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Segregation and Mapping Analysis of Polymorphic HLA Class I Restriction Fragments: Detection of a Novel Fragment

Abstract. *An HLA-B7 complementary DNA clone was used as a hybridization probe to analyze the segregation pattern of polymorphic class I restriction fragments in several families whose HLA types had been determined by serological techniques. In one family in which a crossover in the HLA region had occurred, a specific genomic fragment was mapped with respect to the crossover. In another family, a novel genomic fragment present in one child and absent in all other family members was observed. With the exception of this novel fragment, all polymorphic class I fragments observed in this study segregated with a serologically defined parental haplotype, a result consistent with HLA linkage.*

The human major histocompatibility complex (MHC) is a highly polymorphic genetic region (HLA) on the short arm of chromosome 6; this region encodes products involved in various immunological functions [reviewed in (*1*)]. The class I genes encode the serologically defined transplantation antigens, *HLA-A*, *B*, and *C* as well as the human analogs to the murine Qa and T1 antigens (*2*, *3*). The transplantation antigens—composed of a polymorphic glycoprotein of 45,000 daltons associated with an invariant polypeptide of 12,000 daltons, β-2 microglobulin, which is encoded on chromosome 15—are present on the surface of all nucleated cells. Population studies have shown significant associations—and family analyses have demonstrated linkage—between specific serologically defined HLA antigens and a large number of human diseases [reviewed in (*4*)]. The genetic complexity of the class I genes revealed by recombinant DNA analysis is significantly greater than that predicted by serological analysis (*5*, *6*), with estimates of more than 30 class I genes in the mammalian MHC. Consequently, the identification and characterization of DNA sequence polymorphisms in the HLA region within normal (*7*, *8*) and diseased populations and their relation to the HLA alloantigens defined by classical serological and immunochemical techniques is of interest. DNA sequence polymorphisms can be detected as polymorphisms in the length of restriction fragments. We report the segregation analysis of polymorphic class I restriction fragments in two families. The HLA-B7 complementary DNA (cDNA) clone (*9*) was used as a hybridization probe. In one family, in which a crossover in the HLA region had occurred, a specific genomic fragment was mapped with respect to the crossover; in another family, a new fragment present in one child and absent in all other family members was observed.

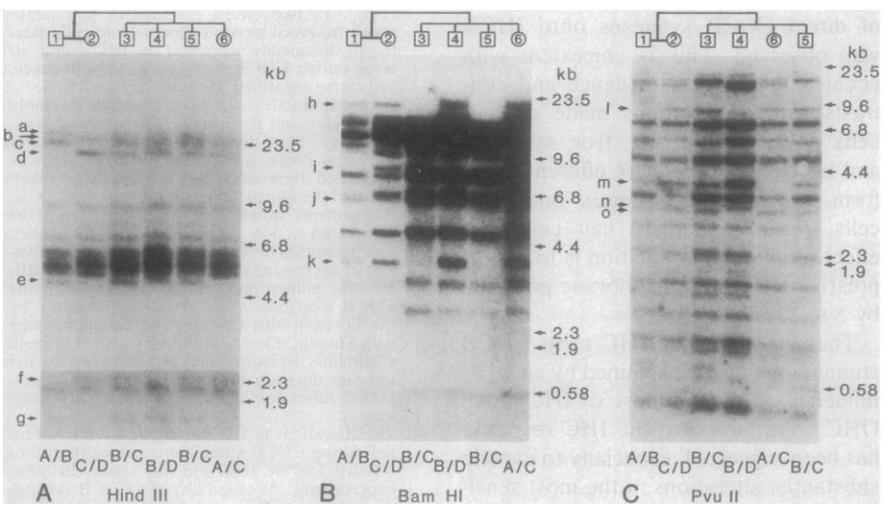

Fig. 1. Genomic blots of restriction endonuclease–digested DNA from an HLA-typed family hybridized with the HLA-B7 cDNA probe (*9*). High molecular weight DNA was prepared from approximately 2×10^7 frozen lymphocytes derived from blood samples of the individual family members as described (*12*). Each genomic DNA was digested with the restriction endonucleases Hind III (A), Bam HI (B), and Pvu II (C); subjected to electrophoresis; transferred to nitrocellulose (*14*, *15*); and hybridized with the nick-translated probe (*16*) as described (*17*). The filters were washed at 20°C in double-strength standard saline citrate and 0.1 percent sodium dodecyl sulfate, and subsequently at 50°C in 0.1-strength standard saline citrate and 0.1 percent sodium dodecyl sulfate, and analyzed by autoradiography. Polymorphic restriction fragments are denoted *a* to *o*. The large arrows represent specific size markers (Hind III digest of λ DNA). The HLA haplotype of the father [A2, C(−), Bw50 (Bw6), DR3, BfS1, Glo1/A11, Cw6, B37 (Bw4), DR5, BfS, Glo1) is designated *A/B* and of that of the mother [A3, C(−), B18 (Bw6), DR3, BfF1, Glo2/A25, C(−), B7 (Bw6), DR2, BfS, Glo1], *C/D*. The fragment n appears in the DNA of only one child and is absent from both parental DNA's. The print displayed in (A) is a composite of two different autoradiograms of the same blot; longer exposure time (bottom) was necessary to reveal the smaller fragments.

Genomic DNA from members of family Stk was digested with Hind III, Bam HI, and Pvu II; transferred to nitrocellulose; and hybridized with the ^{32}P-labeled HLA-B7 probe (Fig. 1). All autoradiographic bands clearly showing polymorphism were denoted by a lowercase letter and are listed in Table 1 along with the segregation pattern and inferred HLA haplotype assignments. Three different segregation patterns could be distinguished. In pattern 1 (exemplified by fragment a), a fragment present in DNA from only one parent appears in the DNA of some but not all of the children, allowing assignment to a unique parental haplotype. In pattern 2 (exemplified by fragment b), a fragment present in DNA from only one parent is present in the DNA of each child; this pattern is consistent with derivation of the fragment from both chromosome 6 homologs of the parent (that is, the mother is homozygous for the fragment, which segregates with both maternal haplotypes). In pattern 3 (exemplified by fragment h), a fragment is present in the DNA of both

parents and of some but not all of the children; this pattern is consistent with derivation of the fragment from one maternal and one paternal haplotype (that is, each parent is heterozygous). Analysis of the Pvu II digest pattern revealed the presence of a novel fragment, 3.4 kilobases (kb) long (designated n), in the DNA of one child (lane 4 in Fig. 1C); this fragment was absent from the DNA of all other family members. In addition, a 3.3-kb and a 0.8-kb Pvu II fragment present in the DNA of all other family members were absent from this individual. The possible significance of this observation is discussed below. A Hind III digest of genomic DNA from members of family Sti was also analyzed (autoradiogram not shown); the segregation pattern of a polymorphic fragment e′ is listed in Table 1. The Hind III fragment e′ from family Sti has the same electrophoretic mobility (~ 5 kb) as the fragment e from family Stk.

Families in which a crossover in the HLA region has occurred provide opportunities for mapping polymorphic restriction fragments with respect to the crossover (*8*). Genomic DNA was extracted from members of family Riv (*10*) in which one member has a recombinant HLA haplotype, HLA-A1, Cw(−), Bw 39 (Bw6), BfS (designated GH), resulting from a crossover between the HLA-C and HLA-A loci of the maternal haplotypes, HLA-A1, Cw3, Bw58 (Bw4), BfF (designated G) and HLA-A2, Cw(−), Bw39 (Bw6), BfS (designated H). The DNA was digested with Pvu II, subjected to electrophoresis, blotted, and hybridized with the ^{32}P-labeled HLA-B7 probe (Fig. 2). Six polymorphic bands (designated p, q, r, s, t, and u) are shown on the autoradiogram and are listed in Table 1. Fragments p and s segregated with the paternal haplotype F. The polymorphic fragment q is apparently homozygous in the father (lane 13 in Fig. 2), segregating with both paternal haplotypes. The fragments t and u are present in both parents (lanes 13 and 14) but absent in children 16, 17, and 18, an indication that both parents were heterozygous and that the fragment was derived from the paternal F haplotype and the maternal H haplotype. A polymorphic restriction fragment present in the mother's DNA and absent from the father's DNA can be mapped with respect to the crossover between the two maternal haplotypes. Fragment r displays the requisite pattern. In the five children who inherited an unrecombined maternal haplotype, r segregates with the maternal haplotype H. DNA from child 15, who inherited the maternal recombinant haplotype GH, contains fragment r. The map order on the short arm of chromosome 6 is centromere, glyoxylase 1, the HLA-D region, Bf, HLA-B, HLA-C, and HLA-A. Fragment r, therefore, is derived from the portion of the recombinant haplotype centromeric to the crossover; that is, r maps centromeric to the HLA-A locus on chromosome H. These

Table 1. Intrafamilial distribution of polymorphic class I restriction fragments. Criteria for inclusion in the table were (i) The presence or absence of the band is clearly discernible in every lane. (ii) The band is polymorphic within the family because at least one member lacks the band. The bands are identified in Fig. 1 or Fig. 2. This number of polymorphic bands represents a minimum estimate since the resolution of some genomic fragments whose mobilities could not be clearly distinguished might be achieved with other electrophoretic conditions. Bands were assigned to the haplotypes with which they showed cosegregation. The likelihood of cosegregation, which is the likelihood that the distribution of the band within the family is consistent with any possible HLA haplotype assortment (given the observed family phenotypes), is calculated by dividing the number of band distribution patterns consistent with HLA cosegregation (given the parental band phenotypes) by the number of possible band distribution patterns in the children.

Endonuclease	Band	Presence of band						Haplotype of band	Likelihood of HLA cosegregation
		Parents		Children					
		Family Stk							
		1 (A/B)	2 (C/D)	3 (B/C)	4 (B/D)	5 (B/C)	6 (A/C)		
Hind III	a	+	−	+	+	+	−	B	3/16
	b	−	+	+	+	+	+	C, D	3/16
	c	+	−	+	+	+	−	B	3/16
	d	−	+	+	+	+	+	C, D	3/16
	e	+	−	+	+	+	−	B	3/16
	f	−	+	+	−	+	+	C	3/16
	g	+	−	+	+	+	−	B	3/16
Bam HI	h	+	+	−	+	−	+	A, D	5/16
	i	−	+	+	−	+	+	C	3/16
	j	+	−	+	+	+	−	B	3/16
	k	+	+	−	+	−	+	A, D	5/16
Pvu II	l	−	+	+	−	+	+	C	3/16
	m	+	+	+	+	+	−	B, D	5/16
	n*	−	−	−	+	−	−		0
	o	+	+	+	−	+	+	A, C	5/16
		Family Sti							
		7 (I/J)	8 (K/L)	9 (J/K)	10 (I/L)	11 (I/K)	12 (J/K)		
Hind III	e′	+	−	+	−	−	+	J	3/16
		Family Riv							
		13 (E/F)	14 (G/H)	15 (F/GH)	16 (E/G)	17 (E/G)	18 (E/G)	19 (F/G)	20 (E/H)		
Pvu II	p	+	−	+	−	−	−	+	−	F	3/64
	q	+	−	+	+	+	+	+	+	E, F	3/64
	r	−	+	+	−	−	−	−	+	H, GH	5/64
	s	+	−	+	−	−	−	+	−	F	3/64
	t	+	+	+	−	−	−	+	+	F, H	5/64
	u	+	+	+	−	−	−	+	+	F, H	5/64

*See text for a discussion of band n.

data demonstrate the feasibility of mapping polymorphic restriction fragments from HLA recombinant families.

With the exception of n, all of the polymorphic restriction fragments segregated with parental HLA haplotypes; this result is consistent with linkage of the segregating genomic fragments to the HLA region. Although classical linkage analysis (lod scores) for a particular fragment cannot be applied to these data, the likelihood (*11*) that by chance alone all except band n of the polymorphic fragments identified here would have shown intrafamilial distributions consistent with HLA haplotype segregation is $(3/64)^3 \times (5/64)^3 \times (3/16)^{11} \times (5/16)^4 = 4.7 \times 10^{-18}$. This result is consistent with the finding of Erlich *et al.* (*12*) that all of the class I genomic fragments are localized on a discrete region, defined by a deletion mutant, on the short arm of chromosome 6. In one family, however, Pvu II digestion revealed the presence, in one child, of a new fragment apparently resulting either from the loss of a Pvu II site by a serologically undetected mutation or from a recombination outside the region detected by serologic and enzymatic markers. The presence of a novel 3.4-kb fragment as well as the absence of a 3.3-kb and a 0.8-kb fragment has been confirmed in the analysis of DNA from an Epstein-Barr virus–transformed cell line derived from this individual. The absence of these two fragments could be attributed to segregation (with the A and C haplotypes), in which case their absence in DNA from the child (haplotype B/D) would be unrelated to the appearance of the novel 3.4-kb fragment. Alternatively, the absence of these fragments might have resulted from the event that gave rise to the new fragment. Recent data on the amino acid sequence of mutant class I antigens (*13*) and nucleotide sequence analysis of mutant genomic clones (*14*) have led to the hypothesis that an event analogous to gene conversion in fungi may be involved in the generation and maintenance of MHC polymorphism (*13, 14*). It is possible that the newly arisen Pvu II fragment observed in this pedigree may have resulted from such an event.

The study of restriction endonuclease fragment polymorphism in families has yielded several important findings. First, the generation of an apparently new fragment, an event revealed by comparing parental and progeny patterns, was observed in family Stk. Second, recombinant haplotypes detected serologically, as in family Riv, have been useful in mapping polymorphic restriction fragments. The analysis of such crossover families can also help to define the specificity of putative locus-specific probes. Third, the segregation analysis of genomic fragments described in this report shows that the genes homologous to the probe we used are part of the HLA region. This methodology is a powerful new tool for the study of MHC gene organization and polymorphism. The DNA polymorphisms defined in these analyses are likely to subdivide some serological specificities and to be correlated with others. Their continued study may help to elucidate the nature of susceptibilities to HLA-linked diseases.

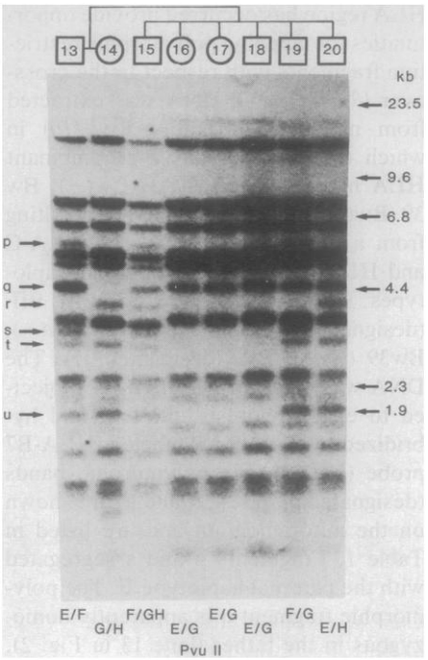

Fig. 2. Genomic blot analysis with class I probe of Pvu II–digested DNA from family with HLA crossover. DNA samples were prepared, digested with Pvu II, and analyzed as described in the legend to Fig. 1. The HLA haplotype of the father is designated *E/F* and that of the mother *G/H*. DNA from the child with the recombinant haplotype (*GH*) is in lane 15. The slightly increased mobility of fragment r in lane 15 relative to its mobility in lanes 14 and 20 is probably due to the slightly decreased amount of genomic DNA located in lane 15.

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Eye Movements of Preschool Children

Kowler and Martins (*1*) have reported that the eye movements of two preschool children are considerably less accurate than adult eye movements under identical viewing conditions. Although we do not dispute the validity of the eye movement recordings obtained from these children, we do disagree with the interpretation that normal children are significantly deficient in oculomotor control and with the conclusion that these presumed deficiencies "limit a child's ability to use eye movements to acquire visual information" (*1*, p. 997).

1) Young children are notoriously poor at maintaining attention during a task, even for brief periods. Despite the authors' opinion that the children were attentive and cooperative, the children's expectations for accurate performance, as well as their level of attention and motivation, were likely to have been lower than the adults'. Furthermore, studies of visual abilities in preschoolers have shown that training and feedback are essential to optimize performance (*2*). Thus, although the two children showed larger mean saccade vector magnitudes during fixation of a stationary target than the adult did, this difference