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Immunology

cDNA clone for the heavy chain of the human B cell alloantigen DC1: Strong sequence homology to the HLA-DR heavy chain

(molecular cloning/differential screening/major histocompatibility complex/membrane protein)

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ABSTRACT **A cDNA library has been constructed from a B cell mRNA fraction enriched for HLA-DR sequences, and cDNA clones corresponding to sequences specifically expressed in B lymphocytes have been isolated by a differential screening procedure. Analysis of these clones with probes specific for the HLA-DR heavy chain gene allowed the characterization of HLA-DR heavy chain-related sequences. One clone, pDCH1, was demonstrated to encode the DC1 heavy chain because the amino acid sequence predicted from its nucleotide sequence matches eight out of nine residues available for comparison in the amino-terminal sequence of the DC1 heavy chain. The heavy chain of the DC1 alloantigen is composed of 232 amino acids and can be divided into two external domains, α1 (amino acids 1–87) and α2 (amino acids 88–181), a connecting peptide (amino acids 182–194), a hydrophobic transmembrane region (amino acids 195–217), and an intracytoplasmic region (amino acids 218–232). Comparison with the HLA-DR heavy chain reveals strong sequence homology in the second external Ig-like domain (α2) and the transmembrane region. In contrast, the first external domain, the connecting peptide, and the intracytoplasmic region are less conserved.**

The major histocompatibility complex (MHC) in man contains a number of loci involved in the regulation of the immune response (1). Among these, the HLA-D/DR region expresses polymorphic molecules that are presently identified by using cellular typing reagents or alloantisera and whose expression is mainly restricted to B lymphocytes. The known products of the HLA-DR region are membrane glycoproteins composed of two noncovalently associated polypeptide chains, an invariant heavy or α chain and polymorphic light or β chains of 34,000 and 29,000 daltons, respectively (2). Both chains span the membrane. Because the products encoded by the HLA-DR region stimulate in the mixed lymphocyte reaction, this region is assumed to correspond to the I region of the mouse H-2 complex, where the genes controlling the immune responsiveness to various antigens are located (3). By using functional markers and serologically defined Ia alloantisera, gene products that have sizes similar to the two chains of the HLA-DR antigens have been characterized for the I-A and I-E subregions. Three of these chains (Aα, Aβ, Eβ or Ae) are encoded in the I-A subregion whereas the fourth chain (Eα) is encoded in the I-E subregion (4). Although these studies have not demonstrated significant homology between AαAβ and EαEβ products, the similarities in function, size, and subunit structure suggest that they might be related.

In man, HLA-DR antigens have been characterized in several laboratories (for a review, see ref. 5); they are homologous to the I-E alloantigens. Multiple HLA-DR region loci have been suggested on the basis of serological and functional studies (6–9). The best characterized of the additional HLA-DR region products is the DC1 alloantigen found in linkage disequilibrium with DR1,2 and w6, which has been shown to correspond to a molecule similar but distinct from the HLA-DR antigen (6, 8). Amino-terminal sequence analysis of the DC1 heavy chain revealed homology to the mouse I-Aα chain (10). Recently, cDNA clones for the HLA-DR heavy chain have been isolated after immunoprecipitation of polysomes with a monoclonal antibody (11). Subsequently, the HLA-DR heavy chain gene has been isolated and studied in detail (12). As an approach to the isolation of HLA-DR heavy chain-related sequences, a cDNA library enriched for B cell-specific sequences has been screened by using HLA-DR heavy chain gene probes under relaxed conditions of hybridization. One clone has been shown to encode the DC1 heavy chain and is the subject of the present report.

MATERIALS AND METHODS

Construction of a cDNA Library. Polysomal RNA was prepared from the lymphoblastoid B cell line JY (DR4,w6) as described (13). The RNA was fractionated by oligo(dT)-cellulose chromatography and sucrose gradient centrifugation. The mRNA fractions enriched for HLA-DR sequences were identified by *in vitro* cell-free translation by using the mRNA-dependent rabbit reticulocyte lysate; this was followed by immunoprecipitation by using the heteroserum anti-p34 (13) and analysis of the precipitates by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography. All procedures were performed as described (13, 14). Double-stranded cDNA was synthesized from the size-fractionated mRNA by using reverse transcriptase (obtained from J. Beard, Petersburg, FL) and *Escherichia coli* DNA polymerase I (P-L Biochemicals), treated with S1 nuclease (Boehringer Mannheim), and inserted into the *Pst* I site of the plasmid vector pBR322 as described (15).

Screening of the cDNA Library. Colonies were grown onto nitrocellulose filters. After amplification on plates that contained chloramphenicol, the filters were prepared for hybridization as described (16, 17). Hybridization was performed for 10–16 hr at 65°C in 0.9 M NaCl/0.09 M sodium citrate, pH 7/5× Denhardt's solution/10% dextran sulfate/0.2% $NaDodSO_4$ with radioactively labeled DNA ($2–5 \times 10^5$ cpm per filter); the filters were finally washed four times (30 min each time) at 65°C in 0.3 M NaCl/0.03 M sodium citrate, pH 7.

For differential screening, ^{32}P-labeled cDNAs (5×10^7 cpm/μg) were transcribed from the HLA-DR enriched fraction or from poly(A)-containing RNA extracted from the T cell line Molt 4. The 3.2-kilobase (kb) *Eco*RI fragment containing the HLA-DR heavy chain gene has been isolated from a human DNA library (11). The *Pst* I/*Eco*RI fragments corresponding to the 5′

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Abbreviations: MHC, major histocompatibility complex; kb, kilobase(s); bp, base pair(s).

end (1.4 kb) and 3′ end (1.8 kb) of the gene were isolated from a polyacrylamide gel and labeled by nick-translation (18) to a specific activity of 10^8 cpm/μg.

DNA Sequence Analysis. DNA fragments were labeled at their 5′ ends by using [γ-^{32}P]ATP (3,000 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels; New England Nuclear) and polynucleotide kinase in the exchange reaction or at their 3′ ends by using either 3′-deoxyadenosine 5′-[α-^{32}P]triphosphate (3,000 Ci/mmol; New England Nuclear) and terminal deoxynucleotidyltransferase or α-^{32}P-labeled deoxynucleotides (>3,000 Ci/mmol; Amersham) and the Klenow fragment of DNA polymerase I. The chemical degradation reactions were performed using the method of Maxam and Gilbert (19) by using 60% formic acid for the A+G reaction. The reaction products were analyzed on 20%, 8%, and 6% thin polyacrylamide/urea gels (20). Nucleotide sequence comparison was performed on a BBNC-70 computer by using a homology matrix program with high filtering capacity written by J. Pustell (personal communication).

RESULTS

Isolation of B Cell-Specific Sequences Related to the HLA-DR Heavy Chain. HLA-DR sequences have been shown to represent ≈0.02% of the total mRNA in lymphoblastoid B cell lines (13). To enrich for such sequences, JY poly(A)-containing RNA was fractionated by sucrose gradient centrifugation. When the mRNA fractions were analyzed by cell-free translation followed by immunoprecipitation and polyacrylamide gel electrophoresis, HLA-DR and HLA-A, -B, -C mRNAs were found in different fractions and the purification factor was estimated to be ≈10-fold (data not shown). A cDNA library of 6,000 clones was constructed from the HLA-DR enriched fraction. To select sequences specifically expressed in B cells, as is the case for HLA-DR chains, 4,500 clones were analyzed by a differential screening procedure. Replicas of the same filters were hybridized with ^{32}P-labeled cDNAs transcribed from the HLA-DR mRNA-enriched fraction or from mRNA extracted from the T cell line Molt 4, which does not express HLA-DR antigens (21). The colonies hybridizing only to the B cell-specific cDNA were grouped in a sublibrary of 420 clones. Both the whole library and the B cell-specific clones were further screened with specific probes corresponding to the 5′ and 3′ ends of the HLA-DR heavy chain gene. The combination of the two screening procedures led to the isolation of two categories of sequences specifically expressed in B cells and related to the HLA-DR heavy chain gene (Fig. 1). An example of the first category is pDRH-3 which hybridizes strongly to both the 5′ end and the 3′ end probes. pDRH-3 was shown by nucleotide sequence analysis (12) to encode amino acids 121–193 of the HLA-DR heavy chain. Clones falling in the second category hybridized more efficiently to the 3′ end probe than to the 5′ end probe. In fact, the hybridization with the 5′ end probe was barely above background and a longer time of exposure was required to detect it (e.g., pDCH1; Fig. 1).

pDCH1 Encodes the Heavy Chain of the B Cell Alloantigen DC1. The nucleotide sequence of the pDCH1 insert [780 base pairs (bp)] was determined by using the procedure of Maxam and Gilbert with the strategy presented in Fig. 2. The nucleotide sequence is presented in Fig. 3. The sequence derived from the reconstructed *Pst* I site bordering the insert on the right side that contains a track of five A residues preceded 13 nucleotides upstream by the sequence A-A-T-A-A-A, indicating that this part of the cDNA insert corresponds to the 3′ end of the mRNA and the beginning of the poly(A) (22). The sequence obtained from the *Pst* I site on the left side contains an open reading frame of 216 codons before a termination codon.

Recently, the DC1 antigen has been purified from the B cell line LB (DRw6,6) by using the matrix-bound monoclonal antibody genox 3.53. Amino-terminal sequence analysis of the heavy chain allowed the determination of 25 of the first 28 residues. These experiments demonstrated that the DC1 heavy chain is the human equivalent of the I-A α chain and has no homology in the amino-terminal part with the HLA-DR heavy chain (10). The amino acid sequence predicted from the nucleotide sequence of pDCH1 was compared to the amino-terminal sequence of the DC1 heavy chain. As shown in Fig. 3, when the two sequences are aligned by matching the first codon of the pDCH1 insert with amino acid 17 of the DC1 heavy chain, 8 of 9 residues available for comparison are identical. Therefore, pDCH1 corresponds to an almost full-length cDNA transcript of the DC1 heavy chain mRNA.

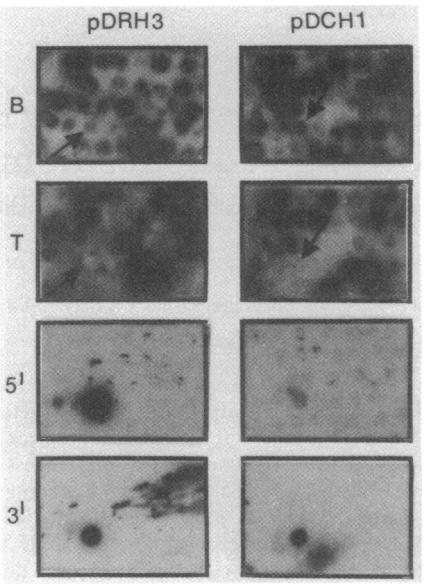

FIG. 1. Isolation of B cell-specific sequences related to the HLA-DR heavy chain gene. *In situ* colony hybridization was performed with ^{32}P-labeled cDNA transcribed from the HLA-DR enriched mRNA fraction (B), poly(A)-containing RNA from the T cell line Molt 4 (T), *Eco*RI/*Pst* I nick-translated DNA fragments from the HLA-DR heavy chain gene (11) containing the α1 exon and part of the α2 exon (5′) and the remaining part of the α2 exon, the transmembrane exon, and the 3′ untranslated region exon (3′). The positions of pDRH3 and pDCH1 colonies are indicated by arrows. Autoradiography with an intensifying screen was for 16 hr (B, T, 5′) or 4 hr (3′).

The DC1 and HLA-DR Heavy Chains Share Common Structural Features and Homologous Regions. The complete structure of the HLA-DR heavy chain gene has been recently

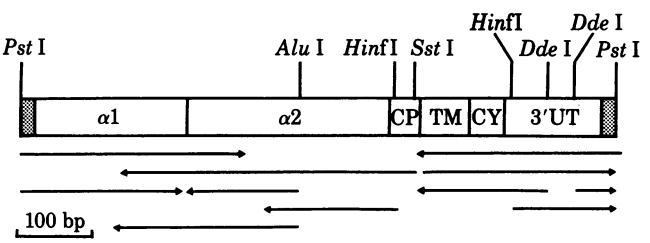

FIG. 2. Strategy of nucleotide sequence analysis of the pDCH1 insert. The restriction sites used for labeling DNA fragments are indicated. Arrows indicate the extent of reading for each fragment. α1 and α2, Regions coding for the first and second extracellular domains, respectively; CP, connecting peptide; TM, transmembrane region; CY, intracytoplasmic region; 3′UT, 3′ untranslated region of the mRNA. The stippled boxes represent the oligo(dC)·oligo(dG) tails introduced during the cloning procedure next to the reconstructed *Pst* I sites on each side of the insert.

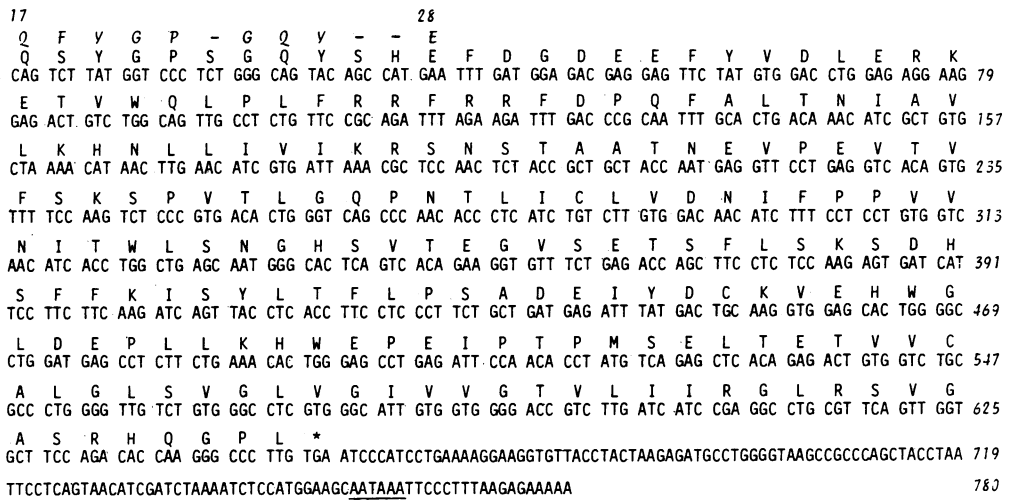

FIG. 3. Nucleotide sequence of the pDCH1 insert. The nucleotide sequence of the coding strand is shown with the translation of the coding region with the single-letter code for amino acids. The termination codon is indicated by a star. The sequence A-A-T-A-A-A in the 3′ untranslated region is underlined. The amino acid sequence on the top of the first line is taken from ref. 10.

determined, showing that the polypeptide chain contains 229 amino acid residues (12). The DC1 heavy chain contains 232 amino acids and is therefore three residues larger than the HLA-DR heavy chain. Alignment of the two protein sequences was performed by matching the first amino acid of the HLA-DR heavy chain with the third amino acid of the DC1 heavy chain and introducing a single gap at position eight in the HLA-DR heavy chain sequence (Fig. 4). A good alignment is obtained without introducing other gaps, pointing out that the two chains are highly homologous. By analogy to the domain structure of the HLA-DR heavy chain that was inferred from the intron/exon structure of the corresponding gene, the DC1 heavy chain is composed of two extracellular domains, α1 (amino acids 1–87) and α2 (amino acids 88–181), connected by a short peptide (amino acids 182–194) to a 23-amino acid hydrophobic transmembrane region (amino acids 195–217) and ending with a 15-amino acid intracytoplasmic region (amino acids 218–232). In both chains, the α2 domain contains a disulfide loop of 55 amino acids homologous to that of Ig domains (see below).

Biochemical studies demonstrated that the HLA-DR and DC1 heavy chains both contain one high mannose oligosaccharide moiety and one complex N-linked oligosaccharide moiety (unpublished results). Correspondingly, two glycosylation sites are found at Asn-81 (Asn-Ser-Thr) and Asn-121 (Asn-Ile-Thr) in the DC1 heavy chain which are located at the same place as those found in the HLA-DR heavy chain (Fig. 4).

Homology in each domain of the two heavy chains was measured as percentage of identical amino acids. The results presented in Fig. 4 and Table 1 indicate that the different domains present quite different levels of homology. Although the α1

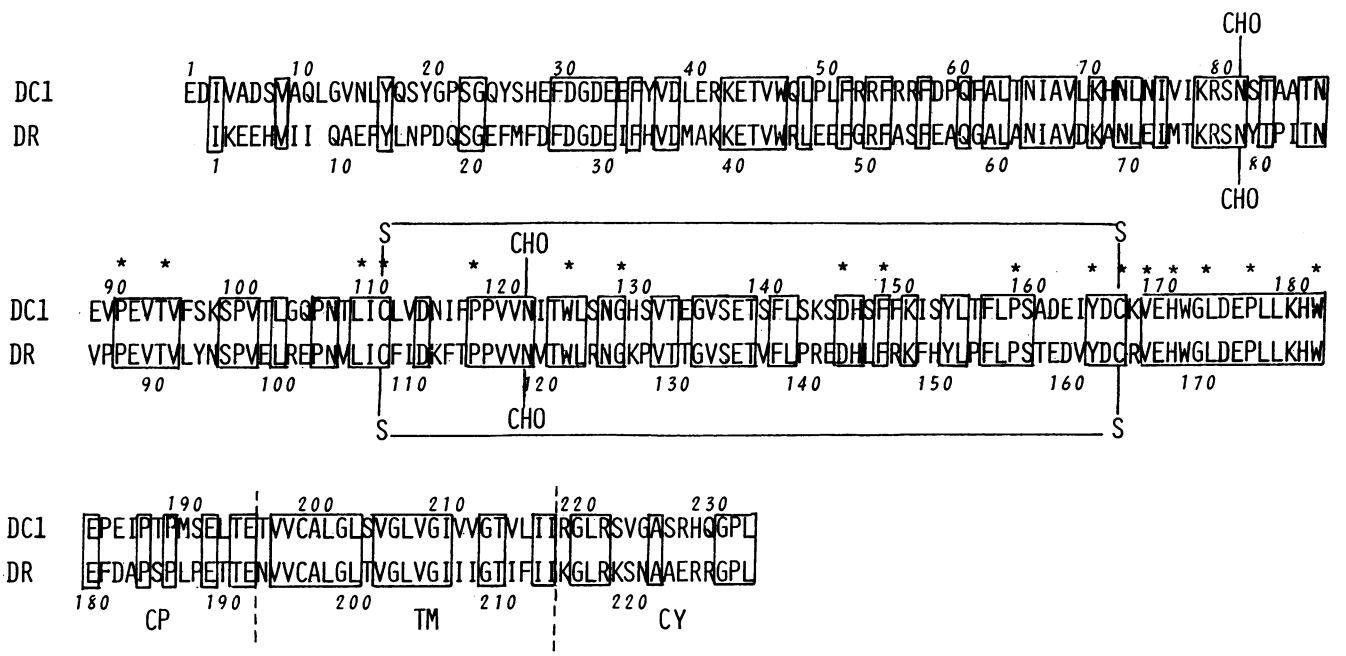

FIG. 4. Comparison of DC1 and HLA-DR heavy chain sequences. The amino acid sequence and the domain structure of the HLA-DR heavy chain are derived from ref. 11 except for the first three amino acids which are taken from ref. 2. Amino acids 1–16 of the DC1 heavy chain are taken from ref. 10. Identical residues are boxed. The limit of the transmembrane region is indicated by dashed lines. Stars indicate positions in the α2 domain conserved among Ig-like domains (see text). CP, TM, and CY, see legend to Fig. 2.

domains have only 47.1% homology, it is worth noting that only 5 of the first 28 amino acids are identical. Homology in the $\alpha 2$ domain is scattered all along the sequence with the last 14 residues being identical. The limit of the $\alpha 2$ domain has been assigned according to the exon/intron structure of the HLA-DR heavy chain gene in which the connecting peptide, the transmembrane region, and the intracytoplasmic region are all encoded in one exon (12). However, it appears that those three segments are not conserved to the same extent. Therefore, the homology was scored independently for the three regions (Table 1), showing that the transmembrane region is much more conserved than the other two regions. The intracytoplasmic region shows common features with similar regions of other transmembrane molecules—namely, the presence of multiple positively charged amino acid residues (three arginines) and a possible phosphorylation site (Arg-Ser at positions 221–222).

Homology of the $\alpha 2$ Domain with Ig-Like Domains. Scores obtained by statistical alignment have pointed out that the $\alpha 2$ domain of the HLA-DR heavy chain has significant homology to Ig constant region domains (12). Because this domain is highly conserved in the DC1 heavy chain, we further investigated its relationship to other Ig-like domains, such as β_2-microglobulin (23), the $\alpha 3$ domain of HLA-B7 (24), the $\beta 2$ domain of HLA-DR light chain (25), and the C_H3 domain of the Ig $\gamma 1$ heavy chain (26). At 17 positions, at least four of the five sequences compared have identical residues to that found in the DC1 $\alpha 2$ domain (Fig. 4), including the two cysteines and several hydrophobic amino acids found at alternate positions in the β-pleated strands of a typical Ig domain (27).

Nucleotide Sequence Homology: No Conservation Is Found in the 3′ Untranslated Region. The comparison between the DC1 and HLA-DR heavy chain was extended to their nucleotide sequence by using a homology matrix program that scores the homology over an adjustable length and prints a score for each position as a letter indicating the homology. The flexibility of this program permits the comparison of distantly related sequences with a high filtering of noise (J. Pustell, personal communication). The comparison of the DC1 and HLA-DR heavy chain nucleotide sequences by using this program is presented in Fig. 5. It illustrates the close relationship of the two coding sequences from positions 29 to 221. It also shows that the homology decreases dramatically in the intracytoplasmic region. Even when lower scores were printed, no homology was found in the 3′ untranslated regions (data not shown).

DISCUSSION

Different approaches have proved to be useful in the isolation of MHC encoded sequences, including hybrid selection of mRNA (28–32), use of synthetic oligonucleotides (33, 34), and immunopurification of polysomes (11). Here we have taken advantage of the fact that HLA-DR sequences are expressed in

Table 1. Homologies between HLA-DR and DC1 heavy chain domains

	Amino acid position		Homology	
	DC1	HLA-DR	Number of identical positions	%
First domain ($\alpha 1$)	1–87	1–84	41/87	47.1
Second domain ($\alpha 2$)	88–181	85–178	61/94	64.9
Connecting peptide (CP)	182–194	179–191	6/13	46.1
Transmembrane region (TM)	195–217	192–214	17/23	73.9
Intracytoplasmic region (CY)	218–232	215–229	7/15	46.6
Complete sequence	1–232	1–229	132/232	57.3

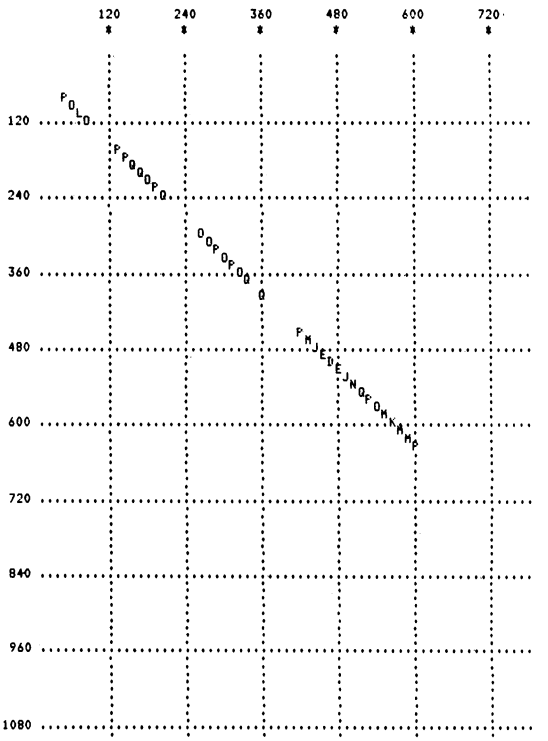

FIG. 5. Comparison of DC1 and HLA-DR heavy chain nucleotide sequences. The nucleotide sequence of the pDCH1 insert is plotted against the nucleotide sequence corresponding to the coding and 3′ untranslated regions of the HLA-DR heavy chain mRNA obtained from the HLA-DR heavy chain gene (11) by using a homology matrix program (J. Pustell, personal communication). Homology is measured over a 41-nucleotide string. A letter is assigned to each 2% range of homologies—i.e., A = 100% and 99%; B = 98% and 97%; . . . P = 70% and 69%; Q = 68% and 67% (minimal value plotted). The matrix has been compressed 12 times for presentation, and the highest value of each group of 12 positions is printed. The termination codons are located at nucleotide 651 in the sequence of pDCH1 and at nucleotide 679 in the HLA-DR heavy chain sequence.

B lymphocytes but not in nonactivated T lymphocytes to perform a differential screening of a cDNA library. The cDNA library was constructed from a B-lymphoblastoid cell line mRNA fraction of 12–15S encoding polypeptides in the 20,000- to 40,000-dalton range. The use of the differential screening procedure showed that the cDNA clones isolated by using specific probes for the HLA-DR heavy chain gene had their expression restricted to the B cell. In fact, the same cDNA clones were isolated independently from the whole library and the sublibrary.

The identification of pDCH1 as encoding the DC1 heavy chain relied on the comparison of the predicted amino acid sequence with the amino-terminal sequence of the DC1 heavy chain. The overlap of the two sequences corresponds to 12 residues, 9 of which have been determined for the DC1 heavy chain. Only one difference was found (Ser/Phe at position 18) which is compatible with a single nucleotide change. It is worth noting that the DC1 heavy chain sequence was derived from material purified from the LB cell line (DRw6,6), whereas the cDNA library was constructed from JY (DR4,w6) mRNA. Therefore, the difference detected could correspond to an allelic or individual variation. The three positions not determined in the amino acid sequence correspond to two serine residues and one histidine residue in the cDNA sequence. The methodology employed for amino acid sequence analysis did not allow the discrimination between serine, threonine, arginine, and histidine residues at these positions which could therefore represent matches. The determination of the complete sequence

of the DC1 heavy chain was obtained by combining the amino-terminal sequence obtained from the protein and the amino acid sequence predicted from the pDCH1 insert for the remaining part of the molecule. The analysis of the sequence demonstrates that the DC1 heavy chain has the same general domain organization as the HLA-DR heavy chain.

The $\alpha 2$ domains of the DC1 and HLA-DR heavy chains are highly homologous not only to each other but also to Ig constant region domains as well as to other HLA molecules containing Ig-like domains, such as the $\alpha 3$ domain of HLA-A, -B, -C antigens and β_2-microglobulin. A similar homology has been described by Larhammar *et al.* (35) for the second extracellular domain of a HLA-DR-like light chain. Thus, it is possible that the second extracellular domains of the heavy and light chains of an HLA-DR antigen fold and interact in a manner similar to that of Ig domains.

Because no detectable polymorphism has been detected for the HLA-DR and DC1 heavy chains, it was not demonstrated that they are encoded in the HLA region. Recently, the availability of HLA-DR heavy chain probes led to the demonstration that the HLA-DR heavy chain gene is present on chromosome 6 where the HLA region is located (31). The close relationship existing between the HLA-DR and DC1 heavy chain sequences favors the idea that the DC1 heavy chain gene is also encoded in the HLA region. Because the amino-terminal sequence of the DC1 heavy chain is homologous to that of the I-A α chain, the DC1 antigen appears to be the human equivalent of the murine I-A antigen. Therefore pDCH1 will be useful in the identification of a new subregion in the HLA-DR region.

The transmembrane region appears to be highly conserved in HLA-DR and DC1 heavy chains. Out of 23 residues, the 6 differences found are neutral in nature and maintain the highly hydrophobic character of this segment. The high degree of homology in this region suggests a requirement for some quaternary interaction with other membrane proteins. In contrast, both the connecting peptide and the intracytoplasmic region are much less conserved—an unexpected feature because the three segments are encoded in the same exon in the HLA-DR heavy chain gene. Moreover, the 3′ noncoding region of the DC1 heavy chain mRNA is only 123 nucleotides long compared with 407 nucleotides for the HLA-DR heavy chain mRNA, and no homology was found between the two sequences in this region. This suggests that the DC1 heavy chain gene could have in this region an intron/exon organization different from that of the HLA-DR heavy chain gene, as is the case for the class I heavy chain genes in mouse and man (36, 37). The determination of the structure of the DC1 heavy chain gene will allow a test of this hypothesis.

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