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Genetics

cDNA clones coding for the heavy chain of human HLA-DR antigen

(histocompatibility antigen/B-cell Ia-like antigen/chromosome 6/membrane protein)

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ABSTRACT **Two cDNA clones, pDRH1 and pDRH2, containing sequences specific for human HLA-DR antigens were isolated from a bank of cDNA clones made from partially purified HLA-DR mRNA from the human lymphoblastoid cell line Maja. The clones were specific for the M_r 34,000 HLA-DR antigen glycoprotein chain. The identity of these clones was established by (*i*) their ability to hybridize specifically to HLA-DR mRNA in a positive selection assay; (*ii*) mRNA species hybridizing to the cDNA clones were expressed in B-cell but not in T-cell or fibroblast cell cultures; and (*iii*) a nucleotide sequence in the longer clone, pDRH2, could be translated into an amino acid sequence that is identical to the limited NH_2-terminal amino acid sequence available for the purified HLA-DR antigen M_r 34,000 chain. Analysis of DNA from human, mouse, and human–mouse somatic cell hybrid lines by Southern transfer of restriction endonuclease digests indicated that the HLA-DR heavy chain is encoded in chromosome 6. This finding is compatible with the location of at least one of the HLA-D/DR heavy chain genes within the HLA region. In addition, the sequences coding for HLA-DR heavy chain appear to be present in only one or a few copies in the genome and to be relatively simple in structure.**

The HLA region in humans, sometimes also called the major histocompatibility system or complex, codes for a series of cell surface determinants involved in cellular interactions in the immune system and for certain of the complement components. Conventional genetic analysis together with serological and biochemical studies of the gene products suggests a complex structure for this large gene cluster whose detailed understanding will depend on analysis at the DNA level. Several groups have already described DNA clones and sequences for HLA-ABC-related products and their mouse equivalents H-2 K and D (1–4). We have chosen to concentrate on obtaining cDNA clones corresponding to HLA-DR-associated products, the human equivalents of the mouse Ia antigens.

The structure and composition of the HLA-D/DR antigens are not as well understood as those of the HLA-ABC products. The HLA-DR molecule appears to be composed of two noncovalently associated integral membrane glycopeptides: a heavy chain of $M_r \approx 34,000$, which is largely invariant, and a light chain of $\approx 28,000$, which appears to carry the major polymorphic determinants (5, 6). Recent evidence indicates, however, that there are at least two and possibly three different HLA-D/DR-associated products (7–9). Two are expected by analogy with the mouse I-E/C and I-A products (10). Analysis of the DNA should help to answer questions about the complexity of these proteins and provide complete amino acid sequence data for the protein products.

Our approach to obtaining HLA-DR cDNA clones was to obtain RNA enriched for HLA-DR mRNA from a homozygous lymphoblastoid cell line by isolating polyadenylylated RNA from membrane-bound polyribosomes and fractionating this RNA by size (11). Double-stranded cDNA was synthesized from the resulting enriched mRNA and inserted into the *Pst* I site of pAT153. Seventy-six ampicillin-sensitive tetracycline-resistant clones were derived from a single transformation experiment. Two of these clones contained HLA-DR heavy chain sequences.

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MATERIALS AND METHODS

Avian myeloblastosis virus reverse transcriptase (RNA-dependent DNA nucleotidyl transferase) was supplied by J. W. Beard (National Institutes of Health). DNA polymerase I (Klenow fragment) and restriction endonucleases were from New England BioLabs. RNase and nuclease S1 were from Sigma. Merck proteinase K was supplied by British Drug House (Poole, England). [α-^{32}P]dCTP and [α-^{32}P]dGTP (2000–3000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) were from the Radiochemical Centre (Amersham, England) and calf liver tRNA was from Boehringer Mannheim.

Cell Lines and Antisera. The B-lymphoblastoid cell line Maja, an Epstein–Barr virus-transformed line from a normal donor of a known HLA type, was used as our major source of mRNA. Other cell lines used are described in Table 1. Antiserum 254 is a polyspecific heteroanti-DR serum and was obtained from M. Crumpton. Recombinant DNA work was carried out under the appropriate conditions specified by the British Genetic Manipulation Advisory Group.

Construction of cDNA Clones. Enriched HLA-DR mRNA from Maja cells was obtained by isolation of poly(A)$^+$RNA from membrane-bound polyribosomes followed by sedimentation in a sucrose gradient as described by Lee *et al.* (11). This enriched RNA fraction was then used as a template for oligo(dT)-primed cDNA synthesis with reverse transcriptase (18), and the second cDNA strand was synthesized with the Klenow fragment of DNA polymerase I. The resulting double-stranded cDNA molecules were treated with nuclease S1, tailed with deoxycytidine, and chromatographed on Bio-Gel A-150 in 0.1 M NaCl/0.1 mM EDTA/10 mM Tris·HCl, pH 7.4, to isolate the molecules >500 nucleotide pairs long (19). Appropriate fractions from the column were hybridized directly with *Pst* I-cleaved pAT153 and used to transform *Escherichia coli* χ1776.

Plasmid Purification. Plasmid DNA was purified from groups of $\approx$40 clones or from single clones. First the colonies were grown overnight on agar plates; then, the plates were flooded with 10 ml of nutrient broth and incubated for 6 hr, and the cells were harvested by centrifugation at 5000 × *g* for 5 min. The bacterial pellets were washed with 10 ml of 10 mM NaCl and then drained and either frozen at −20°C or suspended in 50 mM glucose/1 mM EDTA/10 mM Tris:HCl, pH 7.4, and lysed with $\approx$1 μg of lysozyme for 5 min at room temperature. Lysis was completed by addition of 2 vol of 0.2 M NaOH/1%

Abbreviations: Pipes, 1,4-piperazinediethanesulfonic acid; kb, kilobase(s).

Table 1. Cell lines used

Name	Source	Remarks
Maja	Genetics Dept., Univ. Oxford	Human B-cell line (Epstein–Barr virus-transformed lymphocytes) primary
HFF	Genetics Dept., Univ. Oxford	Human fibroblast
Molt-4	Ref. 12	Human T-cell line
RPMI 1788	Ref. 13	Human B-cell line (Epstein–Barr virus-transformed lymphocytes)
G3.32.2	Ref. 14	Burkitt lymphoma
PCC4	Ref. 15	Mouse teratocarcinoma
Somatic cell hybrids (human–mouse)*		Human chromosomes
MCP-6	Ref. 16	X/6 translocation
G3X-11	M. Quintero	4,5q⁺,6/X,7,9,10,13,14,17,20, 21
ThyB-1.33	Ref. 17	X,21
Geoc 4	P. Goodfellow	X deletion (Xp.21-Xp.ter)
Horl 9D2	P. Goodfellow	X,11,15
MCP-6R8	P. Goodfellow	Revertant of MCP-6

* Mouse parents were PCC4 (MCP-6, Geoc 4, MCP-6R8), XG3-NS1 (G3X-11), Bw5147 (ThyB-1.33), and 1R (Horl 9D2).

NaDodSO$_4$ for 10 min on ice, and chromosomal material and cell debris were precipitated by addition of 1.5 vol of 5 M KOAc for 5 min on ice and centrifugation for 5 min in an Eppendorf centrifuge. The plasmid in the supernatant was precipitated by addition of isopropanol at −20°C, keeping the precipitate at this temperature for at least 2 hr and then pelleting for 5 min in an Eppendorf centrifuge. This procedure was developed by D. Ish-Horowicz (20). We found it necessary for our purposes to treat the crude plasmid preparations, in addition, with RNase at 100 μg/ml in 0.1 M NaCl/5 mM EDTA/0.1 M Tris:HCl, pH 7.9, for 10 min at 37°C. The RNase step was followed by digestion with proteinase K at 200 μg/ml in the presence of 0.5% NaDodSO$_4$ for 15 min at 37°C, extraction with 1 vol of phenol, pH 7, and precipitation with 2 vol of EtOH after addition of NaOAc, pH 5, to a final concentration of 0.2 M. Average yields of plasmid DNA were 5–10 μg from one plate.

Positive Selection for Plasmids Containing HLA-Related Sequences. Plasmids purified as above were suspended in 50 mM NaCl/6 mM MgCl$_2$/6 mM 2-mercaptoethanol/6 mM Tris·HCl, pH 7.4, and treated with ≈1 unit of *Hha* I restriction enzyme per μg of DNA at 37°C for 10 min. After further proteinase K digestion, phenol extraction, and EtOH precipitation, the DNAs were suspended in 10 mM sodium phosphate, pH 6.5, and the suspension was boiled for 2 min, quick frozen in dry ice/EtOH and then applied to diazophenylthioether paper suitably marked before activation (footnote 51 in ref. 21). For groups of 40 plasmids, ≈5 μg of plasmid DNA in 50 μl of buffer was applied to 1-cm squares and, for individual plasmids, ≈1 μg was applied to 3-mm squares. After incubation at 0°C overnight, the paper squares were washed four times in 0.4 M NaOH, twice in H$_2$O, and twice in three-times recrystallized formamide. They were maintained for ≈1 hr in 25 μl per filter of prehybridization buffer [0.4 M NaCl/5 mM EDTA/40 mM 1, 4-piperazinediethanesulfonic acid (Pipes), pH 6.4/50% formamide/1% glycine containing poly(A) at 50 μg/ml and calf liver tRNA at 250 μg/ml]. The filters were then washed in prehybridization buffer lacking poly(A), tRNA, and glycine and hybridized with 5 μl per filter of 0.4 M NaCl/5 mM EDTA/40 mM Pipes, pH 6.4/50% formamide containing Maja poly(A)$^+$ RNA at 300 μg/ml overnight at 41°C. After hybridization, batches of ≈20 filters were washed 10 times with 10 ml of 0.15 M NaCl/1 mM EDTA/0.1% NaDodSO$_4$/10 mM Tris·HCl, pH 7.4, at 50°C. They were then washed twice for 5 min individually with 0.1 mM EDTA/10 mM Tris·HCl, pH 7.9, at 50°C. The RNA specifically hybridized to each filter was eluted by boiling the filter in 0.2 ml of sterile double-distilled H$_2$O containing 5 μg of calf liver tRNA, washing a second time with 0.2 ml of H$_2$O, and then precipitating with 1 ml of EtOH after the addition of 50 μl of 2.0 M NaOAc, pH 5. After incubation overnight at −20°C, the RNAs were collected by centrifugation for 15 min in an Eppendorf centrifuge and air dried for ≈ 1 hr. This procedure is a synthesis of published techniques (22, 23). The RNA pellets were suspended in 3 μl of sterile double-distilled H$_2$O, boiled for 2 min, and quick frozen in dry ice/EtOH.

Cell-Free Translation. The RNAs were translated in a rabbit reticulocyte lysate (24) supplemented with dog pancreas microsomes, a gift from M. Owen (25). After translation of 1 μl of RNA in 29 μl of supplemented lysate at 34°C for 90 min, an aliquot of the mixture was removed for electrophoresis in polyacrylamide gels. To the remainder was added 0.1 ml of 0.15 M NaCl/1 mM EDTA/0.1% NaN$_3$/1.0%Nonidet P-40/0.5% Na deoxycholate/50 mM Tris·HCl, pH 8.3, containing bovine serum albumin at 1 mg/ml and 0.5 μl of antiserum 254. Each mixture was spun on a Vortex briefly and then incubated overnight at 4°C. Next, 20 μl of a 10% suspension of formalin-fixed *Staphylococcus aureas* (26) was added, and the mixtures were spun on a Vortex, incubated for another 15 min at 4°C, and then underlayed with 0.5 M NaCl/2 mM EDTA/1% Nonidet P-40/0.5% Na deoxycholate/0.1% NaN$_3$/30% (wt/vol) sucrose/50 mM Tris·HCl, pH 8.3. The immune complexes were centrifuged through this layer for 5 min in an Eppendorf centrifuge, suspended in 0.5 ml of 0.1% NaDodSO$_4$/50mM NaCl/2% Nonidet P-40/0.125 M Tris·HCl, pH 6.8, and centrifuged again. The resulting pellets were suspended in 25 μl of sample buffer [2% NaDodSO$_4$/50% (vol/vol) glycerol/0.125 M Tris·HCl, pH 6.8], heated at 90°C for 2 min and centrifuged as before. The supernatants were subjected to electrophoresis in 12.5% polyacrylamide gels as described (27).

RNA Analysis. Total cytoplasmic RNA was isolated from various cell lines by the procedure of Favaloro *et al.* (28). The concentrations of the RNAs were determined by A_{260} measurement. Ten micrograms of RNA from each cell type was treated with 20 mM methyl mercury hydroxide and then subjected to electrophoresis in a 2.5% agarose/5 mM methyl mercury hydroxide gel at 30 V for 12 hr (29). The gel was removed, stained for 30 min with ethidium bromide at 1 μg/ml in 0.5 M NH$_4$OAc, destained with 0.5 M NH$_4$OAc for 30 min, and photographed. Next, the gel was soaked in several changes of 10 mM sodium phosphate, pH 6.5, for 60 min and then blotted onto nitrocellulose in 3.0 M NaCl/0.3 M Na citrate, baked, and hybridized as described by Thomas (30). pDRH1, pDRH2, and pHLA-A plasmid DNAs were nick translated as described (31). cDNA clone pHLA-A was obtained by positive selection following the same approaches as described here for the HLA-DR clones. It was validated as a probable HLA-A clone by comparison of its sequence with published HLA and H-2 amino acid sequences and nucleotide sequence data (1, 4, 32). Details concerning this clone and its characterization will be published elsewhere.

DNA Sequence Analysis. Plasmid inserts were isolated after cleavage with *Pst* I under standard conditions and electrophoresis in 9% polyacrylamide gels (33). The inserts were cut out of the gels and eluted from them (34). The inserts were then cleaved with *Sau*3A or *Taq* I and labeled with [α-^{32}P]dGTP or [α-^{32}P]dCTP. The labeled fragments were cleaved a second time if necessary with an appropriate enzyme and the separation and elution steps were repeated. The sequences of the isolated fragments were determined as described (34).

Southern Blot Analysis. Restriction digests of high molecular weight DNAs were subjected to electrophoresis in 0.8% agarose

gels, denatured, neutralized, and blotted onto nitrocellulose. The filters were baked and hybridized as described (35).

RESULTS

Isolation of pDRH1 and pDRH2. A total of 76 plasmids containing cDNA from Maja cell mRNA were grown in pools, isolated, bound to diazophenylthioether paper, and hybridized. One filter specifically selected mRNA that stimulated the translation of HLA-DR heavy chain according to molecular weight and serum specificity (Fig. 1). On testing the plasmids from the individual colonies of the positive pool by the same method, pDRH1 was isolated. The insert from pDRH1, ≈900 nucleotides long, was purified, nick translated, and hybridized to blots containing the 76 plasmids. One other plasmid was found to hybridize specifically with pDRH1; the insert of this plasmid, pDRH2, was ≈1300 nucleotides long. In fact, pDRH2 had been detected in the positive-selection assay, but the signal for the HLA-DR heavy chain was barely above background. pDRH1 and pDRH2 were later shown to share sequence identity (see below). Preliminary results suggested that both clones encode HLA-DR heavy chain sequences, and further work was needed to confirm this.

Structures of pDRH1 and pDRH2. Inserts from both clones were excised with *Pst* I restriction endonuclease and separated from the plasmid sequences. Each insert contained one internal *Pst* I cleavage site. As shown in Fig. 2, the larger *Pst* I fragments of each insert appeared to be identical in size, ≈700 nucleotides long, while the small fragment of pDRH2, ≈600 nucleotides long, appeared to be an extension of the smaller 230-nucleotide *Pst* I fragment of pDRH1. Further analysis of the inserts by cutting end-labeled fragments with restriction enzymes that cleave DNA more frequently (those with four nucleotide restriction sites) indicated that pDRH1 contained a subset of pDRH2 sequences. Full details of restriction endonuclease sites will be published elsewhere.

Size and Cellular Distribution of RNA Sequences Matching pDRH1 and pDRH2. RNAs from several different cell lines were analyzed by electrophoresis in denaturing gels, followed by blotting of the size-separated RNAs onto nitrocellulose filters and hybridization of the filters with ^{32}P-labeled pDRH2 and pHLA-A (used as a positive control). The results showed that the mRNAs hybridizing with pDRH2 and pHLA-A differ

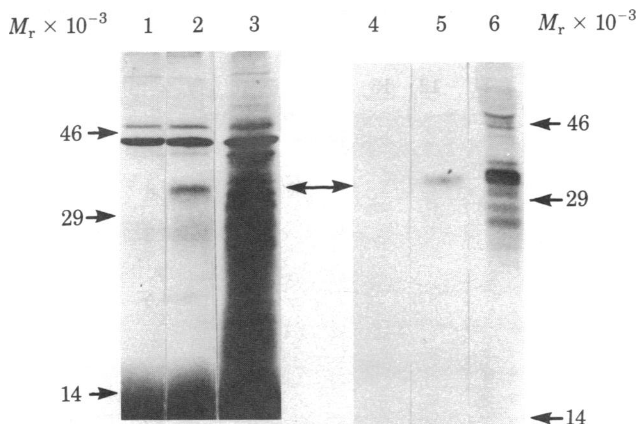

FIG. 1. Translation and immunoprecipitation of products synthesized by mRNA selected by pDRH1. Recombinant plasmids, immobilized on diazophenylthioether paper, were used to select mRNA from total Maja poly(A)$^+$RNA. Translation products and immunoprecipitates were analyzed by electrophoresis in 12.5% NaDodSO$_4$/polyacrylamide gels. Lanes: 1 and 2, translation products of mRNA selected by pAT153 and pDRH1, respectively; 3, total translation products; 4–6, immunoprecipitation of samples 1–3, respectively, with 254 antiserum. Arrows, position of band selected by pDRH1.

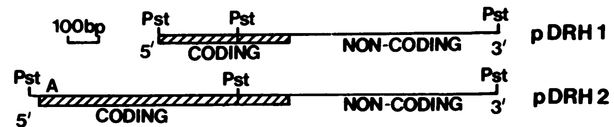

FIG. 2. pDRH1 and pDRH2 inserts. Proposed locations of the coding regions (5′ to 3′) of HLA-DR mRNA complementary to the pDRH1 and pDRH2 inserts are shown. Full sequences of both clones will be given elsewhere. A, location of the sequence in clone pDRH2 sequence that matches protein sequence data. bp, Base pair(s).

slightly in size, being ≈1500 nucleotides and 1700 nucleotides long, respectively (Fig. 3). These sizes are consistent with earlier estimates (1, 11). Fig. 3 also shows that only RNA extracted from the B-cell lines in this experiment hybridized to pDRH2. Fibroblasts and T cells, along with several other human lines tested (data not shown), while they contain similar quantities of HLA-ABC-related mRNA, do not express the putative HLA-DR heavy chain mRNA, as expected. These findings strengthen the conclusion that pDRH1 and pDRH2 are cDNA clones containing HLA-DR heavy chain sequences.

Nucleotide Sequence Encoding NH_2-Terminal Amino Acids of the HLA-DR Heavy Chain. The most definitive evidence that pDRH1 and pDRH2 encode HLA-DR heavy chain sequences is the demonstration that one or both clones include DNA sequences that can be translated into the known NH_2-terminal amino acid sequence of an HLA-DR heavy chain. The region in pDRH$_2$ that was thought to correspond to the 5′ terminus of the mRNA, and hence the NH_2-terminal region of the protein, was therefore chosen for analysis. Because the restriction analysis suggested that pDRH1 sequences were a subset of those of pDRH2 and poly(A) tracts had been located at the common end of the two cDNA inserts, the 600-nucleotide *Pst* I fragment of pDRH2 was the most probable candidate to contain the region encoding the NH_2-terminus of the protein (Fig. 2). The relevant DNA sequence, present in pDRH2 but not in pDRH1, and its translation into amino acids is shown in Fig. 4. This sequence is in complete agreement with the amino acid sequence data so far obtained for the NH_2-terminal region of the HLA-DR heavy chain (ref. 36; C. Kelly, M. Waterfield, and M. Crumpton, personal communication). These data provide the best evidence that pDRH2 and pDRH1, because of its similarity to pDRH2, contain HLA-DR heavy chain sequences.

HLA-DR Heavy Chain Sequences in Genomic DNA and Mapping to Human Chromosome 6. When total human DNA from several sources was digested with restriction enzymes and

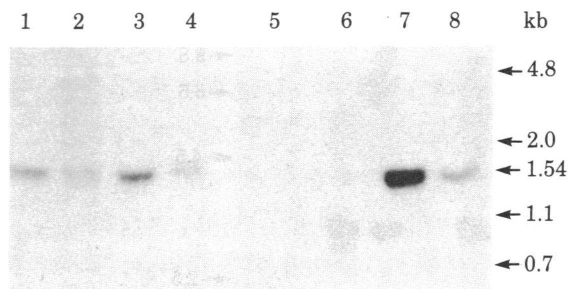

FIG. 3. Expression of HLA-DR mRNA in fibroblast, T-cell, and B-cell lines. Total RNA samples were denatured and subjected to electrophoresis on 2.5% agarose/5 mM methyl mercury hydroxide gels (29). RNAs in the gels were blotted onto nitrocellulose and hybridized with ^{32}P-labeled nick-translated pDRH2 insert (lanes 5–8). A duplicate blot was hybridized with the pHLA-A cDNA clone (lanes 1–4). mRNA samples: HFF (lanes 1 and 5), Molt-4 (lanes 2 and 6), Maja (lanes 3 and 7), RPMI 1788 (lanes 4 and 8). Size markers were rRNA and rRNA cleavage products isolated from an extract of mouse L cells incubated with ppp(A2′p)$_2$A (R. Silverman, personal communication).

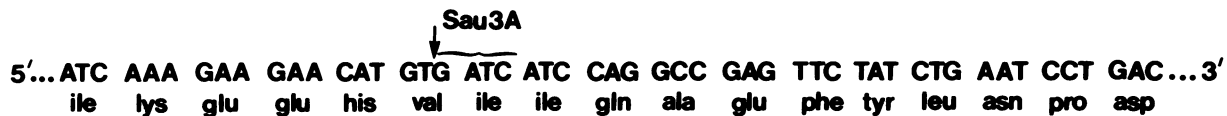

FIG. 4. Comparison of the partial nucleotide sequence and translation into amino acids of the pDRH2 insert with the first (NH_2-terminal) 17 amino acids of HLA-DR heavy chain determined by amino acid sequence analysis (ref. 36; C. Kelly, M. Waterfield, and M. Crumpton, personal communication).

analyzed by agarose gel electrophoresis, Southern transfer, and hybridization with ^{32}P-labeled pDRH1 and pDRH2, relatively simple genomic restriction patterns were observed (Fig. 5, lanes 1, 7, and 11). The simplicity of the digestion patterns, especially with *Eco*RI, indicates that there are few copies of this sequence in the haploid genome. The earlier experiments suggest that pDRH2 contains almost all of the sequences present in the mRNA—i.e., ≈1300 of the estimated 1500 nucleotides for the complete mRNA, which probably includes poly(A) of ≈200 residues at its 3′ end. The data taken together imply a relatively simple structure for the HLA-DR heavy chain genes. Several human DNAs were tested and, so far, no evidence for restriction endonuclease site polymorphism has been found. These results are in marked contrast to those obtained from HLA-ABC or H-2 K and D sequences, which appear to be highly complex when analyzed in a similar way (4, 37, 38).

The evidence so far for the chromosomal location of HLA-DR heavy chain sequences is inconclusive, although indirect evidence favors the view that the HLA-DR heavy chain is encoded in the HLA region (39–41). Attention was focused, therefore, on the issue of whether sequences hybridizing to pDRH1 and pDRH2 are present on chromosome 6. DNAs from human cells, mouse cells, and six different human–mouse somatic cell hybrids, containing single or only a small number of human chromosomes in a mouse background, were analyzed for restriction digest patterns of sequences hybridizing to pDRH1 and pDRH2. Fig. 5 shows that the pDRH1 and pDRH2 hybridizing patterns with human DNA differed significantly from that obtained with a similar digest of mouse DNA, and this difference forms the basis for detection of the human sequences in human–mouse hybrids. In the *Eco*RI digests, for example, both clones detected a band of 3.4 kb in human DNA. In mouse DNA, there was a faint band at 3.1 kb. When DNA from the somatic cell hybrids MCP-6 and G3X-11, which are known to contain part of human chromosome 6 including the HLA region (ref. 16; M. Quintero, personal communication), were analyzed, the human pattern of hybridization with HLA-DR heavy chain sequences was observed (Fig. 5, lanes 3, 4, 9, and 13). In some cases, both human and mouse bands were visible in the hybrids (lane 4). Hybrids containing only the X chromosome, or revertants of MCP-6 that had lost the X/6 translocation chromosome, did not contain the 3.4-kb band (lanes 5, 6, 10, and 14–16). Thus, the human HLA-DR heavy chain sequences are not encoded in the X chromosome and so must be present in that part of chromosome 6 carried on MCP-6 and G3X-11. We have also noted the 3.4-kb human band in somatic cell hybrids containing a normal human chromosome 6 (data not shown). These results, therefore, map an HLA-DR heavy chain to chromosome 6.

DISCUSSION

The possibility that we had identified HLA-DR heavy chain cDNA clones by using positive selection and cell-free translation of mRNA was supported by the limited data on tissue distribution (Fig. 3) and confirmed by comparison of a partial nucleotide sequence with the currently available amino acid sequence data (Fig. 4). Thus, our data show that the positive-selection assay can be used successfully to identify cDNA clones for mRNAs that comprise <0.1% of the total mRNA in the cell. Our earlier estimate of the enrichment for HLA-DR mRNAs in the RNA we used as template for cDNA synthesis was 20- to 50-fold (11). This appears to have been reasonably accurate based on finding HLA-DR heavy chain sequences in 2 of 76 cDNA clones in our bank, if the level of HLA-DR heavy chain mRNA is <0.1%. [Estimates are 0.01–0.05% for HLA-ABC mRNA in total cell mRNA (2).]

The mapping of the HLA-DR heavy chain sequences to chromosome 6 suggests that at least one of the HLA-D/DR heavy chains is encoded in the HLA region. It would be surprising to

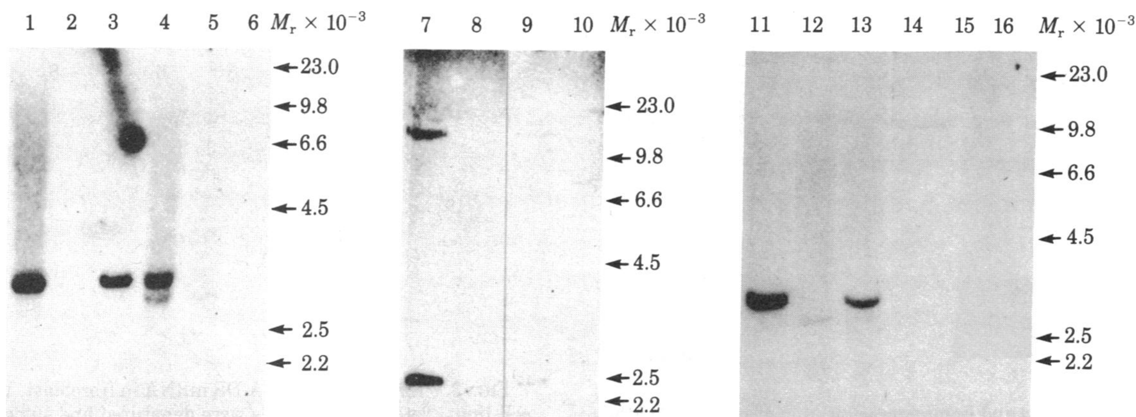

FIG. 5. Hybridization of pDRH1 and pDRH2 to genomic DNA and localization of the sequences on chromosome 6 *Eco*RI (lanes 1–6 and 11–16) or *Hin*dIII (lanes 7–10)-digested DNA. Samples (40 μg per lane) were loaded onto 0.8% agarose gels. Electrophoresis was at 20 V for 36 hr and then the DNA was denatured and transferred to nitrocellulose. The filters were hybridized to nick-translated human DNA fragments from *Pst* I digests of pDRH1 (lanes 1–10) and pDRH2 (lanes 11–16). DNA samples from the following cell lines were used: G3.32.2 (human; lanes 1, 7, and 11), PCC4 (mouse; lanes 2, 8, and 12), MCP-6 (hybrid with human X/6 translocation chromosome; lanes 3, 9, and 13), G3X-11 (hybrid with human X/6 translocation chromosome; lane 4), ThyB-1.33 (hybrid with human X and 21; lane 5), Geoc 4 (hybrid with human X; lanes 6 and 15), Horl 9D2 (hybrid with human X, 11, 15; lanes 10 and 14), MCP-6R8 (revertant of MCP-6; lane 16). Size markers: *Hin*dIII-digested bacteriophage λ DNA.

find the HLA-DR heavy chain mapped to chromosome 6 but not in the HLA region itself. Further work is, however, needed to localize these sequences with respect to the HLA region. The simplicity of the restriction digest pattern detected with these HLA-DR cDNA clones is striking, especially in comparison with the pattern obtained using HLA-ABC- or H-2 K- and D-related clones. The simplest explanation is that there is just one or a few copies of the sequences coding for HLA-DR heavy chains in the genome. Our data do not rule out the possibility of multiple HLA-DR heavy chain genes, with identical restriction patterns, but we find this unlikely based on structures and understanding of other higher eukaryotic genes such as globin and immunoglobulin genes (42, 43).

Several groups have reported recently that there are at least two types of Ia-like or HLA-D/DR-associated antigens with different heavy and light chains on human B cells (7–9). Our data imply that the heavy chains do not share a high degree of homology, because we find no cross-hybridization to a second possible gene under the conditions used. However, we have not yet tested the possibility that there are other Ia-like heavy chain gene(s) with a lower degree of homology, which would be detected under relaxed conditions of hybridization. The limited amino acid sequence and peptide map data available for human and mouse Ia heavy chains indicate that the two sequenced heavy chains are quite distinct (41).

Our initial work with pDRH1 and pDRH2 has already provided answers regarding tissue distribution of HLA-DR antigens, complexity in the genome, and location of the gene in the human genome. Continued analysis of the gene and its adjacent sequences may provide indications of other HLA-D/DR or human Ia-like antigens and other genes that may function as immune response genes. Eventually, we hope to determine the size and relationship of the HLA-D/DR region to the other parts of the major histocompatibility complex.

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