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HLA-DR Typing "at the DNA Level": RFLPs and Subtypes Detected with a DRβ cDNA Probe

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Summary

The HLA-DRβ gene, used as a hybridization probe, detects RFLPs that correlate with HLA-DR specificities. Using genomic DNA from more than 200 individuals, we have carried out a population study with a cDNA probe for the DRβ chain, which, under appropriate conditions, does not cross-hybridize with genes from other HLA-D subregions (e.g., DP and DQ). We first assessed the correspondence between serologically defined HLA-DR types and DNA patterns obtained after digestion with *TaqI* and found that DNA patterns allowed us to identify most specificities. Only two pairs of antigens are not distinguishable: with the DRβ probe alone we cannot distinguish DR3 from DRw6 or DR7 from DRw9. However, the correct assignment can always be made for the first pair by hybridizing the same digests with a DQα or DQβ probe. Thus DR typing from the DNA patterns is practical and accurate. We also looked for serologically undetectable subtypes. RFLPs revealed high-frequency subtypes for the specificities DR 2, 3, 5, w6, 7, and w9. Some of these are more accurately viewed as variant haplotypes, since the relevant variation is probably not at the DRβ locus that determines the serological specificities but rather at other closely linked and highly homologous DRβ loci such as DRβ-III. Nevertheless, the existence of variant haplotypes for so many specificities indicates a wealth of polymorphic variation beyond that detected serologically and provides more specific markers for studies of various diseases associated with HLA-DR specificities.

Introduction

The class II or HLA-D region antigens of the human major histocompatibility complex (MHC) play a central role in the regulation of the immune response. These antigens are heterodimeric molecules consisting of a heavy (α) and light (β) chain noncovalently associated on the cell surface (for review, see Trowsdale et al. 1985). The best-studied of these molecules, and the genes that code for them, are the members of two closely linked allelic series: HLA-DR and -DQ. The HLA-DR and -DQ products (homologous to mouse I–E and I–A, respectively) were originally defined by polyclonal antisera, and serological studies have revealed an extraordinary wealth of allelic variation at these loci. More recently, many of the genes of the HLA-D region have been cloned, and it has become clear that, when they are used as probes in Southern hybridization, a high level of variation is revealed at the DNA level as well (Wake et al. 1982). It is therefore of considerable interest to determine how well the serologically defined alleles correspond to variation detected as DNA polymorphisms (RFLPs) in the vicinity of the HLA-D region genes.

Numerous studies have shown that RFLP patterns detected with probes from the DR region (Wake et al. 1982; Andersson et al. 1984; Kohonen-Corish and Serjeantson 1986; Bell et al. 1987) or DQ-region (Owerbach et al. 1983; Andersson et al. 1984; So et al. 1984; Spielman et al. 1984) are strongly correlated with HLA-DR types. Furthermore, these studies have suggested that variation detected by RFLPs is far more extensive than that detected serologically. However, in most of these studies, each DR specificity is represented by only two to five individuals, usually homozygous for the an-

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tigen. The extent of variation *within* DR specificities cannot be accurately determined with such small samples. Although some studies have used larger samples (Cohen et al. 1984; Boehme et al. 1985; Tilanus et al. 1986), there has been little systematic assessment of the extent of variation in RFLP patterns corresponding to a single DR antigen. This type of variation is of special interest because it indicates the possible existence of "subtypes" not detected when the DR antigens are defined serologically. From a technical viewpoint, the conclusion has been reached (Cohen et al. 1984; Tilanus et al. 1986) that several restriction enzymes and possibly several probes will be required for accurate "DNA typing," or, more pessimistically, that RFLPs are simply not informative enough and that hybridization with multiple sequence-specific oligonucleotides will be needed (Angelini et al. 1986).

We have studied a large number of unrelated individuals and families typed for HLA-A, -B, and -C and -DR alleles. After pilot studies with several restriction endonucleases, we concluded that *Taq*I provides the best resolution; that is, it reveals the most RFLP variation among and within DR (and DQ) types. In the present study we describe in detail the correspondence between DR specificities and RFLPs defined with *Taq*I, and the extent of subtype variation revealed within DR types. We show that, except for two pairs, all DR specificities can be distinguished by their *Taq*I patterns, and we illustrate how variation in the DQ subregion, as detected with a DQα probe, allows discrimination in one of these cases. The results allow a thorough assessment of the practicality of DR typing "at the DNA level," and show that subtypes of most of the common DR specificities can be defined by DNA polymorphisms.

Material and Methods

Subjects

Subjects were recruited without regard to HLA type from the donor panel of the Tissue-Typing Laboratory at the Hospital of the University of Pennsylvania. The sample studied includes 55 blacks and eight Orientals as well as 152 Caucasians. In the present study, however, we report results for Caucasians only, because of the well-known difficulties in typing non-Caucasians with conventional antisera. DNA was also available from members of four normal control families. In addition, we studied DNA from 30 cell lines derived from individuals homozygous (in most cases as products of consanguineous matings) for the entire HLA region and representing various DR specificities.

To enlarge the sample studied, we also made use of DNA obtained from patients with insulin-dependent diabetes mellitus (IDDM) or multiple sclerosis (MS) and from their family members. Because of the well-established associations between these diseases and HLA, the DR specificities of these patients are clearly not a random sample from the Caucasian population. We therefore used these materials only where we found uniformity of DNA band patterns *within* DR specificities (see Results) and to confirm segregation within families.

Laboratory Methods

HLA typing was carried out by the standard two-stage microcytotoxicity test (Ray 1980) with reagents capable of recognizing most of the known HLA-A and -B specificities and -DR antigens DR1-w10.

DNA was extracted from 20 or 30 ml of heparinized blood (Kunkel et al. 1977). A sample of DNA (6 μg) was digested to completion with *Taq*I, using conditions specified by the manufacturer (BRL or Boehringer). DNA fragments were separated according to size by electrophoresis for 20–26 h at 40–70 V in 0.7% agarose in Tris-acetate buffer (Maniatis et al. 1982) and were blotted to Zetabind (AMF Cuno) or Nylon 66 (MSI) filters. DNA fragments of known size from phage lambda (*Hin*dIII digest) or phi X 174 (*Hae*III digest) were included in the gel as size markers.

Two probes for HLA-D-region molecules were used. For HLA-DR we used the ~520-bp *Pst*I fragment from a cDNA ("β1") for the DRβ chain (Long et al. 1983) provided by Eric Long. This fragment contains the coding region for the 3′ part of the first and most of the second domain. For HLA-DQα, we used a 2.1-kb genomic DQα probe (Trowsdale et al. 1983). Filters were prehybridized overnight at 65 C in 6 × SSC (1 × SSC = 150 mM NaCl, 15 mM trisodium citrate) containing 10 × Denhardt's solution (1 × Denhardt's solution = 0.02% BSA, 0.02% Ficoll, 0.02% polyvinylpyrrolidine), 10% (wt/vol) dextran sulfate, 0.5% SDS, and denatured salmon sperm DNA (100 μg/ml). Filters were hybridized for 16 or 40 h at 65 C in fresh hybridization buffer to which probe labeled with ^{32}P (Feinberg and Vogelstein 1983) had been added at a concentration of ~2–5 × 10^6 cpm/ml. Filters were washed twice for 1 h at 65 C in 0.1 × SSC, 0.1% SDS. Autoradiograms were prepared by exposing XAR-5 film to filters at −70 C for several days in cassettes containing intensifying screens.

Band positions on autoradiograms were converted to estimated DNA fragment sizes as follows. The third-

order polynomial regression of size as a function of position (migration from origin) was determined for the phage fragments. This regression equation was then used to "predict" the size of the genomic bands from their positions. The FORTRAN computer program written for this purpose (available on request) runs on an IBM microcomputer equipped with a math coprocessor.

Results

DRβ Chain as Probe

Figure 1 shows a typical autoradiogram of a Southern transfer of DNA digested with *Taq*I and hybridized with the DRβ probe. Despite the inevitable variation in size estimates taken from different gels, there is very high consistency of band *patterns* seen in different gels (see below). For this reason, instead of using estimated fragment sizes, we prefer simply to number bands from largest to smallest as shown in figure 1 and use these numbers as identifiers. Table 1 shows the sizes of the fragments that give rise to the bands identified in this way. The same band number is used for fragments that appear to be the same size, even when they are found in different DR specificities. It is possible that in some cases these bands actually represent different DNA fragments that happen to be approximately the same size (see below). The variable number of bands per track in figure 1, as well as the large number of band positions, make the band patterns quite complex. For this reason, simpler autoradiograms obtained with homozygotes for the DR specificities are often presented (see also figure 3 below). Here, however, we prefer to demonstrate the interpretation of band patterns in random individuals who are usually heterozygotes, as will be necessary in practice.

When the band patterns for a number of individuals, all sharing at least one DR specificity, are compared, it is straightforward to identify the band pattern they share. This was done for each antigen by systematically comparing the appropriate individuals. Where possible, a prior idea was obtained from the simpler band patterns found in individuals homozygous for DR. For example, by starting with the homozygote for DR1

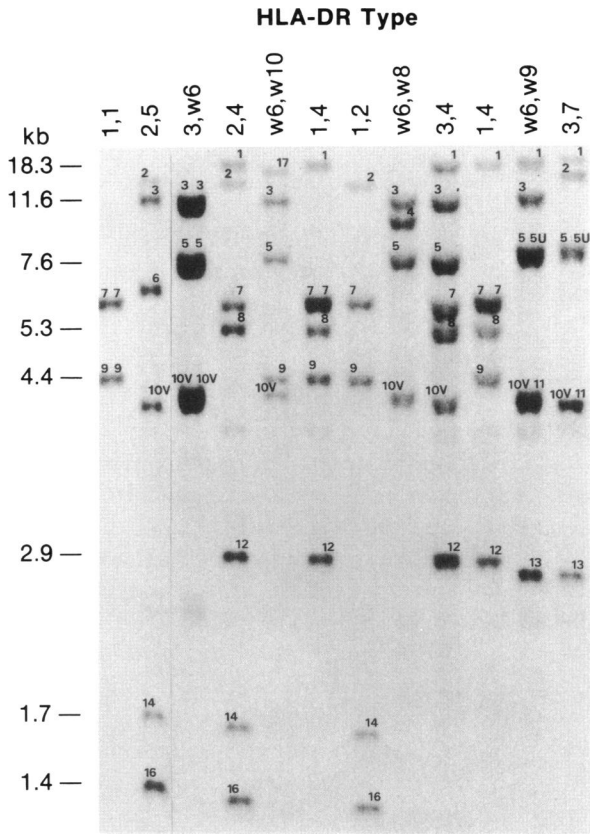

Figure 1 Representative *Taq*I DNA band patterns detected with the DRβ probe after Southern blotting. The numbers on the bands are arranged in two columns to indicate the band patterns (haplotypes) found with each DR antigen. The sizes of the fragments are given in table 1.

Table 1

Sizes (in kb) of *Taq*I Fragments (bands) Detected with the DRβ Probe

	Band																	
	1	17	2	3	4	5	6	7	8	9	10	10V	11	12	13	14	15	16
Size	18.3	15.0	13.6	11.6	9.5	7.6	6.3	5.8	5.3	4.4	4.2	(4.2–3.9)	4.0	2.9	2.7	1.7	1.6	1.4
SE	.6	.5	.4	.2	.26	.12	.13	.09	.07	.07	.05	.06	.05	.09	.05	.03	.03	.03

NOTE.—Estimates were obtained by polynomial regression (see Material and Methods) from at least 15 gels for most antigens (at least five gels for DRw8, DRw10, and DN1). SE = standard error.

(bands 7, 9) on the left in figure 1, we deduced the patterns for DR2 (2, 14, 16) and DR4 (1, 7, 8, 12) from the heterozygous DR1,2 and DR1,4 individuals. These patterns can then be confirmed in the DR2,4 individual, etc. This process of identifying shared band patterns was carried out for all the DR specificities, DR1–DRw10. With the exception of those for the rare specificity DRw10, all band patterns deduced in this way and described below have also been confirmed by segregation in at least one family. Figure 2 shows segregation of band patterns in a representative family. With practice, it is easy to identify the band patterns on an autoradiogram, and it is much easier to remember a group of four integers (e.g., 1,7,8,12 for DR4) than the corresponding set of estimated fragment sizes in kilobase pairs.

Several complications arose as we designed a numbering system for *Taq*/DRβ bands. First, the distinctive band for DRw10 was recognized after the other band numbers had been assigned, so it bears the number 17 despite its position between bands 1 and 2. Second, for the fragments originally labeled bands 5 and 14, we now recognize two alternative bands, and in each case we indicate the less common variant with the letter U (upper) or L (lower), reflecting its position relative to the more common variant as appropriate. We thus have additional bands 5U and 14L. Third, in the positions for bands 10 and 11, we found a seemingly continuous, and initially bewildering, distribution of sizes. Careful inspection showed that the largest of these fragments, ~4.2 kb, is found with the specificity "DN1" (a new specificity on Terasaki trays) and does not vary among individuals who type as DN1; we call this band 10. Similarly, DR7 and DRw9 have a smaller fragment, ~4.0 kb, that does not vary detectably in size; we call this band 11. Any other band that occurs in this region is called 10V ("10 variable"). Finally, it appears that the band 3 in the 3, 6, 10V pattern of DR5 may be larger than the band 3 in the 3, 5, 10V pattern of DR3 and DRw6. We have studied so few individuals with the 3, 6, 10V pattern, however, that this difference cannot be considered as established. Most of the band patterns described above are shown in figure 1; those that are not are discussed below and illustrated in figure 3.

We note that the "identical" migration of bands does not necessarily imply that the fragments are identical in size or origin. For example, we do not know whether the same DNA fragment gives rise to the band 2 in the DR2 pattern (bands 2, 14, 16) and the band 2 in the DR3 and DRw6 pattern (bands 2, 5, 10V). The variability noted above with respect to bands 5 and 14 (upper and lower), 10V, and possibly band 3, reinforces this caution. We have not, however, detected this kind of subtle variation in size for any other bands.

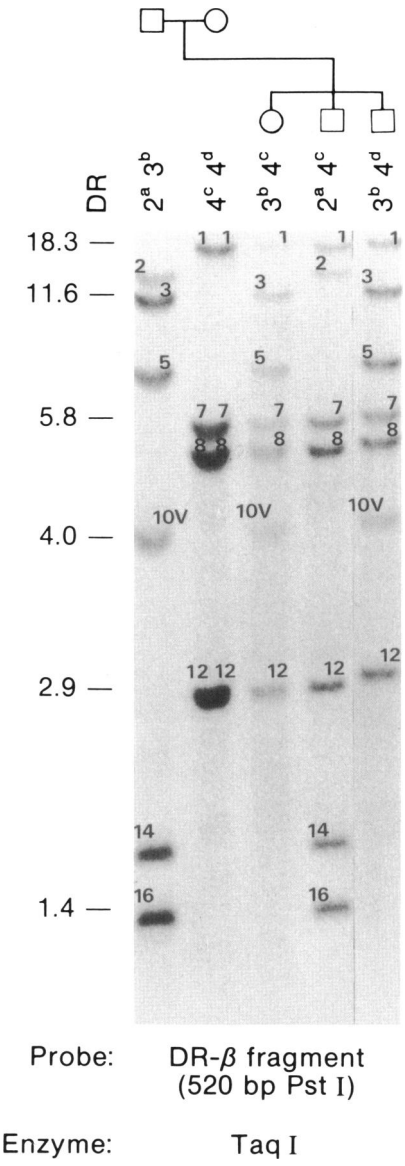

Figure 2 Mendelian segregation of *Taq*I DNA band patterns, detected in Southern blots, with the DRβ probe. Columns of numbers on the bands indicate patterns specific for each HLA-DR specificity.

For each HLA-DR specificity, we have tabulated the number of occurrences in our sample and the number and distribution of band patterns found. Table 2 shows the results for the specificities whose band patterns are "distinctive"; that is, their patterns are never found with

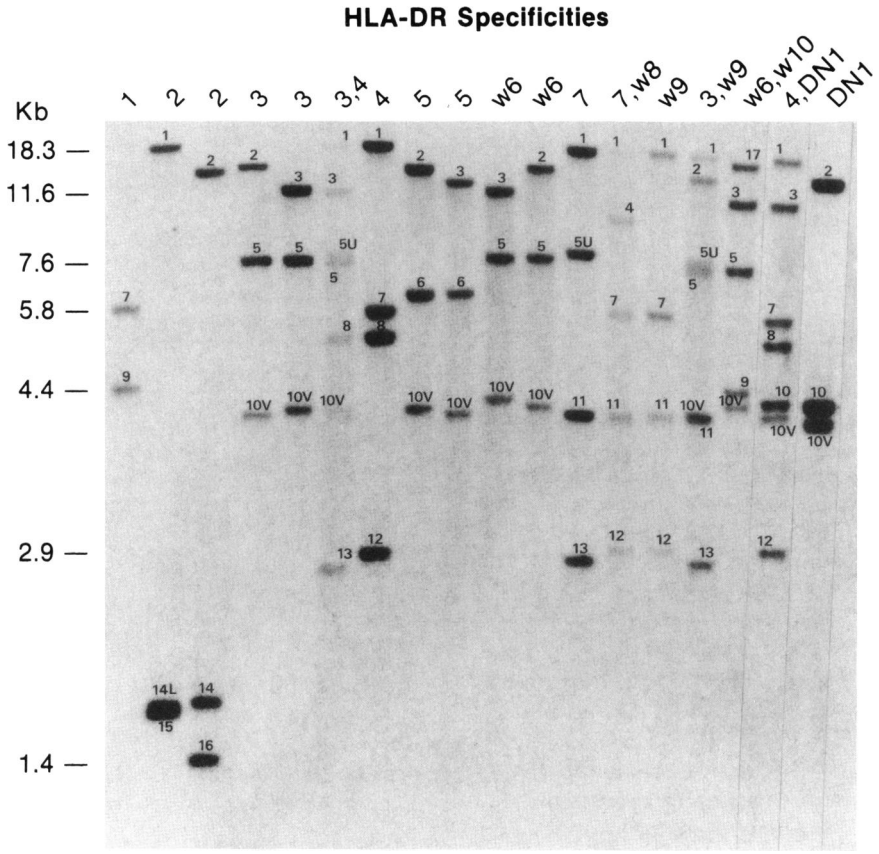

Figure 3 *TaqI* DNA patterns detected with the DRβ probe after Southern blotting. The autoradiogram shows *every* combination of DR specificity and DNA pattern we have observed. The lane labeled DN1 (*far right*) contains DNA from the cell line Herluf (Jakobsen et al. 1986).

any other antigen, and the specificity can therefore be reliably identified from the band patterns. These specificities are DR1 (bands 7, 9), DR2 (bands 2, 14, 16 or 1, 14L, 15), DR4 (bands 1, 7, 8, 12 or very rarely 1, 5U, 8, 13), DR 5 (bands 2, 6, 10V or 3, 6, 10V), DRw8 (band 4 only), DRw10 (bands 17, 9), and DN1 (bands 2, 10, 10V or 3, 10, 10V). Note that the last three antigens in table 2 have multiple band patterns (subtypes) but that the patterns are nonetheless distinctive. For DR1, 4, and w10, specificities that do not show polymorphic variation in associated band pattern, we have included data from patients with IDDM or MS.

DN1 is a new specificity that has only recently appeared on commercial typing trays, and not all our subjects were typed for it. Because it appears to be more common than DRw10 in our sample and has distinctive band patterns which have been demonstrated to segregate in families, we have included it in our studies. DN1 has cross-reactivity with some DR5 and some DRw8 antisera, and Schreuder et al. (1984) have found that some DN1-positive cells are also positive for DRw12. The homozygous cell line Herluf (Jakobsen et al. 1986) has the DN1 band pattern 2, 10, 10V; see figure 3 below.

The specificities DR5 and DRw6 required special consideration. It is well-known that DRw6 is a heterogeneous and poorly defined specificity. Among 31 individuals whose antigen would be designated DRw6 by conventional criteria, we found four with the *Taq*/DRβ band 6 (3, 6, 10V pattern), which is otherwise found only with DR5 (see table 2). Reexamining the serological reactivity of cells from these individuals, we found evidence for reactivity with a subset of the DR5 antisera, reflecting a known serum "cluster" consisting of DR5 plus the DRw13 subtype of DRw6 (Schreuder et al. 1984). The DNA band pattern, in conjunction with this observation, leads us to designate these antigens as DR5—not as DRw6—in table 2, and results with

Table 2

Distribution of DNA Band Patterns for HLA-DR Antigens with "Distinctive" *TaqI* Band Patterns after Hybridization with a DRβ Probe

Antigen	Bands	No. Observed[a]	Frequency
A. No Polymorphic Subtypes[b]			
DR 1	7, 9	59	1.0
DR 4	1, 7, 8, 12	143	1.0[c]
DR w8	4	19	1.0
DR w10	17, 9	5	1.0
B. Polymorphic Subtypes Present[d]			
DR 2a	2, 14, 16	25	.81
DR 2b	1, 14L, 15	6	.19
DR 5a	2, 6, 10V	24	.83
DR 5b	3, 6, 10V	5	.17
DN 1a	2, 10, 10V	3 + 2[e]	.83
DN 1b	3, 10, 10V	1	.17

NOTE.—See text for derivation of band patterns. The more frequent subtype is designated "a" (e.g., DR2a).

[a] Number of antigens (haplotypes) observed.

[b] All Caucasian subjects (blood donors, unrelated diabetic and MS patients).

[c] Variant DR4 pattern 1, 5U, 8, 13 found segregating in one IDDM family and in one black MS patient; not found among 143 haplotypes of Caucasian controls, MS patients, or other IDDM patients.

[d] Caucasian controls (blood donors) only.

[e] DN1 by DNA pattern (DN1 serum not available when typed). The 3, 10, 10V pattern was also seen with DN1 in two non-Caucasians and in one MS family.

a DQα probe (see below) also provide support for this conclusion. Among the four DR5s identified in this way, all had the usual DR5-associated DQα pattern.

Four HLA-DR specificities do *not* give rise to distinctive band patterns when the DRβ probe is used after *TaqI* digestion. As shown in table 3, DR3 is associated with two band patterns (2, 5, 10V and 3, 5, 10V) that are also found with DRw6, and DR7 is associated with two band patterns, 1, 7, 11, 12 and 1, 5U, 11, 13) that are also found with DRw9. Thus these four antigens can be grouped into two pairs, reflecting recognized cross-reacting clusters defined by serology (Albert et al. 1984). The members of each pair differ somewhat with respect to the relative frequencies of subtypes. However, given the modest sample sizes, it is not clear whether these differences are significant. Consequently the band patterns do not aid appreciably in discriminating between DR3 and DRw6 or between DR7 and DRw9.

Table 3

Distribution of DNA Band Patterns for HLA-DR Antigens Not Distinguishable by *TaqI* Band Patterns (DR3 and w6, DR7 and w9)

Antigen	Bands	No. Observed	Frequency
DR 3a	3, 5, 10V	15	.79
DR 3b	2, 5, 10V	4	.21
DR w6a	3, 5, 10V	14	.52
DR w6b	2, 5, 10V	13	.48
DR 7a	1, 7, 11, 12	20	.61
DR 7b	1, 5U, 11, 13	13	.39
DR w9a	1, 7, 11, 12	1	. . .[a]
DR w9b	1, 5U, 11, 13	0	. . .

NOTE.—Data are for Caucasian blood donors only. See text and table 2 for details.

[a] Although the single blood donor with DRw9 had the 1, 7, 11, 12 pattern, the 1, 5U, 11, 13 pattern was also observed with DRw9 in one MS patient and in two IDDM patients and may be considered a common variant. The 1, 7, 11, 12 pattern was seen with DRw9 in two MS patients and in one IDDM patient.

Subtypes Detected with DRβ

Using the DRβ probe, we have found subtypes—that is, more than one DNA band pattern—for the specificities DR2, DR3 and DRw6, DR4, DR5, DR7 and DRw9, and DN1. Figure 3 shows the band pattern for both subtypes of each of these specificities and also includes examples of the specificities (DR1, DRw8, and DRw10) where we have not encountered subtype variation. Thus, figure 3 illustrates every band pattern we have observed in DRβ hybridizations with *TaqI* digests of DNA from Caucasians. The subtypes defined by DNA band patterns result in a high frequency of polymorphism *within* some specificities. The frequency of the minority subtype for a single specificity varied from a maximum of 0.48 for one of the DRw6 subtypes to less than 1% for the rare DR4 subtype. This DR4 subtype (bands 1, 5U, 8, 13) was not found among a total of 143 DR4-positive haplotypes in unrelated Caucasian controls, MS patients, or IDDM patients (table 2) but was segregating in one Caucasian family with two IDDM sibs.

DQα Chain as Probe

Although the DRβ probe used with *TaqI* digests fails to distinguish within two pairs of DR antigens, it is possible to achieve complete discrimination within one of these pairs by using a DQα probe with the same digests. Despite the similarity of DR3 and DRw6 in serology and DRβ/*TaqI* pattern, it is possible to distin-

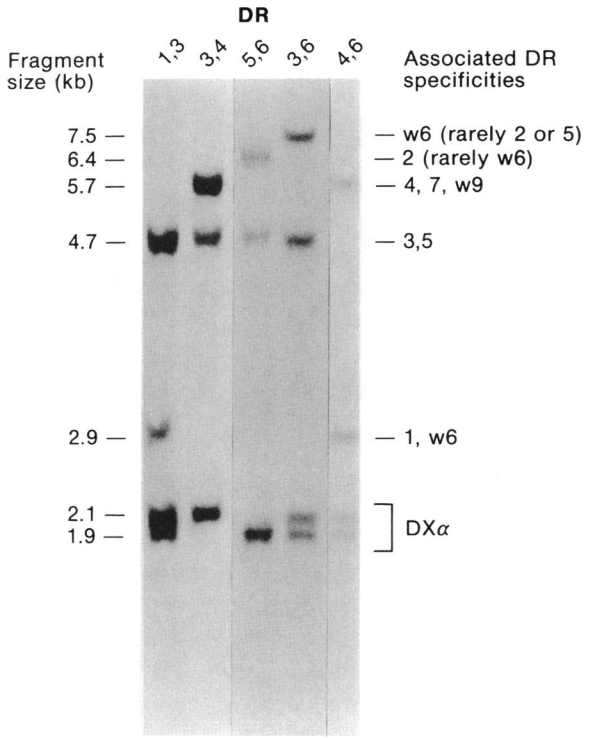

Figure 4 Discrimination between HLA specificities DR3 and DRw6 by *Taq*I DNA patterns detected with a DQα probe after Southern blotting. *All* the DR specificities associated with each DNA band position are shown; the DXα bands represent a separate but closely linked two-allele locus (Spielman et al. 1984). The 4.7-kb band always found with DR3 is easily distinguished from the bands of 7.5 kb, 6.4 kb, or 2.9 kb associated with DRw6 and shown in various heterozygous combinations.

guish these specificities by using the DQα chain gene as a probe, since they differ with respect to their DQα-associated RFLP allele (Spielman et al. 1984; Rosenshine et al. 1986). Figure 4 shows the result of hybridizing, with the DQα probe, a filter containing *Taq*I-digested genomic DNA from representative DR3- and DRw6-positive individuals. We have studied 81 DR3-positive haplotypes and found the DQα 4.7-kb band (see fig. 4) corresponding to the DR3-bearing haplotype in every case. We have not seen this band with any of 73 DRw6 antigens studied. Instead, one of three DRw6-associated bands is always found: 7.5 kb (most common), 6.4 kb (rarer), or 2.9 kb (intermediate in frequency). Thus, in our material there is no overlap in the bands found, and it is possible to distinguish between DR3 and DRw6 with perfect accuracy by using a DQα chain probe. However, there is a rare subtype of DRw6 (Dw16), associated with DQw3 and found by Bosch et al. (1987) to have the DQα 4.7-kb band. The DRβ pattern for Dw16 has not been reported, to our knowledge.

For DR7 and DRw9, discrimination using DRβ or DQα with *Taq*I digests is not possible; DR7 and DRw9 give rise to the same single band in DQα/*Taq*I hybridizations and to the same two band patterns (table 3) in DRβ/*Taq*I hybridizations. In quantitative terms the problem is not very serious because DRw9 is quite rare in Caucasians. In any case, however, some DR7s and DRw9s can be distinguished by using the DQβ chain as a probe (K. J. Gogolin, personal communication).

Discussion

We have used a cDNA probe for a β-chain of HLA-DR to screen DR alleles from several hundred HLA haplotypes for RFLPs. Using the band patterns detected by Southern hybridization after *Taq*I digestion, we can easily and reliably distinguish most of the known DR antigens. Furthermore, subtypes, defined by polymorphism *within* a DR specificity, were found for more than half the specificities studied. The dual goals of DR typing at the DNA level and expanding the resolution of DR typing by identifying subtypes have been met. On a practical level, this makes possible HLA-DR typing from DNA or filters prepared earlier, even if cells or sera for conventional typing are not available. Similarly, linkage studies involving the HLA region can be carried out using these RFLPs, and HLA-DR types can be obtained as a bonus.

Subtypes

We found subtypes—that is, more than one DNA pattern for a single specificity—in eight of the 11 DR specificities we studied: DR2, 3, 4, 5, w6, 7, w9, and DN1. Some of these subtypes are also recognizable by Dw typing or other methods. For example, it appears likely that most DR2a alleles are Dw2 while most DR2b alleles are Dw12 (Bell et al. 1987). Similarly, the serologically defined DRw6 specificity is known to be heterogeneous and includes the Dw alleles Dw9, Dw16, Dw18, and Dw19. It appears, from the small number of homozygotes that have been studied, that these subtypes can be distinguished by *Taq*I RFLP patterns detected with DQα or DQβ probes (Spielman et al. 1984; Rosenshine et al. 1986; Bosch et al. 1987).

There are two general explanations for the finding of more than one band pattern with a single DR specificity. The first, or "classical," explanation is that alleles that are homogeneous when defined serologically are heterogeneous at the DNA sequence level. In

this case, the subtypes are true alleles of the locus that determines the serological variation. The corresponding variation is not detected serologically because it is not expressed (e.g., because it is not in a coding region) or, if expressed, does not alter determinants recognized by available typing sera.

Furthermore, serological specificities continue to be refined, and some subtypes defined by DNA patterns will correspond to newly discovered or future antigen "splits." This appears particularly likely for the rarer DR5b antigens with the 3,6,10V DNA band pattern, whose pattern of *serological* reactivity is clearly different from that of individuals with the common DR5 DNA band pattern (2,6,10V).

Subtypes can also be defined by nonalleleic variation, and this provides a second possible explanation when multiple DNA bands are found with a single specificity. Bands that account for subtype variation may arise from genes that cross-hybridize with—but are not allelic to—those coding for the serologically detected products. In the HLA-DR region, such bands could arise from the closely linked loci DRβ-II (a pseudogene) or β-III, which codes for DRw52 and DRw53 (Gorski and Mach 1986). The subtypes would therefore be defined by variant haplotypes rather than by alleles of a single locus. Gorski and Mach (1986) have shown that the restriction-site difference responsible for bands 2 and 3, associated with DR3 and DRw6, is located in the vicinity of DRβ-III and corresponds to what they call DRw52a and DRw52b. In the case of band 10V, the apparently continuous variation in fragment size suggests that insertion/deletion or tandem duplication is the cause. The locus responsible for the hybridizing fragment may be the pseudogene DRβ-II.

In any case, although the variation in the DNA pattern allows us to identify DR specificities and subtypes, it is not likely that the RFLPs are due to variation in the DNA that codes for serologically detected HLA-DR products. Instead, the DNA variation we observe is probably located in noncoding regions but occurs in near-complete linkage disequilibrium with the serologically detected variation. (For DQβ, see Holbeck and Nepom 1986; for DRβ, see discussion in Bell et al. 1987.)

Discrepancies between Serology and DNA Pattern

Discrepancies—that is, exceptions to the correspondence between DR specificity and DNA pattern as given in tables 2 and 3—were extremely rare. Among all Caucasian subjects, 19 individuals who typed serologically for only one antigen had DNA patterns suggestive of two. Eight of these had band patterns corresponding to specificities (DN1 and DR5b in table 2) not recognizable when the DR typing was done. Of the remaining 11, seven were available for retyping. In six of these, serological evidence for the second antigen predicted by the DNA pattern was found. In the one who remained serologically "blank" for a second antigen, the DNA pattern for the missing or untyped antigen was bands 7 and 9, otherwise found only with DR1. In three of the four who were unavailable for repeat typing, the DNA pattern of the "missing" antigen was also bands 7 and 9, suggesting that this band pattern may be associated with DR1 and a DRβ allele(s) whose product we could not detect serologically. A similar suggestion was made by Keller et al. (1987) in RFLP analyses of DR blank alleles. In the fourth subject unavailable for retyping, the missing antigen was DR2.

Only two individuals appear to be truly discordant for DNA band pattern and serological type. One types serologically for DR4 and DR5, but his DRβ DNA band pattern indicates DR4 and DR1. His DQα/*Taq*I and DQβ/*Taq*I DNA patterns, obtained by rehybridizing the same filter (K. J. Gogolin et al., personal communication), are consistent with DR4 and DR5, not with DR1. One additional individual, serologically DR5,w6, has the DRβ/*Taq*I DNA band pattern 2,3,6,10V, suggesting heterozygosity for DR5a (2,6,10V) and DR5b (3,6, 10V). However, her DQα and DQβ patterns agree with the type DR5,w6. Thus, if we ignore the discrepancies due to serologically untyped products, we have only two discrepancies between serology and DNA pattern among 367 (0.5%) individuals studied (152 controls, 103 IDDM patients or their parents, 112 MS patients or their parents) or 2/734 (0.3%) among haplotypes.

Non-Caucasians

Although we have not included non-Caucasians in our summary tables, our RFLP analyses of a sample of black Americans and of a small sample of Oriental Americans have revealed no patterns not already seen in Caucasians. In addition, the data suggest that the correspondence between DNA band pattern and DR serological type is the same in these ethnic groups as in Caucasians, although the frequencies of the subtypes may differ. The few non-Caucasians with discordance between DNA band pattern and DR serology cluster in the rarer DR antigens (DRw8 and DRw10) and in a subset of individuals who had the 3,6,10V DNA band pattern but were not assigned DR5 by serology (as described for Caucasians in Results).

Assigning TaqI Fragments to Particular DRβ Genes

It is possible to make plausible inferences regarding the assignment of bands to genes (Boehme et al. 1985; Bell et al. 1987). For this purpose, we separate the bands we have identified into two categories, those that are unique to one specificity (or two that are closely related, such as DR1 and DRw10) and those that occur with apparently unrelated specificities, such as DR2 and DR7. We have been unable to resist the temptation to use this distinction in trying to assign bands to particular DRβ loci. We find that with each DR specificity there are one or two bands that are unique to that specificity. It seems likely (but not necessary; see below) that these "diagnostic" bands arise from the locus responsible for the serologically detected DR specificities, DRβ-I. For example, band 9 is found only in DR1 and DRw10, which are closely related specificities (and band 17 is found only in DRw10). Bands 14, 14L, 15, and 16 are found in the two variants of DR2 but with no other antigens. Band 5 is unique to the variants of DR3 and DRw6, while band 6 is unique to the two variants of DR5. Band 8 is unique to DR4, and band 4 is found only with DRw8. Band 11 defines a position unique to DR7 and DRw9. Similarly, band 10 is unique to the two variants of DN1. On the other hand, all the other bands (1, 2, 3, 5U, 7, 10V, 12, and 13) are found with more than one antigen, and, provided we assume that apparent identity of bands is not due to chance comigration, this suggests that they may arise from loci other than DRβ-I, the locus responsible for the serological variation.

Comparing DNA patterns for different DR types, we find that serologically related DR specificities tend to share bands; this is also true for the bands that generate the variation within specificities. Thus, we see a parallel set of variants for DR4 and the pair DR7 and DRw9. For DR4 (bands 1, 7, 8, 12), band 8 is the distinctive band, while band 1 is shared with DR7 and DRw9. Variation within the DR 4, 7, w9 "family" (all DRw53) is due to the additional presence of either bands 7 and 12 or bands 5U and 13. We do not know which of these bands, if any, is due to variation in DRβ-II and which is due to DRβ-III. Similarly, DR3, DR5, DRw6, and DN1 share a set of variants: all may have band 2 or 3, and all have the variable band 10V. DR5, and possibly DN1, belong to the same serologic supertypic cluster as DR3—namely, DRw52—suggesting that bands 2 and 3 in all these specificities have the same origin; this must be DRβ-III, since it is known to be the case (see above) for DR3 and DRw6.

The variation in band number probably reflects, in part, variation in the number of DRβ-like loci present, as has been suggested, e.g., by Boehme et al. (1985) Bell et al. (1987). DRw8, for example, may lack both the DRβ-II and DRβ-III loci, DR2 may lack DRβ-II, and DR1 and DRw10 may lack DRβ-III. Thus, we could speculate that bands 1, 2, and 3 are due to variation attributable to DRβ-III and that band 10V, band 7, band 17, and the 7,12 and 5U,13 variations are due to the DRβ-II locus (or multiple copies of the pseudogene), while bands 4, 5, 6, 8, 9, 10, and 14 (and possibly 17) are DRβ-I bands. Ultimately these uncertainties will be resolved by identifying specific sequences for the relevant DRβ (and DQβ) chains in the bands, for instance by using locus-specific oligonucleotide probes.

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