The Genetics of LD_2. I. Gene Frequency: An Estimation

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THE existence of LD_2 has been inferred from crossing-over families in which weak MLC stimulation occurs between the recombinant (R) and his HLA-D identical siblings (S).[1-9]

This locus, which controls weakly stimulatory determinants, appears to be located in the proximity of HLA-A, as indicated by the finding that some HLA-A/C or A/B recombinants respond to and/or stimulate slightly lymphocytes from siblings that differ only for the HLA-A region.[1-9] We have suggested that the LD_2 locus is of restricted polymorphism, since LD_2 differences were absent in a large proportion of previously reported HLA-A/B and in all HLA-B/D crossing-over families.[8]

Our present report on 16 recombinant families informative for LD_2 (8 HLA-A/B and 8 HLA-B/D) and on the results of MLCs between unrelated HLA-D-identical individuals is consistent with the above hypothesis.

MATERIALS AND METHODS

All reported families were completely typed, on at least two different occasions, for HLA-A, B, C, D, DR, and Bf and tested in intrafamilial mixed lymphocyte cultures (MLCs) using previously described procedures.[10-13] The following criteria were used for ascertaining LD_2 differences between R and S: (1) following the triple normalization procedure, the response in one or in both directions had to be at least threefold higher than the autocontrol values, yet consistently lower than that induced by one-haplotype (HLA-D) difference; (2) when R had more than one HLA-D-identical sibling, all of the latter ones had to display the same pattern of response to R; (3) HLA-D identity between R and S had to be confirmed by HTC-typing, DR typing, or, in the case of unidentified HLA-D alleles, by PLT typing with reagents prepared within the family against each parental haplotype.[11]

Ten blood group systems and 15 biochemical markers were determined by standard techniques and legitimacy of offspring was confirmed in all recombinant families.[14]

Unrelated HLA-D-identical individuals were selected from an HLA-A, B, C, D, DR genotyped panel of 100 families. The strength of MLC responses in checkerboard experiments was quantitated by cluster analysis.[12] Responses in cluster 0 are considered negative, cluster 1 weak positive, clusters 2 and 3 positive.

RESULTS

Estimation of LD_2 Gene Frequency in Recombinant Families

Sixteen crossing-over families (8 HLA-A/B and 8 HLA-B/D) in which the recombinant had at least one HLA-D-identical sibling are included in this analysis. The MLC stimulation patterns between the recombinant and one of his HLA-D-identical siblings were: (A) reciprocal nonstimulation; (B) one-way stimulation; (C) two-way stimulation. The following distribution was observed:

Pattern A		
	HLA-A/B : 3	HLA-B/D : 5
Pattern B		
	HLA-A/B : 2	HLA-B/D : 2
Pattern C		
	HLA-A/B : 3	HLA-B/D : 1

Since the HLA-D-identical, LD_2-different children share one nonrecombinant haplotype, carrying, for example, the allele a, the other parent in which the recombination occurred should be: (1) aa or bb for pattern A; (2) ab for pattern B; and (3) bc for pattern C. Thus a minimum of three LD_2 alleles should be considered.

For n LD_2 alleles, pattern A is expected in $n(n+1)/2$; B in $n(n-1)$; and C in $n(n-1)\ (n-2)/2$ LD_2-informative sib

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Table 1. Estimation of LD_2 Gene Frequency: Recombinant Families

Pattern of MLC	Type of R-S Combination	Number of Possible Combinations for n LD_2 Alleles	Frequency of Patterns for n Alleles
(A) No stimulation	aa-aa	n	$P_i \times P_i^2$
	ab-ab	$\binom{n}{2} = \frac{n(n-1)}{2}$	$P_i \times P_j^2$
Total		$n + \frac{n(n-1)}{2} = \frac{n(n+1)}{2}$	$\sum_{i=1}^{n} P_i\left(P_i^2 + \sum_{j\neq i} P_j^2\right) = \sum_{i=1}^{n} P_i \sum_{j=1}^{n} P_j^2 = \sum_{i=1}^{n} P_i^2$
(B) One-way stimulation	aa-ab	$n(n-1)$	$P_i \times 2P_jP_k$
Total			$2\sum_{i=1}^{n} P_i^2\left(\sum_{j\neq i} P_j\right) = 2\sum_{i=1}^{n} P_i^2(1-P_i)$
(C) Two-way stimulation	ab-ac		$P_i \times 2P_jP_k$
Total		$n\binom{n-1}{2} = \frac{n(n-1)(n-2)}{2}$	$2\sum_{i=1}^{n} P_i\left(\sum_{\substack{j<k \\ j,k\neq i}} P_jP_k\right) = \sum_{i=1}^{n} P_i\left(1 - \sum_{i=1}^{n} P_i^2 - 2P_i\sum_{j\neq i} P_j\right)$
			$= 1 - \sum_{i=1}^{n} P_i^2 - 2\sum_{i=1}^{n} P_i^2(1-P_i)$

pairs (Table 1). Assuming n LD_2 alleles $(A_1, \ldots, A_n)$ with frequencies $P_1, \ldots, P_n$ and $\sum_{i=1}^{n} P_i = 1$, the three patterns will fit into the following equations:

(A) $\sum_{i=1}^{n} P_i^2$

(B) $2 \cdot \sum_{i=1}^{n} P_i^2 (1 - p_i)$

(C) $1 - \sum_{i=1}^{n} P_i^2 - 2 \cdot \sum_{i=1}^{n} P_i^2 (1 - P_i)$

The distribution of the stimulation patterns in our crossing-over series cannot fit these equations unless one LD_2 allele has an $rf > 0.5$

Estimation of LD_2 Gene Frequency in Unrelated HLA-D-Identical Individuals

It is possible that LD_2 also contributes to MLC stimulation between pairs of HLA-D-identical unrelated individuals. In this case, reciprocal stimulation (pattern C) would include not only LD_2 half-identical

Table 2. Estimation of LD_2 Gene Frequency: Nonrecombinant Families

Pattern of MLC	Type of MLC Combination	Number of Possible Combinations for n LD_2 Alleles	Frequency of Patterns for n Alleles
(A) No stimulation			
Type 1	aa-aa	n	$P_i^2 \times P_i^2 = P_i^4$
Type 2	ab-ab	$\binom{n}{2} = \frac{n(n-1)}{2}$	$2P_iP_j \times 2P_iP_j = 4P_i^2P_j^2$
Total		$n + \frac{n(n-1)}{2} = \frac{n(n+1)}{2}$	$\sum_{i=1}^{n} P_i^4 + 4\sum_{i<j} P_i^2P_j^2$
(B) One-way stimulation			
Type	aa-ab	$n(n-1)$	$P_i^2 \times 2P_iP_j = 2P_i^3P_j$
Total			$2\sum_{i=1}^{n}\left(P_i^3\sum_{j\neq i} P_j\right) = 2\sum_{i=1}^{n} P_i^3(1-P_i)$
(C) Two-way stimulation			
Type 1	aa-bb	$\binom{n}{2} = \frac{n(n-1)}{2}$	$P_i^2 \times P_j^2$
Type 2	ab-ac	$n\binom{n-1}{2} = \frac{n(n-1)(n-2)}{2}$	$2P_iP_j \times 2P_iP_k = 4P_i^2P_jP_k$
Type 3	aa-bc	$n\binom{n-1}{2} = \frac{n(n-1)(n-2)}{2}$	$P_i^2 \times 2P_jP_k$
Type 4	ab-cd	$3\binom{n}{4} = \frac{n(n-1)(n-2)(n-3)}{8}$	$2P_iP_j \times 2P_kP_l = 4P_iP_jP_kP_l$
Total		$\frac{n(n-1)(n^2+3n-6)}{8}$	$1 - \sum_{i=1}^{n} P_i^4 - 4\sum_{i<j} P_i^2P_j^2 - 2\sum_{i=1}^{n} P_i^3(1-P_i)$

pairs (ab with ac), as in crossing-over families, but also individuals differing by both LD_2 alleles.

Table 2 shows the type of LD_2 combinations expected to result in the three patterns of MLC stimulations, the number of possible combinations for n LD_2 alleles, and the frequency of the different patterns.

This model has been tested in two different ways. The first one consisted of determining the frequency of the 3 patterns of MLC stimulation in 90 pairs of unrelated individuals heterozygous for the same HLA-D alleles (Table 3). No stimulation occurred in 44%, one-way stimulation in 33%, and two-way stimulation in 23% of MLCs within HLA-D-identical pairs.

The second approach consisted of testing 14 unrelated individuals, homozygous for Dw3 (Dw3-HTCs) in a single checkerboard MLC.

Each of these HTCs was previously documented to be Dw3-DRw3 homozygous by complete family segregation studies. In addition, they were all tested as stimulators on a selected panel comprising 40 Dw3-heterozygous individuals.

Four Dw3-heterozygous individuals were included as responders and stimulators in the checkerboard experiment. Table 4 illustrates the results obtained by triple normalization and cluster analysis of MLC responses between the 14 Dw3 homozygotes. Reciprocal nonstimulation was observed in 44/182 (24%) reactions, one-way stimulation or responsiveness in 72/182 (39%), and reciprocal stimulation in 66/182 (36%) reactions. Three groups of reciprocally nonstimulatory Dw3 HTCs were found. The first comprises 6 individuals (nos. 1–6); the second, 4 individuals (nos. 7–10); the third, only 2 (nos. 11 and 12). Two HTCs (nos. 13 and 14) were reciprocally stimulatory in MLC with all the other Dw3 homozygotes. The first group reacted against, yet failed to stimulate, all HTCs from the second and third groups. This observation, in conjunction with the level of stimulation seen in MLCs between cells from different groups, is consistent with the possibility that the LD_2 type is xx for individuals 1–6, xy for 7–10, xz for 11 and 12, yz for no. 13, and zw for no. 14. The frequency of the x, y, z, and, w LD_2 alleles would then be .64, .18, .14, and .036, respectively, with no significant differences between the observed and expected number of LD_2-homozygous and

Table 3. MLC Responses Within Pairs of HLA-D-Identical Unrelated Individuals

	No. of Individuals	No. of Pairs	Patterns of MLC Stimulation (A) None	(B) One-way	(C) Two-way
Dw1, Dw2	3	6	4	0	2
Dw1, Dw3	4	12	12	0	0
Dw1, Dw4	2	2	2	0	0
Dw1, Dw7	4	12	2	2	8
Dw2, Dw3	4	12	12	0	0
Dw2, Dw4	3	6	0	2	4
Dw2, Dw5	3	6	2	4	0
Dw3, Dw4	2	2	2	0	0
Dw3, Dw5	4	12	0	8	4
Dw4, Dw5	4	12	0	8	4
Dw4, Dw6	2	2	2	0	0
Dw4, Dw7	3	6	2	2	2
Dw5, Dw7	3	6	2	4	0
Dw5, Dw11	3	6	2	4	0
Dw10, Dw11	2	2	2	0	0
Total	46	104	46 (44%)	34 (33%)	24 (23%)

Table 4. Clusters of MLC Responses in a Checkerboard Between Dw3 HTCs

		Stimulators													
HLA(−A,B) Haplotypes	Responders	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1,8/28,8	1	0	0	0	0	0	0	1	1	1	1	1	1	2	2
1,8/28,8	2	0	0	0	0	0	0	1	1	1	1	1	1	2	2
1,8/1,8	3	0	0	0	0	0	0	1	1	1	1	1	1	2	2
1,8/1,8	4	0	0	0	0	0	0	1	1	1	1	1	1	2	2
1,8/1,8	5	0	0	0	0	0	0	1	1	1	1	1	1	2	2
1,8/2,35	6	0	0	0	0	0	0	1	1	1	1	1	1	2	2
11,8/23,50	7	0	0	0	0	0	0	0	0	0	0	1	1	1	2
1,8/1,8	8	0	0	0	0	0	0	0	0	0	0	1	1	1	2
1,8/1,8	9	0	0	0	0	0	0	0	0	0	0	1	1	1	2
1,8/1,8	10	0	0	0	0	0	0	0	0	0	0	1	1	1	2
1,8/2,8	11	0	0	0	0	0	0	1	1	1	1	0	0	1	1
1,8/24,8	12	0	0	0	0	0	0	1	1	1	1	0	0	1	1
1,8/1,8	13	1	1	1	1	1	1	1	1	1	1	1	1	0	1
1,8/30,8	14	1	1	1	1	1	1	2	2	2	2	1	1	1	0

-heterozygous individuals:

	xx	*xy*	*xz*	*xw*
Expected	5.74	3.22	2.50	.64
Observed	6.00	4.00	2.00	0
	yy	*yz*	*yw*	
Expected	.45	.71	.18	
Observed	0	1.00	0	
	zz	*zw*	*ww*	
Expected	.27	.14	.018	
Observed	0	1.00	0	

These results fit with the equations presented in Table 2. They are also in general agreement with the frequency of cluster-0 stimulation induced in the Dw3-heterozygous panel by the Dw3 HTC from the first group (≃90%), second group (≃40%), HTC 13 (≃25%), and 14 (<5%). Nevertheless, it is equally possible that stimulation between unrelated HLA-D-identical individuals reflects the complexity of the HLA-D region, in which distinct, but closely linked, (sub)loci or multiple alleles at a single locus might code for antigenic determinants constituting the same HLA-D cluster.

CONCLUSION

Data obtained from HLA-A/B and B/D recombinant families, which were informative for LD_2, as well as from MLCs between HLA-D-identical unrelated individuals, suggest that LD_2 is a locus of a restricted polymorphism, possibly having 4 alleles of which one has a frequency > .50.

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