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The DR3(w18),DQw4 Haplotype Differs from DR3(w17),DQw2 Haplotypes at Multiple Class II Loci

Carolyn Katovich Hurley, Peter K. Gregersen, Jack Gorski, Noriko Steiner, Fu Meei Robbins, Robert Hartzman, Armead H. Johnson, and Jack Silver

ABSTRACT: *The polymorphism of HLA class II molecules in man is particularly evident when comparisons between population groups are made. This study describes a DR3 haplotype commonly present in the American black population. Unlike the Northern European population, in which almost all DR3 individuals are DQw2, approximately 50% of DR3-positive American blacks express a DQw4 allelic product. This study characterizes the DR subregion of that haplotype. cDNA sequence analysis has revealed a DRβ gene which differs at several positions from previously described DR3β1 genes. It is postulated that a gene-conversion-like event with a DRw52β gene as donor has generated some of these differences. The haplotype carries a DRw52a allele as defined by oligonucleotide hybridization studies. DNA restriction fragment analysis using a family and several unrelated individuals has allowed us to identify DRα and β fragments associated with the DR3(w18),DQw4 haplotype. The most striking observation is that the DR3(w18),DQw4 haplotype differs from DR3(w17),DQw2 haplotypes at multiple class II loci. Several genetic mechanisms including reciprocal recombination, gene conversion, and point mutation were involved in generating the differences between these haplotypes. Once established, the DR3(w18),DQw4 haplotype appears to be relatively stable in the population.*

ABBREVIATIONS

B-LCL B-lymphoblastoid cell line
HTC homozygous typing cell

INTRODUCTION

Class II molecules encoded by the human major histocompatibility complex are cell surface α/β heterodimers which exhibit extensive polymorphism. This polymorphism, localized to specific segments or variable regions of the amino-terminal domains of DR, DQ, and DP molecules (reviewed by [1]), is important in the function of these molecules in the immune response [2–4]. Since all of the

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class II molecules function in antigen presentation [5–8] and serve as targets of allorecognition [9,10], an individual contributes a variety of class II molecules to an immune response. This diversity is limited, however, in that particular combinations of class II alleles predominate in the population, a phenomenon known as linkage disequilibrium. These particular combinations of class II genes represent unique haplotypes.

As the class II genes have evolved, families of related haplotypes have developed and have been detected using serology, T-cell specificity analysis, DNA restriction fragment length polymorphism studies, and DNA sequencing. All of the haplotypes in the DRw52 family carry one of a group of closely related DRβIII genes which control the expression of the DRw52 molecule [11]. The DRβIII gene is closely linked to a polymorphic DRβI locus controlling the expression of DR3, 5, or w6 allelic products and an invariant DRα gene locus. The DR subregion also contains a DRβ pseudogene [12]. DR alleles in the DRw52 family are often found in linkage disequilibrium with specific DQ alleles encoded by polymorphic DQα and β genes [for example, DR3(w17),DQw2; DR5(w11),DQw3; and DRw6,DQw1 are common haplotypes in Northern European populations].

Haplotypes which express DR3, members of the DRw52 family, are especially important because of the association of DR3 with a number of autoimmune diseases such as insulin-dependent diabetes, myasthenia gravis, and Graves' disease [13]. We have begun a study of a DR3 haplotype, DR3(w18), commonly found in the American black population. This haplotype expresses a DQw4 allele in contrast to the DQw2 allele commonly associated with Caucasian DR3(w17) haplotypes. Previous studies by our group have determined that the DQ subregion of this haplotype differs from that of the Caucasian DR3(w17) haplotypes in several important ways. Reciprocal recombination between DQα and β genes has generated the DQw4 phenotype. The DQw4β gene encoded in this subregion represents a very different allele from the DQw2β gene. In addition, point mutation has produced differences in the DR3(w18), DQw4-associated DQw2α gene. Finally, additional recombination in the DQ subregion has resulted in the association of a unique DXβ gene DNA restriction fragment with the DQw4 allele. Thus, several genetic mechanisms appear to have generated the DQ subregion diversity in this haplotype [14].

An important question addressed in this study is the extent of divergence of the DR subregion of the DR3(w18),DQw4,D− haplotype from that of the DR3(w17),DQw2,Dw3 haplotype. Thus, we have characterized the genes and proteins encoded by the DR subregion associated with the DR3(w18),DQw4 haplotype using cDNA sequence analysis and DNA/RNA hybridization analysis. These studies reveal a number of important differences in the DR subregion.

MATERIALS AND METHODS

Description of DR3 haplotype. Serology and HTC typing of this population have been previously described [14]. The DR3(w18) and DQw4 specificities [15] were identified using reagents from the Tenth International Histocompatibility Workshop and/or the Third Asia-Oceania Histocompatibility Workshop. DR3 has recently been split into DR3(w17) and DR3(w18) by the Tenth International Histocompatibility Workshop. The presence of the LB-Q1 specificity [16] was analyzed using T-cell clone L4C40 (F. Robbins and R. Hartzman, manuscript in preparation).

Cells and reagents. B-lymphoblastoid cell lines (B-LCLS) (Tables 1 and 2) were established by transforming purified peripheral blood B lymphocytes with

TABLE 1 HLA typing of DR3-positive B-LCL from the American black population

Cell	HLA specificity[a]					
	A	B	D	DR	DQw	LB-Q1
1563	30,28	w42,45	–[b],–	3(w18),w11	4,3	+[c]
2041	w19,2	w50,44	–,w2	3(w18),2	4,1	NT[d]
1014[e]	1,23	w53,45	–,w2	3(w18),2	4,1	NT
1568[e]	30,w34	w42,w71	–,–	3(w18),1	4,1	–
1559[e]	2,25	w35,27	–,w8	3(w18),w8	4	–
1401	w36,31	w42,w35	–,–	3(w18),–	4,3	–

[a] DR3 haplotype underlined.

[b] Undefined specificity.

[c] Based on family analysis, the DR3(w18) haplotype is LB-Q1–.

[d] NT, not tested.

[e] Informative family not available to define haplotypes.

Epstein-Barr virus [14]. HLA-D region homozygous cell lines (HTC) used in the study were: PGF (DR2,DQw1); AVL [DR3(w17),DQw2,Dw3]; QBL [DR3 (w17),DQw2,Dw3]; 3164 (DR4,DQw3); IDF (DR5,DQw3) (all preceding cell lines were obtained from the Mutant Cell Repository, Camden, NJ); and ARC (DRw8,DQw4) (gift from E. Mickelson, Puget Sound Blood Center, Seattle, WA). AVL expresses an HLA-B8 allele, while QBL expresses an HLA-B18 allele.

Isolation of class II cDNAs. A cDNA library was prepared from B-LCL 2041 [DR3(w18),2;DQw4,w1;D–,w2] in the Okayama and Berg vector [17] and screened for DRβ clones as previously described [14,18]. cDNA was prepared from B-LCL 1563 [DR3(w18),w11;DQw4,w3;D–,–] [19] and was subjected to two sets of 15 cycles of *Taq* DNA polymerase-catalyzed DNA amplification [20,21] using primers containing conserved sequences found in the DRβ leader sequence and second domain. The amplified products were purified using low melting agarose (IBI/IBC Corp., Rockland, ME), digested with *Sst*I and ligated into the pBluescript vector (Stratagene, La Jolla, CA) which had been previously digested with *Sst*I and *Sma*I (BRL, Gaithersburg, MD).

TABLE 2 014 Family

	Haplotype					B-LCL				
						2707	2708	2704	1066	2710
a:	D–[a]	DRw8	(w52)[b]	LB-Q1–	DQw3	+		+	+	
b:	D–	DR3(w18)	(w52)	LB-Q1–	DQw4	+				+
c:	D–	DR–[a]	(w52)	LB-Q1–	DQw2		+	+		
d:	D–	DRw13	(w52)	LB-Q1+	DQw1		+		+	+

[a] *Undefined.*

[b] Since all individuals are DRw52, the assignment of DRw52 to individual haplotypes cannot be made.

TABLE 3 Oligonucleotides used for typing of DR3-positive B-LCL

Oligo	Gene detected	Allele detected	Codon position
6/1[a]	DRβI	DR3,5,w6	9–14
3/2[b]	DRβI	DR3	74–79
6/2[a]	DRβIII	DRw52a	9–14
52a/2	DRβIII	DRw52a	35–40
52b/2	DRβIII	DRw52b	26–32
6/3[a]	DRβIII	DRw52b+c	9–14
52c/3	DRβIII+ DRβI	DRw52c+ DR3(w18)	26–32

[a] From [24].
[b] From [23].

DNA sequencing. Sequencing was performed by the Sanger dideoxy chain termination method [22]. The cDNA clones were sequenced directly in the vectors using internal primers as previously described [14,18].

Hybridization with an oligonucleotide probe. Total RNA (20 μg) was spotted onto a Genescreen Plus membrane (New England Nuclear, Boston, MA) and probed with radiolabeled oligonucleotides as previously described [23–25]. The DRβ nucleotide sequences which hybridize to the oligonucleotides are indicated in Figure 1, and the oligonucleotides are described in Table 3.

Southern blotting. Isolation of DNA, Southern blotting protocol, and hybridization conditions have been previously described [14]. Filters were probed with radiolabeled DRα (a gift from E. Long, NIH, Bethesda, MD) and β [26] cDNAs. The DRβ probe used for hybridization contains only the 3′ untranslated region of the DR1β gene and detects all three DRβ loci [27].

RESULTS

Population Analysis

Only half of the DR3-positive American blacks possess the DR3(w17),DQw2 haplotype commonly observed in Caucasians. The remainder of DR3 individuals express a DQw4 allele [28]. (This allele was previously reported as DQw− [14].) None of these DR3(w18),DQw4 individuals type for any established HLA-D specificity (i.e., D undefined or blank) using mixed lymphocyte typing. This haplotype has been observed in South African blacks and is expressed by the homozygous typing cell RSH [29].

cDNA Sequence Analysis of a DR3-like βI Gene

In order to characterize the DR3βI gene from the DR3(w18),DQw4 haplotype, the *Taq* DNA polymerase-catalyzed DNA amplification technique [20] was used to obtain the first domain nucleotide sequence of the DRβI gene from B-LCL 1563 [DR3(w18),w11;DQw4,w3] (Figure 1, Table 1). In order to confirm the identity of the DR3-associated βI gene and to obtain the sequence of the second domain, a size-selected cDNA library was constructed in the Okayama and Berg expression vector [17] from an unrelated individual expressing the same

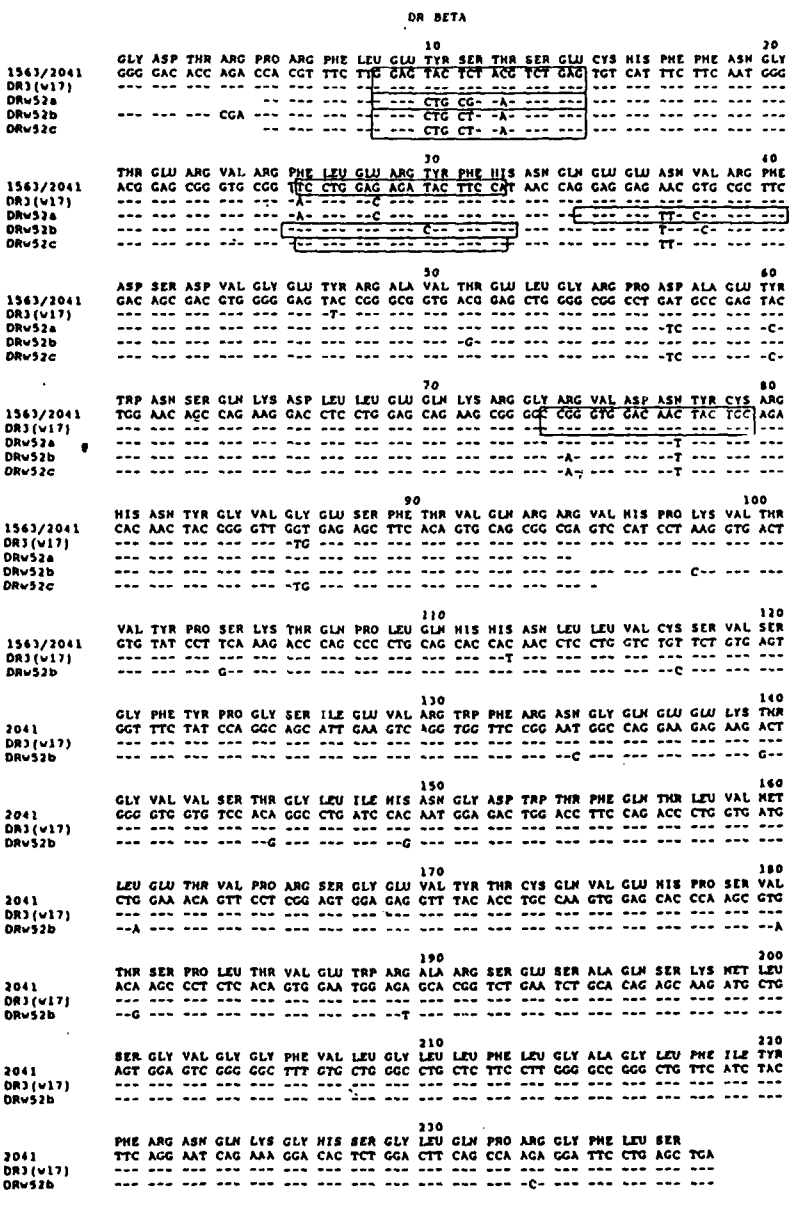


FIGURE 1 Nucleotide and predicted amino acid sequence of DRβ genes from: DR3(w18),DQw4βI (1563 and 2014); DR3(w17),DQw2βI [30–33]; DRw52aβIII [32]; DRw52bβIII [34]; DRw52cβIII [35]. The DRβI sequence from B-LCL 1563 includes the codons for amino acids 1–104. The sequence of the DRβI clone from the B-LCL 2041 library is truncated beginning at the codon for amino acid 15. Sequences which hybridize to the oligonucleotide probes are boxed.

haplotype, B-LCL 2041 [DR3(w18),2;DQw4,w1] (Table 1). The nucleotide sequences of the DR3-associated βI cDNAs from the two cell lines are identical and are most similar to the DR βI sequence previously described from several DR3(w17),DQw2 haplotypes [30–33] (Figure 1). The derived DR βI protein

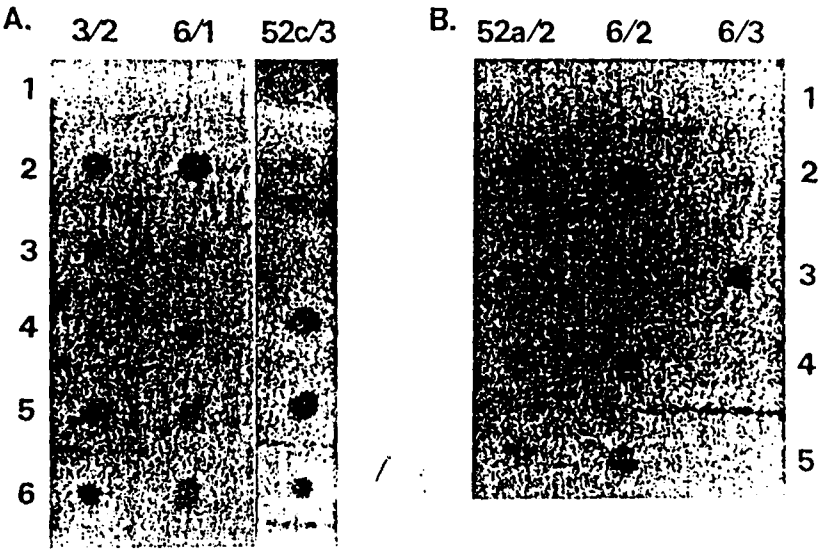


FIGURE 2 Hybridization of RNA with oligonucleotide probes described in Table 3. A. Probes 3/2, 6/1, and 52c/3 with specificity for DR3βI genes. B. Probes 52a/2, 6/2, and 6/3 with specificity for DRw52a and DRw52b genes. Cell lines: 1, 3164 (DR4,DQw3); 2, AVL [DR3(w17),DQw2]; 3, QBL [DR3(w17),DQw2]; 4, 1014 [DR3(w18),2; DQw4,w1]; 5, 2041 [DR3(w18),2;DQw4,w1] and 6, 1568 [DR3(w18),1;DQw4,1].

sequence from the DR3(w18),DQw4 haplotype differs from the DR3(w17) protein sequence by only four replacement substitutions in the first domain at positions 26 (Phe vs. Tyr), 28 (Glu vs. Asp), 47 (Phe vs. Tyr), and 86 (Gly vs. Val). All of the amino acid substitutions are relatively conservative and involve one or two nucleotide substitutions. The substitutions are located at positions that are variable in other DRβ genes. A single silent substitution is found in the second domain at position 112 (CAC vs. CAT). The cDNA clones differ from DRw52βIII sequences [32,34,35] at a number of positions throughout the coding sequence (Figure 1). A cDNA clone encoding a DR5βI gene was identified in the B-LCL 1563 amplified gene products (unpublished data). Two other DRβ cDNA clones isolated from the B-LCL 2041 library are identical to sequences derived from a DR2 cell [36].

RNA from B-LCL 2041 and two other individuals expressing the DR3 (w18),DQw4 haplotype, B-LCL 1014 and 1568 (Table 1), hybridized with an oligonucleotide probe specific for the region encoding amino acids 9–14 of the DRβI gene (probe 6/1) (Figure 2A, Table 3) (Table 3 and Figure 1 describe the oligonucleotides), suggesting conservation of the DR3βI sequence in this region which was missing in the cDNA clone from the B-LCL 2041 library. In addition, RNA from all three cell lines hybridized with probe 3/2, which is specific for the 3′ end (codons 74–79) of the third variable region (codons 68–75) of the DRβI gene. This sequence is so far unique to DR3. Both oligonucleotides also hybridized to RNA from DR3(w17) HTC, AVL and QBL, and failed to hybridized to RNA from a DR4 HTC, 3164. Finally, RNA from all three DR(w18),DQw4 cell lines hybridized to a probe (52c/3) which corresponds to a sequence found either in the DR3-encoding βI gene of the DR3(w18),DQw4 haplotype or in the DRw52c β gene (codons 26–32) [35]. As these cells are

DRw52a (described below), this hybridization suggests that B-LCL 1014 and 1568 contain the same sequence polymorphism of DR3 as B-LCL 1563 and 2041, which were the sources of the DR3(w18),DQw4 cDNA sequence. RNA from AVL, QBL, and 3164 did not hybridize to the 52c/3 probe.

The DR3(w18),DQw4 Haplotype Expresses a DRw52a Allele

RNA oligonucleotide hybridization was used to identify the DRw52 allele in three DR3(w18),DQw4 unrelated individuals who do not express two DRw52 haplotypes (B-LCL 1014, 2041, and 1568) (Table 1). Figure 2B shows the hybridization data from B-LCL 1014 and 2041. The RNA from the cells was positive with both the DRw52a probes (52a/2 and 6/2) and negative with two DRw52b probes (6/3 and 52b/2) (data from 52b/2 is not shown), indicating that these individuals express DRw52a. One of the DRw52c probes, 52c/2, also hybridized as discussed above. As expected [37], the DRw52a-specific probes hybridized to RNA from AVL, and the DRw52b-specific probes hybridized to RNA from QBL. None of the probes hybridized to a DR4 HTC, 3164. The hybridization studies are supported by the T-cell clone typing of the DR3 (w18),DQw4 haplotype as LB-Q1 negative (Tables 1 and 2). DRw52-positive cells which lack the LB-Q1 specificity usually express DRw52a or DRw52c alleles [37].

DRβ Restriction Fragments Associated with the DR3(w18),DQw4 Haplotype

Polymorphism in DNA restriction fragments was used to assess the homogeneity of the DR3(w18),DQw4 haplotype in the American black population. Polymorphism in the DRβ region was probed using the restriction enzyme *Taq*I and a DRβ 3′ untranslated region probe. Two DRβ fragments associated with the DR3(w18),DQw4 haplotype can be identified using family 014 (Table 2; Figure 3). An 11.5-kb fragment is observed in father (a/b), mother (c/d), and two siblings (a/c and b/d). Sibling a/d lacks this fragment, thus assigning the fragment to the b [DR3(w18),DQw4] and c (DR−,DQw2) haplotypes. A second fragment at 6.3 kb shows a similar segregation. The 11.5- and 6.3-kb DNA fragments associated with the DR3(w18),DQw4 haplotype in the family are also observed in several DR3(w18),DQw4-positive unrelated individuals, B-LCL 1559, 1401, 1563, 1014, and 2041 (Table 1; Figure 4). (Other DNA fragments observed in Figure 4 can be tentatively assigned to the non-DR3 haplotypes in the unrelated individuals by comparison with fragments from DR homozygous cells with similar DR specificities. For example, B-LCL 2041 and 1014, which express a DR2 haplotype, exhibit fragments which coelectrophorese with fragments from the DR2 HTC, PGF.)

The two DR3(w17),DQw2 HTC, AVL and QBL, share a 7.4-kb *Taq*I DRβ restriction fragment but differ in the presence of a higher-molecular-weight fragment associated with the DRw52βIII gene [38,39]. The 11.5-kb fragment in AVL is shared with the b and c haplotypes in family 014, while the 14.2-kb fragment in QBL is shared with the d haplotype. A DNA fragment at 2.2 kb is shared by all cells and likely represents a DRβ pseudogene.

Therefore, all of the DR3(w18),DQw4 individuals share 11.5- and 6.3-kb bands associated with DRβIII and DRβI, respectively. The 6.3-kb band is associated with this haplotype and two related haplotypes [DR−,DQw2; DR5,DQw3 (HTC IDF)] and differs from DR3(w17) HTC, AVL and QBL. The

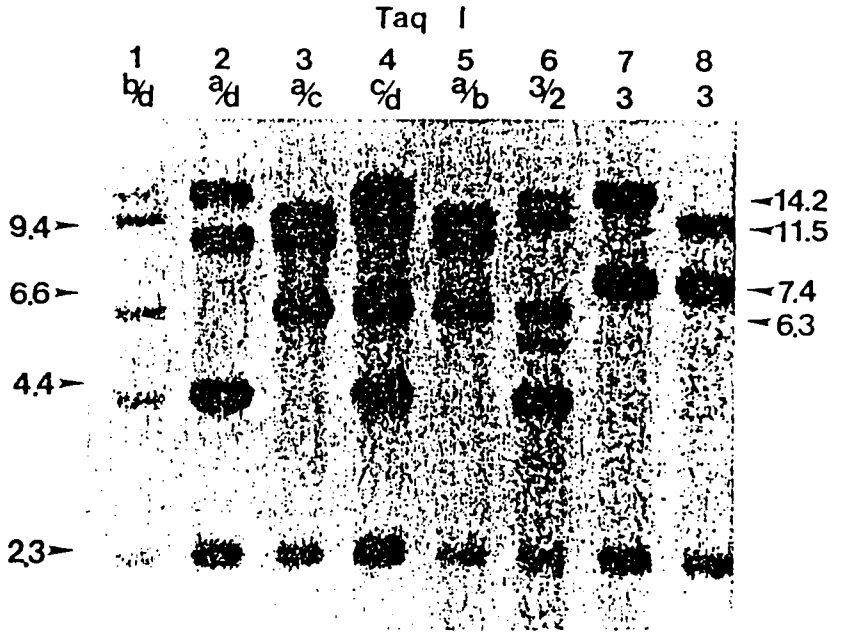


FIGURE 3 DNA digested with *Taq*I and probed with a DRβ 3' probe. Lanes 1–5 are family members described in Table 2. 1, 2710 [DR3(w18),w13;DQw4,w1]; 2, 1066 (DRw8,w13;DQw3,w1); 3, 2704 (DRw8,−;DQw3,w2); 4, 2708 (DR−,w13;DQw2,w1); 5, 2707 [DRw8,3(w18);DQw3,w4]; 6, 1014 [DR3(w18),2;DQw4,w1]; 7, QBL [DR3 (w17),DQw2]; 8, AVL [DR3(w17),DQw2]. The positions of λ *Hind*III DNA fragments are indicated on the left side of the figure; the right side lists sizes of fragments discussed in the text.

11.5-kb fragment observed in the DR3(w18),DQw4 individuals is shared with some DR3 haplotypes (e.g., AVL). This correlates with the oligonucleotide hybridization data which demonstrate that the DR3(w18),DQw4 haplotype, like AVL, carries a DRw52a allele.

DRα DNA Restriction Fragments Associated with the DR3(w18),DQw4 Haplotype and Shared with the HLA-B8–associated DR3(w17) Haplotype

DNA sequence polymorphisms closely linked to the 3' end of the DRα gene [40] were used to define DRα DNA restriction fragments associated with the DR3(w18),DQw4 haplotype and to analyze the homogeneity of the DR3 (w18),DQw4 haplotype in the American black population. DNA from family 014 (Table 2) digested with *Bgl*II and probed with a DRα cDNA exhibited one or two fragments (Figure 5). All of the family members shared a 4.5-kb fragment. All except sibling a/d expressed a 4.2-kb fragment. This assigns the 4.2-kb fragment to the b [DR3(w18),DQw4] and c (DR−,DQw2) haplotypes. Analysis of DNA digested with *Bgl*II from six unrelated individuals (Table 1) also reveals one or two fragments (Figure 5 and data not shown). All of the DR3(w18),DQw4 individuals exhibit the 4.2-kb fragment associated with the DR3(w18),DQw4 haplotype of family 014. B-LCL 1563, 1014, and 1568 also show a 4.5-kb

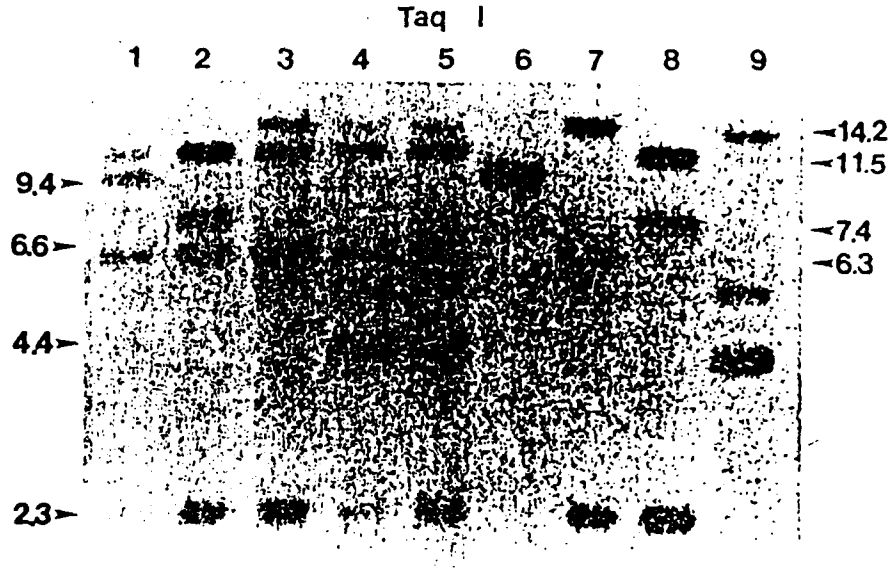
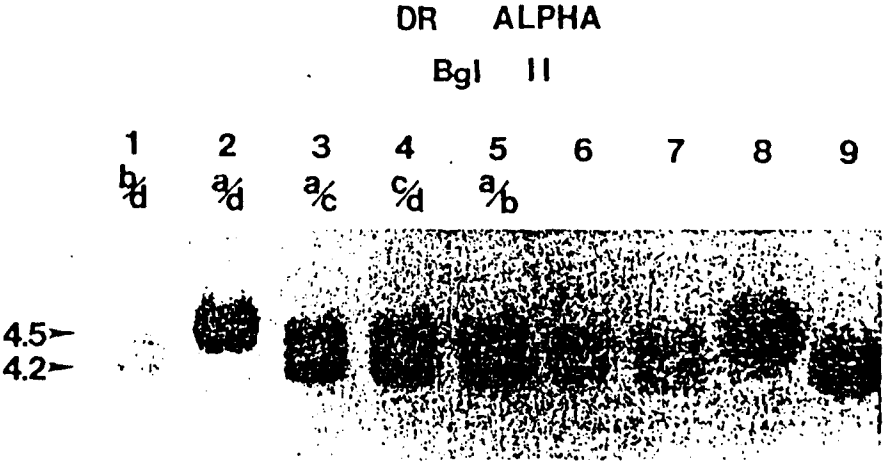


FIGURE 4 DNA digested with *Taq*I and probed with a DRβ 3' probe. 1, 1559 (DR3(w18),w8;DQw4); 2, 1401 [DR3(w18),−;DQw4,w3]; 3, 1563 [DR3(w18), w11;DQw4,w3]; 4, 1014 [DR3(w18),2;DQw4,1]; 5 2041 [DR3(w18),2;DQw4,w1]; 6, ARC (DRw8,DQw4); 7, IDF (DRw5,DQw3); 8, AVL [DR3(w17),DQw2]; 9 PGF (DR2,DQw1). The positions of λ *Hind*III DNA fragments are indicated on the left side of the figure; the right side lists sizes of fragments discussed in the text.

FIGURE 5 DNA digested with *Bgl*II and probed with a DRα probe. 1, 2710 [DR3(w18),w13;DQw4,w1]; 2, 1066 (DRw8w13;DQw3,w1); 3, 2704 (DRw8,−; DQw3,w2); 4, 2708 (DR−,w13;DQw2,w1); 5, 2707 [DRw8,3(w18),DQw3,w4]; 6, 2041 [DR3(w18),2;DQw4,w1]; 7, 1559 [DR3(w18),w8;DQw4]; 8, QBL [DR3(w17), DQw2]; 9, AVL [DR3(w17),DQw2].



fragment. Similar results were observed with *Eco*RV (data not shown) in which a 9.2-kb *Eco*RV fragment is associated with the DR3(w18),DQw4 haplotype. As demonstrated in a previous study [40], 4.2-kb *Bgl*II and 9.2-kb *Eco*RV fragments were associated with DR3(w17),DQw2 haplotypes which express HLA-B8 (AVL), while other DR3(w17),DQw2 haplotypes which do not express HLA-B8 (QBL) exhibited 4.5-kb *Bgl*II and 13-kb *Eco*RV fragments. The Southern blot hybridization studies with DRα suggest homogeneity within the individuals expressing the DR3(w18),DQw4 haplotype and a similarity to the allele of DRα carried by the HLA-B8–associated DR3(w17),DQw2 haplotype.

DISCUSSION

The polymorphism within the DR3 haplotypes is only beginning to be appreciated. Not only can the DR3 specificity be associated with at least two DRw52 alleles, DRw52a (LB-Q1 negative) and DRw52b (LB-Q1 positive) [16,37], but it has also been observed in conjunction with a variety of DQ alleles, DQw2, DQw3, and DQw4 [14,41]. Using restriction fragment length polymorphism analysis, unexpressed polymorphisms associated with the DXβ [14] and DRα [40] genes have also been found in DR3 haplotypes. This study describes two DRβI alleles associated with the DR3 haplotype. The DR3(w17) allele associated with DQw2 has been found in four different DR3(w17) cells [30–33] in combination with several different HLA-B alleles including B8 and B17. The second DR3 allele [DR3(w18)], associated with DQw4, is described in this study.

The derived protein sequences of the two DRβI genes from the DR3 haplotypes differ by several conservative amino acid substitutions which are spread throughout the first domain (amino acids 1–94). Two of these differences lie in the second variable region (amino acids 26–38). The other two differences are in positions outside of variable regions that vary in other DR alleles. Again, as observed among other class II allelic products [1,42], the majority of nucleotide differences between the two DRβI genes result in amino acid substitutions. All four variant codons in the first domain result in substitutions, while the only nucleotide difference in the second domain is silent.

The DR3 haplotype is postulated to have arisen from a DRw6(13) haplotype through gene conversion between DRβI and βIII genes [32]. An exchange in the region encoding the third variable region (codons 68–75) has been proposed as the basis of the event which generated an ancestral DR3βI sequence. Since the DR3(w17) (AVL) and the DR3(w18) DRβI sequences share the region around amino acid 70, it is likely that the DR3(w18) DRβI gene arose from the same event. Additional gene-conversion-like events involving the region encoding amino acids 26–30 could have generated some of the differences between the DR3(w18)βI and DR3(w17)βI genes from an interaction between the ancestral DR3βI gene and donor DRw52βIII genes (Figure 1) [32]. Such an event would involve a donor DRw52cβIII gene to generate the DR3(w18) sequence.

None of the differences in the DR3βI gene identified in B-LCL 1563 and 2041 affect the broad DR3 serologic determinant(s) carried by the haplotype. It is difficult, however, to determine the structural basis of this shared serologic determinant since there are no regions of the DRβ protein sequence unique to the DR3 haplotypes. Although both DRβI polypeptides appear to have identical sequences in the first (amino acids 9–13) and third (amino acids 68–75) variable regions, these sequences are also shared with other DR and DRw52 alleles. This implies that the broad DR3 serologic determinant(s) is not localized on a linear sequence but is conformational in nature. The differences in the DRβI polypep-

tide may, however, affect the mixed lymphocyte typing response contributing to the undefined HLA-D specificity of this haplotype.

The DR3(w18),DQw4 haplotype is similar to the HLA-B8–associated DR3(w17) haplotype (represented by AVL) in that it carries a DRw52a allele and shares DRα *Bgl*II and *Eco*RV DNA restriction enzyme fragments. Other DR3(w17) haplotypes such as the B18,DR3(w17) HTC QBL express a DRw52b allele [37] and carry a different DRα gene fragment [40,43]. The DRα gene, the most telomeric of the known class II genes [44], is located adjacent to the less polymorphic DRβ gene (DRw53) in a DR4 haplotype [45]. The close linkage of a particular DRw52 subtype to DRα gene polymorphisms suggests that this α-β gene organization is also present in the DR3 haplotype. The association of DRw52 and DRα alleles also suggests that little if any recombination has occurred between DRβIII and DRα genes. This is in contrast to the recombination which may have occurred between DRβ loci to generate different DR3/DRw52 combinations [37].

These observations allow some speculation as to the possible evolutionary relationships among the various DR3 haplotypes. The divergence of the DR3(w17),DQw2 and the DR3(w18),DQw4 haplotypes is probably an old event, possibly coinciding with the separation of the Negroid and Caucasian racial groups. This is supported by the presence of multiple differences in both the DR and DQ subregions of these two haplotypes—differences which appear to have arisen by multiple genetic mechanisms including reciprocal recombination, gene coversion, and point mutation. If this view is correct, it would imply that the gene conversion event which gave rise to the ancestral DR3 haplotype from a DRw6 haplotype may have occurred prior to the divergence of the racial groups. Presumably, subsequent divergence of the HLA-B8–associated DR(w17),DQw2 haplotype from other DR3(w17),DQw2 haplotypes occurred more recently by recombination within the DR subregion.

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