any given class I locus independent of other family members, at either the RNA or DNA level. This paper describes the construction and characterization of probes from the 3′-untranslated region that can circumvent these problems. These probes provide a means for rapid assignment of cloned class I genes to either the HLA-A or -B locus. Restriction fragment-linked polymorphisms can be studied for either the HLA-A or -B locus. This may be especially useful for examining the basis of disease associations that have been identified for several class I alleles (reviewed in ref. 29). As the two probes described were constructed from the 3′-untranslated region, they will also be useful for studying the expression of either HLA-A or -B genes at the mRNA level.

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