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Characterization of Recombination in the HLA Class II Region

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Summary

Studies of linkage disequilibrium across the HLA class II region have been useful in predicting where recombination is most likely to occur. The strong associations between genes within the 85-kb region from DQB1 to DRB1 are consistent with low frequency of recombination in this segment of DNA. Conversely, a lack of association between alleles of TAP1 and TAP2 (~15 kb) has been observed, suggesting that recombination occurs here with relatively high frequency. Much of the HLA class II region has now been sequenced, providing the tools to undertake detailed analysis of recombination. Twenty-seven families containing one or two recombinant chromosomes within the 500-kb interval between the DPB1 and DRB1 genes were used to determine patterns of recombination across this region. SSCP analysis and microsatellite typing yielded identification of 127 novel polymorphic markers distributed throughout the class II region, allowing refinement of the site of crossover in 30 class II recombinant chromosomes. The three regions where recombination was observed most frequently are as follows: the 45-kb interval between HLA-DNA and RING3 (11 cases), the 50-kb interval between DQB3 and DQB1 (6 cases), and an 8.8-kb segment of the TAP2 gene (3 cases). Six of the 10 remaining recombinants await further characterization, pending identification of additional informative markers, while four recombinants were localized to other intervals (outliers). Analysis of association between markers flanking HLA-DNA to RING3 (45 kb), as well as TAP1 to TAP2 (15 kb), by use of independent CEPH haplotypes indicated little or no linkage disequilibrium, supporting the familial recombination data. A notable sequence motif located within a region associated with increased rates of recombination consisted of a $(TGGA)_{12}$ tandem repeat within the TAP2 gene.

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Introduction

The human major histocompatibility complex (MHC) contains genes encoding highly polymorphic cell surface glycoproteins that mediate both humoral and cell-mediated immune responses. The strong linkage disequilibrium among loci in the HLA class II region (DR, DQ, and DP) indicate that they are likely to interact synergistically (i.e., there is selection for specific combinations of alleles of these linked loci) in their response to foreign agents. Studies of recombination and linkage disequilibrium can provide insight into the selective pressures on those DR-DQ and DR-DQ-DP haplotypes that may be beneficial in mediating an effective immune response against relatively old, common pathogens.

Mechanisms for generating polymorphism within genes of the MHC include recombination, gene conversion, and, to a lesser extent, mutation. Recombination provides a means of generating novel haplotypes, which may eventually prove to be beneficial to a population against a recently introduced pathogen. The frequency of recombination between DR-DQ and DP is 0.74% (Begovich et al. 1992; Martin et al. 1995), which is within the expected range, given 1% recombination per megabase of DNA per meiosis. However, it is reasonable to expect that, within the class II region, there may be segments of DNA where recombination occurs more or less frequently. High frequencies of recombination may indicate selection for haplotypic diversification, just as regions of low recombination frequency may reflect selective pressure favoring specific allele associations at two or more genes. For instance, *cis* association of specific DQA1 and DQB1 alleles ensures a functional DQ heterodimer (Kwok et al. 1993). It is also likely that maintenance of specific DQ-DR associations influences resistance to disease, as exemplified in the insulin-dependent diabetes mellitus–protective haplotypes DRB1*1501/DQA1*0102/DQB1*0602 and DRB1*1301/DQA1*0103/DQB1*0603 (Altmann et al. 1991; Nepom and Erlich 1991). On the other hand, frequency of recombination in a given region may reflect the presence or absence of proper recombination signal sequences. Reciprocal recombination occurs more readily in regions of high homology in yeast (Sugawara and Haber 1992), and this requirement, as well as other

putative signal sequences, may be required for recombination in vertebrate DNA. A thorough understanding of recombination within the human MHC will enhance linkage studies and provide insight into the role recombination has on MHC diversity.

The mouse MHC contains at least six defined recombinational hot spots within the 1,500 kb spanning from *H2-K* to *H2-D*, two of which are located within the *H2-S* region (class III) (Lafuse et al. 1992; Yoshino et al. 1994A) and four of which are located within the *H2-I* region (class II) (Steinmetz et al. 1982, 1986; Lafuse and David 1986; Uematsu et al. 1986; Shiroishi et al. 1990, 1991). These studies have provided information regarding the reciprocal/nonreciprocal nature of the crossover, the preferential participation of certain haplotypes in the recombination process, the preference for crossover specific to female meiosis, and the role of nucleotide identity in the recombination process (Kobori et al. 1984; Lafuse and David 1986; Saha and Cullen 1986; Saha et al. 1986; Steinmetz et al. 1986; Uematsu et al. 1986; Passmore et al. 1987; Shiroishi et al. 1990, 1991; Sant'Angelo et al. 1992; Yoshino et al. 1995). The hot spots of recombination located within the second intron of the *Eb* gene and between the A_{B3} and A_{B2} genes have both been mapped to physical distances of $\leq$36 bp (Uematsu et al. 1986; Shiroishi et al. 1991; Bryda et al. 1992). Fine mapping of crossover events in the mouse MHC by DNA sequencing has allowed identification of sequences putatively involved in the recombinational process, such as dinucleotide and tetrameric repeats, the mouse middle repetitive mouse transcript (MT)–family sequence, and the long terminal repeat–insertion (LTR-IS) element (Kobori et al. 1986; Saha et al. 1986; Uematsu et al. 1986; Shiroishi et al. 1990, 1993; Padgett et al. 1991). *Cis*-acting elements located in close proximity to recombinational breakpoints may enhance binding of the necessary recombination machinery to the target DNA or induce a conformational change in the DNA that promotes DNA exchange (Treco and Arnheim 1986; Uematsu et al. 1986; Satyanarayana and Strominger 1992).

Recombinational hot spots in humans have been proposed near or within the Duchenne muscular dystrophy, insulin, collagen, β-globin and T-cell–receptor β-chain genes, the immunoglobulin heavy-chain gene cluster, and the MHC, on the basis of observed recombination events or linkage equilibrium between pairs of loci (Lebo et al. 1983; Chakravarti et al. 1984, 1986; Bowcock et al. 1988; Grimm et al. 1989; Charmley et al. 1990; Benger et al. 1991; Oudet et al. 1992; Crouau-Roy et al. 1993; Eiermann et al. 1993; Bouissou et al. 1995; Cullen et al. 1995; Klitz et al. 1995). Familial recombination events identified in class II have been mapped by molecular typing to intervals including DPB1–TAP1, TAP2, and DOB–DQB1, with implications of a hot spot for recombination within intron 2 of TAP2 (Cullen et al. 1995). Eierman et al. (1993) have suggested that the interval from HLA-DNA to TAP2 contains a hot spot for recombination. In spite of our knowledge regarding regions of recombination in the human genome, very few breakpoints have been analyzed at the sequence level. A $(GT)_n$ dinucleotide repeat and bacterial χ sequence (Miesfeld et al. 1981; Chakravarti et al. 1984) have been identified between the δ- and β-globin genes corresponding to the site of the β-globin hot spot. Historic recombination breakpoints located between DQB1 and DQA1 were found to be closely linked to a $(CA)_{22}$ repeat and minisatellite core sequences (Satyanarayana and Strominger 1992). A 350-kb segment of HLA class II genomic sequence reported recently (Beck et al. 1996) will facilitate refinements in mapping of recombinational breakpoints in class II and may allow a more productive study of recombinational signal sequences in vertebrates.

The HLA class II region provides an excellent model system for examining recombination, given our knowledge of its well-characterized polymorphic genes, near-complete sequence data (Beck et al. 1996), linkage disequilibrium patterns (Klitz et al. 1995), abundant polymorphic markers (reported herein), and data regarding recombination in the mouse MHC (Steinmetz et al. 1982, 1986; Kobori et al. 1986; Lafuse and David 1986; Saha et al. 1986; Uematsu et al. 1986; Shiroishi et al. 1990, 1991; Padgett et al. 1991; Bryda et al. 1992; Yoshino et al. 1994*a*, 1994*b*). In the present study, molecular typing of DP, DQ, and DR loci in family data resulted in the identification of 30 recombinant chromosomes within the HLA class II region. By use of microsatellite typing and SSCP analysis, 127 novel polymorphic markers distributed throughout the class II region were used to delineate the site of crossover in each recombinant chromosome. Results indicate that there are three main regions where recombination occurs in class II: HLA-DNA to RING3, within TAP2, and DQB3 to DQB1.

Families and Methods

Recombinant Families

Twenty-seven HLA class II recombinant families, including 7 CEPH families and 20 other families, were previously identified by typing DR, DQ, and DP loci. Families analyzed were Caucasian, predominantly of northern European descent, with the exception of one Mexican family. All families included both parents and at least three offspring.

CEPH Families

DNA from 59 CEPH pedigrees was typed at markers flanking the three regions where increased recombination was observed in order to determine whether levels of association (linkage disequilibrium) between these markers supported the recombination frequency data.

Loci typed included DPB1, DQB1, DRB1 (Begovich et al. 1992), TAP1, TAP2 (Carrington et al. 1994), G25238, RING3CA, and G496 (reported herein).

PCR Amplification

Genomic DNA (100–500 ng) was amplified in one of two ways. For SSCP analysis, [α^{32}P] dCTP was incorporated directly into amplified products. Amplification reactions were performed in a total volume of 20 μl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each of dATP, dTTP, and dGTP, 100 μM dCTP, 0.7 μCi of [α-^{32}P] dCTP (3,000 Ci/mole) (Dupont/NEN), 80 ng of each oligonucleotide primer, and 1.5 U *Taq* polymerase (Perkin Elmer). Amplification conditions were as follows: 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 60 s, and extension at 72°C for 90 s, followed by a final extension at 72°C for 5 min. For microsatellite typing, the 5′ oligonucleotide primer was end labeled prior to amplification. End-labeling involved incubating at 37°C for 30 min 333 ng of primer, 100 μCi of [γ-^{32}P] dATP (6000 Ci/mole) (Dupont/NEN), with 10 U of T4 polynucleotide kinase in 1× kinase buffer (Boehringer Mannheim). Amplification reactions were performed in a total volume of 10 μl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dGTP, dATP, dTTP, dCTP, 10 ng of cold 5′ primer, 14 ng of 3′ primer, 0.7 ng of end-labeled 5′ primer, and 0.5 U of *Taq* polymerase. The amplification conditions were the same as those noted for SSCP analysis. Prior to resolution by gel electrophoresis, 5 μl of PCR product was diluted into 10 μl of dye solution (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol). Oligonucleotide primer sequences will be made available, on request.

SSCP Analysis and Microsatellite Typing

SSCP analysis of the radiolabeled amplification products involved thermal denaturation (95°C for 2 min) followed by chilling on ice and separation of products by electrophoresis on a 6% nondenaturing polyacrylamide gel (37.5:1, acrylamide:bis-acrylamide). Electrophoresis proceeded at 50 W for 4 h at 4°C. Gels were dried and placed on Kodak X-Omat AR X-ray film overnight. Analysis of radiolabeled amplification products from microsatellite typing proceeded as described for SSCP analysis, with two exceptions: the use of a 6% denaturing gel containing 50% urea and electrophoresis at 1,500 V for 4 h at room temperature.

HLA Class II Typing

HLA Class II typing for DRB1 and DPB1 was performed on all samples with the PCR/sequence-specific oligonucleotide probe method reported elsewhere (Bugawan et al. 1990; Scharf et al. 1991). In brief, genomic DNA (100–500 ng) was amplified by PCR with primers specific for the second exon of the relevant locus. The PCR product was then dot blotted and crosslinked onto a nylon membrane. Membranes were subsequently hybridized with horseradish peroxidase–labeled, sequence-specific oligonucleotide probes. Following stringent washing, bound probe was detected by means of a colorimetric detection system utilizing the substrate tetramethylbenzidine. The pattern of probes hybridizing to a given sample indicated the identity of the alleles in the PCR product.

HLA class II typing for DQB1 and DQA1 was performed on all samples using the SSCP method as described by Carrington et al. (1992). Oligonucleotide primers used to amplify DQA1 exon 2 are as follows: 5′-GTGCTGCAGGTGTAAACTTGTACCAG and 5′-CACGGATCCGGTAGCAGCGGTAGAGTTG (DQA1*0601, -0501, -0401, -0300, -0103, -0102, and -0101). Oligonucleotide primer sets used to amplify DQB1 exon 2 are as follows: 5′-CCCGCGGTACGCCACCTCG and 5′-GACTCTCCCGAGGATTTCGTG (DQB1*05 and -06); 5′-CGTGCGGAGCTCCAACTGG and 5′-GACTCTCCCGAGGATTTCGTG (DQB1*02, -03, and -04).

Identification of Polymorphic Markers

Localization of recombinational breakpoints required identification of numerous polymorphic markers within the genomic interval containing the crossover event. Oligonucleotide primers were designed to target noncoding regions situated at the center of each genomic interval in which recombination had occurred. Identification of polymorphic markers entailed the amplification of DNA from a reference panel of cell lines (homozygous typing cells [HTCs]), many of which are homozygous for class I and II genes (Yang et al. 1989). Ten HTCs representing different DP-DQ-DR haplotypes were used in the identification of polymorphic sites, and all markers with two or more alleles were typed in the appropriate families.

Disequilibrium Statistics

The pairwise disequilibrium statistic (D) was calculated for each of eight marker pairs typed in CEPH

$$D = x - pq ,$$

where x is the observed haplotype frequency, and p and q are the observed allele frequencies at each of the loci. The normalized disequilibrium statistic is

$$D' = D/D_{\max} ,$$

where $D_{\max}$ is the lesser of pq or $(1 - p)(1 - q)$ when $D < 0$, or $D_{\max}$ is the lesser of $p(1 - q)$ or $q(1 - p)$, if $D > 0$. The global disequilibrium statistic is

$$W = (\Sigma\Sigma\, D^2/pq)^{1/2} ,$$

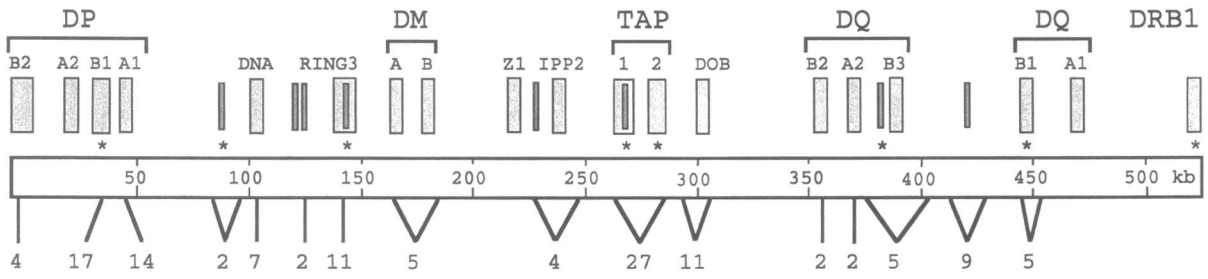

Figure 1 Distribution of 127 polymorphic markers across the HLA class II region. The approximate location of class II loci have been adapted from Beck et al. (1996). The location and abundance of all markers employed is displayed under the map, and dark boxes represent the site of seven microsatellites and one tandem repeat (between DQA2 and DQB3). The location of eight markers employed in the analysis of association between markers flanking regions associated with increased or decreased rates of recombination are indicated by an asterisk (*).

where p and q are the observed allele frequencies at each of two loci having k and l alleles (Cohen 1988). The normalized global disequilibrium statistic,

$$W_n = W[\min(k, l) - 1]^{1/2}$$

is a statistic between 0 and 1, with values of 1 reflecting strong disequilibrium and 0 reflecting equilibrium. A more thorough discussion of disequilibrium statistics can be found in Klitz et al. (1995).

Results

Mapping Regions of Recombination

DNA from 30 individuals having recombinant chromosomes within the 500-kb interval between DPB1 and DRB1 was used to define more specifically where recombination occurred. The recent availability of 350 kb of genomic sequence from DPA2 to DQB1 (Beck et al. 1996) enabled the synthesis of oligonucleotide primers for PCR amplification and identification of polymorphic markers. By use of SSCP and microsatellite typing, 127 novel polymorphic markers distributed throughout the class II region were identified on a standard panel of DNA from 10 HTCs (fig. 1). These, plus a number of previously characterized markers, were used to delineate the site of crossover in the 30 recombinant chromosomes. The three regions where recombination occurred most frequently are as follows: the 45-kb interval between HLA-DNA and RING3 (11 cases), the 50-kb interval between DQB3 and DQB1 (6 cases), and an 8.8-kb segment of the TAP2 gene (3 cases) (fig. 2). Of the remaining 10 recombinant chromosomes, 6 require further resolution (recombinants 15, 19–22, and 30) and could potentially fall in one of the three intervals listed above. The remaining four (recombinants 1, 13, 14, and 23) represent outliers, that is, were localized to other intervals (fig. 2). Two of the three TAP2 recombinant chromosomes have been mapped previously to overlapping 138-bp and 850-bp segments of intron 2 (Cullen et al. 1995). Six of the 11 HLA-DNA to RING3 recombinants have been further localized to a 4-kb interval. Three regions in class II where recombination occurred rarely, if at all, in the sample tested here include the 70-kb interval between DPB1 and HLA-DNA, the 120-kb interval between RING3 and TAP1, and the 85-kb interval between DQB1 and DRB1 (fig. 2).

A single HLA class II recombinant chromosome was observed in 24 families. Two class II recombinants were observed in separate siblings in each of three families (recombinants 8 and 9 in one family, 12 and 25 in a second family, and 18 and 28 in a third family). The only recombinant siblings in which the crossover occurred in the same recombination interval (HLA-DNA to RING3) were recombinants 8 and 9.

Haplotype and Frequency Characteristics of Recombinant Chromosomes

Twenty-one of the 30 recombinant chromosomes studied were maternally derived (table 1), which is consistent with the observation of a higher recombination frequency in females relative to males in humans and, more specifically, HLA (Donis-Keller et al. 1987; Thomsen et al. 1989; Thomas and Rothstein 1991). Recombination within the HLA-DNA–to–RING3 interval displayed a strong female preference (9/11 crossover events) relative to the DQB3 to DQB1 interval (3/6 crossover events), but the difference in parental sex associated recombination is not significant (one-tailed Fisher's exact test $P = .21$). A sex preference at specific sites was described in mice (Shiroishi et al. 1987, 1990; Yoshino et al. 1994*a*).

Studies of recombination in mouse H2 also suggest that certain haplotypes are more prone to recombination than others (Shiroishi et al. 1982; Lafuse and David 1986; Steinmetz et al. 1986; Yoshino et al. 1994*a*). DR, DQ, and DP haplotypes of the recombinant individuals and donating parent were determined to test whether a similar phenomenon occurs in humans (table 1). Ten of the 30 DR-DQ-DP haplotypes generated by recombination were not observed in the panel of CEPH class II haplotypes published previously (Carrington et al.

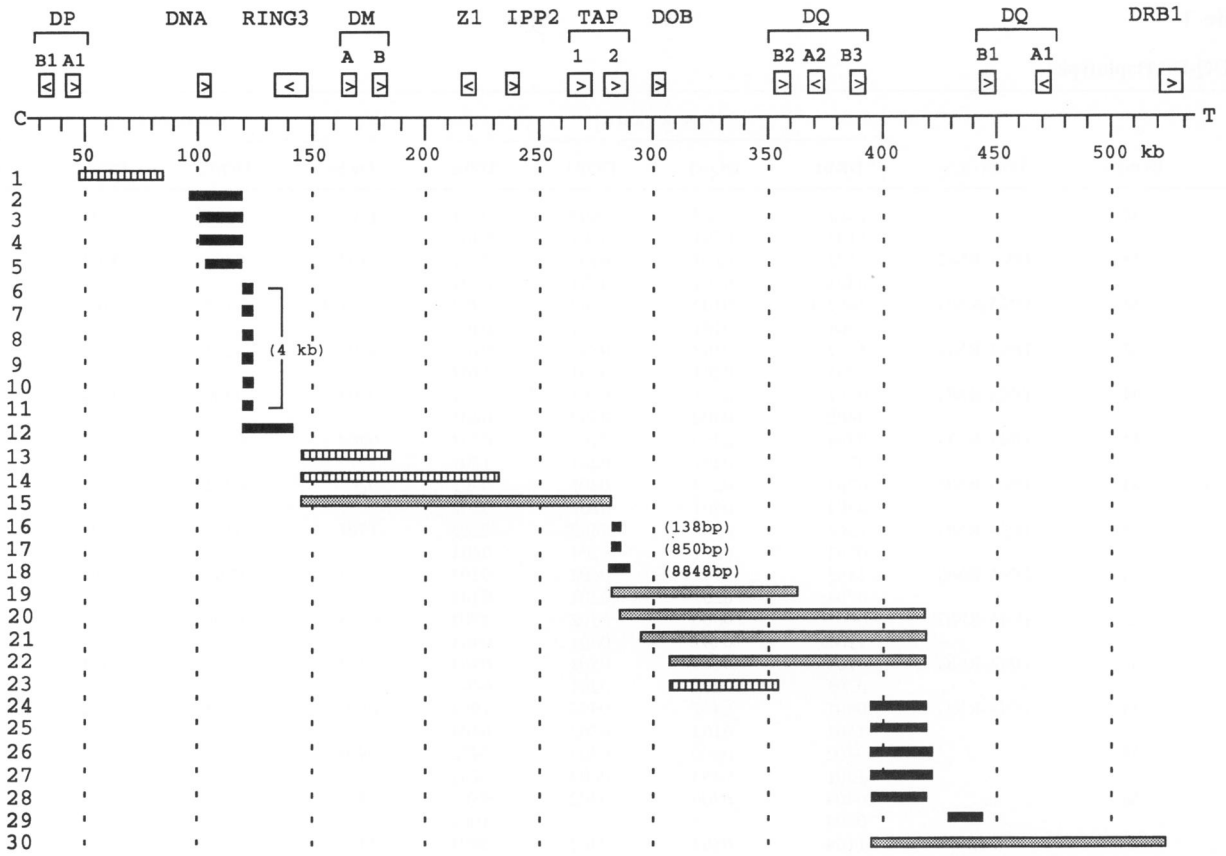

Figure 2 Five hundred thirty–kilobase genomic map of the HLA class II region in which 30 recombinant chromosomes were mapped by molecular typing. The approximate locations of loci, relative to DPB2 (not shown), have been adapted from Beck et al. (1996). The location of each recombination breakpoint is depicted as a horizontal bar relative to the map of the class II region. The black bars represent crossover events that occurred within one of three regions associated with an increased frequency of recombination. The gray bars represent crossover events that await additional informative markers before they can be further localized. The striped bars represent outliers in which the crossover event did not fall within one of the three regions associated with increased rates of recombination. Recombinant chromosomes 16 and 17 have been mapped previously to overlapping 138-bp and 850-bp segments of the second intron of TAP 2 (Cullen et al. 1995). Oligonucleotide primer sequence and map position (relative to DPB2) for markers flanking the HLA-DNA to RING3 and DQB3 to DQB1 recombinants are as follows: DN2229 = 5′-CCCGATCAAGTCCAGTTCTG and 5′-GAGTGTGGCTCTTCCATAAC; DN5200 = 5′-CCTCAGTGGAGTCAGTCATG and 5′-CCCAAGCAGTTCTTATGTGC (102 kb telomeric to DPB2); DNRNG4031 = 5′-TCTGCACAATGGAGTAATGC and 5′-TGTTGGCAACATCTCAAGTC (120 kb telomeric to DPB2); DNRNGCA = 5′-AGGAATCTAGTGCTCTCTCC and 5′-CTCTAGCAAAAGGAAGAGCC (125 kb telomeric to DPB2); RING3CA = 5′-TGCTTATAGGGAGACTACCG and 5′-GATGGGAAGTTTCCAGAGTG (140 kb telomeric to DPB2); G412348 = 5′-AAAGACCAAGGCTGGATAGG and 5′-TATCACCAGAGTTCCTGGAG (395 kb telomeric to DPB2); G57530 = 5′-TCCTTCTTGGGTGCTCTATC and 5′-TTTATCCTAGTGGTGCTGGC; and G511525 = 5′-GGTAAAATTCCTGACTGGCC and 5′-GACAGCTCTTCTTAACCTGC (422 kb telomeric to DPB2). Additional information is available on request.

1994), and these haplotypes may represent novel haplotypes. Ten parental haplotypes were involved in more than one recombination event, and the haplotype DRB1*01-DQA1*0101-DQB1*0501-DPB1*0401 was involved in five events, one of which represented the only crossover between chromosomes homozygous for DR, DQ, and DP.

Linkage Disequilibrium Analysis

Recombination within an interval of DNA diminishes the strength of association between alleles at loci flanking that interval. Therefore, we have examined the degree of association between markers flanking the recombination intervals HLA-DNA to RING3, DQB3 to DQB1, and TAP2 in order to determine whether linkage disequilibrium values and recombination frequency is concordant for these three intervals. The level of association between the following pairs of loci, three of which flank the recombination intervals listed above, was determined using data from 248 independent CEPH chromosomes: DPB1-G25238, G25238-RING3CA, RING3CA-TAP1CA, TAP1-TAP2, G496-DQB1, DQB1-DRB1, DPB1-DRB1, and DPB1-RING3CA (W_n = .59, .23, .19, .11, .51, .89, .32, and .36, respectively) (fig. 3). Genotyping of DPB1, DQB1, and DRB1 (Begovich et al. 1992), as well as TAP1 and TAP2 (Carrington et al. 1994), in CEPH were reported previously and are used here in the association analysis. The level of associ-

Table 1

DR-DQ-DP Haplotypes

			Donor Parent				Recombinant			
No.	Donor	Location	DRB1	DQA1	DQB1	DPB1	DRB1	DQA1	DQB1	DPB1
1	M	...[a]	*1301*	*0103*	*0603*	*0201*	1101	0501	0301	*0201*
			1101	0501	0301	0401				
2	M	DNA-RNG	*0101*	*0101*	*0501*	*0401*	*0101*	*0101*	*0501*	0101
			0301	0501	0201	0101				
3	M	DNA-RNG	*1601/3*	*0102*	*0502*	*1301*	*1601/3*	*0102*	*0502*	0401
			0101	0101	0501	0401				
4	M	DNA-RNG	*1601*	*0102*	*0502*	*0401*	0701	0201	0201	0401
			0701	0201	0201	1101				
5	M	DNA-RNG	*0701*	*0201*	*0201*	*0301*	0402	0300	0302	*0301*
			0402	0300	0302	0401				
6	M	DNA-RNG	*0404*	*0300*	*0302*	*0301*	*0404*	*0300*	*0302*	1701
			07	0201	0201	1701				
7	M	DNA-RNG	*0701*	*0201*	*0201*	*0401*	*0701*	*0201*	*0201*	0202
			0301	0501	0201	0202				
8	M	DNA-RNG	*1501*	*0102*	*0602*	*0201*	*1501*	*0102*	*0602*	0401
			0701	0201	0201	0401				
9	M	DNA-RNG	*1501*	*0102*	*0602*	*0201*	*1501*	*0102*	*0602*	0401
			0701	0201	0201	0401				
10	P	DNA-RNG	*0405*	*0300*	*0302*	*1701*	*0405*	*0300*	*0302*	0401
			1101	0501	0301	0401				
11	P	DNA-RNG	*0101*	*0101*	*0501*	*0401*	*0101*	*0101*	*0501*	0401[c]
			0701	0201	0201	0401				
12	M	DNA-RNG	*0801*	*0401*	*0402*	*1901*	*0801*	*0401*	*0402*	0401
			1501	0102	0602	0401				
13	M	...[a]	*0402*	*0300*	*0302*	*0402*	*0402*	0300	0302	0201
			1301	0103	0603	0201				
14	M	...[a]	*0404*	*0300*	*0302*	*0501*	*0404*	*0300*	*0302*	1001
			0404	...	...	1001				
15	P	...[b]	*1104*	*0501*	*0301*	*0401*	*1104*	*0501*	*0301*	0301
			1301	0103	0603	0301				
16	M	TAP2	*0404*	*0300*	*0302*	*1501*	*0404*	*0300*	*0302*	0401
			1501	0102	0602	0401				
17	M	TAP2	*0701*	*0201*	*0201*	*0201*	*0701*	*0201*	*0201*	1301
			1602	0501	0301	1301				
18	M	TAP2	*DR3*	*0501*	*0201*	*0101*	*DR3*	*0501*	*0201*	0401
			DR13	0103	0603	0401				
19	M	...[b]	*0404*	*0300*	*0302*	*0301*	*0404*	*0300*	*0302*	0401
			0701	0201	0201	0401				
20	P	...[b]	*0404*	*0301*	*0302*	*0601*	1302	0102	0604	*0601*
			1302	0102	0604	0401				
21	M	...[b]	*0401*	*0300*	*0302*	*0402*	*0401*	*0300*	*0302*	0402[c]
			1501	0102	0602	0402				
22	M	...[b]	*0901*	*0300*	*0303*	*0402*	*0901*	*0300*	*0303*	0401
			0801	0401	0402	0401				
23	P	...[a]	*07*	*0201*	*0201*	*0201*	1302	0102	0604	*0201*
			1302	0102	0604	0301				
24	M	DQB3-B1	*0401*	*0300*	*0302*	*0401*	1104	0501	0301	*0401*[c]
			1104	0501	0301	0401				
25	M	DQB3-B1	*0801*	*0401*	*0402*	*1901*	1501	0102	0602	1901
			1501	0102	0602	0401				
26	P	DQB3-B1	*1103*	*0501*	*0301*	*0401*	*1103*	*0501*	*0301*	0101
			0301	0501	0201	0101				
27	M	DQB3-B1	*1503*	*0102*	*0602*	*1801*	0301	0501	0201	*1801*
			0301	0501	0201	1701				
28	P	DQB3-B1	*DR1*	*0101*	*0501*	*0401*	*DR1*	0101	*0501*	1101
			dr7	0201	0201	1101				
29	P	DQB3-B1	*1201*	*0501*	*0301*	*0202*	*1201*	*0501*	*0301*	0401
			0301	0501	0201	0401				
30	P	...[b]	*0103*	*0101*	*0501*	*0401*	*0103*	*0101*	*0501*	0401[c]
			0101	0101	0501	0401				

NOTE.—All recombinant DR-DQ-DP haplotypes consist of alleles from parental haplotype one (italic) and parental haplotype two (roman).

[a] Represents outlier.

[b] Awaits further characterization pending additional informative markers.

[c] Crossover event occurred between DRB1 and DPB1.

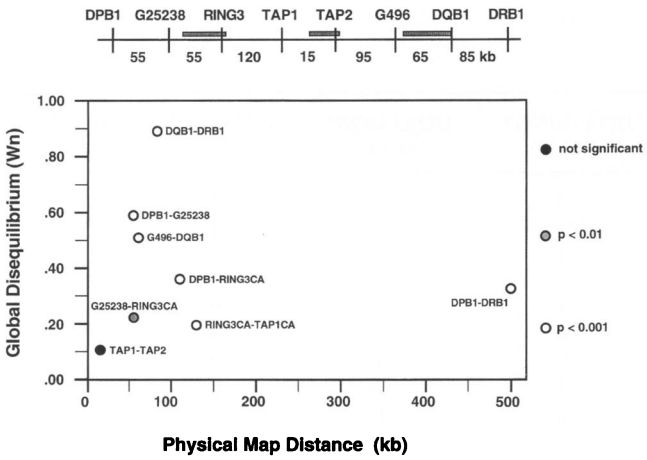

Figure 3 Relationship between pairwise global disequilibrium (W_n) and physical map distance in the HLA class II region for eight pairs of loci. The distance between loci, as well as the relative position of the three recombination intervals (represented as three shaded horizontal bars), is indicated on the map above. Alleles of DPB1, DQB1, and DRB1 reflect polymorphism at exon 2. Alleles of TAP1 and TAP2 reflect polymorphism within the coding region. The dinucleotide repeats G25238, RING3CA, and TAP1CA and the tandem repeat G496 reflect polymorphism at sites outside coding regions. The significance level for each pairwise comparison is indicated for each data point. Oligonucleotide primers used in the analysis of association are as follows: G25238 = 5′-GGTGACTCATGCCTTTAATCC and 5′-TTGCATTCTAGCCTCCATGG; G496 = 5′-AGTCCCAGCTACTACTCAGG and 5′-CAACGGCATGTCAATTAGTGC; RING3CA = 5′-TGCTTATAGGGAGACTACCG and 5′-GATGGGAAGTTTCCAGAGTG; and TAP1CA (Carrington and Dean 1994). Genotyping of DPB1, DQB1, and DRB1 (Begovich et al. 1992), as well as TAP1 and TAP2 (Carrington et al. 1994), in CEPH were reported previously and used here in the association analysis.

ation between markers flanking the HLA-DNA–to–RING3 interval was measured using G25238 (20 kb centromeric to HLA-DNA) and RING3CA (RING3 intron 1), and that for the DQB3 to DQB1 interval was measured using G496 (5 kb centromeric to DQB3) and DQB1 (fig. 3). Analysis of association across the TAP1 to TAP2 interval has been reported elsewhere (Klitz et al. 1995). Association between pairs of loci were examined using the global disequilibrium statistic, W_n. Five of the eight marker pairs analyzed displayed intermediate to high levels of disequilibrium that were statistically significant at $P < .001$: DQB1-DRB1 ($W_n = .89$), DPB1-G25238 ($W_n = .59$), G496-DQB1 ($W_n = .51$), DPB1-RING3CA ($W_n = .36$), and DPB1-DRB1 ($W_n = .32$). Greatly reduced or no disequilibrium was observed for the following marker pairs: TAP1-TAP2 ($W_n = .11$; P = not significant), G25238-RING3CA ($W_n = .23$; $P < .01$) and RING3CA-TAP1CA ($W_n = .19$; $P < .001$).

Potential Recombination Signal Sequences

Recent studies suggest the presence of eukaryotic recombination signal sequences that enhance recombination in specific regions of the genome (Treco and Arnheim 1986; Edelmann et al. 1989; Schuchert et al. 1991; Satyanarayana and Strominger 1992). Regions of class II were screened for the presence of putative recombination signal motifs to determine whether a correlation could be made between the presence of such sequences and high levels of recombination. Sequence-homology analysis was accomplished using the Genetics Computer Group Wisconsin sequence analysis package programs Bestfit and Findpattern (Wisconsin Package 1994). Allowable mismatches programmed into the search parameters corresponded with a sequence identity of ~80% or better. Genomic intervals screened for potential recombination signal sequences included a 38-kb segment between HLA-DNA and RING3, a 9-kb segment of TAP2, and a 48-kb segment between DQB3 and DQB1, all of which are located in a region having a relatively high frequency of recombination. Sequences from β-tubulin (8.8 kb), a segment between DMB and IPP2 (48 kb), and a segment between DPA1 and HLA-DNA (48 kb) were used as control sequences (those not known to be associated with an increased frequency of recombination) to distinguish candidate from ubiquitous sequence motifs (table 2) (Gwo-Shu Lee et al. 1984; Beck et al. 1996) (GenBank accession numbers X00734, X87344, and MHCDB at http://www.hgmp.mrc.ac.uk/). The six genomic intervals were screened for the following potential recombination signal sequences: the bacterial χ sequence (GCTGGTGG) (Smith et al. 1981), the *Schizosaccharomyces pombe ade6*-M26 heptanucleotide sequence (ATGACGT) (Schuchert et al. 1991), the LTR-IS motif (TGGAAATCCCC) (Edelmann et al. 1989), the telomeric repeat sequence $(TTAGGG)_6$ (Pace et al. 1990; Zhong et al. 1992), the retroposon LTR sequence (TCATACACCACGCAGGGGTAGAGGACT) (Zimmerer and Passmore 1991), the XY32 homopurine-pyrimidine H-palindrome motif (AAGGGAGAA[A/G]GGGTATAGGG[A/G]AAGAGGGAA) (Rooney and Moore 1995), tandem repeats of the $(AGGC)_n$, $(TCTG)_n$, and $(TGGA)_n$ tetramers (Steinmetz et al. 1986; Uematsu et al. 1986; Fischer-Lindahl 1991), $(pur:pyr)_n$ dinucleotide repeats (Gillies et al. 1984; Treco and Arnheim 1986; Satyanarayana and Strominger 1992), the human minisatellite core sequence $(GGGCAGGA[A/G]G)_1$, two human hypervariable minisatellite sequences $(GGAGGTGGGCAGGA[A/G]G)_5$ (Jeffreys et al. 1985), and $(AGAGGTGGGCAGGTGG)_5$ (Wahls et al. 1990), the 289-bp consensus sequence of the middle-repetitive MT family (Heinlein et al. 1986; Shiroishi et al. 1990), and the 588-bp LTR-IS-L element (Edelmann et al. 1989) (table 2).

Telomeric repeats, retroposon LTR sequences, XY32 homopurine-pyrimidine H-palindrome motifs, and human hypervariable minisatellite sequences were not observed in any of the six genomic intervals screened. The significance of all sequence motifs identified in the three recombination intervals is questionable, because of their

Table 2

Candidate Recombination Signal Sequences in Six Genomic Locations

	TAP2 (9 kb)	β-*Tubulin* (8.8 kb)	DNA[c]-RING3 (38 kb)[a]	DQB3-DQB1 (48 kb)[a]	*DMB-IPP2* (48 kb)	*DPA-DNA*[c] (48 kb)
Chi sequence (0)	4	4	2	0	2	3
M26 heptanucleotide (0)	0	0	2	3	1	1
LTR-IS motif (1)	1	0	1	1	0	3
Tandem repeats (3):						
$(AGGC)_4$	0	0	1	0	0	1
$(TCTG)_4$	2	0	0	3	4	1
$(TGGA)_4$	3[b]	0	0	0	2	1
Human minisatellite core sequence (0)	1	1	2	0	0	1
$(pur:pyr)_n$ dinucleotide repeats[d]	0	0	3	1	2	2
Middle-repetitive MT family (% identity)	72	66	70	79	73	76
LTR-IS element (% identity)	63	70	70	70	73	70

NOTE.—Other sequences tested: homopurine-pyrimidine, homopurine-palindrome, 2 human minisatellites, retroposon LTR, and telomeric repeat. Recombination intervals are given in bold, and control intervals are in italics. Number of mismatches allowed (equivalent to ≈20% nonidentity given in parentheses.

[a] Incomplete sequence data.

[b] Includes one $(TGGA)_{12}$ tandem repeat.

[c] HLA-DNA.

[d] $(pur:pyr)_n \geq 10$.

abundance in the three control regions (table 2). Alignment with larger sequence motifs (middle-repetitive MT and LTR-IS element) revealed reasonable sequence identity in the class II recombination intervals, but the quality of the alignment was no better than when two random (negative control) sequences of equivalent size were used. A $(TGGA)_{12}$ tandem repeat (87% identity) within intron 6 of TAP2, 5 kb downstream of the observed recombination sites in intron 2, was identified, and this sequence could possibly influence recombination in the TAP2 gene. Additional notable matches included three $(pur:pyr)_n$ dinucleotide repeats between HLA-DNA and RING3 and one between DQB3 and DQB1. However, two $(pur:pyr)_n$ dinucleotide repeats were identified in the DMB to IPP2 and DPA-to-DNA intervals, which were used as a control.

Discussion

Evidence indicates that recombination does not occur randomly across a chromosome but is restricted to specific regions. The distribution of crossover events reported here also suggests a nonrandom pattern of recombination. Molecular typing of 30 familial class II recombinant chromosomes has identified three regions where recombination occurs preferentially: the 45-kb interval between HLA-DNA and RING3, the 50-kb interval between DQB3 and DQB1, and, to a lesser extent, the TAP2 gene (fig. 2). Alternatively, three regions exhibit recombination rarely if at all. These include the 70-kb interval between DPB1 and HLA-DNA, the 120-kb interval between RING3 and TAP1, and the 85-kb interval between DQB1 and DRB1. Selection favoring particular haplotypes may explain the lack of recombination between DRB1 and DQB1, since the genes in this region have similar functions and are, therefore, likely to behave in a cooperative manner. On the basis of sequence data (Beck et al. 1996), it is now known that a total of 16 genes are present within the class II region from DPB1 to DQB1. The limited extent of polymorphism and the distribution of those polymorphic sites in all class II region genes apart from DR, DQ, and DP loci suggest that the polymorphism has no functional significance. If the polymorphism in these genes is nonfunctional, the selection to keep certain alleles of these genes in phase does not explain the lack of recombination in the DPB1-to-HLA-DNA or RING3-to-TAP1 regions. Chromatin structure, potentially high sequence variability in noncoding regions, lack of appropriate signal sequences, or some other physical deterrent of crossing over may account for the relatively low occurrence of recombination in these regions.

The involvement of 38/60 different DR-DQ-DP and 24/60 different DR-DQ parental haplotypes shows that recombination cannot be strongly haplotype dependent. This observation contrasts with suggestions of inferred haplotype-dependent crossover events mapped within DQB1 to DQA1 and DQA1 to DRB1 based on sequence data from independent chromosomes (Gregersen et al. 1988; Satyanarayana and Strominger 1992; Haas et al. 1994). Identification of sufficiently large numbers of recombinant chromosomes to address haplotype dependent recombination is difficult to accomplish with family samples. Single-sperm typing of DNA may allow a more

efficient means to identify recombination events from donors of varied DR-DQ-DP haplotype backgrounds to test more rigorously the possibility of haplotype-dependent recombination.

Low levels of linkage disequilibrium between a pair of markers may result, to a large degree, from high rates of recombination in the segment of DNA between the markers. Predictions based on this relationship have been proposed for the region separating TAP1 and TAP2 (van Endert et al. 1992; Carrington et al. 1993; Powis et al. 1993; Klitz et al. 1995), and recent familial studies have confirmed a proposed recombinational hot spot between TAP1 and TAP2 (Cullen et al. 1995). Levels of association were high in DNA segments where recombination rarely occurs, including DPB1 to G25238 and DQB1 to DRB1 (W_n = .59 and .89, respectively). Conversely, levels of association between markers flanking the three recombination intervals (G496 to DQB1, G25238 to RING3CA, and TAP1 to TAP2) were either lower than adjacent intervals or absent (W_n = 0.51, 0.23, and 0.11, respectively), consistent with the recombination data. Basically, linkage disequilibrium data and recombination data correlated convincingly for all but one segment of the class II region. A weak association was observed between markers flanking the 120-kb RING3-TAP1 interval, which was unexpected, given the small number of crossover events observed in this region. The markers used in association analysis across this interval are both $CA_{(n)}$ dinucleotide repeats, which are known to have a mutation rate of $\sim 10^{-3}$ in cell lines (Banchs et al. 1994). Thus, mutation rates rather than recombination may account for the weak association between these two markers. Accordingly, such an effect on any pairwise association in which a dinucleotide repeat (G25238, RING3CA, and TAP1CA; fig. 3) is involved must be kept in mind. Additional association analyses using polymorphic markers neighboring the RING3CA and TAP1CA repeats may clarify the inconsistency between linkage disequilibrium and recombination frequency across the RING3 to TAP1 segment.

Numerous sequence motifs found in a variety of organisms may operate as recombination enhancement elements based on their close proximity to recombinational breakpoints. These sequences are thought to render DNA accessible to the machinery involved in recombination. For example, dinucleotide repeats are thought to promote meiotic recombination in yeast, possibly by inducing a Z-DNA conformation (Treco and Arnheim 1986). Screening for sequences implicated in the recombination process has identified numerous sequences, including dinucleotide repeats, in the three recombination intervals. However, similar sequences were identified in the three control regions, and identification of many more recombinational breakpoints in vertebrates is necessary before ubiquitous motifs can be distinguished from actual recombination signal sequences.

Together, familial recombination studies and linkage disequilibrium analysis provide insight into the generation of diversity of the HLA complex. Understanding the dynamics of this process may yield useful information regarding functional relationships among the loci within the complex as well as the role of HLA in disease resistance and susceptibility.

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