CHECKERBOARDS BETWEEN 8WHTCs: POLYMORPHISM OF LD2 OR HETEROGENEITY OF HLA-D ANTIGENS?

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There are many uncontrollable variables that make it difficult to obtain a meaningful interpretation of 'broad and narrow,' 'supertypic and subtypic' HLA-D specificities within a cluster. In this respect clustering techniques for quantitation of MLC responses, which offer the advantage of a robust algorithm without resorting to nonparametric estimators, may be a useful tool for testing the degree of homogeneity of HLA-D groups (1). Using this statistical approach for the analysis of panel responses to 8W HTCs we have identified 12 HLA-D clusters with r values between HTCs in any given group ranging from 0.4 to 1 (2). The typing cells formed mutually exclusive clusters except for DW7 which was positively correlated to DW11 (Table 1). Possible 'splits' of other clusters were also suggested by the behavior of certain HTCs as 'leading' cells and of others as 'inclusions.' To further evaluate the relationships between HTCs of the same cluster, cells that were in sufficient amounts were tested in reciprocal MLCs including as controls HLA-D heterozygotes which differed by one and by two HLA-D antigens. The magnitude of MLC responses was expressed as clusters 0, 1, and 2 corresponding to negative, intermediate, and positive stimulation (Figs. 1-10).

Table 2 gives the frequency of: (a) Reciprocal nonstimulation (pattern A), (b) one-way stimulation (pattern B), and (c) two-way stimulation (pattern C) between pairs of HLA-D-identical HTCs.

If alleles at an HLA locus other than HLA-D, such as LD2, contribute to MLC stimulation, the type of MLC combinations in which these patterns would occur is aa-aa or ab-ab for pattern A, ab-aa for pattern B, and aa-bb, ab-ac, and ab-cd for pattern C.

For n alleles with corresponding frequencies $P_1,\ldots,P_n$ and

$$\sum_{i=1}^{n} P_i = 1$$

the expected frequency of these patterns from the equations: (3):

$$A)\ \sum_{i=1}^{n} P_i^4 + 4\sum_{i<j} P_i^2 P_j^2 \qquad B)\ 2\sum_{i=1}^{n} P_i^3(1-P_i)$$

C) difference to 1. 8W HTCs defining the DW1, DW2, DW3, and DW10 specificities were, with few exceptions (which made no pattern), reciprocally nonstimulatory in MLC (Figs. 1-3 and 10). Reactions observed within DW4, DW5, and DW7 x 11 checkerboards could be interpreted, however, as possibly due to four LD2 alleles, a, b, c, and d.

Thus, if HTCs are assigned the corresponding phenotypes (aa, ab, etc.) and gene frequency is determined by the counting method, one can infer from the DW4 checkerboard the existence of an allele *'a'* with a frequency of 0.65 and *'b'* with a frequency of 0.35. Similarly, four alleles, *a, b, c,* and *d* with a corresponding frequency 0.44, 0.39, 0.11, and 0.06, respectively, can be derived from the DW5 checkerboard. The DW6, DW8, and DW9 checkerboards show at least four, two, and four different types of HTCs, respectively (Figs. 6, 8, and 9). Reciprocal MLCs between DW7 and DW11 HTCs suggest that the DW7 HTCs might be LD2 (*aa*) homozygous whereas the DW11 HTCs are in fact DW7 homozygotes which are heterozygous for LD2. From the DW7 x DW11 checkerboard three alleles with a frequency of 0.67, 0.28, and 0.05, respectively, can be inferred.

Within each of the HLA-D clusters which was informative for LD2 there was no great deviation in Hardy-

Table 2.
MLC Responses* within Pairs of HLA-D identical HTCs.

HLA-D Specificity of HTCs	No. of Pairs	Patterns of MLC Stimulation (A) none	(B) one-way	(C) two-way
DW1	56	40	16	0
DW2	56	56	0	0
DW3	30	27	3	0
DW4	71	50	14	7
DW5	72	12	28	32
DW6	12	2	2	8
DW7 & DW11	72	26	36	10
DW8	6	2	0	4
DW9	30	6	6	18
DW10	6	6	0	0
Total	411	227	105	79
Observed Frequencies:		0.552	0.256	0.192
Expected Frequencies:		0.305	0.221	0.474

* Only cluster 0 responses were considered negative

Fig. 1 Dw1 Checkerboard

responders \ stimulators	103	104	105	108	403	101	102	106
103	0	0	0	0	0	0	1	1
104	0	0	0	0	0	1	1	1
105	0	0	0	0	0	0	1	0
108	0	0	0	0	0	1	1	1
403	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0

Fig. 2 Dw2 Checkerboard

responders \ stimulators	109	110	111	112	113	114	115	117
109	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0

Fig. 3 Dw3 Checkerboard

responders \ stimulators	118	120	121	123	125	119
118	0	0	0	0	0	1
120	0	0	0	0	0	0
121	0	0	0	0	0	0
123	0	0	0	0	0	1
125	0	0	0	0	0	1
119	0	0	0	0	0	0

Fig. 4 Dw4 Checkerboard

responders \ stimulators	127 aa *	128 aa	130 aa	129 aa	135 ab	133 ab	132 ab	126 ab	131 ab	134 bb
127	0	0	0	0	1	ND	1	2	2	1
128	0	0	0	0	1	1	1	0	2	1
130	NOT DONE									
129	NOT DONE									
135	0	0	0	0	0	0	0	0	0	0
133	0	0	0	0	0	0	0	0	1	0
132	0	0	0	0	0	0	0	0	0	0
126	0	0	0	0	0	0	0	0	0	0
131	0	0	0	1	0	0	0	0	0	0
134	1	2	1	1	1	1	1	1	2	0

Fig. 5 Dw5 Checkerboard

responders \ stimulators	137 bb *	136 ab	139 ab	141 ab	143 ab	138 bc	140 aa	6W 3005 aa	311 cd	321
137	0	1	1	1	2	2	2	2	2	2
136	0	0	0	0	0	1	0	0	1	1
139	0	0	0	0	0	1	0	0	1	1
141	0	0	0	0	0	2	0	0	1	1
143	0	0	1	0	0	2	0	0	2	1
138	0	1	2	2	1	0	2	2	1	2
140	1	1	1	1	1	2	0	0	1	1
6W 3005	1	1	1	1	1	2	0	0	1	1
311	2	1	2	1	2	2	2	1	0	1
321	NOT DONE									

Fig. 6 Dw6 Checkerboard

responder \ stimulators	144 aa *	145 aa	326 ab	146	148 aa	149 aa	152
144	0	0	2	2	0	0	0
145	0	0	1	2	0	0	1
326	1	0	0	1	0	0	2
146	2	2	2	0	2	2	2

* suggested LD2 phenotype

Fig. 8 Dw8 Checkerboard

stimulators	201 *	202 ax	204 ax	203 ax
201	0	2	2	2
202	2	0	0	0
204	2	0	0	0

Fig. 9 Dw9 Checkerboard

stimulators	205 *	206 ab	207 aa	208 aa	209 aa	306
205	0	2	2	2	2	1
206	2	0	0	0	0	2
207	2	2	0	1	1	2
208	2	2	0	0	0	1
209	2	1	0	0	0	1
306	1	2	2	1	2	0

Fig. 10 Dw10 Checkerboard

stimulators	210	211	213	212
210	0	0	0	0
211	0	0	0	0
213	0	0	0	0

Weinberg distribution, but the numbers are small. Assuming that the *a* and *b* alleles inferred from the three checkerboards were the same and had a frequency of about 0.59 and 0.34, there is, however, a significant difference between observed (pattern A 0.552, pattern B 0.256, pattern C 0.192) and expected (pattern A 0.305, pattern B 0.221 pattern C 0.474) frequencies of none, one-way, and two-way MLC stimulation for the entire experiment (Table 1). This could be attributed to linkage disequilibrium between certain HLA-D and LD2 alleles (a proposition that is hardly consistent with our study on crossover families which have positioned 'LD2' on the A side of HLA (3,4). Alternatively, if LD2 is a locus of restricted polymorphism (3,4) it is possible that most often HLA-D homozygotes which have common LD2 types are selected as typing reagents and that rarer types are, if not missed in family studies, then misinterpreted as 'new HLA-D' in population studies.

The above considerations do not exclude the possibility that MLC stimulation is through two separate but closely linked loci, or that HLA-D antigens are 'complex rather than simple' or that the pattern observed could have changed on repeat experiments with fresh and/or frozen cells from another bleeding.

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Table 1. Pairwise r values (x100) for HTCs forming the DW7 and DW11 Clusters.

	HTC	154	161	162	163	164	166	301	153	156	157	158	159	160
DW11	154		61	54	71	71	52	58	49	50	41	43	45	53
DW11	161			63	61	63	61	61	52	57	59	58	63	59
DW11	162				53	58	53	57	61	57	48	54	56	49
DW11	163					71	71	57	44	48	48	48	53	48
DW11	164						71	59	51	51	51	55	58	51
DW11	166							61	49	52	59	41	58	42
DW11	301								47	50	51	47	52	51
DW7	153									67	61	65	66	61
DW7	156										66	58	65	65
DW7	157											54	69	60
DW7	158												61	55
DW7	159													56
DW7	160													

Figure 7. DW7-DW11 Checkerboard.

			stimulators										
	HTC	154	161	162	163	164	166	153	156	157	158	159	160
		*	ab	ab	ab	ab	ab	ac	aa	aa	aa		
r	DW11 154												
e	DW11 161	1	0	0	0	0	0	0	0	0	0	2	0
s	DW11 162	2	0	0	0	0	0	1	0	0	0	2	0
p	DW11 163	2	0	0	0	0	0	2	0	0	0	1	1
o	DW11 164	1	0	0	0	0	0	0	0	0	0	1	0
n	DW11 166	1	0	0	0	0	0	1	0	0	0	2	1
d	DW7 153	2	1	1	1	1	2	0	0	0	1	ND	1
e	DW7 156	2	1	2	2	2	2	1	0	0	0	1	0
r	DW7 157	2	2	2	2	2	2	1	0	0	0	1	0
s	DW7 158	2	1	2	1	1	2	1	0	0	0	1	0

* suggested LD2 phenotype