Brief Definitive Report

PUTATIVE AMINO ACID SEQUENCE OF HLA-DRB CHAIN CONTRIBUTING TO RHEUMATOID ARTHRITIS SUSCEPTIBILITY

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Rheumatoid arthritis (RA) is a common autoimmune disease showing a variety of immune abnormalities. Although the etiology of RA is still unknown, the association between HLA-DR4 and RA has been well established. In Caucasians, many reports confirmed that >60% of the patients have DR4 (1-3). In Japanese, the reported frequencies of DR4 in the patients range from 60 to 70% (4-6). The strong association between DR4 and RA is also reported in many other ethnic groups, such as American Blacks (3, 7, 8), Indians (3, 9), Venezuelan (10), Mexican (3, 8), and Chippewa Indians (11).

In this report, we have focused on the β chain sequence of the DR4 molecule and investigated which part of the chain plays a major role in developing RA. A specific segment of the HLA-DR β (DRB) gene in human genomic DNA was amplified in vitro and then the sequence variation was analyzed with synthetic oligonucleotide probes. A particular DRB1 sequence showed a strong association with RA. Moreover, a comparison of the published sequences of DRB alleles in relation to RA susceptibility enabled us to deduce a putative consensus sequence that contributes to RA susceptibility.

Materials and Methods

Patients. Patients were 31 unrelated Japanese suffering from RA. Controls were 33 randomly selected healthy unrelated Japanese and most of them had been previously HLA typed.

Oligonucleotide Primers and Probes. The primers used were GLPDRB1 (5'-TTCTTCAATGGGACGGAGCG-3') and GAMPDRB1 (5'-GCCGCTGCACTGTGAAGCTCTC-3'), which anneal to the sequences encoding the amino acids 17-23 and 87-94, respectively, of the DRB genes (12). Four probes were designed in this study (Table I). Probe 1 hybridizes with the sequence that is commonly found in the DR4 DRB1 genes (Figure 1). Probe 2 detects the DRB1 sequence that codes serine at the 57th amino acid position. Probe 3 hybridizes with the sequence encoding amino acid residues 70-74 of the DRB1 chain. This sequence

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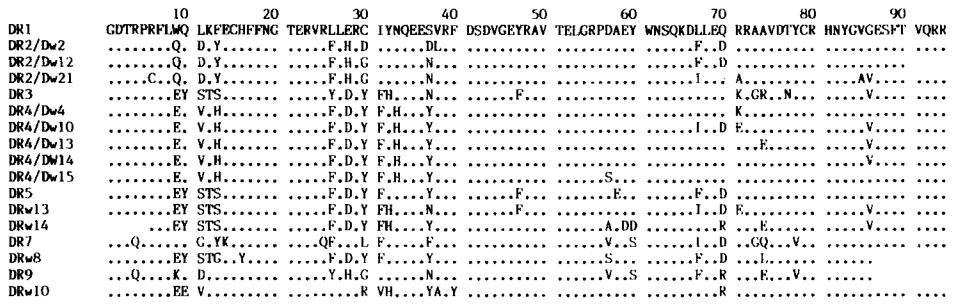

FIGURE 1. Amino acid sequences of the first domain of the HLA-DRB1 chain. The numbers above the sequence refer to the positions of the amino acid. A period indicates identity with the DR1 sequence. Blanks indicate that no sequence information is available. The references of the sequences are as follows: DR4/Dw13 from Cairns et al. (22); DR4/Dw15 from Gregersen et al. (23); DRw10 from Merryman et al. (24); the others from Todd et al. (12) and references therein.

is found in DR4/Dw14, DR4/Dw15, and DR1 alleles. Probe 4 is specific for DR4/Dw10 and DRw13 specificities.

In Vitro DNA Amplification. In vitro DNA amplification was carried out by the polymerase chain reaction (PCR) method according to Saiki et al. (13) with minor modifications. 2 μg of genomic DNAs were dispensed in 100 μl of PCR mixture (50 mM Tris-HCl (pH 8.8 at 25°C), 10 mM $MgCl_2$, 10 mM ammonium sulfate, 1.5 mM of each dNTP, and 0.5 μg of each primer). 2.5 U of Taq DNA polymerase (Stratagene, La Jolla, CA) and 40 μl of liquid paraffin were added to each sample. Then they were incubated at 65°C for 1.5 min, at 95°C for 1 min, and at 55°C for 2 min. This cycle was repeated 25 times, then followed by an additional incubation at 65°C for 5 min to complete the extension.

Dot Blotting and Detection of Specific Sequences. After the completion of the PCR amplification, samples were mixed with 1 ml of 0.5 M NaOH/25 mM EDTA. 200-μl aliquots of the mixture were blotted to a nylon filter (BioTrace RP, Gelman, Ann Arbor, MI) using a slot-blotter. The hybridization was carried out at 46, 50, 55, and 60°C for probes 1, 2, 3, and 4, respectively. Filters were prehybridized in 5× SSPE (0.9 M NaCl, 0.05 M sodium biphosphate, 5 mM EDTA, pH 7.4), 5× Denhardt's solution (0.1% BSA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone), 0.5% SDS for 1 h. The oligonucleotide probe (10 pmol) was 3' end-labeled with α-[^{32}P]ddATP and hybridized with filters for another 1 h. Then the filters were washed twice in 2× SSPE/0.5% SDS for 10 min and exposed with two intensifying screens at −70°C for time periods from 1 h to overnight.

Results and Discussion

The analysis of PCR-amplified DNA segments from 31 Japanese RA patients and 33 controls with four sequence-specific probes (Fig. 2) revealed that the frequency

TABLE I

Synthesized Oligonucleotide Probes

Probe	Specificity	Sequence	aa*
1	DR4	5'-TACTTCTATCACCAAGAGGA-3'	30–36
2	DR4/Dw15, DRw8	5'-CGGCCTAGCGCCGAGTAC-3'	55–60
3	DR4/Dw14, DR4/Dw15, DR1	5'-GCAGAGGCGGGCCGCGGT-3'	70–74
4	DR4/Dw10, DRw13	5'-GAAGACGAGCGGGCCGCG-3'	69–74

* Amino acid position.

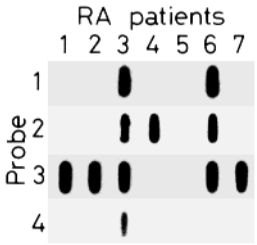

FIGURE 2. Representative dot blot analysis of seven RA patients. A specific segment of the HLA-DRB gene was amplified by the PCR method and hybridized with four oligonucleotide probes.

of the sequence defined by probe 3 was significantly increased in the patients (Table II): 52% in patients while only 15% in controls ($p < 0.005$, relative risk 6.0). The frequencies of the sequences defined by the other probes were slightly increased in the patients, but the differences were not statistically significant.

These data indicate that among the various sequences characterizing DR4 and its subspecificities, the amino acid residues 70–74 of the DRB1 chain defined by probe 3 are most important in developing RA in the patients studied. The amino acid sequence of DR4/Dw15, DR4/Dw14, and DR1 alleles is Gln^{70}-Arg^{71}-Arg^{72}-Ala^{73}-Ala^{74}. If this is a real disease susceptibility sequence to RA, then all the DR alleles that have this particular sequence should have an increased chance of developing RA. Previous studies generally support this possibility. In Japanese, the HLA-DR4/Dw15 specificity (HLA-DYT) is the susceptibility factor to RA (6). Nepom et al. (14) found that DR4/Dw14 is significantly associated with RA in Caucasians. Several reports have described the significant association of DR1 with RA (15, 16). Furthermore, Duquesnoy et al. (17) reported that MC1, which is a supertypic specificity associated with DR1 and a part of DR4, is more strongly associated with RA than DR4.

In Caucasians the association between DR4/Dw4 and RA is well established (1, 18). The DR4/Dw4 sequence differs by only one amino acid from the particular sequence in DR4/Dw15, DR4/Dw14, and DR1 alleles: Dw4 has lysine instead of arginine at the 71st position (Table III). Since both amino acids are basic, this substitution does not significantly change the net charge and may not alter the function of this portion.

The DRw10 has arginine at the 70th position instead of glutamine. In regard to the net charge, this region of DRw10 is also positively charged. Therefore, DRw10

TABLE II

Dot Blot Analysis of RA Patients and Controls

	RA (%)* (n = 31)	Control (%)* (n = 33)	Relative risk	p
Probe 1 (DR4)	13 (42)	9 (27)	1.9	NS‡
Probe 2 (Dw15, DRw8)	12 (39)	8 (24)	2.0	NS
Probe 3 (Dw14, Dw15, DR1)	16 (52)	5 (15)	6.0	<0.005
Probe 4 (Dw10, DRw13)	5 (16)	2 (6)	3.0	NS

* Percent shown in parentheses.

‡ NS, Not significant.

TABLE III

Comparison of the Amino Acids 70–74 of the DRB1 Chain in Relation to RA Susceptibility

Association	Amino acid position				
	70	71	72	73	74
Positive association					
DR1, DR4/Dw4, DR4/Dw14, DR4/Dw15	U	B	B	N	N
(DRw10	B	-	-	-	-)*
Neutral or negative association					
DR2/Dw2, DR2/Dw12, DR5, DRw8	A	-	-	-	-
DR2/Dw21	-	N	-	-	-
DR3	-	-	-	U	B
DR4/Dw10, DRw13	A	A	-	-	-
DR4/Dw13	-	-	-	-	A
DRw14, DR9	B	-	-	-	A
DR7	A	-	-	U	U

A hyphen indicates identity with the DR1 sequence in terms of charge and polarity. A, acidic amino acids; B, basic amino acids; N, nonpolar amino acids; U, uncharged polar amino acids.

* Association of DRw10 with RA has not yet been well established (see text).

could be associated with RA, although few association studies have been reported thus far.

Amino acid substitutions that alter the net charge of this portion clearly affect the RA susceptibility of this allele (Table III). For example, DR4/Dw10 has aspartic acid and glutamic acid instead of glutamine and arginine at positions 70 and 71, respectively. In the Israeli population, in which DR4/Dw10 is the preponderant DR4 subtype, no association between DR4 and RA was observed (16). In the case of DR2, neutral glutamine at the 70th position is substituted by aspartic acid, and a neutral or even negative association with RA has been reported (4, 7).

These findings support that the amino acid residues 70–74 of the DRB1 chain are crucial in RA susceptibility. This conclusion is basically consistent with the shared epitope hypothesis by Gregersen et al. (19). Todd et al. (20) also reached a similar conclusion that amino acid residues 70 and 71 in particular may be important.

Of great interest is that this region of the DR molecule may participate in the antigen binding according to the structural model by Brown et al. (21). It is possible that the alteration of charge, polarity, or conformation in this region may affect the interaction between the DR molecule and putative triggering agent(s) of RA and/or TCRs, resulting in changes of immune responsiveness to the trigger(s) or self molecules.

Summary

The association between HLA-DR4 and rheumatoid arthritis (RA) has been established in many ethnic groups. To clarify the determinant of susceptibility to RA, a polymorphic segment of the HLA-DRB gene was amplified in vitro by polymerase chain reaction and analyzed with oligonucleotide probes specific for the HLA-DR4 DNA sequences. A particular sequence encoding amino acids Gln^{70}-Arg^{71}-Arg^{72}-Ala^{73}-Ala^{74} showed a strong association with RA ($p < 0.005$, relative risk 6.0). This

amino acid sequence occurs in the DRB molecules with three RA-associated specificities, DR4/Dw14, DR4/Dw15, and DR1. DR4/Dw4, which is common in Caucasian RA patients, has a strikingly similar amino acid sequence Gln^{70}-Lys^{71}-Arg^{72}-Ala^{73}-Ala^{74} in terms of polarity and charge profiles. Other RA nonassociated sequences differ from this sequence by at least one amino acid substitution that causes the change of the net charge. The composition of amino acid residues at the positions 70–74 may play a crucial role in the pathogenesis of RA.

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