Excess of DR3/4 in Type I Diabetes

What Does It Portend?

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SUMMARY

The HLA-DR genotypes of 158 new type I diabetic probands from simplex families are compared with those of 43 multiply affected sibships. There were no significant differences in the gene frequencies of the insulin-dependent diabetes mellitus (IDDM)–associated alleles, DR3 and DR4, and whereas the DR3/4 heterozygotes were as frequent among simplex probands as among the first affected of multiplex sibships, subsequently affected sibs displayed lower frequencies of this genotype in this as well as in previously reported samples, indicating that the excessive risk associated with DR3/4 heterozygosity depends on the order of affection and thus on environmental factors. It is proposed that the penetrance of the susceptibility gene is enhanced by epistatic effects of this genotype and that this enhancement is strongest under conditions of low environmental liability. Thus, the excessive risk for DR3/4 individuals appears to depend on secondary interactions between DR and the environmental factors that trigger the onset of this disease and does not in itself indicate the existence of distinct susceptibility alleles in coupling with these genes, i.e., of genetic heterogeneity or overdominance. DIABETES 1986; 35:985–89.

The existence of associations between insulin-dependent diabetes mellitus (IDDM) and certain HLA alleles, especially DR3 and DR4, is widely recognized[1–3] and genetic linkage between HLA and the putative IDDM-susceptibility genes has been formally proven by family studies.[3–7]

The mode of inheritance, however, has remained controversial. Thus, although the HLA genotypes of affected sib pairs are compatible with recessive inheritance,[3,8–10] family studies have produced estimates of gene frequency and penetrance that fail to accurately predict the population prevalence of this disease.[3,11] This inconsistency, coupled with the demonstration of excessive risks for the compound heterozygotes DR3/4 in some samples,[3,7,12,13] has been taken to disprove the hypothesis of recessivity[14] and to indicate the existence of a more complex genetic background for IDDM. Several models have been proposed,[12,15–17] usually based on one set of data, that result in numerical estimates that predictably fit that one set but that may not fit other patient samples. One aspect of IDDM that has been largely overlooked in these calculations is that environmental factors such as viral infections affect the liability of genetically susceptible individuals, as established by the lack of concordance in at least half of identical twin pairs.[19,20] By their nature, such viral infections are variable, and therefore the effects of the environment on individual liability to IDDM may not be taken to be constant. Given that viruses are among the environmental agents most widely suspected of this role, it is conceivable that HLA genes may have epistatic effects on the genetic susceptibility to IDDM, e.g., by regulating immune responsiveness against and perhaps autoimmune reactivity triggered by viral infections. This hypothesis is not incompatible with the close linkage between HLA and the IDDM-susceptibility genes; however, if epistasis is demonstrated, the analysis of linkage will have to be repeated with corrections appropriate to the type of interaction and its consequences. Some theoretical aspects of epistasis have been discussed, for example, by Hodge and Spence[18] and Clerget-Darpoux and Bonaiti-Pellie.[21]

In this report, we present data suggesting a double role for HLA: as a marker for the susceptibility gene and as a factor in the determination of the penetrance.

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MATERIALS AND METHODS

IDDM probands and their families. Diagnosis of IDDM was based on standard criteria.[22] Probands with insulin-dependent ketosis-prone disease were ascertained consecutively at

the Division of Pediatric Endocrinology, Department of Pediatrics, Mt. Sinai Hospital (New York, NY). Age at diagnosis for the first-affected sibling in each family was <16 yr; a few of the second-affected sibs exceeded this age limitation. They and their families were studied after granting informed consent without regard to the structure of the pedigree. We analyzed 158 simplex families not previously studied and 43 multiply affected sibships, including 16 of those in the series studied by Suciu-Foca et al. (pedigree numbers 40, 41, 43–53, 55, and 59).[23] Thirty-five of these families have two affected sibs and eight have three.

HLA typing. The HLA-A, -B, -C, and -DR antigens of the new probands and of all their available first-degree relatives were identified with sera standardized according to the criteria of the 8th International Histocompatibility Testing Workshop.[24] Contrast-fluorescence and two-color–fluorescence techniques were used for class I and class II antigens, respectively.[25,26] Class III and GLO allotypes were also determined, as well as C3, Kidd, Gm, Km, and a number of other non-HLA–linked genetic markers.

RESULTS

HLA-DR frequencies in IDDM patients. We first approached the question of possible genetic heterogeneity of IDDM with regard to the HLA-linked genetic susceptibility by comparing the gene frequencies of the probands in simplex families with those of multiplex ones. The data in Table 1 include the DR frequencies of the patients and their parents and, for affected sib pairs, of the first- and second-affected sib separately. In addition, the DR haplotypes encountered in affected sib pairs have been summed so that the table identifies all haplotypes present in at least one member of the sibling pair ("affected haplotypes").

The DR3 gene frequency is lower and that of DR4 is higher in the probands of simplex compared with duplex families, especially in the first sib affected, but the difference is not significant. The frequency of blank genes has been monitored by testing additional family members in cases of ambiguity. In all, 141 second- and third-degree relatives of 38 diabetic probands have been tested, and some but not all of the blanks have been proven by segregation.

Inheritance of DR3, DR4, and DRX by probands in simplex families. The relevant parental combinations are shown in Table 2. Families with only one DR3 gene in the parents indicate that this allele is inherited by the proband in 81% of such cases, whereas for the DR4 gene the proportion is 85%. Families with both parents heterozygous for DR3 had DR3 homozygous probands in 12 of 16 cases; for DR4, there were 8 of 14 homozygous probands. These figures are not different from the expectations of a recessive situation, assuming linkage disequilibrium between DR3 and DR4 and the susceptibility to IDDM: expected DR3/3 = 0.81^2 = 0.66 = 11/16; expected DR4/4 = 0.85^2 = 0.72 = 10/14. There were also 17 multiplex families with one DR3 and one DR4 heterozygous parent: DR3/4 was observed in 13 of 17 cases. The expectation on the same basis is 0.81 × 0.85 × 17 = 11.7 (Table 3).

DR3/4 excess: frequency in probands of simplex and multiplex sibships. Families in which DR3/4 probands are possible are analyzed here. To eliminate the possibility of more than one DR3/4 combination, the selection disallows families with homozygous parents or with two DR3/4 heterozygous parents. All other combinations in this group have been designated "conducive" and are presented in Table 3.

The frequency of the DR3/4 genotype was the same in the first affected of multiplex sibships as in the probands of simplex ones (76 and 77%, respectively), whereas it was lower in the subsequently affected siblings (40%). This difference was significant within the multiplex families ($P \sim .02$) and for the whole set ($P \sim .001$). The DR3/4 frequency in second- and later-affected sibs (10/25) is still somewhat higher than expected in a random segregation (6.25/25), but the difference is not significant ($\chi^2 = 2.25$; $.2 > P > .1$) given the small numbers.

Linkage between HLA and IDDM: inheritance of DR3, DR4, and DRX by affected sibling pairs. The segregation of the relevant haplotypes from informative parents is given in Table 4. The second-affected sibs shared the DR3, DR4, or DRX haplotypes received by the respective index cases with very similar probability ($\chi^2 = 0.16$; $P \sim .95$), indicating that the DRX (i.e., not DR3, not DR4) haplotypes received by the index cases are as likely to transmit the susceptibility gene as are the DR3- or DR4-carrying ones.

TABLE 1
DR gene frequencies in patients and their parents

	Simplex		Duplex families			
DR type	Parents (N = 617)	Probands (N = 316)	Parents (N = 140)	First affected (N = 70)	Second affected (N = 70)	Affected haplotypes (N = 95)
1	.106	.072	.10	.114	.143	.132
2	.075	.042	.093	.043	.043	.044
3	.177	.260	.250	.357	.257	.297
4	.243	.357	.250	.243	.286	.264
5	.091	.057	.079	.043	.029	.033
6	.083	.048	.071	.029	.057	.055
7	.089	.069	.064	.10	.071	.088
8	.011	.006	.0	.0	.0	.0
9	.01	.016	.007	.014	.014	.01
10	.006	.006	.0	.0	.0	.0
4 × 5	.018	.012	.007	.014	.014	.01
Blank	.082	.054	.079	.043	.086	.066

N, sum of haplotypes included in column.

TABLE 2
Segregation of DR3 and DR4 to type I diabetic probands in simplex families (N = 158)

Parental DR genotypes	N (%)	Proband DR type	N	%
(DR3/X) × (DRX/X)	21 (13)	DR3 positive	17	81
		negative	4	19
(DR4/X) × (DRX/X)	27 (17)	DR4 positive	23	85
		negative	4	15
(DR3/X) × (DR4/X)	35 (22)	DR3/4	28	80
		DR3/X	1	3
		DR4/X	5	14
		DRX/X	1	3
(DR3/X) × (DR3/X)	16 (10)	DR3/3	12	75
		DR3/X	4	25
		DRX/X	0	0
(DR4/X) × (DR4/X)	14 (9)	DR4/4	8	57
		DR4/X	5	36
		DRX/X	1	7

DISCUSSION

Svejgaard and Ryder[12] were the first to notice that the compound heterozygotes for the two IDDM-associated HLA alleles (initially B8 and B15, later DR3 and DR4) constituted a disproportionately large fraction of the patients. Although the amount of this excess varies from sample to sample,[3] in general DR3/4 heterozygotes seem to exceed Hardy-Weinberg expectations and thus to be inconsistent with a recessive mode of inheritance for the susceptibility status.[3,12,14]

We have presented evidence that the preponderance of the DR3/4 type is characteristic of the probands of simplex families and of the first-affected but significantly less so of the later-affected sibs of multiplex sibships. Our own data are consistent with previous findings: the report by Contu et al.[27] indicates that in their families with conducive parents, all 10 probands of simplex sibships and all 5 first-affected but only 3 of the 5 second-affected sibs of multiplex families carried the DR3/4 genotype. Similarly, the first proband in 5 of 9 Canadian affected sib pairs belonged to the DR3/4 class and only 3 of the second-affected sibs belonged.[28] Furthermore, among 19 multiplex Israeli families (ref. 29 and personal communication) there were 9 with conducive parents. Eight of the first-affected and 6 of the second-affected sibs were DR3/4. Thus, we have information on 36 multiply affected sibships with conducive parents: 31 of 36 first-affected and 22 of 42 later-affected sibs inherited both DR3 and DR4 [the difference is significant (χ^2 = 10.13; .001 < P < .005)]. Furthermore, all DR3/4 second- (or later-) affected sibs are thus far DR identical to their first-affected sibs (obviously exceptions should be expected). Thus, the predominance of this genotype decreases in the second- and later-affected sibs of probands. The consistency of this finding is contrary to expectations stemming from a purely genetic explanation of the DR3/4 excess, according to which the genotypes of the first- and later-affected sibs in families with conducive parents should differ by chance alone. The earlier observations of age-at-onset influence on DR-antigen frequency (e.g., see ref. 30), which might explain this phenomenon because DR3/4 and especially DR4 appear to be more frequent in patients with early onset, are probably not relevant to these data because all our patients had onset before the age of 20 and most, including all probands, had onset before age 16. The decrease of DR3/4 in second- and later-affected sibs toward the frequencies expected from a recessive HLA-linked gene[11,12,14] suggests that the exaggerated frequency of this genotype in type I diabetes results from an interaction with environmental factors that is most noticeable for the first proband in a sibship. Because the risk increases for genetically susceptible individuals who are siblings of probands, especially when they have two affected siblings,[31] we suggest that the excessive risk to DR3/4 genotypes relative to other DR genotypes is associated with conditions of comparatively low environmental liability. When this liability is higher, as in siblings of probands, the penetrance for genetically susceptible individuals of other DR genotypes should increase, re-

TABLE 3
Frequency of DR3/4 heterozygotes among index cases and affected sibs in families with conducive parents

	Simplex (N = 158)	Multiplex (N = 43)	All (N = 201)
Conductive parents*	43 (27%)	17 (40%)	60 (30%)
DR3/4 indices	33 (77%)	13 (76%)†	46 (77%)‡
DR3/4 later-affected sibs		10/25 (40%)	10/25 (40%)

*One parent DR3/X and the other DR4/X. However, one may be DR3/4.
†χ^2 = 5.43; P ~ .02.
‡χ^2 = 10.56; P ~ .001.

TABLE 4
Segregation of relevant DR alleles to index cases and affected sibs

Parental DR genotypes	N	Proband genotypes: Index	Proband genotypes: Affected sibling
DRX/DRY	15	DRX = 15	DRX = 10 DRY = 5
DR3 or DR4/DRX	42	DRX = 13	DRX = 9 DR3 or DR4 = 4
		DR3 or DR4 = 29	DR3 or DR4 = 21 DRX = 8

For DRX, X is not 3 or 4.
For DRY, Y is not 3, 4, or X.

ducing the degree of DR3/4 excess. We further suggest that variations in environmental liability underlie the different incidences of IDDM in ethnically related countries,[32] even in the same region at different times.[33] As a consequence, such environmental variations would contribute to the reported differences in the frequency of the DR3/4 genotype in racially similar patient samples and explain the DR frequency changes observed after a few years in one and the same region.[10,34] These observations imply that HLA genotypes contribute to the probability of expression of the susceptibility genes and that the system is not merely a marker for the presence of such genes. Furthermore, they have important implications for the correct evaluation of the inheritance patterns.

If HLA-DR does exert epistatic influence on susceptibility, the sharing of HLA alleles by affected-sib pairs might be a consequence of epistasis rather than of linkage between the corresponding loci.[18,21] We have therefore reinvestigated the joint inheritance of HLA and susceptibility in affected-sib pairs by partitioning the families according to the DR alleles of the parents. Our results indicate that the sharing of DR alleles is independent of the specificity. Thus, if the first-affected sib received DRX from a DR3/X or a DR4/X parent, the second-affected sib shared DRX with the same probability (9/13) that sharing occurred when the first-affected sib received the DR3 or DR4 alleles (21/29): $\chi^2 = 0.04$. Sharing an X allele from an XY parent occurred with the same frequency (10/15). Therefore, in our data, HLA and IDDM susceptibility are inherited in coupling irrespective of whether the DR allele is 3, 4, or X.

A final point refers to the DR gene frequencies in probands from simplex and multiplex families. According to the intermediate model of inheritance,[35] the probability of homozygotes for the susceptibility gene should be significantly higher in familial (i.e., multiplex) cases than in nonfamilial (i.e., simplex) cases. As a consequence, the model requires that the frequency of the IDDM-associated DR genes be higher in familial than in sporadic cases, which are assumed to be mostly heterozygous for the susceptibility gene. Our data are inconsistent with this requirement: in fact, the sum of the frequencies of DR3 and DR4 is slightly, although not significantly, higher for the simplex than for the multiplex cases, which agrees with previous studies that failed to encounter significant differences.[3] Similarly, if most of the simplex probands were heterozygotes for the susceptibility gene, the probability of DR3 or DR4 homozygosity should be low enough that the frequency of either of these antigens should not differ much in probands with one as opposed to two positive parents. In fact, all but one of the probands from intercross families [(DR3/X) × (DR3/X) or (DR4/X) × (DR4/X)] present the corresponding antigen (16 of 16 are DR3 and 13 of 14 are DR4). In these intercross simplex families, the frequency of homozygous probands fits very closely with expectations on the basis that each of the DR3 (and each of the DR4) alleles has the same chance to be inherited by the proband that exists when only one such gene segregates. If most of these probands possessed a single IDDM-susceptibility gene, the expected homozygosity would be only about half of the total positives. The numbers are small but consistent in this series with those reported earlier by us in an independent series of patients[23] and by Contu.[27] In all, 8 DR3- or DR4-intercross families were reported in those studies, and 7 of the probands were homozygotes; the 8th one was a DR3/X heterozygote. Together with our new data, consisting of 30 families with 20 homozygotes and 9 heterozygote positives, the total ratio of homozygotes is 27 of 37 (73%). This is significantly higher than the expected 50% of the positives, assuming that a single HLA-linked gene is involved ($\chi^2 = 7.81$; $P \sim .005$) [for the new data alone: 20 homozygotes and 9 heterozygotes ($\chi^2 = 4.17$; $P < .05$)].

Therefore, it appears that two HLA-DR–linked genes are required by sporadic probands (from simplex families) and that this requirement is present whether the paired DR alleles are identical or different. This view of the data, therefore, does not support the hypothesis of intermediate, as opposed to recessive inheritance of the susceptibility unless the penetrance for the heterozygote is extremely low.

The hypothesis that the mechanisms of IDDM association with DR3 are different from, and additive in terms of risk to, those associated with DR4 (genetic heterogeneity) appears inconsistent with the evidence that the excessive risk to DR3/4 heterozygotes varies in a nongenetic fashion. We conclude that the putative IDDM-susceptibility gene *1*) behaves as a recessive trait; *2*) is linked to HLA; and *3*) maintains linkage disequilibrium with specific HLA-DR haplotypes. Also, the penetrance, which depends mainly on environmental factors, is further influenced by specific DR genotypes.

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