No Excess of DR*3/4 in Ashkenazi Jewish or Hispanic IDDM Patients

PABLO RUBINSTEIN, MARY WALKER, NORMAN MOLLEN, CAROL CARPENTER, SVETLANA BECKERMAN, NICOLE SUCIU-FOCA, ROBERT McEVOY, AND FREDDA GINSBERG-FELLNER

The gene frequencies, haplotype relative risks, and zygotic assortments of HLA-DR in three ethnically defined samples of insulin-dependent diabetes mellitus (IDDM) patients were determined in a prospective family study. Although DR3 and DR4 were positively associated with IDDM in the probands of 123 northern European, 94 Ashkenazi Jewish, and 49 New York Hispanic families, significant excess of DR*3/4 heterozygotes was observed only among the probands from families of northern European ancestry. There was also a significant decrease in the frequency of Bw62,DR4 haplotypes derived by northern European patients from their mothers compared with their fathers. This difference, together with data reported in the literature, suggests that the expressivity of the susceptible genotype(s) in IDDM patients may be modified by protective maternal effects associated with Bw62,DR4 and probably other DR4 haplotypes. Samples of IDDM patients from populations with high frequencies of these modifiers should have different DR-gene frequencies contributed by fathers and mothers, capable of accounting for the observed Hardy-Weinberg disequilibrium. We postulate that, because the mechanism of action of these modifiers is distinct from that of the susceptibility gene, the difference must be considered in devising strategies for elucidation of the mode of inheritance of the disease and for understanding the molecular nature of the susceptibility. *Diabetes* 39:1138–43, 1990

Since the discoveries of association (1–3) and linkage (4–8) between the HLA region and genetic susceptibility to insulin-dependent diabetes mellitus (IDDM), diverse HLA variants have been used as markers to explore the nature of the susceptibility and its mode of inheritance. Early in those studies, Svejgaard et al. (3) noted that, although the antigens B8 and B15 were each statistically associated with susceptibility, the relative risk was even higher for the HLA-B*8/B15 genotype. A stronger IDDM association was later uncovered with the class II antigens DR3 and DR4, and again the DR*3/4 heterozygote had the highest relative risk of all class II genotypes. Similar data have been obtained by most investigators (3,9,10), and the consensus holds that susceptibility is encoded by at least two distinct disease alleles in linkage disequilibrium with either DR3, DR4, or other HLA haplotypes (11,12). In work toward identifying the disease locus, Nepom et al. (13) and Todd et al. (14) showed that the risk associated with DR4 haplotypes is contributed by a DQB1 allele now termed *DQw8*. Low risk seems to associate with aspartic acid at position 57 in DQB1 (14–17). The finding of mixed-isotype dimers of relevant DQA1 and DQB1 molecules by Nepom et al. (18) is consistent with DQ as a likely disease-susceptibility region of HLA.

The identification of HLA haplotypes as genetic markers for IDDM, however, has not led to clarification of the mode of inheritance of the disease. Although the segregation data are consistent with the recessive inheritance of a disease allele at an HLA-linked locus (3,8,19), its penetrance is incomplete as shown by the lack of concordance among HLA-identical siblings of probands (3–5) and, remarkably, among identical twins (20,21). Furthermore, at the DR locus, IDDM patients are out of the Hardy-Weinberg equilibrium expected under simple recessive and intermediate models of inheritance. (Hardy-Weinberg equilibrium should occur, however, only if the penetrance of the IDDM-susceptibility genotype[s] would be independent of any differential effects associated with distinct alleles of the closely linked HLA immune-response genes. Given the involvement of autoimmune components in IDDM pathogenesis, such independence may not

From the Fred H. Allen Laboratory of Immunogenetics, The New York Blood Center, Lindsley F. Kimball Research Institute; the Division of Pathology, College of Physicians and Surgeons of Columbia University; and the Division of Pediatric Endocrinology, Mount Sinai School of Medicine, New York, New York.

Address correspondence and reprint requests to Pablo Rubinstein, MD, Laboratory of Immunogenetics, The New York Blood Center, 310 East 67th Street, New York, NY 10021.

Received for publication 20 November 1989 and accepted in revised form 27 April 1990.

be expected a priori.) Other observations, also incompatible with simple hypotheses of IDDM genetics, have been reported. If a (recessive) disease-gene frequency and its average penetrance are calculated from segregation data (i.e., on disease prevalence among siblings of probands), they predict a much higher population prevalence than is actually found (3,8,12). Also unexpected of a disease inherited as a simple autosomal trait is the excessively frequent inheritance of DR4 haplotypes from fathers compared with mothers (22,23) and the lower prevalence of IDDM among the offspring of affected mothers than of affected fathers (24). The latter suggests the presence of modifiers of IDDM expression.

The above data were gathered in samples overwhelmingly integrated by White patients of mostly northern European descent. In contrast, in our ongoing studies of New York City diabetic patients of diverse ethnic origin, the genotypic classes defined by DR*3, DR*4, and DR*X (DR*X is all DR*non-3,non-4 alleles), among White patients, are closer to Hardy-Weinberg predictions (25). The excessive frequency of the DR*3/4 heterozygous class is much less marked here than in other White data sets. This suggests that one or more of the populations represented in our study might be exempt from the genetic distortion typical of the northern European samples, a suggestion confirmed by the analysis reported herein.

RESEARCH DESIGN AND METHODS

The patients, who are index cases to the families in this study, all have classic IDDM according to the current definition (26). They were sequentially ascertained between 1977 and 1989, and their families were invited to participate in this research without regard to the number of additional family members affected or healthy. If informed consent was granted, a genetic questionnaire containing information on the national origin and religious background of the four grandparents of the respective proband was completed by each family. Assignment of the family to one of the three specific ethnic groups to be studied was reportedly unambiguous in 266 cases, although 13 probands had grandparents of more than one of the study groups and were excluded. For assignment, non-Jewish individuals who gave only countries of northwestern Europe as the origin of their families were designated as of northern European ancestry, Jewish people of European descent as Ashkenazi Jewish, and individuals whose native language is Spanish and whose families originated in Central and/or South American countries as New York Hispanics. Southern European, American Black, and Oriental families typed in this study were not included because their respective numbers were low ($n < 35$ in each case).

Class I and class II antigens were identified with the contrast-fluorescence test (27) and the two-color fluorescence method (28), respectively. Antiserums were standardized with reference to reagents from the 8th, 9th, and 10th International Workshops on Histocompatibility Testing (29–31). All families were studied by intrafamilial mixed-lymphocyte culture tests. Class III allotypes of the Bf locus in all families and those of C2 and C4 in selected pedigrees were determined with standard electrophoretic techniques to help resolve cases of HLA-genotype ambiguity. Grandparents and uncles and/or aunts were also typed in most of these cases. Cell lines were prepared from all members of a subset of the families in this sample and were studied for their class II molecular variants at the protein and DNA levels by high-resolution isoelectric focusing (32) and restriction-fragment–length polymorphism typing (33). As reported, these tests confirmed the serological typing of DR and DQ alleles and also increased the information for segregation analysis.

The child first diagnosed was chosen as the index in all cases, because differences in DR frequencies between the first and later affected siblings have been encountered in multiplex families (25,34). In simplex families, the number of haplotypes inherited by the index cases, designated *affected,* is equal to that of the *unaffected* haplotypes. In samples that do not exclude multiplex sibships, the number of affected haplotypes is generally larger than that of unaffected haplotypes, because not all affected sibling pairs are HLA identical. This complicates some types of analyses, because multiplex pedigrees may include two, three, or four affected haplotypes, some of which are present in all siblings and others in only one or some, but not all, of the affected siblings (35). Therefore, in this study, although the affected haplotypes were the two haplotypes from the index cases of each sibship, the control haplotypes were the two not inherited by the probands from single-case families only. Eighty-four of the 123 northern European, 40 of the 49 Hispanic, and 81 of the 94 Ashkenazi families were single case.

Affected and control haplotypes were used in the computation of antigen and gene frequencies within each ethnic group. From these were derived the haplotype relative risks (HRRs) (36,37) and the gene frequencies for the study of Hardy-Weinberg equilibrium. For the comparison between the numbers of paternally and maternally derived unaffected haplotypes, however, the unaffected haplotypes from all families, including the multiplex pedigrees, were used.

The standard χ^2-test and Fisher's exact test were used to determine the significance of deviations from Hardy-Weinberg equilibrium and for simpler comparisons, respectively.

RESULTS

Table 1 shows the DR-allele frequencies of the index cases according to ethnic background. Because there were no significant differences between the DR frequencies of the index cases from simplex and multiplex families, they were analyzed as a single group. The DR frequencies of the control haplotypes and the corresponding HRRs are also given in Table 1. Because these families were studied during a period >10 yr, Table 1 shows only DR specificities that were typeable throughout and not the subtypic antigens defined more recently. The data show consistent increases in the frequencies of DR3 and DR4 and decreases of DR2 and DR5 haplotypes in patients from all three groups. DQ typing (not shown) revealed that DR3 haplotypes consistently carried DQw2, and DR4 haplotypes were associated with either DQw7 or DQw8 (formerly DQw3.1 and DQw3.2). The frequency of these DQw3 subtypes varied with the affection status; 60–70% of unaffected DR4 haplotypes and 80–90% of the affected haplotypes had DQw8 in the three populations.

Table 2 shows the gene frequencies, obtained by direct gene counting, of DR*3, DR*4, and DR*X in the three patient

TABLE 1
DR antigens and haplotype relative risks (HRRs) according to ethnic background

	Northern European			Ashkenazi			Hispanic		
HLA antigens	Affected	Control	HRR	Affected	Control	HRR	Affected	Control	HRR
DR1	22	18	0.81	18	19	0.79	6	7	0.64
DR2	12	30	0.23	3	16	0.15	2	10	0.14
DR3	82	10	7.80	49	11	4.77	19	9	1.79
DR4	85	22	3.46	78	32	2.84	38	12	3.38
DR5	11	23	0.29	6	19	0.25	6	9	0.49
DR6	15	22	0.43	8	18	0.35	8	7	0.88
DR7	9	19	0.29	12	18	0.54	10	11	0.67
Other*	10	22	0.27	14	24	0.46	9	13	0.49
Total	246	166		188	157		98	78	

For details, see RESEARCH DESIGN AND METHODS.
*Other specifications and blanks.

samples. These frequencies were used to calculate the expected numbers of patients of the six genotypic classes and were compared with those actually observed by use of χ^2-tests (Table 3). The results indicate that Hardy-Weinberg conditions do not exist in the 123 northern European White probands (χ^2 = 13.17, 2 df, $0.0025 > P > 0.001$). As in previously published samples (3,8–12), the largest deviations are an excess of DR*3/4 and a deficit of DR*4/4 genotypes. In contrast, the Hispanic and Ashkenazi samples were in Hardy-Weinberg equilibrium (n = 49, χ^2 = 2.37, 2 df, $P \sim 0.35$; and n = 94, χ^2 = 1.37, 2 df, $P \sim 0.55$, respectively). Because the sample sizes are not large, particularly in the case of the Hispanic IDDM sample, and an undeclared population admixture probably exists in the three groups, significant disequilibrium remains a possibility. The magnitudes of the possible deviations are much lower, however, than in northern Europeans.

Because the DR genotypes are in Hardy-Weinberg equilibrium in the Hispanic and Ashkenazi IDDM patient samples, we investigated whether the difference from the northern Europeans is attributable to differences in the affected HLA-B, DR haplotypes. As shown in Table 4, the frequencies of the relevant B,DR3 haplotypes are similar in all three ethnic groups and virtually identical in the northern European and Ashkenazi probands. In contrast, the frequencies of the B,DR4 haplotypes are very different, and some are essentially restricted to one (e.g., B38,DR4 in Ashkenazi Jewish) or two of the three populations (Bw62,DR4). Table 5 displays the data for the unaffected haplotypes of all the families. As stated earlier, these data differ from the control data used in the calculation of HRRs, as shown in Table 1, which exclude the unaffected haplotypes from multiplex sibships. Tables 4 and 5 show paternal and maternal haplotypes separately to allow testing of the reportedly unequal transmission of DR alleles from fathers and mothers (22,23). There are no significant differences between the numbers of B,DR3 haplotypes provided to the probands by fathers and mothers. In the case of DR4 probands, northern European mothers contributed significantly fewer Bw62,DR4 haplotypes than did fathers (3 vs. 13, Fisher's exact test, P = 0.0047), and Hispanic mothers may also have done so (2 vs. 6). Across all groups, 17 Bw62,DR4 haplotypes came from fathers and 5 from mothers (P = 0.0081). The opposite trend was observed for the B7,DR4 haplotype: northern European fathers (1 vs. 7, P = 0.031) and perhaps Jewish fathers (2 vs. 6, not significant), but not Hispanic fathers (2 vs. 1), gave this haplotype to probands less often than did the corresponding mothers (overall 5 vs. 14, P = 0.02). Ashkenazi fathers gave, on the aggregate, the less frequent B,DR4 haplotypes to probands (Table 4, Other) more often than did the mothers (P = 0.0095), but this did not occur in the other groups.

DISCUSSION

The data presented herein demonstrate that there is no excess of DR*3/4 heterozygotes among either Ashkenazi or New York Hispanic IDDM patients. In fact, the DR-genotype frequencies in these patients, confirmed by family studies, are in Hardy-Weinberg equilibrium and are therefore consistent with recessive and intermediate modes of inheritance. In agreement with our results, the DR types of an earlier Israeli sample of 50 Ashkenazi patients, reported by Brautbar et al. (38), did not significantly deviate from Hardy-Weinberg equilibrium (total χ^2 = 2.22, 2 df, $P \sim 0.4$). No comparable studies of New York Hispanic IDDM probands have been reported.

In our sample, the Hardy-Weinberg distortion is restricted to the patients of northern European ancestry. We can find no trivial explanation for the difference between this and the other population samples. In addition to being ascertained with identical diagnostic criteria and procedures, all three patient samples disclose the same IDDM-associated DR,DQ complexes: DR3,DQw2 and DR4,DQw8.

The discrepancy with regard to Hardy-Weinberg equilibrium is not readily accounted for by the three-allele hypothesis explanation of the DR*3/4 excess in IDDM; namely, DR4 haplotypes as a set bear a susceptibility gene different from DR3 haplotypes, and these different susceptibility genes or

TABLE 2
DR-gene frequency data for insulin-dependent diabetes mellitus patients

Gene	Northern European	Ashkenazi	Hispanic
DR3	0.333	0.261	0.202
DR4	0.345	0.415	0.383
DRX	0.322	0.324	0.415

DR*X, any allele other than DR*3 or DR*4.

TABLE 3
Observed versus expected DR genotypes used to test for Hardy-Weinberg equilibrium among insulin-dependent diabetes mellitus probands according to ethnic origin

	Northern European			Ashkenazi			Hispanic		
Gene	Observed	Expected	χ^2	Observed	Expected	χ^2	Observed	Expected	χ^2
DR3/X	24	26.38	0.21	18	15.90	0.28	7	8.22	0.18
DR3/3	9	13.64	1.58	4	6.40	0.90	2	1.99	0.00
DR3/4	40	28.26	4.87	22	20.36	0.13	8	7.58	0.02
DR4/X	33	27.33	1.18	24	25.27	0.06	20	15.58	1.25
DR4/4	6	14.64	5.09	16	16.19	0.00	5	7.19	0.67
DRX/X	11	12.75	0.24	10	9.87	0.00	7	8.44	0.25
Total	123	123.00	13.17	94	93.99	1.37	49	49.00	2.37

Data represent simplex cases and first affected cases in multiplex families. Expected values were determined with gene frequency data presented in Table 2.

their products interact in the heterozygote (11,12). The existence of equilibrium in one population and its absence from another must therefore result either from more complex differences between the various susceptibility genotypes or from the modulation of one and the same susceptibility genotype by HLA-linked genetic modifiers associated with ethnicity.

We prefer the second hypothesis. If the DR*3/4 excess were due to the presence of different IDDM-susceptibility genes in the DR3 and DR4 haplotypes of northern Europeans, the absence of such an excess in the other populations would indicate that their DR3 and DR4 haplotypes bear the same susceptibility gene. It is more plausible, therefore, that the distortions of HLA-DR Hardy-Weinberg equilibrium in IDDM result from epistatic effects of other HLA-linked genes (25). The frequency of the relevant alleles of these genes and their linkage disequilibrium with DR-defined haplotypes probably vary with the population and thus explain the population differences in Hardy-Weinberg equilibrium reported herein.

In attempting to suggest a possible mechanism of action for the genetic modifiers proposed herein, we considered whether the same modifiers might also account for the lower prevalence of IDDM in the children of IDDM mothers than fathers and for the higher frequency of paternal origin of DR4 haplotypes in IDDM patients.

If a subset of mothers, both affected and unaffected (including but probably not limited to those carrying the Bw62,DR4 haplotype), induced in their offspring an immunological effect capable of reducing the penetrance of the IDDM-susceptibility genotype, the resulting protection could account for all three observations. It would lead to reduced prevalence of IDDM in children who derive their affected Bw62,DR4 haplotypes from their mother. Hence, fewer DR4 homozygous probands would be expected. Because the mothers would contribute to probands fewer DR4s and thus proportionately more of the other susceptibility-bearing haplotypes, the DR-allele frequencies in fathers and mothers would not be the same, as required by Hardy-Weinberg conditions, and DR*3/4 excess could easily ensue. Because the frequency of these protective DR4 haplotypes is lower in Ashkenazi individuals (no examples of Bw62,DR4), DR-genotypic classes would be in equilibrium in that population.

The maternal effect is probably immunological in nature, given the evidence that autoimmune reactivity to pancreatic β-cells contributes to the pathogenesis of the disease. Ma-

TABLE 4
Main HLA-B,DR haplotypes in insulin-dependent diabetes mellitus probands according to ethnic background

	Northern European			Ashkenazi			Hispanic			Total		
Haplotype	Paternal	Maternal	All	Paternal	Maternal	All	Paternal	Maternal	All	Paternal	Maternal	All
DR3												
B8	29	22	51	12	19	31	4	2	6	45	43	88
B18	7	2	9	2	4	6	1	1	2	10	7	17
Other	12	10	22	8	4	12	4	7	11	24	19	43
Total	48	34	82	22	27	49	9	10	19	79	69	148
DR4												
Bw62	13	3	16	0	0	0	4	2	6	17	5	22
B44	5	5	10	1	2	3	4	5	9	10	12	22
B40	5	6	11	1	1	2	3	0	3	9	7	16
B7	1	7	8	2	6	8	2	1	3	5	14	19
B35	1	5	6	5	4	9	0	0	0	6	9	15
B38	1	3	4	19	21	40	0	0	0	20	24	44
Other	16	14	30	13	3	16	7	10	17	36	27	63
Total	42	43	85	41	37	78	20	18	38	103	98	201

Paternal and maternal refer to origin of affected haplotypes.

TABLE 5
Main HLA-B,DR unaffected haplotypes in insulin-dependent diabetes mellitus families according to ethnic background

	Northern European			Ashkenazi			Hispanic			Total		
Haplotype	Paternal	Maternal	All	Paternal	Maternal	All	Paternal	Maternal	All	Paternal	Maternal	All
DR3												
B8	9	5	14	3	2	5	1	0	1	13	7	20
B18	0	1	1	0	0	0	0	2	2	0	3	3
Other	1	6	7	4	2	6	6	2	8	11	10	21
Total	10	12	22	7	4	11	7	4	11	24	20	44
DR4												
Bw62	3	4	7	0	0	0	0	0	0	3	4	7
B44	2	2	4	2	2	4	4	0	4	8	4	12
B40	2	0	2	1	0	1	0	1	1	3	1	4
B7	3	1	4	0	0	0	0	1	1	3	2	5
B35	2	3	5	1	3	4	0	0	0	3	6	9
B38	1	1	2	9	3	12	0	0	0	10	4	14
Other	7	7	14	7	6	13	6	2	8	20	15	35
Total	20	18	38	20	14	34	10	4	14	50	36	86

Paternal and maternal refer to origin of unaffected haplotypes.

ternal influence on the immunoreactivity of the offspring has been described in mice: experimental neonatal tolerance to allogeneic *H-2* antigens modifies the cytotoxic T-lymphocyte responsiveness to vaccinia virus under specific Ir-gene control (39). Similar effects have been found in humans, where Claas et al. (40) encountered a remarkable difference in the B-lymphocyte response to maternally and paternally derived class I HLA antigens. A state of partial tolerance of the non-inherited maternal antigens appears to be induced, presumably during fetal life, in some but not all polytransfused patients. It is therefore conceivable that the maternal effect is mediated by intrauterine or perinatal exposure to antigens differentially expressed by mothers of different HLA genotypes, such as maternal alloantigens, viruses, or idiotypes of specific immune T lymphocytes or antibodies.

To cause measurable genetic distortions, the proposed protective mechanism should accompany a significant fraction of the DR4 haplotypes of the population sampled, which is consistent with the sample studied by the 5th Genetic Analysis Workshop (77.3% of the maternal and 91.5% of the paternal DR4s were inherited by IDDM probands; $P = 0.02$; 23). Among the northern Europeans in our data, the proposed differences in paternal or maternal origin only materialize in the case of Bw62,DR4, a preponderant DR4 haplotype in northern Europeans. The magnitude of this difference for this haplotype alone (i.e., 13 of 16 vs. 3 of 7) may be, however, somewhat small to explain the overall Hardy-Weinberg disequilibrium involving DR4. Therefore, we expect that the protective effect is not uniquely associated with strictly HLA-B–defined DR4 haplotypes.

In conclusion, the available data are consistent with the existence of two types of HLA-linked genes that influence the apparent mode of inheritance of IDDM. One yields the true IDDM-susceptibility genotype, whose presence is absolutely necessary for disease expression and which may be inherited as a recessive. The other(s), including the maternal protective effect proposed in this article, would modulate the probability of IDDM expression without constituting an essential prerequisite for IDDM. Success in the discrimination between the susceptibility gene and the HLA-associated modifier(s) may help focus study of the mechanisms of IDDM pathogenesis.

ACKNOWLEDGMENTS

This work was supported in part by Grant DK-19631-13 from the National Institutes of Health and Grant 1891232 from the Juvenile Diabetes Foundation International.

We are grateful to V. Moore for help in the preparation of this article.

REFERENCES

1. Singal DP, Blajchman MA: Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes* 22:429–32, 1973
2. Nerup J, Platz P, Ortved-Andersen O, Christy M, Lyngsoe J, Poulsen JE, Ryder LP, Nielsen LS, Thomsen M, Svejgaard A: HLA antigens and diabetes mellitus. *Lancet* 2:289–91, 1974
3. Svejgaard A, Platz P, Ryder LP: Insulin-dependent diabetes: joint results of the 8th workshop study. In *Histocompatibility Testing, 1980*. Terasaki PI, Ed. Los Angeles, Univ. of California Press, 1980, p. 638–56
4. Rubinstein P, Suciu-Foca N, Nicholson JF, Fotino M, Molinaro A, Harisiadis L, Hardy MA, Reemtsma K, Allen FH: The HLA system in the families of patients with juvenile diabetes mellitus. *J Exp Med* 143:1277–82, 1976
5. Barbosa J, Chern MM, Noreen H, Anderson VE, Yunis EJ: Analysis of linkage between the major histocompatibility system and insulin-dependent diabetes in multiplex families. *J Clin Invest* 62:492–95, 1978
6. Green A, Morton NE, Iseluis L, Svejgaard A, Platz P, Ryder L, Hauge M: Genetic studies of insulin-dependent diabetes mellitus: segregation and linkage analyses. *Tissue Antigens* 19:213–21, 1982
7. Green JR, Woodrow JC: Sibling method for detecting HLA-linked genes in disease. *Tissue Antigens* 9:31–35, 1977
8. Rubinstein P, Ginsberg-Fellner F, Falk CT: Genetics of type 1 diabetes mellitus: a single recessive predisposition gene mapping between HLA-B and GLO. *Am J Hum Genet* 33:865–82, 1981
9. Morton NE, Green A, Dunsworth T, Svejgaard A, Barbosa J, Rich SS, Islius L, Platz P, Ryder LP: Heterozygous expression of insulin-dependent diabetes mellitus (IDDM) determinants in the HLA system. *Am J Hum Genet* 35:201–13, 1983
10. Rotter JI, Anderson CE, Rubin R, Congleton JE, Terasaki PI, Rimoin DL: HLA genotypic study of insulin-dependent diabetes: the excess of DR3/DR4 heterozygotes allows rejection of the recessive hypothesis. *Diabetes* 32:169–74, 1983
11. Baur MP: Genetic analysis workshop IV: insulin dependent diabetes mellitus—summary. *Genet Epidemiol* 3 (Suppl. 1):299–312, 1986
12. Thomson G, Robinson WP, Kuhner MK, Joe S, MacDonald MJ, Gottschall JL, Barbosa J, Rich SS, Bertrams J, Baur MP, Partanen J, Tait BD, Schober E, Mayr WR, Ludvigsson J, Lindblom B, Farid NR, Thompson C, Deschamps I: Genetic heterogeneity, modes of inheritance and risk estimates for a joint study of Caucasians with insulin-dependent diabetes mellitus. *Am J Hum Genet* 43:799–816, 1988
13. Nepom BS, Palmer J, Kim SJ, Hansen JA, Holbeck SL, Nepom GT: Spe-

cific genomic markers for the HLA-DQ subregion discriminate between DR4+ insulin-dependent diabetes mellitus and DR4+ seropositive juvenile rheumatoid arthritis. *J Exp Med* 164:345–50, 1986
14. Todd JA, Bell JI, McDevitt HO: HLA-DQ-beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* (Lond) 329:599–604, 1987
15. Todd JA, Bell JI, McDevitt HO: HLA antigens and insulin-dependent diabetes (Letter). *Nature* (Lond) 333:710, 1988
16. Todd JA, Bell JI, McDevitt HO: A molecular basis for genetic susceptibility to insulin-dependent diabetes mellitus. *Trends Genet* 4:129–34, 1988
17. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M: Aspartic acid at position 57 of the DQ beta chain protects against type I diabetes: a family study. *Proc Natl Acad Sci USA* 85:8111–15, 1988
18. Nepom BS, Schwarz D, Palmer JP, Nepom GT: Transcomplementation of HLA genes in IDDM: HLA-DQ α- and β-chains produce hybrid molecules in DR3/4 heterozygotes. *Diabetes* 36:114–17, 1987
19. Thomson G, Bodmer W: The genetic analysis of HLA and disease associations. In *HLA and Disease*. Dausset J, Svejgaard A, Eds. Copenhagen, Munksgaard, 1977, p. 84–93
20. Tattersall RB, Pyke DA: Diabetes in identical twins. *Lancet* 2:1120–24, 1972
21. Cahill GF Jr: Current concepts of diabetic complications with emphasis of hereditary factors: a brief review. In *Genetic Analysis of Common Diseases: Application to Predictive Factors in Coronary Heart Disease*. Sing CF, Skolnick M, Eds. New York, Liss, 1979, p. 113–25
22. Field LL: Genes predisposing to IDDM in multiplex families. *Genet Epidemiol* 6:101–106, 1989
23. Klitz W, Kuhner MK, Robinson W, Esposito M, Thomson G: Clues to IDDM pathogenesis from genetic and serological traits in multiple affected families. *Genet Epidemiol* 6:117–22, 1989
24. Warram JH, Krolewski AS, Gottlieb MS, Kahn CR: Difference in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N Engl J Med* 311:149–52, 1984
25. Rubinstein P, Walker M, Ginsberg-Fellner F: Excess of DR3/4 in type I diabetes: what does it portend? *Diabetes* 35:985–89, 1986
26. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–57, 1979
27. Martel JL, Jaramillo S, Allen FH Jr, Rubinstein P: Serology for automated cytotoxicity test. *Vox Sang* 27:13–20, 1974
28. Van Rood JJ, van Leeuwen A, Ploem JS: Simultaneous detection of two cell populations by two-colour fluorescence and application to the recognition of B-cell determinants. *Nature* (Lond) 262:795–97, 1976
29. Terasaki PI (Ed.): *Histocompatibility Testing 1980*. Los Angeles, Univ. of California Press, 1980
30. Albert ED, Baur MP, Mayr WR (Eds.): *Histocompatibility Testing 1984*. New York, Springer-Verlag, 1984
31. Dupont B (Ed.): *Histocompatibility Testing 1987. Immunobiology of HLA*. New York, Springer-Verlag, 1989
32. Rodriguez de Cordoba S, Nunez-Roldan A, Winchester R, Marshall P, Carrier C, Mollen N, Walker M, Ginsberg-Fellner F, Rubinstein P: Molecular characterization by high-resolution isoelectric focusing of the products encoded by the class II region of the major histocompatibility complex in humans. I. DR and DQ gene variants. *Hum Immunol* 20:71–93, 1987
33. Carrier C, Mollen N, Rothman WC, Rodriguez de Cordoba S, Rey-Campos J, Ginsberg-Fellner F, Carpenter C, Rubinstein P: Definition of IDDM-associated HLA DQ and DX RFLPs by segregation analysis of multiplex sibships. *Hum Immunol* 24:51–63, 1989
34. Falk CT: HLA haplotype sharing in IDDM affected sib pairs as a function of HLA DR genotype. *Genet Epidemiol* 3 (Suppl. 1):353–58, 1986
35. Falk CT: Characteristics of a multiplex IDDM sample: unexplained differences with other samples. *Genet Epidemiol* 6:95–100, 1989
36. Falk CT, Rubinstein P: Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. *Ann Hum Genet* 51:227–33, 1987
37. Ott J: Statistical properties of the haplotype relative risk. *Genet Epidemiol* 6:127–30, 1989
38. Brautbar C, Karp M, Amar A, Cohen O, Cohen I, Sharon R, Topper E, Levene C, Cohen T, Albert ED: Genetics of insulin dependent diabetes mellitus in Israel: population and family study. *Hum Immunol* 3:1–12, 1981
39. Mullbacher A, Blanden RV, Brenan M: Neonatal tolerance of major histocompatibility complex antigens alters Ir gene control of the cytotoxic T cell response to vaccinia virus. *J Exp Med* 157:1324–38, 1983
40. Claas FHJ, Gijbels I, van der Velden-de Munck J, van Rood JJ: Induction of B cell unresponsiveness to noninherited maternal HLA antigens during fetal life. *Science* 241:1815–17, 1988