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Evolutionary and genetic implications of sequence variation in two nonallelic HLA-DR β-chain cDNA sequences

(HLA class II antigens/genetic polymorphism/DNA sequence homology)

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ABSTRACT **Most HLA haplotypes carry two expressed DR β-chain genes; in the DR4 haplotype, the polymorphic locus has been called DR β_1 and the apparently nonpolymorphic locus has been called DR β_2. We have isolated nearly full-length DR β-chain cDNA clones representing each of these two loci from a cell line homozygous for DR4 and Dw4. The clones have been sequenced and the sequences compared with published DR β cDNA sequences derived from other haplotypes. A comparison of our sequences with other published cDNA sequences did not allow assignment of these other sequences to either the β_1 or β_2 locus. Comparison of our DR4 β_1 sequence with DR β_1 sequences isolated from other DR4-positive cells suggests that the alleles of DR4 β_1 may have recently diverged from a common ancestor. The apparent lack of polymorphism of DR β_2 may in part be a reflection of this recent divergence.**

HLA class II molecules are members of a family of polymorphic cell surface proteins that are involved in the regulation of immune responses and are found primarily on macrophages, B lymphocytes, and activated T lymphocytes. They are present on the cell surface as glycosylated heterodimers consisting of an α chain with a molecular weight of approximately 34,000 and a β chain with molecular weight varying from 25,000 to 29,000. Class II products have been subdivided by immunological and biochemical criteria into three families, called DR, DQ, and DP (1–4).

In the HLA-DR family, there appears to be only one DR α-chain gene, while the number of DR β-chain genes varies from one to four among the various serologically defined haplotypes (1, 2). A DR4/DRw53 haplotype has been shown by Southern blotting and genomic cloning to have three complete DR β genes and one apparently incomplete DR β gene (5); one of the complete genes is a pseudogene (6). The DR α gene has been shown by biochemical analyses and DNA sequencing to be essentially invariant (1), whereas the two expressed DR4 β genes encode one chain that is variable and one that is constant, as determined by two-dimensional gel electrophoresis (7). The variable DR β chain is here designated DR β_1 and carries the antigenic determinant(s) responsible for the DR4 serological classification (8). The "nonvariant" chain is here designated DR β_2 and carries the DRw53 serological specificity, a so-called "supertypic" specificity also found on cells with the DR7 and DR9 haplotypes (9).

Molecular cloning and sequencing have shown that overall DNA sequence homology among α or β chains within a class II family is 85% or greater and between families is in the range of 70% (1). Genes belonging to different families can be readily differentiated, especially by sequence differences in the 3′ untranslated portion of those genes. The existence of multiple DR β-chain genes and, in some cases, the study of cells that are not homozygous for DR have made it difficult to assign a DNA sequence to a particular locus within the DR family and to determine to what degree the variation seen among DR β sequences represents allelic variation versus interlocus (isotypic) differences. To address these questions we report here a sequence of a nearly full-length DR β_2 cDNA clone derived from a DR4 homozygous cell, which allows sequence comparisons to be made with known DR β_1 sequences of DR4 and with DR β sequences from other haplotypes.

Within the DR4 classification there is a further polymorphism, referred to as Dw-subtype polymorphism, which is detected by T-lymphocyte proliferative responses rather than by serological assays (10, 11). The mobility of a particular DR β_1 protein in isoelectric focusing correlates with the Dw subtype of the cell from which it was derived (12–15). The extent of polymorphism of the DR β_1 gene and the degree of conservation of the DR β_2 gene of the DR4, DR7, and DR9 haplotypes are areas of some uncertainty. Thus, to obtain additional information relevant to DR β_1 gene polymorphism we sequenced a DR β_1 cDNA clone derived from the DR4 Dw4 cell line that was the source of the DR β_2 clone. The possible contributions of these sequences to an understanding of the evolution of the DR4 haplotype are also discussed.

MATERIALS AND METHODS

Construction and Screening of the cDNA Library. A cDNA library was constructed from poly(A)$^+$ RNA isolated from the DR4, Dw4-homozygous cell line MJ4 (A2, Bw62, Bw35, Cw3, DR4, Dw4, DQw3, DRw53), as described (16). Two nearly full-length positive clones were selected for sequencing because they had certain restriction enzyme sites characteristic of DR β genes but also differed slightly from each other in their restriction site maps. These clones are designated MJ5.4 (1022 base pairs) and MJ8.2 (935 base pairs).

Sequencing of DR β-Chain cDNA Clones. The inserts from clones MJ5.4 and MJ8.2, or fragments thereof, were subcloned into bacteriophage vector M13mp19. Subclones were sequenced directly or, alternatively, truncated subclones were generated by the method of Dale *et al.* (17). Sequencing was done using the dideoxy chain-termination method (18).

RESULTS AND DISCUSSION

Sequence of cDNA Clones Derived from β_1 and β_2 Loci of the DR4 Dw4 Haplotype. To obtain additional data about sequence variation among alleles of DR β_1 within the DR4 haplotype and to obtain a complete sequence known to be DR β_2, we isolated and sequenced two DR β cDNA clones, MJ8.2 (935 base pairs) and MJ5.4 (1022 base pairs), both generated from RNA isolated from the DR4-homozygous lymphoblastoid cell line MJ4. The nucleotide and predicted amino acid sequences for clone MJ5.4 are given in Fig. 1. The sequence of clone MJ8.2 is identical to the sequence of a clone derived

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                                   -1|+1
                                     |
                         17          | 32              47              62              77
MJ5.4     Val Leu Ser Ser Pro Leu Ala Leu Ala Gly Asp Thr Gln Pro Arg Phe Leu Glu Gln Ala Lys Cys Glu Cys His Phe Leu (18)
       CG GTG CTG AGC TCC CCA CTG GCT TTG GCT GGG GAC ACC CAA CCA CGT TTC TTG GAG CAG GCT AAG TGT GAG TGT CAT TTC CTC

        92             107             122             137             152             167
Asn Gly Thr Glu Arg Val Trp Asn Leu Ile Arg Tyr Ile Tyr Asn Gln Glu Glu Tyr Ala Arg Tyr Asn Ser Asp Leu Gly Glu Tyr Gln (48)
AAT GGG ACG GAG CGA GTG TGG AAC CTG ATC AGA TAC ATC TAT AAC CAA GAG GAG TAC GCG CGC TAC AAC AGT GAC CTG GGG GAG TAC CAG

        182            197             212             227             242             257
Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu Glu Arg Arg Arg Ala Glu Val Asp Thr Tyr (78)
GCG GTG ACG GAG CTG GGG CGG CCT GAC GCT GAG TAC TGG AAC AGC CAG AAG GAC CTC CTG GAG CGG AGG CGG GCC GAG GTG GAC ACC TAC

        272            287             302             317             332             347
Cys Arg Tyr Asn Tyr Gly Val Val Glu Ser Phe Thr Val Gln Arg Arg Val Gln Pro Lys Val Thr Val Tyr Pro Ser Lys Thr Gln Pro (108)
TGC AGA TAC AAC TAC GGG GTT GTG GAG AGC TTC ACA GTG CAG CGG CGA GTC CAA CCT AAG GTG ACT GTG TAT CCT TCA AAG ACC CAG CCC

        362            377             392             407             422             437
Leu Gln His His Asn Leu Leu Val Cys Ser Val Asn Gly Phe Tyr Pro Gly Ser Ile Glu Val Arg Trp Phe Arg Asn Gly Gln Glu Glu (138)
CTG CAG CAC CAC AAC CTC CTG GTC TGC TCT GTG AAT GGT TTC TAT CCA GGC AGC ATT GAA GTC AGG TGG TTC CGG AAC GGC CAG GAA GAG

        452            467             482             497             512             527
Lys Ala Gly Val Val Ser Thr Gly Leu Ile Gln Asn Gly Asp Trp Thr Phe Gln Thr Leu Val Met Leu Glu Thr Val Pro Arg Ser Gly (168)
AAG GCT GGG GTG GTG TCC ACA GGC CTG ATC CAG AAT GGA GAC TGG ACC TTC CAG ACC CTG GTG ATG CTG GAA ACA GTT CCT CGG AGT GGA

        542            557             572             587             602             617
Glu Val Tyr Thr Cys Gln Val Glu His Pro Ser Met Met Ser Pro Leu Thr Val Gln Trp Ser Ala Arg Ser Glu Ser Ala Gln Ser Lys (198)
GAG GTT TAC ACC TGC CAA GTG GAG CAT CCA AGC ATG ATG AGC CCT CTC ACG GTG CAA TGG AGT GCA CGG TCT GAA TCT GCA CAG AGC AAG

        632            647             662             677             692             707
Met Leu Ser Gly Val Gly Gly Phe Val Leu Gly Leu Leu Phe Leu Gly Thr Gly Leu Phe Ile Tyr Phe Arg Asn Gln Lys Gly His Ser (228)
ATG CTG AGT GGA GTC GGG GGC TTT GTG CTG GGC CTG CTC TTC CTT GGG ACA GGG CTG TTC ATC TAC TTC AGG AAT CAG AAA GGA CAC TCT

        722            737             753        763        773        783        793        803
Gly Leu Gln Pro Thr Gly Leu Leu Ser  *  (238)
GGA CTT CAG CCA ACA GGA CTC TTG AGC TGA AGTGCAGATG ACCACATTCA AGGAAGAACC TTCTGCCCCA GCTTTGCAAG ATGAAAAGCT

      813        823        833        843        853        863        873        883        893        903
TTCCCACTTG GCTCTTATTC TTCCACAAGA GCTTTGTCAG GACCAGGTTG TTACTGGTTC AGCAACTCTG CAGAAAATGT CCTCCCTTGT GGCTTCCTTA

      913        923        933        943        953        963        973        983        993       1003
GCTCCTGTTC TTGGCCTGAA GCCTCACAGC TTTGATGGCA GTGCCTCATC TTCAACTTTT GTGCTTCCCT TTACCTAAAC TGTCCTGCCT CCCGTGCATC

     1013       1023
TGTACTCCCC TTGTGCCACA
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FIG. 1. Nucleotide and derived amino acid sequences of the DR β-chain cDNA clone MJ5.4. The nucleotide numbers, given above the sequence, are for MJ5.4. The 5′ end of the MJ8.2 cDNA clone corresponds to base number 89 of MJ5.4. The amino acid number of the mature protein is noted in parentheses at the right of each line; the last residue of the leader peptide (−1) and the first residue of the mature protein (+1) are indicated. An asterisk is shown above the termination codon TGA (amino acid position 238).

from a cell with the DR4 Dw4 haplotype (E. Long, personal communication), which is also identical to the sequence of a clone derived from a cell with the DR4 Dw14 haplotype, with the exception of three nucleotides in the first domain, as described (16). By comparison with the genomic sequences of Spies *et al.* (5), we were able to determine that the sequences of MJ8.2 and MJ5.4 were derived from the DR β_1 and DR β_2 loci, respectively. By comparison with published sequences we can determine that, at its 5′ end, clone MJ8.2 lacks the 5′ untranslated region as well as nucleotides coding for the signal sequence and the first 20 amino acids of the mature DR β polypeptide. At its 5′ end, clone MJ5.4 lacks the 5′ untranslated region and the nucleotides coding for the first 22 amino acids of the signal sequence. By comparison with published sequences we estimate that, at their 3′ ends, clones MJ8.2 and MJ5.4 end approximately 13 nucleotides 5′ of the polyadenylylation signal.

Grouping of DR β Sequences by Homologies. A primary goal of these studies was to evaluate whether some portions of the DR4 β_1 or β_2 genes would be quite different from each other yet very similar to the corresponding regions of other, previously published, DR β-chain gene sequences derived from cells of other haplotypes. The discovery of such characteristic regions of homology might allow future assignment of any sequenced DR β gene to the DR β_1 or β_2 locus. To this end, we have compared the sequence (E. Long, personal communication) of a DR4 Dw4 β_1 cDNA clone obtained by Long and coworkers (group II clones described in ref. 19), since it is identical to ours but includes all of the first domain, and the sequence of the DR4 Dw4 β_2 cDNA clone MJ5.4 with each other and with other published DR β sequences (19–21) (Table 1).

The four other DR β sequences, derived from cells with DR1, DR3, and DR6 haplotypes, are more homologous with each other, in pairwise comparisons, than with either DR4 β_1 or β_2. These four sequences all differ from each other by 15 to 25 nucleotides in the first 282 bases of the 3′ untranslated region; for ease of discussion we call these group I sequences. In contrast, group I sequences differ by 38–45 nucleotides from DR4 β_1 and by 29–42 nucleotides from DR4 β_2 in their 3′ untranslated regions; the DR4 β_1 and β_2 sequences differ by 42 nucleotides in their 3′ untranslated regions. Thus, by comparison of 3′-untranslated-region homologies alone one may place the DR1, -3, and -6 sequences in one homology group, with these sequences appearing to be more homologous with others within the group than with either DR4 β_1 or β_2 and equally different from both DR4 β_1 and β_2. In regions coding for the first domain, group I sequences differ from each other by 16–27 nucleotides; these sequences differ from DR4 β_1 to a comparable degree in the first domain—i.e., by 17–22 nucleotides. In contrast, group I sequences differ from DR4 β_2 by 30–38 nucleotides in the first domain, and DR4 β_1 and β_2 sequences differ from each other by 29 nucleotides in the first domain. Thus, inclusion of the first-domain se-

Table 1. Comparison of DR β sequences by domain

Clone* and domain†	Number of nucleotides different				
	DR4 Dw4	HLA-DR β_1	pIIβ3	pIIβ4	2918.4
MJ5.4					
First domain	29	33	33	30	38
Second domain	10	12	13	10	13
TM + CYT	6	5	6	6	6
3′ untranslated	42	42	29	36	39
DR4 Dw4					
First domain		20	22	17	22
Second domain		9	10	11	12
TM + CYT		1	2	2	0
3′ untranslated		43	45	38	43
HLA-DR β_1					
First domain			16	21	18
Second domain			12	13	11
TM + CYT			3	3	1
3′ untranslated			17	15	23
pIIβ3					
First domain				27	27
Second domain				11	13
TM + CYT				2	2
3′ untranslated				15	25
pIIβ4					
First domain					24
Second domain					9
TM + CYT					2
3′ untranslated					17

*MJ5.4 is, as reported here, a DR β_2 cDNA sequence derived from a DR4 Dw4 haplotype. DR4 Dw4 is taken from a DR β_1 cDNA clone, derived from a DR4 Dw4 haplotype, that was obtained by E. Long and coworkers. HLA-DR β_1 is derived from a DR6 haplotype (19); pIIβ3 and pIIβ4 are derived from the cell line Raji, which is heterozygous for DR3 and DR6 or DR1 (20); 2918.4 is derived from a DR1 haplotype (21).

†The first domain includes the nucleotides coding for amino acids 1–94. The second domain includes the nucleotides coding for amino acids 95–188. TM + CYT refers to the transmembrane and cytoplasmic regions of the protein and includes nucleotides coding for amino acids 189–238. The first 282 nucleotides of the 3′ untranslated region are included in the comparison.

quences in the comparison suggests that the group I sequences may be somewhat more homologous with DR4 β_1 than they are with DR β_2 of DR4. However, the extent of homology of group I sequences with DR4 β_1 is similar enough to the homology with DR4 β_2 that we cannot confidently suggest that the previously sequenced DR β cDNA clones were all derived from the DR β_1 locus. Thus, we cannot at this time define regions that are characteristic of the β_1 or β_2 locus, outside of the DR4 haplotype.

Possible Contribution of Gene-Conversion-Like Events to Evolution of DR β Alleles. It is difficult to determine whether the groupings discussed above reflect evolutionary relatedness of the various class II genes. Several investigators, for instance, have noted evidence for apparent gene-conversion-like events occurring between related genes (16, 22–26). Our comparison of published DR β sequences suggests that these genes are much like a patchwork of sequences being exchanged among related genes. For example, in the comparison of our DR β sequences with other published DR β sequences, we have noted evidence for a possible gene-conversion-like event involving the DR4 β_2 gene and the gene represented by the DR β cDNA clone pIIβ3, derived from the Raji cell line (20). As shown in Fig. 2, a segment of the 3′ untranslated region of pIIβ3, otherwise very similar to others of our group I sequences, shows greater homology with the 3′ untranslated region of MJ5.4, the DR β_2 cDNA clone. This possible gene-conversion event might have involved a minimum of 24 nucleotides or a maximum of 65 nucleotides.

The apparently very extensive gene conversion occurring among class I genes in the mouse, even between families, has completely obscured evolutionary relationships; thus, alleles of the H-2K locus may be as different from each other as they are from genes encoded in the H-2D locus. However, it has been recently reported (27) that gene-conversion events, at least in class I genes, occur predominantly in exons of the genes. Therefore, by comparison of introns it has been possible to group sequences derived from the same locus and to construct an evolutionary tree for the family of class I genes (27). When additional studies of class II gene organization and genomic sequences are available, it may be possible to discuss more confidently the extent of allelic variation and interlocus variation, as well as evolutionary relatedness, of these genes.

Comparison of the Limits of Polymorphism of DR4 β Genes as Detected by Several Methods. The extent of the polymorphism of class II products has not yet been determined. The polymorphism defined by T-lymphocyte proliferative responses was found to be more extensive than that defined by serological reagents; the possibility exists that even more polymorphism will be detected at the DNA sequence level. We can now begin to address this possibility directly by comparing multiple DR β_1 sequences derived, by ourselves and others, from cells that have the same haplotype (i.e., DR4 Dw4). We find that the sequence of our DR β_1 cDNA clone MJ8.2 is identical, where comparison is possible, to the sequence of the first-domain exon of a DR β genomic clone derived from the DR4 Dw4 cell line Priess (5) and to the sequence (communicated to us by E. Long) of a DR β cDNA clone derived from a cell that we have determined to have a DR4 Dw4 haplotype (16). Thus, among three DR4 β_1 cDNA sequences from cells expressing the T-lymphocyte-defined Dw4 subtype, base substitutions that alter amino acid sequence but do not alter T-cell reactivity (Dw subtype) have not yet been detected. These comparisons include three first-domain sequences and two sequences of the second domain, transmembrane, cytoplasmic, and 3′ untranslated regions. In addition, a comparison of the first domains of our DR4 Dw14 β_1 cDNA clone (16) with another Dw14 DR β_1 sequence (28) shows no differences. Thus, while variations among Dw-identical DR β_1 chains may be found as more sequence data are gathered, to date all DR β_1 cDNA sequences derived from cells identical for Dw subtypes as defined by T lymphocytes are themselves identical. Also, as previously discussed, T lymphocytes are apparently able to discriminate among DR β gene products that differ by only one to several amino acids. These results suggest that HLA-Dw typing by T-lymphocyte proliferative responses may have substantially defined the limits of HLA-DR gene polymorphism, at least for the polymorphism within DR4. This conclusion is strengthened by data from comparison of DR4 β_1 proteins by two-dimensional gel electrophoresis. The migration of β_1 proteins varies in isoelectric focusing, and the focusing position correlates with the Dw subtype of the cell from which the protein comes. Previous studies in this and several other laboratories indicate that β_1 chains from all cells expressing a particular Dw subtype migrate identically (12–15). These findings support, albeit with a much lower level of discrimination than DNA sequence analysis, the relative lack of additional polymorphism beyond that detected by T cells. In the case of the DR4 β gene, then, the T-cell-recognized Dw polymorphism appears to define the extent of nucleotide polymorphism. To what extent this will apply to other haplotypes, or how much DNA sequence polymorphism that does not alter protein sequence may exist in class II genes in haplotypes other than DR4, is not known.

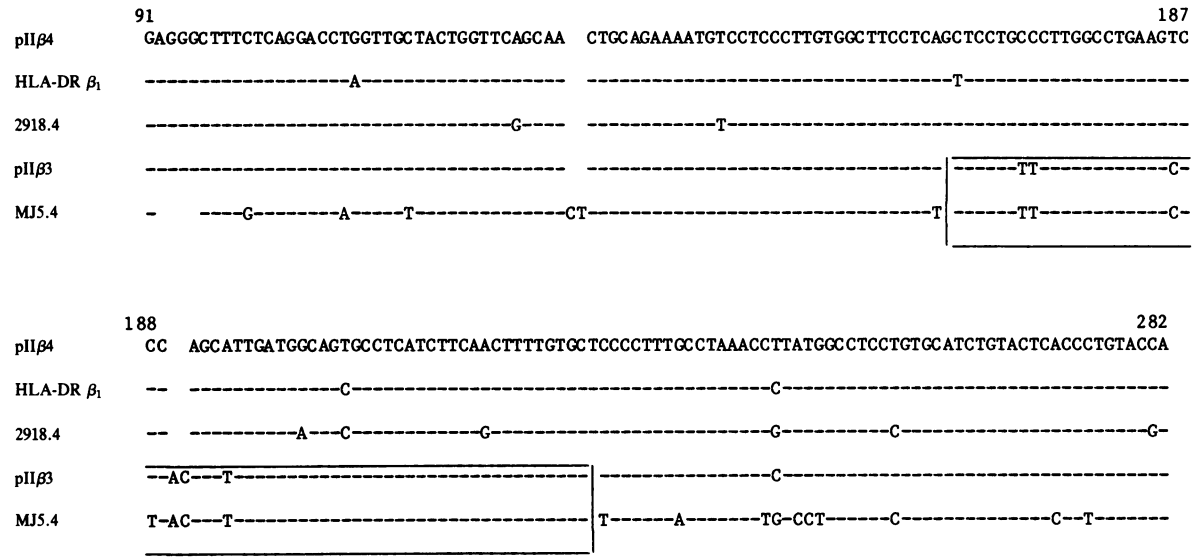

FIG. 2. Evidence for gene conversion among DR β-chain genes. The sequences shown represent nucleotides 91–282 of the 3′ untranslated regions of four sequences comprising group I and the DR4 β_2 sequence MJ5.4 reported here. Dashes indicate identity with the pIIβ4 sequence; gaps were inserted where necessary to give the best alignment. The boxed region represents the maximal number of nucleotides that might have been involved in a possible gene-conversion-like event.

Conjectures Regarding the Evolution of the DR4 Haplotype. Allelic sequence variation is expected to be subject to selective pressures. However, since nucleotide substitutions that do not alter amino acid sequence are most likely neutral with respect to selection, the number of silent nucleotide changes that have accumulated in alleles may reflect the length of time during which these alleles have diverged. In pairwise comparisons of all DR4 β_1 sequences available (Dw4, Dw13, and Dw14), from one to four nucleotide substitutions can be detected, with at most a single silent base substitution (16). These comparisons include all regions of the mature mRNA—including the 5′ and 3′ untranslated regions, which would be expected to be subject to less selective pressure. The limited number of replacement substitutions and the single silent base substitution suggest a very recent evolutionary divergence of the β_1 genes of the various Dw subtypes from the prototype DR β_1 sequence of the DR4 haplotype. The conclusion of a recent evolutionary origin of the Dw polymorphism of DR4 was also suggested by studies showing a very limited restriction fragment length polymorphism (RFLP) of the DR4 haplotypes and no detected RFLP associated with a single Dw subtype (29). One possible mechanism to explain the limited polymorphism within DR4 is that, in recent evolutionary time, the prototype DR4 haplotype was one of a few that passed through an evolutionary bottleneck (10, 29). That haplotype is now conceivably beginning to accumulate mutations, which we detect as Dw-subtype polymorphism.

The issue of pressure to maintain polymorphism versus pressure for conservation of DR β genes found in DR4 haplotypes should be reviewed in light of the very limited polymorphism and probable very recent divergence of the β_1 genes of different DR4 subtypes. Although it might be suggested that evolutionary pressures, possibly due to different functional constraints, select for variation in the β_1 locus and/or for conservation of the β_2 locus, an additional contributing factor may be that variation within the DR4 haplotype appeared recently in evolutionary time and that the DR β_2 locus has not yet accumulated variation. In fact, the evidence for conservation of the DR β_2 sequence is primarily based on its lack of positional variation in isoelectric focusing (7, 30). However, such analysis cannot detect all polymorphism, as shown by the fact that the DR β_1 chains from several Dw4 cells and one Dw14 cell focus identically (12, 13), and yet Dw4 β_1 genes and Dw14 β_1 genes studied to date differ from each other by two amino acids (16); tryptic peptide mapping of DR β_1 gene products derived from other Dw4 and Dw14 cells shows that there are peptides with different elution profiles on high-performance liquid chromatography (14). Thus, the DR β_2 chain of the DR4 haplotype may have variation that has not been detected by the methods used to date. Also, among the different serological haplotypes, the DR β_2 chain may be variable if serological determinants, such as DRw52 and DRw53, represent an allelic series defined by epitopes on DR β_2.

In fact, by at least one line of reasoning, the DR β_2 gene described here may be subject to accumulating mutation. It has been noted that the frequency of CpG dinucleotides in polymorphic exons of class I and class II genes is approximately what would be expected from the nucleotide composition; this is in contrast with the general phenomenon of CpG suppression in genomic DNA of higher vertebrates, where the overall ratio of observed frequency of CpG dinucleotides to that expected from the nucleotide composition is approximately 0.25 (31). In DR β genes, CpG suppression has been shown to occur in the highly conserved second-domain exon but not in the polymorphic first-domain exon (3); analysis of the first- and second-domain sequences of DR4 β_1 and β_2

Table 2. CpG dinucleotides in DR β coding regions

	CpG, % of total dinucleotides*		
	Observed	Expected	Ratio
DR β_1			
First domain	7.8	9.0	0.87
Second domain	1.4	7.9	0.18
DR β_2			
First domain	5.7	8.3	0.67
Second domain	0.4	7.7	0.18

The DR β_1 sequence analyzed is from the DR4 Dw14 cell line LS40 (16); the DR β_2 sequence is from the DR4 Dw4 cell line MJ4, as reported here. The first and second domains of DR β sequences include the coding regions for amino acids 1–94 and 95–188, respectively.

*The percent of CpG dinucleotides that is observed and the percent expected from the base composition are reported. The ratio of observed to expected is a measure of the CpG suppression.

shows that both loci exhibit CpG suppression in the second but not the first domains (Table 2). This is unlike the nonpolymorphic murine I-E α-chain gene, in which CpG dinucleotides are suppressed in both first and second domains (31). Thus, the first domain of the DR β_2 locus does not share the characteristic of CpG suppression with highly conserved sequences and, by this criterion at least, is potentially a polymorphic locus. Additional sequences of both DR β_1 and β_2 genes from cells of the DR4, DR7, and DR9 haplotypes, all of which carry the DR β_2-associated DRw53 determinant, should help to resolve the question of whether the DR β_2 locus is in fact much less polymorphic than the β_1 locus. Although a significant amount of protein analysis supports the concept that the DR β_2 gene of the DR4, -7, and -9 haplotypes is conserved, we suggest that the different haplotypes sharing the DRw53 specificity have recently diverged from a single prototype and that the lack of obvious polymorphism in DR β_2 may in part reflect a lack of sufficient time for mutations to accumulate. This possibility should be considered in attempts to assess a greater selective pressure for conservation of β_2 and/or pressure for variation in β_1.

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