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Genetics

Sequences related to HLA-DRα chain on human chromosome 6: Restriction enzyme polymorphism detected with DCα chain probes

(histocompatibility antigens/SBα chains/gene duplication/DNA sequence homology)

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ABSTRACT **Three sets of cosmid clones—containing the HLA-DRα chain gene and two additional related genes—were isolated from human genomic DNA libraries by using a cDNA probe for the HLA-DRα chain. Southern blot analysis using DNA from somatic cell hybrids indicated that all of the clones mapped to chromosome 6. Partial sequence analysis showed that the two additional related genes were highly homologous to each other, and to the HLA-DRα chain, in parts of the exon that encoded the α2 domain but were more divergent in intron sequences. One of the genes corresponds to the HLA-DR-related DC series. DNA probes made from this gene revealed marked restriction enzyme polymorphism when hybridized to genomic DNA from HLA-DR typed homozygous cell lines. The patterns obtained from a number of homozygous and heterozygous cell lines correlated with the HLA-DR crossreactive serotypes and also indicated that there is a further sequence in the haploid human genome that is closely homologous with the DCα chain sequence. One family was studied and showed the expected HLA-DR-associated inheritance of restriction enzyme patterns. No polymorphism has yet been demonstrated in restriction enzyme fragments that include the other cloned sequence, which may correspond to the SBα chain gene or to a novel HLA-DR-related gene. These experiments indicate that there are at least three sequences in the human genome related, but not identical, to the HLA-DRα chain gene.**

The HLA-D region in man contains genes that are involved in the regulation of the immune response (reviewed in refs. 1 and 2). The known products of this region are composed of dissimilar subunits called α and β chains (M_r approximately 34,000 and 28,000, respectively); the β chains appear to express the majority of the polymorphic determinants. Immunochemical analysis of glycoproteins from HLA-DR-typed cell lines by using monoclonal antibodies has demonstrated at least three different sets of such oligomeric molecules (3, 4). The best characterized are the HLA-DR antigens which are homologous with the mouse I-E products and which correspond to the first serologically defined allelic determinants. Another separate product, DC, initially characterized by sera showing patterns of association with DR1, -2, and -w6, is now clearly defined by certain polymorphic monoclonal antibodies (4, 5). The DCα chain sequence has been shown to have homology with the mouse I-A α chain. DC is thought to be the human equivalent of I-A (6). The extreme NH_2 terminus of the DCα chain shows no detectable homology with the DRα chain but other parts of the sequence are highly homologous with it (6, 7). Another HLA-D region antigen, SB, initially defined by primed lymphocyte typing, is related to the HLA-DRα chain at the NH_2 terminus (8–10).

Recent information from analysis of the protein sequences derived from cDNA clones for α and β chains of HLA-D region antigens has shown that in both cases the protein domains nearest the cell membrane are homologous with the α3 domain of HLA-ABC antigens and to β_2-microglobulin and immunoglobulin domains (11–15). This argues strongly for evolution of the genes for these cell surface molecules from a common ancestral gene by duplication.

We recently isolated cDNA and genomic DNA clones corresponding to the HLA-DRα chain (11, 12). In this paper we show that two additional sets of cosmid clones contain different HLA-DRα-related genes. One of these genes corresponds to a DCα chain, and the other may correspond to the SBα chain or to another novel HLA-DRα-related gene. In addition, our results indicate that a fourth sequence, most closely related to the DCα chain gene, is present in the haploid genome.

MATERIALS AND METHODS

Enzymes and Other Reagents. Restriction enzymes were obtained from Bethesda Research Laboratories, New England Biolabs, and Boehringer Mannheim. Radiochemicals were obtained from Amersham. DNA polymerase (large fragment) was obtained from Boehringer Mannheim.

Cells and Somatic Cell Hybrids. Details of the DR-typed cell lines have been published (4). All of the lines are homozygous for DR except for Daudi. Human–mouse somatic cell hybrids were obtained from P. Goodfellow, E. Solomon, and M. Quintero and are described in detail elsewhere (16). Essentially all of the hybrids used for assigning sequences to chromosome 6 contained a human X/6 translocation chromosome from the cell line G3.32.2 (16). Details of other hybrids and cell lines, where appropriate, are given in the text and the figure legends.

Isolation of DNA. DNA was isolated from tissue culture cells or from blood as described (11).

Southern Blots. Restriction endonuclease digests of high molecular weight DNAs were electrophoresed in 0.7% agarose gels, denatured, neutralized, and blotted onto nitrocellulose. The filters were baked and hybridized as described in the figure legends, according to published procedures, with high specific activity DNA probes made by nick-translation (17).

Isolation of Cosmid Clones from Human DNA Libraries. Two different banks of human genomic DNA clones were probed with the cDNA insert from pDRH2. Both libraries were made from partial *Mbo* I digests of human DNA from a placenta (pre-

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Abbreviation: kb, kilobase(s).

fix JG; DR1,2) and a lung carcinoma (LC) cloned into cosmid vectors (18). Both libraries contained human DNA inserts of about 40 kilobases (kb). Clones were screened at 3× standard saline citrate (NaCl/Cit; 1× is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) containing 0.1% $NaDodSO_4$ at 65°C and the filters were washed with 1× NaCl/Cit/0.1% $NaDodSO_4$ at 65°C.

DNA Sequence Analysis. *Sau*3A or *Sau*3A/*Pst* I digests of fragments of the human DNA inserts in the cosmids were ligated, respectively, into *Sau*3A- or *Bam*HI/*Pst* I- digested bacteriophage M13, MP8 vector. Plaques were then picked, and templates were prepared and subjected to sequence determination by the chain-termination method (19).

RESULTS

Southern Blot Analysis of Human DNA. From our earlier studies it was clear that there were few, if any, DNA sequences closely related to the HLA-DRα chain gene. Under relatively stringent conditions (1× NaCl/Cit at 65°C), the HLA-DRα cDNA probe pDRH2 detected two bands on Southern blots of *Eco*RI-cut human DNA, at 3.4 and 4.5 kb (Fig. 1, lane 3). The 3.4-kb band contained most of the HLA-DRα chain sequences and the 4.5-kb band contained a small part of the 5′ end of the clone, including the signal sequence. Both of these bands were present in somatic cell hybrids containing chromosome 6.

When similar blots were done under less stringent conditions (6× NaCl/Cit, 65°C), further bands were revealed at positions corresponding approximately to 10.0 and 5.0 kb (Fig. 1, lane 7). The sequences in these bands are also encoded on chromosome 6 because they were present in DNA from somatic cell hybrids containing the X/6 translocation chromosome (Fig. 1, lane 9). They were not seen in somatic cell hybrids which included, between them, all of the other human chromosomes (data not shown) and were not present in hybrids containing just the human X chromosome (Fig. 1, lane 10). In *Hin*dIII digests of similar DNA samples, all of the four bands hybridizing to the pDRH2 HLA-DRα chain cDNA probe were also present, as expected, in the X/6-containing hybrid (see below).

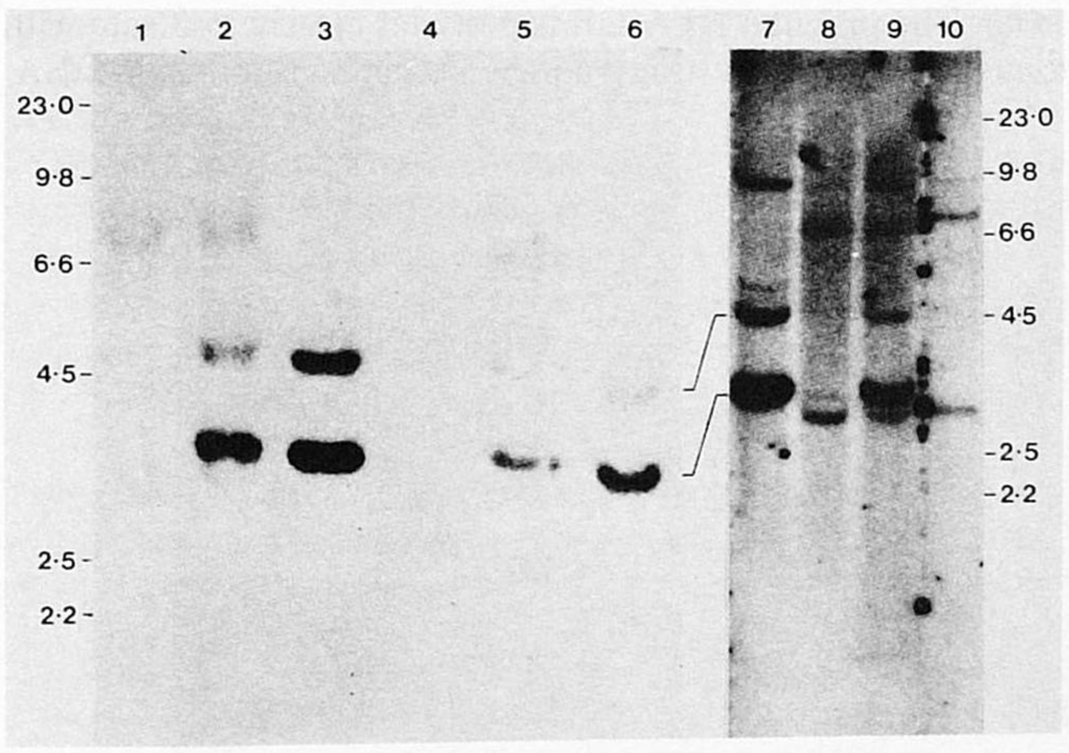

FIG. 1. Southern blots of DNA from human, mouse, and somatic cell hybrids probed with nick-translated pDRH2 insert. *Eco*RI-digested DNA samples (25 μg per lane) were loaded onto 0.7% agarose gels. Electrophoresis was at 20 V for 36 hr; then the DNA was denatured and transferred to nitrocellulose sheets. DNA samples from the following cell lines were used: lane 1, GBS1-R (revertant of GBS1 and so contains only mouse chromosomes); lane 2, GBS1 (hybrid with X/6 translocation); lanes 3 and 7, G3.32.2 (human); lanes 4 and 8, PCC4 (mouse); lane 5, G3X-11 (hybrid with X/6 translocation); lanes 6 and 9, MCP-6 (hybrid with X/6 translocation and no other human chromosomes); lane 10, ThyB1 (hybrid with human X). The conditions were: lanes 1–6, hybridization and washing in 1× NaCl/Cit/0.1% $NaDodSO_4$ at 65°C; lanes 7–10, 6× NaCl/Cit/0.1% $NaDodSO_4$ at 65°C.

Isolation of Cosmid Clones. The above experiments indicated that there are genes on chromosome 6 related to the HLA-DRα chain gene. Of the initial clones isolated from two cosmid libraries of 250,000 clones each, 9 consistently hybridized with the pDRH2 probe on subsequent screening in 3× NaCl/Cit/0.1% $NaDodSO_4$ at 65°C. Only one (JG10ii) contained the strongly hybridizing 3.4-kb *Eco*RI band indicative of the authentic HLA-DRα chain, and it has been described (11). Some of the other clones with different genetic structures were chosen for further analysis (Fig. 2). Clones JG8a and LC11 contained *Eco*RI fragments, hybridizing to the pDRH2 probe, of approximately 10 and 5 kb. These are comparable in size to the bands on Southern blots of human genomic DNA probed with pDRH2 (Fig. 1). Clones LC10 and LC14 contained *Eco*RI fragments, which hybridized to the pDRH2 probe, of about 9 and 1.9 kb, respectively. *Pst* I fragments from LC10 and LC14, hybridizing to the pDRH2 probe, also differed in size (2.5 and 1.3 kb, respectively). These two clones had common fragments and are overlapping, but cosmid LC14 contained an incomplete pDRH2-related sequence which runs into vector sequences (data not shown). Presumably this is the reason why cosmids LC10 and LC14 have different restriction enzyme fragments at this point.

The Cosmid Clones Containing the HLA-DRα-Related Genes Are from Chromosome 6. Nitrocellulose filters containing DNA from human, mouse, and human–mouse somatic cell hybrid cell lines that had been digested with *Eco*RI or *Hin*dIII were probed with fragments of DNA isolated from the cosmid clones as follows. *Pst* I cut cosmid LC14 into pieces ranging in size from about 0.5 to 5.0 kb. DNA corresponding to one band, of about 1.3 kb, that hybridized to the pDRH2 probe was excised and eluted from a preparative agarose gel after electrophoresis. Similarly, *Bam*HI digestion of cosmid JG8a produced a 4.4-kb band that hybridized to both *Pst* fragments A and B of the pDRH2

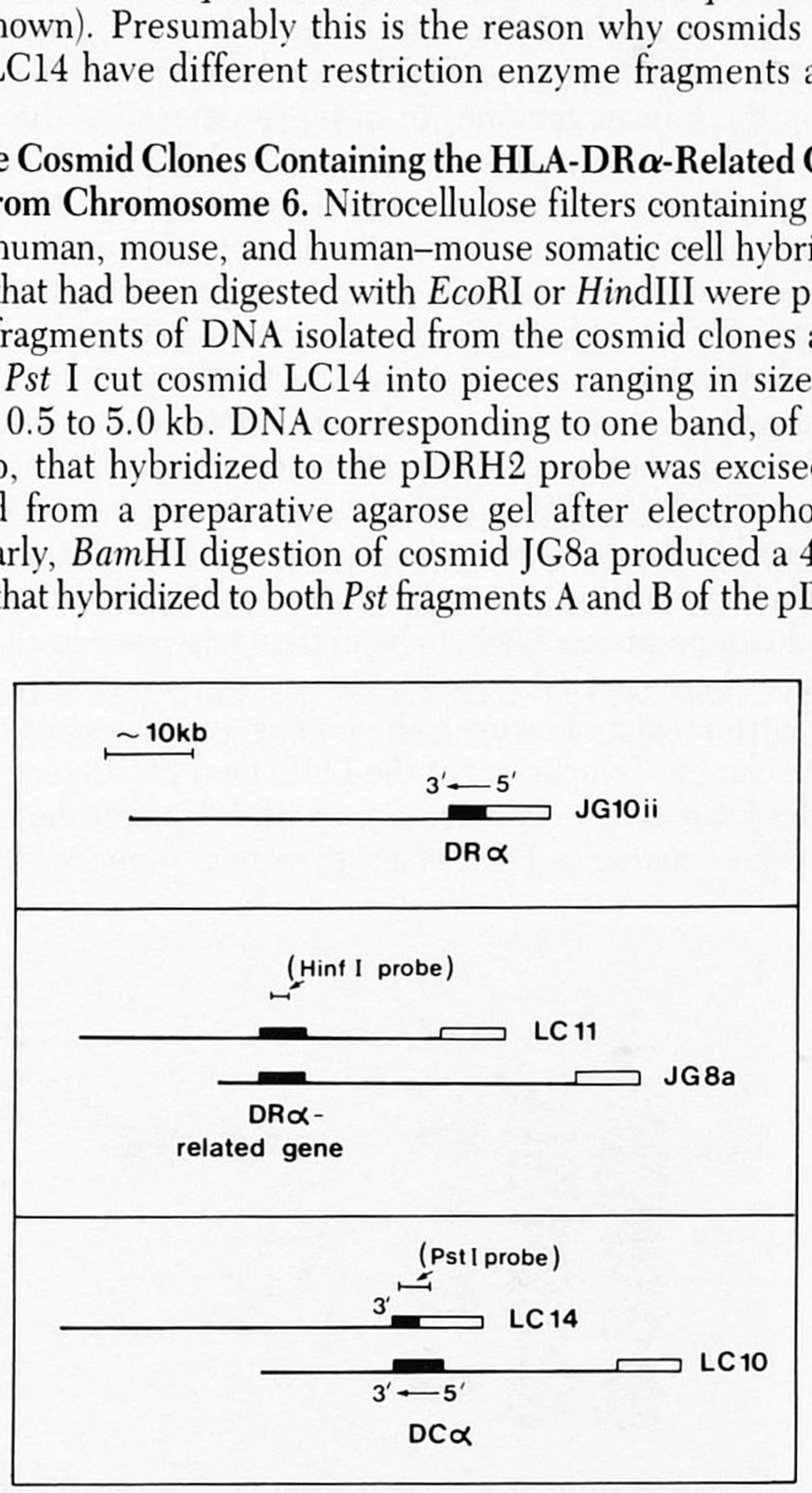

FIG. 2. Provisional maps of the cosmid clones used. Cosmid JG10ii contains most of the DRα chain gene. Its isolation and partial sequence have been described (11). Cosmids JG8a and LC11 are overlapping clones from different DNA libraries. Both contain similar sequences and share restriction enzyme fragments in the area of the DRα-related gene (shown as black boxes). Cosmids LC10 and LC14 are overlapping clones that contain a DCα chain gene (black boxes). The gene is incomplete in cosmid LC14. The 5′-3′ orientation of the genes is indicated, where it is known. The white boxes indicate cosmid vector sequences. The cosmids contain other genes that will be described elsewhere. These provisional maps have been constructed from double digestions with *Bam*HI, *Cla* I, and *Kpn* I. No overlaps have been found so far between cosmids from the three different groups indicated.

probe. This 4.4-kb band was purified and subcloned by ligation into the *Bam*HI site of pAT153 by standard techniques. A smaller probe was obtained by cutting the subcloned 4.4-kb *Bam*HI fragment with *Hin*fI. A 1-kb *Hin*fI band reacted strongly with pDRH2 5′ and 3′ probes, providing a convenient probe for Southern blots as shown in Fig. 3. The JG8a *Hin*fI probe hybridized on human genomic DNA, under stringent conditions, to an *Eco*RI band of ≈10 kb (Fig. 3, lanes 5 and 6) and *Hin*dIII bands of ≈4.0 and ≈4.7 kb (Fig. 3, lane 12), which are similar in size to the bands that were detected with the HLA-DRα cDNA probe (Fig. 1, lane 7; Fig. 3, lane 9). The LC14 *Pst* I probe hybridized to three bands on *Eco*RI-cut human DNA from cell line G3.32.2, all of which were also found in DNA from the somatic cell hybrids with the X/6 translocation (Fig. 3, lanes 15 and 17). A probe made from the LC10 cosmid produced similar bands, confirming that it was related to LC14 and was also on chromosome 6 (data not shown). Thus, all the sequences hybridizing to the two new sets of cosmids shown in Fig. 2 are assigned to chromosome 6 and so presumably to the HLA region.

Sequence Analysis. The results described above indicate that there are at least three gene sequences related to the HLA-DRα chain in the human genome. In order to determine the relatedness of the cloned sequences, *Sau*3A and *Sau*3A/*Pst* I fragments of subclones were ligated into bacteriophage M13 vectors and phages hybridizing with the pDRH2 insert were subjected to sequence analysis. A region in both genomic clones LC14 and JG8a that hybridized well to the pDRH2 probe corresponded to the sequence for the extracellular protein domain nearest to the cell membrane in the HLA-DRα chain—namely, the α2 domain. Sequence data for this region in both clones are shown in Fig. 4. The HLA-DRα-related sequences from clones JG8a and LC14 exhibit considerable homology with the HLA-DRα chain gene throughout the α2 coding region shown. The splice donor points are likely to be in the same place in all three sequences because the sequences are conserved at this point. In the intron region, however, the homology was less than 40% for both clones. Comparison of the LC14 (and LC10) sequences with the DCα chain sequence from a cDNA clone shows that these clones contain a DCα chain gene or a sequence closely related to it (6, 7). This proviso must be added because, as described below, there are two highly related sequences of this class in the human genome and nothing is known about sequences of other DC antigens and its related series, apart from DC1. The gene from clones JG8a and LC11 has not yet been identified but, from the sequence shown in Fig. 4, it clearly is different from the DR and DCα chains.

There Are Two Highly Related Copies of DCα Chain Sequences in the Haploid Human Genome and Both Are Associated with Polymorphism at the DNA Level. In earlier studies, little polymorphism was observed in the restriction enzyme sites in and around the HLA-DRα chain gene (11, 15). It was of interest, therefore, to determine whether the sites around the related genes were polymorphic. DNA from HLA-DR homozygous cell lines was digested to completion with *Pst* I and *Eco*RI, electrophoresed, blotted, and probed with DNA fragments from cosmids JG8a and LC14. With the JG8a probe, no polymorphism was detected when *Eco*RI (or *Pst* I) was used (Fig. 5*A*). In contrast, the *Pst* fragment from LC14 revealed striking polymorphism (Fig. 5 *B* and *C*). Southern blots of DNA from the homozygous cell lines cut with *Eco*RI or *Pst* I always gave two bands when the probes from LC14 and LC10 were used, indicating that there are two sequences corresponding to this gene in the human genome. This finding was confirmed by using short nonoverlapping probes cut from the DCα chain sequence (data not shown). With *Eco*RI, the band pattern corresponded broadly with the HLA-DR serotypes of the cell lines (Fig. 5*B*). Thus, the cell lines examined so far which were HLA-DR1, -2, or -w6 gave bands in similar positions when *Eco*RI was used and it is these three types that are associated with the DC1 variant of the DC series (3–6). Both bands showed polymorphic variation when *Pst* I was used (Fig. 5*C*).

Inheritance of the DC Polymorphism. The Southern blots in Fig. 5 revealed that the patterns with the DCα chain probe were characteristic for different HLA-DR serotypes. A family study was carried out to confirm the expected linkage of the restriction enzyme polymorphism with the associated haplotypes. Fig. 6 shows the results for the one typed family tested so far. The parental HLA-DR haplotypes clearly associate with the corresponding restriction enzyme patterns for their DNA.

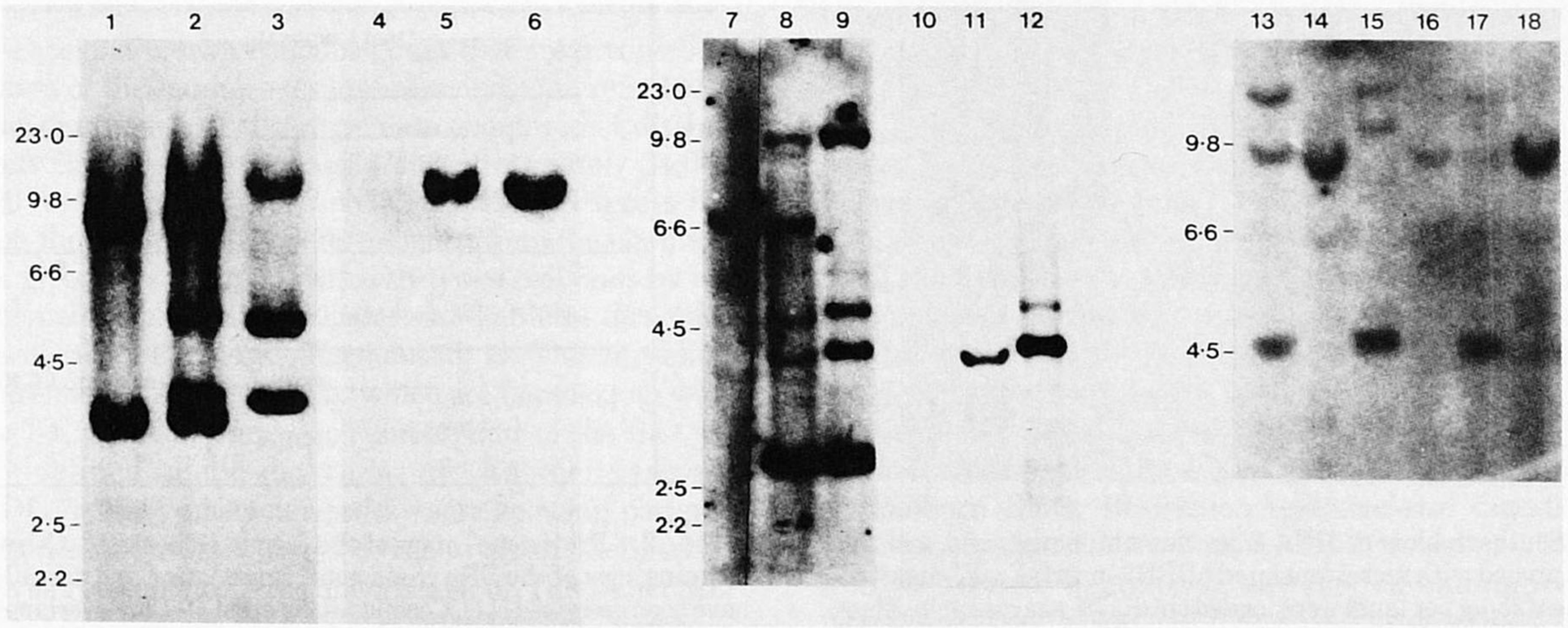

FIG. 3. Southern blots of DNA from human, mouse, and somatic cell hybrids probed with nick-translated *Pst* I DNA fragments from cosmids JG8a and LC14. DNA samples (25 μg per lane) were digested with *Eco*RI (lanes 1–6 and 13–18) or *Hin*dIII (lanes 7–12) and loaded onto 0.7% agarose gels. Electrophoresis was at 20 V for 36 hr; then the DNA was denatured and transferred to nitrocellulose sheets. DNA samples from the following cell lines were used: lanes 1, 4, and 14, GBS1-R (revertant of GBS-1); lanes 2, 5, and 15, GBS1 (hybrid with X/6 translocation); lanes 3, 6, 9, 12, and 13, G3.32.2 (human); lanes 7, 10, and 16, PCC4 (mouse); lanes 8, 11, and 17, MCP-6 (hybrid with X/6 translocation); lane 18, Horl 1 (hybrid without human 6). The probes were as follows: lanes 1–6 and 10–12, a 1-kb *Hin*fI fragment of cosmid JG8a that contained sequences hybridizing to pDRH2; lanes 7–9, pDRH2; lanes 13–18, a 1.9-kb *Pst* I fragment of cosmid LC14 that hybridized to pDRH2 (see text and Fig. 2). Conditions: lanes 1–3 and 7–9, hybridization and washing, 6× NaCl/Cit/0.1% $NaDodSO_4$, 65°C; lanes 4–6, hybridization 1× NaCl/Cit, washing 0.1× NaCl/Cit/0.1% $NaDodSO_4$, 65°C; lanes 10–18, hybridization and washing, 1× NaCl/Cit/0.1% $NaDodSO_4$, 65°C.

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                147                 150                                                 160
        Lys Phe His Tyr Leu Pro Phe Leu Pro Ser Thr Glu Asp Val Tyr
A       AAG TTC CAC TAT CTC CCC TTC CTG CCC TCA ACT GAG GAC GTT TAC

            Ile Ser         Thr                         Ala Asp Glu Ile
B       --- A-- AGT --C --- A-- --- --C --T --T G-- --T --G A-- --T

                        Thr     Val         Ala
C       --- --- --T --C --G A-- --T G-- --- --- G-- --- --- --C --T

                                                170
        Asp Cys Arg Val Glu His Trp Gly Leu Asp Glu Pro Leu Leu Lys
A       GAC TGC AGG GTG GAG CAC TGG GGC TTG GAT GAG CCT CTT CTC AAG

                Lys
B       --- --- -A- --- --- --- --- --- C-- --- --- --- --- --G --A

                                                    Gln
C       --- --- --- --- --- --- --- --- --- --C C-- --G --C --- ---

            178
        His Trp
A       CAC TGG    GGTATGGACCAACACTCAATCTCCTTTATTTCAAGGTTTCCTCCTATGA
B       --- ---    ----A---TG-GTTTCACCC--TT---GA-GCTTTC--GT--GTC-A-T
C       --- ---    ------C-A-TG-TT-TCTCTC-A-AATC-C-TG-CA-C-TCTA-TCC-
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FIG. 4. Sequence around the internal *Pst* site in the HLA-DRα chain gene from cosmid JG10ii (*A*) and the homologous sequences from the genes on cosmids LC14 (*B*) and JG8a (*C*). The top line gives the derived amino acid sequence for part of the α2 domain of HLA-DRα chain (11). The second line shows the nucleotide sequence from which it was derived, continued into the intron sequence (12). – in the nucleotide sequence indicates no change from the HLA-DRα chain sequence. A *Sau*3A fragment of cosmid LC14 was subjected to sequence analysis in the MP8 vector digested with *Bam*HI. *Pst* I/*Sau*3A double-digestion fragments of cosmid JG8a were analyzed from the *Pst* I site in MP8 digested with *Bam*HI and *Pst* I. Arrow, proposed boundary between exon and intron, assumed to be the splice donor point in all three genes.

The *Eco*RI polymorphism shown in Fig. 6*A* appears to involve only one of the two genes; the lower band (≈4 kb) is invariant. The ≈10-kb, ≈8-kb, and ≈5-kb bands segregated with DRw6, DR3, and DR7, respectively. The association of the 10-kb band with DRw6 in this family is consistent with its association with DR1, -2, and -w6 homozygous cell lines, as shown in Fig. 5, emphasizing the marked linkage disequilibrium between restriction site and serological polymorphisms. The *Pst* I polymorphism involved both genes (Fig. 5*C* and Fig. 6*B*). Each haplotype can be associated with two *Pst* I bands that segregate appropriately in the family shown in Fig. 6. An ≈6.5-kb band has been common to all DNAs so far studied, except for some cells typed DR7 (e.g., see Fig. 5*C*, lane 8). The haplotypes in Fig. 6, as derived from the segregation in the children, can be described by two *Pst* I bands thus: father, DRw6 (6.5, 10.0), DR7 (6.0, 2.0); mother, DR3 (6.5, 3.0), DR7 (6.5, 2.0). The maternal DR3 haplotype (6.5, 3.0) and paternal DR7 haplotype (6.0, 2.0) correspond, respectively, to those for the DR3 and DR7 homozygous lines shown in Fig. 5*C*, lanes 4 and 8. Thus, these results also suggest marked linkage disequilibrium between the restriction polymorphism patterns and the DR serotypes. However, more data are needed to establish the basis of the genetic relationship between these DNA markers and the serological specificities.

DISCUSSION

The experiments described in this paper show that there are at least three DNA sequences on human chromosome 6 related to the HLA-DRα chain. Our partial sequence from cosmids LC10 and LC14 matches that for a recently reported DCα chain cDNA clone, pDCH1 (7). That clone was identified by comparison with protein sequence data for the isolated DC1α chain obtained in the same laboratory (6). Absolute confirmation of the identity of the genomic clone must await sequence analysis of the other highly related DCα gene indicated by Southern blots of human DNA (Figs. 5 and 6).

The DRα-related gene on cosmids JG8a and LC11 has not yet been identified. This gene may code for the SB antigen α chain because the SBα chain shows homology with HLA-DRα at the NH_2 terminus (10). The DRα-related sequences in cosmids JG8a and LC11 are highly homologous to the DRα chain in the coding regions for the α1 domain (unpublished data). Alternative explanations are that the sequence belongs to a novel HLA-DRα-related gene or to a pseudogene. However, it is expressed in B-cell mRNA, as detected by RNA blots, as is the DCα chain gene (data not shown). It is of interest to note that

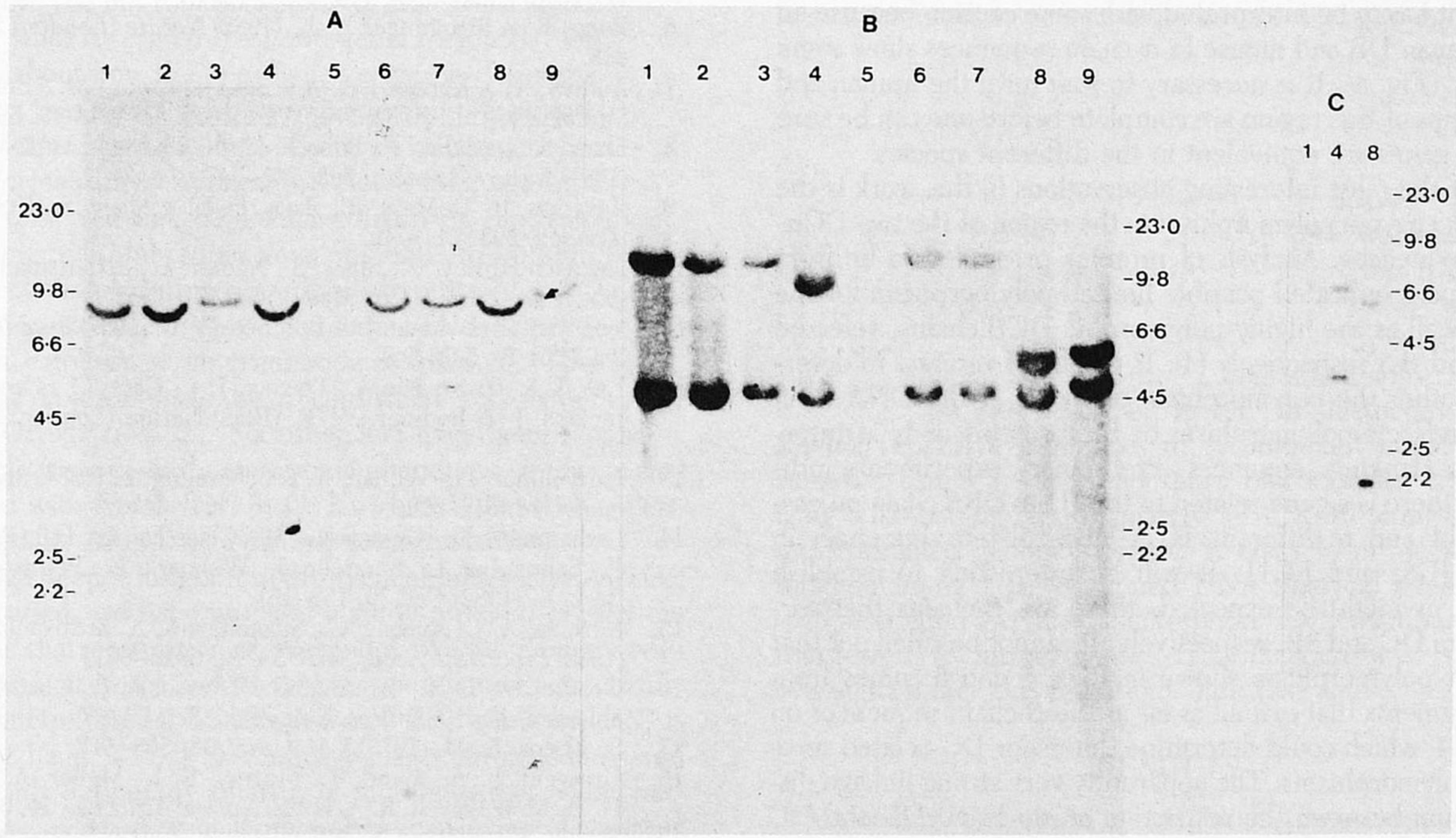

FIG. 5. Southern blot patterns of homozygous DR cell lines (apart from Daudi) probed with the HLA-DRα-related gene fragments. The DNA samples were from the following cell lines: lane 1, Maja (DR1); lane 2, Mette (DR1); lane 3, MST (DR2); lane 4, WT49 (DR3); lane 5, WT51 (DR4); lane 6, Daudi (DRw6); lane 7, WVB (DRw6); lane 8, Mann (DR7); lane 9, WT52 (Dw9). The DNA samples were digested with *Eco*RI (*A* and *B*) or *Pst* I (*C*). Probes: *A*, *Hin*fI fragment from cosmid JG8a; *B* and *C*, *Pst* I fragment from cosmid LC14.

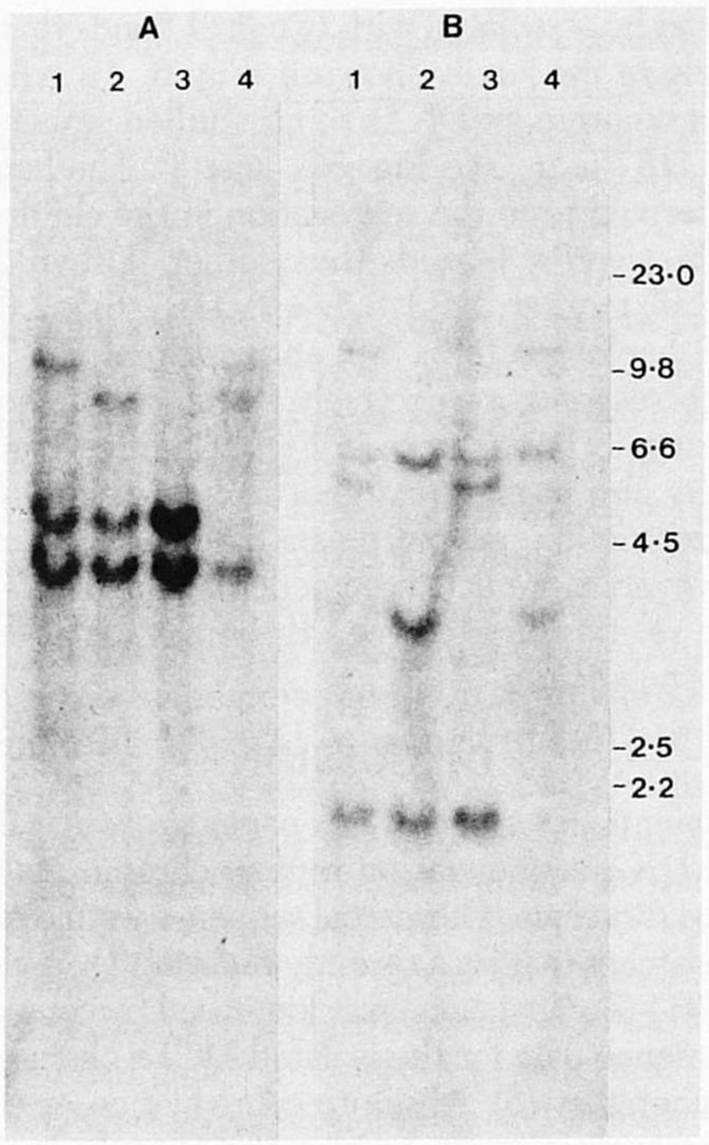

FIG. 6. Southern blots of DNA taken from an HLA-DR-typed family and hybridized with a DCα chain probe. The probe was taken from the *Pst* I fragment of cosmid LC14 as indicated in Fig. 2. (*A*) *Eco*RI digestion; (*B*) *Pst* I digestion. Lanes and HLA-DR phenotypes: 1, father, DRw6,7; 2, mother, DR3,7; 3, son, DR7,7; 4, daughter, DR3,w6. Conditions: hybridization in 6× NaCl/Cit/0.1% $NaDodSO_4$ at 65°C; washing at the same temperature in 0.1× NaCl/Cit/0.1% $NaDodSO_4$.

an equivalent mouse gene has not yet been demonstrated. When filters containing mouse DNA were probed with subcloned fragments of the cosmids, under identical conditions the *Pst* I fragment (DCα) from cosmid LC14 hybridized to mouse DNA but the *Hin*fI probe from cosmid JG8a did not (data not shown). This result has to be interpreted with some caution because all of the human DR and mouse Ia α-chain sequences show some homology (Fig. 4). It is necessary to wait until the human and mouse maps of this region are complete before one can be sure of which genes are equivalent in the different species.

One of the most interesting observations in this work is the restriction enzyme polymorphism in the region of the two DCα-related sequences. Analysis of proteins presumed to be from the DC locus indicated possible limited polymorphism for the DCα as well as the highly polymorphic DCβ chains, referred to as ε and β-3 respectively (4). It will be of interest to determine whether the polymorphism observed at the DNA level directly reflects polymorphism of the proteins or is in intervening or flanking sequences. Preliminary experiments indicate that there is a gene related to the HLA-DRβ chain on cosmid LC14 and a different HLA-DRβ-related sequence on cosmids JG8a and LC11. It will be interesting to establish whether, as might be expected, these are β-chains that correspond to DC and SB, respectively. It cannot be ruled out that the DNA polymorphism shown in Figs. 5 and 6 comes from DNA fragments that extend as far as the β-chain sequences on cosmid 14, which could determine the major DC-related serological polymorphisms. The apparently very strong linkage disequilibrium between the restriction enzyme and DR and DC serological polymorphisms is consistent with the presumed close proximity of the DR and DC loci and the correspondence of the nucleotide sequence of the α-chain genes on clones LC10 and LC14 with that for the DCα chain.

Another four HLA-DRα-related cosmids that we have not yet characterized are related to the cosmids presented in this paper—i.e., they fall into one of the groups shown in Fig. 2, and all are on chromosome 6. This argues that there are not many more copies of sequences related, at this level, to the HLA-DRα chain in the human genome. But, in view of the prevalence of the genetic structures that share features of the α2 domains in these molecules, as discussed here and elsewhere, other related genes could well exist. Out data also indicate that the majority, and perhaps all, of the sequences closely related to the HLA-DRα chain are on chromosome 6 and so most probably in the HLA region. This study identifies potential genes for at least two more molecules that have homologous membrane-proximal protein domains, in addition to HLA-DRα,β chains, HLA-ABCα chains, β_2-microglobulin and immunoglobulin constant domains. It therefore adds weight to the proposal that the HLA region comprises a family of genes that arose by multiple duplication events (20, 21).

Note Added in Proof. Recent experiments indicate that there are two separate DRα-related sequences on cosmids JG8a and LC11, one in the 10-kb *Eco*RI fragment (partial sequence in Fig. 4) and a different gene in the 5-kb *Eco*RI fragment. The two genes are separated by at least 15 kb of DNA.

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