Brief Definitive Report

THE HLA SYSTEM IN THE FAMILIES OF PATIENTS WITH JUVENILE DIABETES MELLITUS*

By P. RUBINSTEIN, N. SUCIU-FOCA, J. F. NICHOLSON, M. FOTINO, A. MOLINARO, L. HARISIADIS, M. A. HARDY, K. REEMTSMA, AND F. H. ALLEN JR.

(From The Lindsley F. Kimball Research Institute of the New York Blood Center and The College of Physicians and Surgeons of Columbia University, New York 10021)

The existence of associations between certain diseases and particular alleles or haplotypes of the HLA system has stimulated considerable interest (1, 2). In the case of juvenile diabetes mellitus (JDM) association with HLA both in families and in the general population have been investigated. Singal and Blachman (3) reported a slight increase in the frequency of W15 among patients, as did Nerup et al. (4) who also found increased frequency of HLA-8. The "risk" conferred by these two antigens was apparently additive. Similar associations were described by Cudworth and Woodrow (5). This "population" approach was refined by Thomsen et al. (6) who included *D*-locus (LD) typing and found the association of JDM to be strongest with LD-8a (DW3). A "family study" approach (2) was followed by Cudworth and Woodrow (7) who reported significant excess of HLA identity in affected siblings within the same family, even when the haplotypes involved did not include either B8 or BW15. Unfortunately, these authors did not give the phenotypes of the complete pedigree, which would be indispensable in assessing the statistical significance of the increased frequency of HLA-identical affected siblings.

We report here the complete HLA and Bf typing of 10 families having one or more children with JDM. In all the patients, the age of onset was 16 yr or less. Several of the normal sibs are below this age and may later become diabetic, so that differences between "affected" and "nonaffected" children may have to be reconsidered in the future. However, the ages of children in both groups are not significantly different.

Materials and Methods

HLA Typing. The serologically defined (SD) antigens were determined with the microcytotoxicity test using multiple sera for 18 HLA-A, 16 HLA-B, and 5 HLA-C specificities. MLC reactivity was tested and analyzed as described previously (8), and typing for *D*-locus antigens (LD antigens) was done with 12 different homozygous cells, two for each of the following: DW1, DW2, DW3, DW4, and DW5, and one for each of two "new" specificities.

Bf Typing. The immunofixation technique of Alper et al. (9) was used with minor modifications.

Other Markers. Blood groups and biochemical markers were determined by standard techniques. The following traits were studied: (*a*) blood groups: ABO, Rh, MNSs, P1, Kell, Duffy, Kidd, Lutheran, Diego, and Xg^a; (*b*) biochemical markers; Hp (haptoglobin), Tf (transferrin),

* Supported by Grant HL-09011 of the National Institutes of Health and by a Grant from the Fay-Hunter Estates.

PGM-1 (phosphoglucomutase 1), AP (acid phosphatase), ADA (adenosine deaminase), Hb (hemoglobin), ED (esterase-D), GPT (glutamic pyruvic transaminase), C3, Gm, Inv, Om (orosomucoid), Gc (globulin component), Ag (lipoprotein marker), and PCE (pseudo cholinesterase).

Results

Table I gives the results of SD, LD, and Bf typing in the 10 families. In families one, two, and seven the father was unavailable and the haplotypes shown were therefore inferred from those found in the rest of the pedigree.

HLA Antigens. No deviations from expected frequencies were found for SD antigens, including HLA-B8 and HLA-BW15; the small size of the sample limits the significance of this fact. On the other hand, in families two and six, the fathers were SD and LD homozygous. Additionally, in family two the mother shared the paternal *A1, B8, DW3* haplotype.

HLA and JDM. 4 of the 10 families (1–4) had only one diabetic child each while the other six (5–10) included two or three. Comparison of the HLA genotype of the diabetic children in each sibship of this second group of families shows no significant increase in identity for HLA-A and -B (Table II) ($P = 0.1$). On the other hand, a significant increase in HLA-D (LD) identity is observed among the diabetic siblings ($P = 0.0013$). The overall association of HLA-D and diabetes can be estimated by a 2×2 comparison, using only the sibs of the first diabetic child of each family.

		Diabetes	
		yes	no
HLA-D identity	yes	6	7
	no	1	13

; $P = 0.03$ (Fisher's exact method).

Thus, the diabetic sibs are HLA-D identical with a significantly increased frequency, but HLA-D identity is not the sole requirement for the appearance of JDM.

Intra-HLA Recombination. Table I shows that 6 out of the 37 children (16%) bear recombinant haplotypes of which 4 are paternal and 2 maternal. The recombination fraction is actually higher than this figure would indicate since in four families with 12 children the father was either HLA-homozygous (families two and six) or missing (one and seven), and the chance to detect a cross-over is thus halved in them. Three of the six recombinations occurred between HLA-A and HLA-B and three between HLA-B and HLA-D.

The recombinant in family 11 showed an unusual HLA sequence, placing the *Bf* locus on the *A* side of the A-B cross-over. This family will be reported on separately.[1]

There is, therefore, a large increase in the frequency of recombination within HL-A in these families. In a separate study of some 600 families with over 2,000 children, we found only six definite intra-HLA recombinants in reasonable agreement with the experience of other groups (10). Even if the overall fre-

[1] Rubinstein, P., N. Suciu-Foca, and F. H. Allen Jr. 1976. HLA: the genetic sequence is not constant. Submitted for publication.

TABLE I

HLA Genotypes of 10 Families With JDM

Family	Father						Mother						1st Recombinant						2nd Recombinant						Nonrecombinant (diabetics/non-diabetics)				Total children	
	Haplotype	HLA A	HLA B	HLA C	HLA D	Bf	Haplotype	HLA A	HLA B	HLA C	HLA D	Bf	Haplotype	HLA A	HLA B	HLA C	HLA D	Bf	Haplotype	HLA A	HLA B	HLA C	HLA D	Bf	ac	ad	bc	bd	Diabetics	Nondiabetics
1. Sm	*a**	[–	W21	–	x	S1]	*c*	9	14	–	W3	S													1*				1	2
	b	[?	?	?	?	?]	*d*	2	W17	–	Y	F													2					
2. Fu	*a**	[1	8	–	W3	F]	*c*	1	8	–	W3	S													1*				1	2
	b	[1	8	–	W3	S]	*d*	2	7	–	W2	S																2		
3. Sm	*a*	9	8	–	W3	S	*c*	2	W15	–	Y	S														1*			1	5
	b	3	14	–	x	S	*d*	W29	12	–	W5	S													3	1		1		
4. Ki	*a*	W30	–	–	–	F1	*c*	2	8	–	W3	S	*a/b**	W30	18	–	W5	F1											1	3
	b	10	18	–	W5	F	*d*	3	7	–	Y	S	*c*	2	8	–	W3	S									1	2		
5. Br	*a*	11	8	–	W3	S	*c*	3	W15	–	Y	S														1*	1*		2	1
	b	–	14	–	x	S	*d*	W32	W40	–	W4	S														1				
6. Ma	*a**	10	12	–	W2	S	*c**	2	18	–	W1	S														3*			3	1
	b	10	12	–	W2	S	*d*	9	x	–	W3	S1													1					
7. Fi	*a*	[19	–	–	x	S]	*c*	2	12	–	Y	F														2*			2	0
	b	[?	?	?	?	?]	*d*	11	27	–	W1	S																		
8. Co	*a*	2	7	–	W2	S	*c*	1	8	–	Y	S	*a/b**	2	7	–	W5	S	*a/b*	2	7	–	W5	S			1*		2	5
	b	2	W35	–	W5	S	*d*	9	7	–	W5	S	*d*	9	7	–	W5	S	*d*	9	7	–	W5	S	1	2	1			
9. He	*a**	1	8	–	W3	S	*c*	9	W27	–	W1	S	*a/b**	2	W40	–	W3	S	*a**	1	8	–	W3	S					2	0
	b	2	W40	–	–	S	*d*	W28	W15	–	Y	F	*c*	9	W27	–	W1	S	*c/d*	W28	W27	–	W1	S						
10. Ma	*a*	2	–	–	x	ND	*c*	3	W35	W4	Y	ND	*a**	10	W16	–	W5	ND										1*	2	1
	b	10	W16	–	W5	ND	*d*	10	8	–	Z	ND	*c/d*	3	8	–	Z	ND									1				

Brackets: individual unavailable, haplotypes deduced; ND, not done.

* JDM patients.

TABLE II

HLA Genotypes of Diabetic Children in Six Families with Two or Three Such Offspring

Family	Sibling 1	Sibling 2	Sibling 3	No. of haplotypes in the family	No. of SD-identical pairs	No. of LD-identical pairs
5. Br	A11,B8,DW3 AW32,BW40,DW4	A-,B14 A3,BW5	–	4	0	0
6. Ma	A10,B12,DW2 A9,B-,D-	A10,B12,DW2 A9,B-,D-	A10,B12,DW2 A9,B-,D-	3	3*	3*
7. Fi	A19,B-,D- A11,B27,DW1	A19,B-,D- A11,B27,DW1	– 3	1	1	
8. Co	A2,BW35,DW5 A1,B8,D-	A2,B7,DW5‡ A9,B7,DW5	–	4	0	1
9. He	A2,BW40,DW3‡ A9,BW27,DW1	A1,B8,DW3‡ AW28,BW27,DW1	–	4	0	1
10. Ma	A10,BW16,DW5 A10,B8,D-	A10,BW16,DW5‡ A3,B8,D-	–	4	0	1
					4§	7‖

* Three pairs are counted for statistical reasons: sib 1–2, 1–3, and 2–3.
‡ Indicates recombinant-bearing individuals.
§ $P = 0.1$, Fisher's exact method.
‖ $P = 0.0013$, Fisher's exact method.

quency of intra-HLA recombination is assumed to be as high as 1%, the data given here differ with a $P = 2.0 \times 10^{-6}$.

Discussion

The genetic component in diabetes, though apparent, is still not clearly understood (11–15). The finding of associations in the Danish and English between early onset diabetes (JDM) and particular HLA antigens may serve to separate this form of diabetes from a heterogeneous group of similar diseases with different etiologies (6). The association with the SD antigens B8 and BW15 are doubtful or nonexistent in other populations (3, 16, 17), suggesting that the HLA system is not directly involved in JDM, but that it maintains linkage disequilibrium with nearby genes that are so involved. The Δ-value for the association with DW3 (formerly LD-8a) is higher than those with the SD antigens (5), supporting the idea of a closely linked gene, located to the *D* side of HLA that is associated with increased risk of JDM.

A very high rate of intra-HLA recombination caused dissociation between SD and LD identity and established that the risk of developing JDM is significantly increased in the sibs of patients that inherit identical LD antigens, whereas no such increase is found among their SD-identical, LD-nonidentical siblings.

The association between LD identity and JDM is not absolute: seven siblings having the same LD antigens as a patient were not diabetic. In only one family, however, was a second diabetic sib LD different from the first. This indicates that LD-linked genes are probably very important but not the sole determinants of the risk to develop JDM.

Most exciting is the high rate of intra-HLA recombination in these families

with JDM. This increased rate of recombination is present in both fathers and mothers and is not dependent on the presence of overt diabetes. The chromosomal instability may, therefore, be caused by a gene similar to *rec-1* which increases about 20-fold the frequency of recombination at the *his-1* locus of *Neurospora crassa* (18). This type of phenomenon may also affect the chromosomal region bearing histocompatibility and other recognition-associated traits in mammals (19). In the mouse's IXth linkage group there are genes (alleles at the *T* locus) that suppress recombination along at least 14 map units, between T and *H-2* (references in 20). The MHC may be subject to some sort of genetic control of its recombination rates. It is tempting to speculate that gene(s) responsible for susceptibility to diabetes may affect this control. Their effect on the pathogenesis of JDM might be related to their capacity to produce these frequent recombinations.

Summary

The HLA and *Bf* genotypes were determined in 10 families with one or more children with JDM. A statistically significant association was found between HLA-D-identity and the chance to present JDM within a sibship. No such association was detectable with the SD antigens. A highly significant increase in the frequency of intra-HLA recombination was also found in these families.

Received for publication 9 February 1976.

References

1. Moller, G. 1975. HLA and disease. *Transplant. Rev.* 22.
2. Levine, B. B., R. H. Stember, and M. Fotino. 1972. Ragweed hay fever: genetic control and linkage to HLA haplotypes. *Science (Wash. D.C.).* 178:1201.
3. Singal, D. P., and M. A. Blachman. 1973. Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies, and tissue antibodies in patients with diabetes mellitus. *Diabetes.* 22:429.
4. Nerup, J., P. Platz, O. Ortved-Andersen, M. Christy, J. Lyngsoe, J. E. Poulsen, L. P. Ryder, L. Staub Nielson, M. Thomsen, and A. Svejgaard. 1974. HLA antigens and diabetes mellitus. *Lancet.* 2:864.
5. Cudworth, A. G., and J. C. Woodrow. 1974. HL-A antigens and diabetes mellitus. *Lancet.* 2:1153.
6. Thomsen, M., P. Platz, O. Ortved-Andersen, M. Christy, J. Lyngsoe, J. Nerup, K. Rasmussen, L. O. Ryder, L. Staub Nielsen, and A. Svejgaard. 1975. MLC typing in juvenile diabetes mellitus and idiopathic Addison's disease. *Transplant. Rev.* 22:125.
7. Cudworth, A. G., and J. C. Woodrow. 1975. Evidence for HL-A linked genes in juvenile diabetes mellitus. *Br. Med. J.* 3:133.
8. Suciu-Foca, N., and J. Dausset. 1975. Mixed lymphocyte cultures in a family with an LD allele shared by the parents. *Tissue Antigens.* 5:137.
9. Alper, C. A., T. Boenich, and L. Watson. 1972. Genetic polymorphism in human glycine-rich B-glycoprotein. *J. Exp. Med.* 135:68.
10. Belvedere, M. C., E. S. Curtoni, J. Dausset, L. U. Lamm, W. Mayr, J. J. van Rood, A. Svejgaard, and A. Piazza. 1975. On the heterogeneity of linkage estimations between LA and four loci of the HL-A system. *Tissue Antigens.* 5:99.
11. Steinberg, A. G. 1955. Heredity and diabetes. *Eugen. Q.* 2:26.
12. Tattersall, R. B., and D. A. Pyke. 1972. Diabetes in identical twins. *Lancet.* 2:1120.

13. Simpson, N. E. 1969. Heritabilities of liability to diabetes when sex and age at onset are considered. *Ann. Hum. Genet.* **32**:283.
14. Smith, C., D. S. Falconer, and L. J. P. Duncan. 1972. A statistical and genetical study of diabetes. II. Heritability of liability. *Ann. Hum. Genet.* **35**:281.
15. Darlow, J. M., C. Smith, and L. J. P. Duncan. 1973. A statistical and genetical study of diabetes. III. Empiric risks to relatives. *Ann. Hum. Genet.* **37**:157.
16. Finkelstein, S., E. Zeller, and R. L. Walford. 1972. No relation between HL-A and juvenile diabetes. *Tissue Antigens.* **2**:74.
17. Seignalet, J., J. Minouze, G. Jaffiol, J. L. Selam, and H. Lapinski. 1975. HL-A in Graves' disease and diabetes mellitus insulin dependent. *Tissue Antigens.* **6**:272.
18. Jessop, A. P., and D. G. Catcheside. 1965. Interallelic recombination at the *his-1* locus in *Neurospora crassa* and its genetic control. *Heredity.* **20**:237.
19. Artzt, K., and D. Bennett. 1975. Analogies between embryonic (T/E) antigens and adult major histocompatibility (H-2) antigens. *Nature (Lond.).* **256**:546.
20. Bennett, D., and L. D. Dunn. 1971. Transmission ratio distorting genes on chromosome IX and their interactions. *In* Proceedings of the Symposium on Immunogenetics of the H-2 System. Karger A. G., Basel, Switzerland. 90.