HLA-D-DR RELATIONSHIPS. III. REACTION PATTERNS OF 8W HTCS

N. Suciu-Foca, M. Godfrey, C. Rohowsky, R. Khan, A. Suciu, M. Sankel, A. Sawczick, M. Hardy, and K. Reemtsma

Columbia University, New York, New York

The use of the Linear Clustering Analysis Program (LCA) for quantitation of MLC responses allowed us to obtain estimates of the correlation between different HTCs in their capacity to stimulate the panel (1-3). Objective statistical criteria were thus utilized for grouping the panel responses to HTCs in: negative (cluster 0), intermediate (cluster 1), and positive (clusters 2+3), after having standardized (by a triple normalization program) the performance in unidirectional MLC of all stimulators and responders used in one experiment.

All 115 8W HTCs, plus an additional eight local HTCs, were used as stimulators in a single experiment for typing 54 unrelated responders. After exclusion of outliers, a 117 x 117 HTC correlation matrix was generated by iterative X^2 analysis of all HTCs with respect to cluster 0, 1, and 2+3 stimulation (3 x 3 comparison) induced in the panel. HTCs with significant correlation ($X^2 > 13$) were then clustered in the same group. The repeat experiment was performed under identical conditions and the results analyzed independently. The two experiments showed good agreement, leading to the following conclusions.

Of 115 8W HTCs, 86 belong to one of the 'classical' clusters. Two other cells (8W309 and 8W322) defined a new cluster which we call LD12. Two cells, 8W116 and 8W304, appear to be heterozygous, DW2,6 and DW4,5 respectively, since they only typed the corresponding heterozygotes. Six cells (8W147, 8W150, 8W212, 8W308,

8W313, and 8W402) were technically unusable while the remaining 18 cells had no specific pattern, largely due to their performance as stimulators.

The HLA-D assignment of 8W HTCs is given in Table 1.

Twenty-eight HTCs (indicated by an asterisk in the Table) were also tested in family studies. The consistent segregation of typing responses (cluster 0 by LCA) to specific groups of HTCs, with the expected haplotype, confirmed the cluster assignment derived from cross-reference typing of unrelated panel members. Typing responses to DW7 and DW11 HTCs segregated in cis in DW11-positive individuals. Less than 10% typing responses shifted from cluster 0 to cluster 1, in repeat family typings, confirming the validity of the LCA program for quantitation of MLC responses.

Associations of HLA-D with HLA-B and DR antigens

For assignment of HLA-D antigens the requirement was made that the responder should type with the majority of HTCs defining any given HLA-D cluster. No individual typing for more than two HLA-D clusters was found (except for DW11 heterozygotes, who typed with both DW7 and DW11 HTCs, in addition to their other antigen). All members of the HLA-D panel were tested on the complete genetic set of sera. The groups of DRW sera used for the definition of DRW1-5, 7, and 8 were previously described (4), while patterns for DRW6 assignments are

Table 1.

```
DW1    : 101*, 102*, 103, 104*, 105, 106, 107, 108, 403.
DW2    : 109*, 110*, 111*, 112, 113, 114, 115, 117.
DW3    : 118*, 119, 120, 121*, 122, 123*, 124, 125, 302, 307.
DW4    : 126, 127*, 128*, 129*, 130, 131, 132, 133, 134, 135.
DW5    : 136*, 137*, 138*, 139, 140, 141, 142, 143, 311, 329, 310, 321.
DW6    : 144*, 145*, 146, 148, 149, 151, 152, 317, 326, 405.
DW7**  : 153*, 156*, 157, 158, 159, 160.
DW8    : 201*, 202*, 203, 204*.
DW9    : 205, 206, 207, 208, 209, 306.
DW10   : 210*, 211*, 213*, 316.
DW11** : 154, 161*, 162*, 163*, 164, 166, 303.
LD12   : 309, 322.
```

* HTCs also used in family segregation studies.

**DW11 positive responders type with both DW7 and DW11 HTCs, DW7 positive
   individuals type with DW7 but not with DW11 HTCs.

given in the accompanying paper. DRW assignment was based on reactivity with most (80%) or all sera in the major groups.

Since the population tested is not random, the gene frequencies, haplotype frequencies, and delta values refer only to this relatively small sample. All significant associations are listed in Table 2.

Confirming our previous data, we found linkage disequilibrium between DRW4 and DW4, DW10 and LD12 (our previous SFN1) and between DRW2 and both DW2 and DW9 (5,6). DW9 also shows significant delta values with B18 and BW39, while LD12 is associated with B40.

REFERENCES

1. Rubinstein P, et al. Complete typing of the HLA region in families. III. Analysis of responses in the primed lymphocyte test (PLT). Transplant Proc 1977, 9:1747.

2. Suciu-Foca N, et al. Quantitation of HLA-D differences. Tissue Antigens 1978 12:94.

3. Suciu-Foca N, Rubinstein P. Immunogenetics of HLA-D: HTC and B-cell typing. In *Histocompatibility Testing 1977*, Bodmer WF, et al, eds, Munksgaard, Copenhagen, 1978, 543.

4. Suciu-Foca N, et al. HLA-D-DR relationships. I. Pattern of reactions of HTCs with the genetic set of 8th Workshop sera. This volume.

5. Suciu-Foca N, et al. Evidence that HLA-D is different from DR and that two different HLA loci code for B-cell antigens. Transplant Proc, In press.

6. Suciu-Foca N, et al. Association of DW9 with DRW2. Transplant Proc, In press.

Table 2. Significant Linkage Disequilibria in the HLA-D panel of responders.

	χ^2	r	Δ	h.f.		χ^2	r	Δ	h.f.
DW1 –DRW1	40.5***	0.30	0.045	0.048					
DW2 –DRW2	39.9***	0.79	0.130	0.171	DW2 –B7	20.3***	0.56	0.083	0.112
DW3 –DRW3	56.5***	0.94	0.067	0.073	DW3 –B8	56.5***	0.94	0.067	0.073
DW4 –DRW4	25.4***	0.63	0.084	0.107	DW4 –BW44	9.0**	0.38	0.044	0.060
DW5 –DRW5	52.1***	0.90	0.080	0.090					
DW6 –DRW6	39.6***	0.79	0.058	0.065					
DW7 –DRW7	37.9***	0.77	0.051	0.056	DW7 –B13	10.0*	0.40	0.014	0.015
DW8 –DRW8	54.0***	1.00	0.060	0.065	DW8 –BW35	5.2*	0.29	0.026	0.036
DW9 –DRW2	7.4*	0.34	0.030	0.040	DW9 –B18	4.7*	0.27	0.012	0.015
					DW9 –BW39	24.4**	0.62	0.015	0.016
DW10–DRW4	6.6*	0.32	0.025	0.032	DW10–BW38	47.2***	0.86	0.023	0.024
DW11–DRW7	20.6***	0.57	0.029	0.032	DW11–BW50	19.6**	0.55	0.015	0.016
LD12–DRW4	6.6*	0.32	0.025	0.032	LD12–BW40	26.1***	0.64	0.029	0.032

Significance levels of χ^2 are: *p<0.05; **p<0.01; ***p<0.001