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Immunology

DNA sequence and characterization of human class II major histocompatibility complex β chains from the *DR1* haplotype

(allelic sequencing/Ia antigens/recombinant DNA/Southern blotting)

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ABSTRACT Two HLA class II β-chain clones from a cell line homozygous for the *DR1* haplotype have been characterized and sequenced. They represent a DRβ chain (2918.4) and a DQβ chain (2918.8). Clone 2918.4 has been used to select mRNA from a lymphoblastoid cell line, and this was injected into *Xenopus* oocytes with mRNA selected with a DRα chain. The translation products were immunoprecipitated with a β-chain-specific monoclonal antibody and electrophoresed on two-dimensional gels. This revealed positive signals in the positions predicted for β and α chains. Sequence comparisons of 2918.4 with previously published DRβ-chain sequences confirm the presence of two regions of variability in the membrane distal domain. Analysis of the sequence of 2918.8 identified it as a DQβ chain identical to one previously published from a DR3,w6 cell line. We speculate, therefore, that the DQβ sequence represents the DQ1 specificity shared by the *DR1* and *DRw6* haplotypes.

The class II region of the human major histocompatibility complex encodes heavy (32 kDa) α- and light (28 kDa) β-chain genes from the DP, DQ, and DR subregions. The association of an α and a β chain as a heterodimer on the surface of B cells, antigen-presenting cells, or activated T cells results in the formation of a molecule referred to as an Ia antigen. These molecules are thought to be responsible, in part, for the genetic control of immune responsiveness and the presentation of antigen for the activation of helper T cells (1). Extensive polymorphism between these class II antigens has been previously defined by the mixed lymphocyte reaction, serological analysis, and two-dimensional gel electrophoresis. Recently, molecular cloning of class II genes has permitted close examination of the organization of class II region and the nature of the polymorphisms at individual loci. Three gene clusters corresponding to the DP, DQ, and DR subregions have been defined, each containing several β chains and at least one α chain. There exists a substantial degree of sequence homology among α and β chains, respectively, of the three subregions (2).

The complexity of the organization of this region and the sizeable amount of sequence homology between loci has made correlation of nucleic acid sequences with serological specificities difficult. This has been further complicated by the use of libraries made from heterozygous cell lines. We have therefore made a cDNA library from a *DR1* homozygous consanguineous line to study a DR and a DQ β-chain sequence from that haplotype.

Final characterization of putative α-chain clones obtained from our library depended on nucleic acid sequencing. Initially, however, an attempt was made to use the techniques of immunoprecipitation and two-dimensional gel electrophoresis to identify translation products of mRNA hybrids selected by the cDNA clones. This approach permitted comparison of already established two-dimensional gel patterns of class II antigens to those obtained from the translated products of the cloned genes.

We therefore report the characterization of two human class II β-chain cDNA clones that can be attributed definitively to the *DR1* haplotype.

MATERIALS AND METHODS

Construction and Screening of the cDNA Library. Poly $(A)^+$ mRNA was extracted from the *DR1* homozygous cell line LG2. This line is homozygous by consanguinity (3). cDNA was ligated into the *Eco*RI restriction site in the phage vector λgt10 by using the method of homopolymer tailing (unpublished results). Library screening was done using conventional techniques (4) with a β-chain-specific 18-base-pair oligonucleotide as ^{32}P-labeled probe (see *Results and Discussion*).

Characterization of Positive Clones. Clones hybridizing to the β-chain-specific oligonucleotide were used as probes in RNA blot and Southern blot analyses. These were performed by established techniques (5). Southern blotting studies were done on DNA from HLA deletion mutant cell lines (6) to help localize the genomic sequence complementary to the cloned cDNA hybridization probes. DNA from consanguineous homozygous typing lines was also probed on Southern blots with the cDNA clones as probes.

Southern blots were hybridized at 42°C in 50% formamide/0.2% $NaDodSO_4$/1%× Denhardt's solution/4 μg of salmon sperm DNA per ml/3× NaCl/Cit, and washed at 55°C, or at 65°C in 0.1× NaCl/Cit/0.1% $NaDodSO_4$ for 1 hr (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone; 1× NaCl/Cit = 0.15 M NaCl/0.015, M Na citrate).

One of the positive clones (2918.4) was used to select mRNA from the human lymphoblastoid cell line CA for studies of its translation products. The mRNA was injected into *Xenopus laevis* oocytes in the presence of [^{35}S]methionine along with mRNA hybrid selected from the same cell line with a clone for the DRα chain (7). Immunoprecipitation of the translation products was performed by using a monoclonal antibody HB10A specific for β chains of class II molecules (E. Clark, personal communication). These immunoprecipitates were then run on nonequilibrium gradient two-dimensional gels as described. Lysates of the cell line CA immunoprecipitated with HB10A, and oocytes injected with the hybrid-selected mRNA and immunoprecipitated with a non-HLA antibody were used as controls. *In vitro* translation using rabbit reticulocyte lysates and immunoprecipitation with B10A indicated that mRNA selected with the DRα clone

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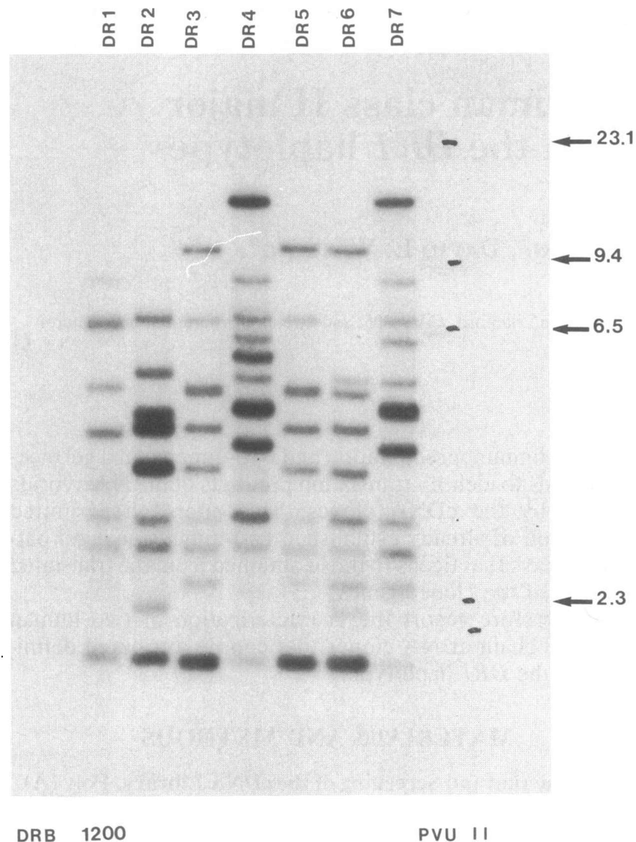

FIG. 1. Autoradiogram of a genomic Southern blot showing the band pattern obtained when probed with the DRβ probe 2918.4. Ten micrograms of DNA from homozygous cell lines representing DR types 1–7 was digested with the restriction enzyme *Pvu* II. The DNA was then run on a 0.8% agarose gel and Southern blotted onto Genetran paper (Plasco). The filter was then probed with 5×10^6 cpm of purified 2918.4 insert labeled with [^{32}P]dCTP by nick-translation.

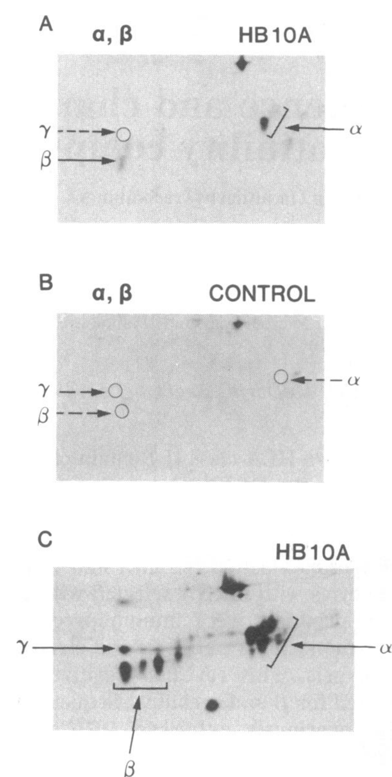

FIG. 2. Autoradiogram of a two-dimensional gel analysis of immunoprecipitations from *Xenopus* oocytes injected with hybrid-selected mRNA. Immunoprecipitations were done by conventional techniques. (*A*) Two-dimensional gel pattern of translation products from oocytes injected with mRNA hybrid selected with 2918.4 and a DRα clone and precipitated with the β-chain-specific monoclonal HB10A. The putative α- and β-chain translation products are indicated by solid arrows. (*B*) Two-dimensional gel analysis of immunoprecipitations of *Xenopus* oocytes injected with DRβ- and DRα-selected mRNA and precipitated with a non-HLA related antibody. Circles indicate where the α and β spots would be found. They are clearly absent in this autoradiogram. (*C*) Example of a two-dimensional gel of an immunoprecipitation with HB10A of the lymphoid cell line CA. The α- and β-chain spots are clearly indicated, as is the spot representing the invariant chain.

alone did not result in β-chain spots on the two-dimensional gels.

Final characterization of the positive clones was obtained by nucleic acid sequencing. Both the chemical degradation procedure of Maxam and Gilbert (8) and the dideoxy-chain termination procedure of Sanger and Cousen (9), modified for use with ^{35}S-labeled dATP were used.

RESULTS AND DISCUSSION

Oligonucleotide Preparation. The 18-mer oligonucleotide chosen to screen the cDNA library had the sequence C-C-C-T-G-T-C-T-C-G-C-G-C-A-C-G-C-A. The selection of this particular sequence has been described in detail elsewhere (10). In brief, available class II β-chain sequences were examined for conserved residues. Three amino-terminal sequences were studied—two protein sequences (11, 12) and one nucleic acid sequence (13). These all shared the same five amino acid residues (Gly-Thr-Glu-Arg-Val-Arg) at positions 20–25. This was the only such conserved sequence in the first 30 residues and, hence, the 18-mer oligonucleotide sequence selected was the complement of the nucleic acid sequence for those residues. When used as a probe in RNA blot experiments, this oligonucleotide hybridized to mRNA derived from B-cell lines but not from a T-cell line.

Characterization of Positive Clones as Distinct HLA Class II β Chains. Initial characterization of positive clones enabled subdivision based on cross-hybridization patterns. Clones were divided into two groups, each cross-hybridizing strongly with other members of its group and weakly with members of the alternate group. One candidate clone was selected from each group for further study. These two clones were 2918.4 and 2918.8.

The two candidate cDNA inserts were subcloned into pBR328 and were used to probe RNA blots of mRNA from human cell lines. Both hybridized to a single band of 1.3 kilobases in mRNA from various B-cell lines but not mRNA from the T-cell line Molt-4 (data not shown). In addition, the clones were used to probe Southern blots of DNA from HLA deletion mutants and from normal human cell lines. Both 2918.4 and 2918.8 had patterns on the deletion mutant Southern blots consistent with their mapping to the major histocompatibility complex. The band patterns produced on genomic Southern blots were different for the two clones 2918.8 and 2918.4, suggesting that they might be distinct class II β chains from a single haplotype.

The Southern blot band pattern seen with 2918.4 (Fig. 1) was complex, even at high stringency. Eight to 12 bands were visible with each haplotype when restriction enzymes with 6-base-pair recognition sites were used. Clone 2918.8 produced a simpler band pattern on Southern blots, with two major bands and three to four weakly hybridizing bands observed in most DR haplotypes.

***Xenopus* Oocyte Microinjection, Immunoprecipitation, and Two-Dimensional Gel Electrophoresis.** Microinjection studies in *Xenopus* oocytes with hybrid-selected messenger RNA

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2918.4    ATG GTG TGT CTG AAG CTC CCT GGA GGC TCC TGC ATG ACA GCG CTG ACA GTG ACA CTG ATG GTG CTG AGC TCC CGA CTG
          Met Val Cys Leu Lys Leu Pro Gly Gly Ser Cys Met Thr Ala Leu Thr Val Thr Leu Met Val Leu Ser Ser Arg Leu
DRβ-1     --- --- --- --- --- --- --- --- --- --- Ser Leu Ala --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- --- --- --- Arg --- --- --- --- --- --- --- Ala Val --- --- --- --- --- --- --- --- --- --- Pro ---
pII-β-4   --- --- --- --- Arg --- --- --- --- --- --- --- Ala Val --- --- --- --- --- --- --- --- --- --- Pro ---

                       1                                                                                     23
2918.4    GCT TTG GCT GGG GAC ACC CGA CCA CGT TTC TTG TGG CAG CTT AAG TTT GAA TGT CAT TTC TTC AAT GGG ACG GAG CGG
          Ala Phe Ala Gly Asp Thr Arg Pro Arg Phe Leu Trp Gln Leu Lys Phe Glu Cys His Phe Phe Asn Gly Thr Glu Arg
DRβ-1     --- --- --- --- --- --- --- --- --- --- --- Glu Leu --- --- Ser --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- Leu --- --- --- --- --- --- --- --- --- Glu Tyr Ser Thr Ser --- --- --- --- --- --- --- --- --- ---
pII-β-4   --- Leu --- --- --- --- --- --- --- --- --- Glu Glu Val --- --- --- --- --- --- --- --- --- --- --- ---
DR2/2                 --- --- --- --- --- --- --- --- --- --- Pro --- Arg --- --- --- --- --- --- --- --- --- ---

                                                                                                             49
2918.4    GTG CGG TTG CTG GAA AGA TGC ATC TAT AAC CAA GAG GAG TCC GTG CGC TTC GAC AGC GAC GTG GGG GAG TAC CGG GCG
          Val Arg Leu Leu Glu Arg Cys Ile Tyr Asn Gln Glu Glu Pro Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala
DRβ-1     --- --- Phe --- --- --- His Phe His --- --- --- --- Tyr Ala --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- --- Tyr --- Asp --- Tyr Phe His --- --- --- --- Asn --- --- --- --- --- --- --- --- --- Phe --- ---
pII-β-4   --- --- --- --- --- --- Arg Val His --- --- --- --- Tyr Ala --- Tyr --- --- --- --- --- --- --- --- ---
DR2/2     --- --- Phe --- Asp --- Tyr Phe --- --- --- --- --- Ser --- --- --- --- --- --- --- --- --- Phe --- ---

                                                                                                             75
2918.4    GTT GAG GAG CTG GGG CGG CCT GAT GCC GAG TAC TGG AAC AGC CAG AAG GAC CTC CTG GAG CAG AAG CGG GGC CAG GTG
          Val Glu Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu Glu Gln Lys Arg Gly Gln Val
DRβ-1     --- Arg --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- Thr --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- Arg ---
pII-β-4   --- Thr --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- Arg Arg --- Ala Ala ---
DR2/2     --- Thr --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- Ile --- --- --- Ala --- Ala Ala ---

                                                                                                            101
2918.4    GAC AAT TAC TGC AGA CAC AAC TAC GGG GTT GGT GAG AGC TTC ACA GTG CAG CGG CGA GTT GAG CCT AAG GTG ACT GTG
          Asp Asn Tyr Cys Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val Glu Pro Lys Val Thr Val
DRβ-1     --- --- --- --- --- --- --- --- --- --- Val --- --- --- --- --- --- --- --- --- His --- Gln --- --- ---
pII-β-3   --- --- --- --- --- --- --- --- --- --- Val --- --- --- --- --- --- --- --- --- His --- --- --- --- ---
pII-β-4   --- Thr --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- Gln --- --- --- --- ---
DR2/2     --- Thr --- --- --- --- --- --- --- --- Val --- --- --- --- --- --- --- --- --- Gln --- --- --- --- ---

                                                                                                            127
2918.4    TAT CCT TCA AAG ACC CAG CCC CTG CAG CAC CAC AAC CTC CTG GTC TGC TCT GTG AGT GGT TTC TAT CCA GGC AGC ATT
          Tyr Pro Ser Lys Thr Gln Pro Leu Gln His His Asn Leu Leu Val Cys Ser Val Ser Gly Phe Tyr Pro Gly Ser Ile
DRβ-1     --- --- Ala --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-4   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- Asn --- --- --- --- --- --- ---
DR2/2     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                                                                            153
2918.4    GAA GTC AGG TGG TTC CGG AAC GGC CAG GAA GAG AAG GCT GGG GTG GTG TCC ACG GGC CTG ATC CAG AAT GGA GAT TGG
          Glu Val Arg Trp Phe Arg Asn Gly Gln Glu Glu Lys Ala Gly Val Val Ser Thr Gly Leu Ile Gln Asn Gly Asp Trp
DRβ-1     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- --- --- --- --- --- --- --- --- --- --- --- Thr --- --- --- --- --- --- --- --- His --- --- --- ---
pII-β-4   --- --- --- --- --- --- --- --- --- --- --- --- Thr --- --- --- --- --- --- --- --- --- --- --- --- ---
DR2/2     --- --- --- --- --- Leu --- --- --- --- --- --- --- --- Gly --- --- --- --- --- --- --- Asp --- --- ---

                                                                                                            179
2918.4    ACC TTC CAG ACC CTG GTG ATG CTG GAA ATA GTT CCT CGG AGT GGA GAG GTT TAC ACC TGC CAA GTG GAG CAC CCA AGT
          Thr Phe Gln Thr Leu Val Met Leu Glu Thr Val Pro Arg Ser Gly Glu Val Tyr Thr Cys Gln Val Glu His Pro Ser
DRβ-1     --- --- --- --- --- --- --- --- --- --- Phe --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-4   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
DR2/2     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                                                                            205
2918.4    GTG ACG AGC CCT CTC ACA GTG GAA TGG AGA GCA CGG TCT GAA TCT GCA CAG AGC AAG ATG CTG AGT GGA GTC GGG GGC
          Val Thr Ser Pro Leu Thr Val Glu Trp Arg Ala Arg Ser Glu Ser Ala Gln Ser Lys Met Leu Ser Gly Val Gly Gly
DRβ-1     --- --- --- --- --- --- --- --- --- Ser --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-4   --- Met --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
DR2/2     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                                                                            231
2918.4    TTC GTG CTG GGC CTG CTC TTC CTT GGG GCC GGG CTG TTC ATC TAC TTC AGG AAT CAG AAA GGA CAC TCT GGA CTT CAG
          Phe Val Leu Gly Leu Leu Phe Leu Gly Ala Gly Leu Phe Ile Tyr Phe Arg Asn Gln Lys Gly His Ser Gly Leu Gln
DRβ-1     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-3   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
pII-β-4   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- Pro

                              237
2918.4    CCA ACA GGA TTC CTG AGC TGA AATGCAGATGAACCACATTCAAGGAAGAACCTTCTGTCCCAGCTTTGCAGAATGAAAAGCTTTCCTGCTTGGCAGTT
          Pro Thr Gly Phe Leu Ser
DRβ-1     --- --- --- --- --- ---
pII-β-3   --- Arg --- --- --- ---
pII-β-4   --- --- --- --- --- ---

2918.4    ATTCTTCCACAAGAGAGGGCTTTCTCAGGACCTGGTTGCTACTGGTTCGGCAACTGCAGAAAATGTTCCTCCCTTGTGGCTTCCTCAGCTCCTGCCCTTGGCCTG

2918.4    AAGTCCCAGCATTGATGACAGCGCCTCATCTTCAGCTTTTGTGCTCCCCTTTGCCTAAACCGTATGGCCTCCCGTGCATCTGTACTCACCCTGTACGACAAACACA

2918.4    TTACATTATTAAATGTTTCTCAAAGATGGAGTTAAAAAAAAAA
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FIG. 3. Nucleic acid and derived amino acid sequence of DRβ clone 2918.4 from the homozygous *DR1* cell line LG2. Amino acid sequences of a DRβ chain from the *DR2* haplotype (11) and clones HLA-DR-β-1 (*DR4,6*) (13), pII-β-3, and pII-β-4 (*DR3,6*) (14) are shown for comparison.

obtained with 2918.4 and the DRα cDNA clone probes were done to obtain putative DRβ translation products for characterization with immunoprecipitation and two-dimensional gel electrophoresis. Because the monoclonal antibody chosen was directed against the isolated β chain (E. Clark, personal communication), we were able to determine whether the putative β-chain translation products reacted with this antibody and whether they associated with the DRα chain. The pattern obtained after autoradiography revealed spots consistent with both class II α and β chains (Fig. 2). These spots were obviously present in immunoprecipitations from a lymphoblastoid cell line using the same antibody but were absent when immunoprecipitations were performed from oocytes using a non-HLA antibody. These studies indicated that 2918.4 was able to select class II β-chain messages that translated into proteins with similar two-dimensional gel migration patterns to classical class II β-chain antigens. That translated β chains were associating with α chains was indicated by the fact that a β-chain-specific monoclonal antibody precipitated both α and β chains (Fig. 2).

Sequence Analysis of 2918.4 and 2918.8. The nucleic acid sequences of 2918.4 and 2918.8 were obtained to finally confirm their identity as class II β chains. In addition, these sequences allow further comparison of allelic variation among the β chains that might be relevant for functional differences between different alleles and different loci.

The sequence of 2918.4 (Fig. 3) showed strong homology to the already published DRβ sequences (15, 16). Previous sequences have been difficult to attribute to particular serological specificities, because they were derived in one case (HLA DR-B1) from a heterozygous *DR4,6* cell line (15) and in the other case (pII-β-3 and pII-β-4) from a *DR 3,6* line (16). Only the original DRβ protein sequence can be clearly attributed to a specific *DR* haplotype (*DR2*) (12). The use of a consanguineous homozygous *DR1* cell line allows us confidently to attribute our sequence to the *DRβ1* haplotype. The presence of at least three DRβ chains, of which at least two are expressed in some haplotypes (unpublished observation), does not permit true allelic comparisons between 2918.4 and other DRβ chains. Comparison of all available DRβ sequences with 2918.4 (Fig. 3) confirms that among DRβ chains there exist two clear regions of variability, one from residue 9 to 13 and the other from residue 26 to 38. These have remarkable similarity to the sites of variation between published mouse Eβ sequences, which show variable regions between residues 2 and 13 and between residues 24 and 35 (17). An additional region of variability is present in one DRβ clone (p-II-β-4) between residues 70 and 77. The regions of polymorphism common to Eβ and DRβ may prove to be particularly relevant to the function of these molecules. The other clearly defined variable regions found in Eβ sequences are less obvious between human DRβ sequences, but they might well appear after more allelic representatives of a single DRβ locus have been sequenced. As with the mouse sequences, the second and third domains are very homologous between alleles.

The amino-terminal protein sequence of a DRβ chain from the same cell line, LG2, has been published (10). Our sequence for these first 30 amino acids clarifies the reported histidine/tyrosine ambiguity at position 16 as being a histidine and confirms the presence of an asparagine at position 19. In addition, our sequence includes a phenylalanine at position 7 rather than a serine and the residues arginine and cysteine at positions 29 and 30, respectively. Several possible explanations exist for these differences. They may indicate that the sequences are of two distinct DRβ chains from the same haplotype. This is made less likely, however, by the striking similarity between the rest of the first variable region. Other explanations include microheterogeneity within the same cell line or errors in sequencing.

The sequence of 2918.8 revealed strong sequence homology with previously published DQβ nucleic acid sequences. The clone was 701 base pairs long and included the 5′ untranslated region, the leader peptide, the first, second, and part of the third domain. A portion of the third domain and the 3′ untranslated region are missing. The sequence is not presented in detail here, as it was identical to the clone pII-β-2 obtained from Raji, a *DR3,w6* cell line (14). This would suggest that the specificity of our DQβ clone is likely to be DQ1, found on *DR* haplotypes *1*, *2*, and *w6*, and the clone pII-β-2 represents the *DQ1* allele from the *Dw6* haplotype. This is supported by the observation that pII-β-1, another *DQβ* allele obtained from the *DR3,w6* cell line (18) is identical in sequence to the DQβ chain associated with the *DR3* haplotype from another cell line, WT49 (19). That at least some of the DQ1 determinants are attributable to 2918.8 and PII-β-2 assumes that these clones do not represent another DQβ common to both haplotypes.

There has been some uncertainty about the amount of heterogeneity one might find at a molecular level between class II genes. Five DQβ-chain genes have now been sequenced from different cell lines. Of these sequences, there are two sets of duplicate sequences (2918.8 and pII-β-2; WT.49 DQβ and pII-β-1). This indicates that, thus far in DQ, there exists a moderate amount of molecular heterogeneity, and this has been fairly well reflected in serological studies. Much further sequencing will have to be done to confirm this trend.

This work has characterized two class II β chains by immunoprecipitation of translation products and sequence analysis. The ability to attribute the sequences obtained to a specific haplotype emphasizes the importance of doing such work with clones from homozygous consanguineous cell lines. Only with similar data from single haplotypes will it be possible to do accurate analyses of allelic variation and to associate the sizeable knowledge of serological specificity with the expanding understanding of structure at a molecular level.

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