HLA Heterogeneity of Insulin-dependent Diabetes Mellitus at Diagnosis

The Pittsburgh IDDM Study

MARK S. EBERHARDT, DIANE K. WAGENER, TREVOR J. ORCHARD, RONALD E. LaPORTE, DRUIE E. CAVENDER, BRUCE S. RABIN, R. WAYNE ATCHISON, LEWIS H. KULLER, ALLAN L. DRASH, AND DOROTHY J. BECKER

SUMMARY

Although some previous studies have suggested that insulin-dependent diabetes mellitus (IDDM) is a heterogeneous condition with variant forms being associated with HLA-DR types, the evidence, thus far, is conflicting. To address this issue, we have examined the presenting characteristics of a consecutive admission series of 200 newly diagnosed cases of IDDM from the Children's Hospital of Pittsburgh. Because HLA-DR frequencies vary by race, data are presented only for the 172 white cases with complete HLA-DR typing. HLA-DR3 was found more frequently among male cases and DR4 among female cases ($P < 0.005$). Generally, patients with DR4 presented with a severer clinical picture, being more likely to have impaired consciousness and significant dehydration. In addition, patients with DR4 were more likely to be acidotic, ketotic, and to more frequently report a recent viral infection. This latter finding was supported by a greater frequency of antibodies to Coxsackie-B viruses in the DR4 cases at presentation. These results therefore suggest that there is considerable heterogeneity in IDDM, at least in presenting characteristics, according to HLA-DR type. DIABETES 1985; 34:1247–52.

Although previous studies support the hypothesis that both genetic and environmental factors appear to be involved in the etiology of type I or insulin-dependent diabetes mellitus (IDDM), the relative importance of the genetic and environmental factors remains unclear. Several authors[1,2] have suggested that more than one etiologically distinct pathway may result in IDDM, and that the human histocompatibility locus (HLA) acts as a marker for different etiologies of IDDM. However, evidence supporting the concept of etiologic heterogeneity by HLA type is conflicting, with some authors reporting different HLA types associated with age at onset,[3–5] while others have not found such an association.[6,7] Furthermore, both associations[2,8] and lack of associations[5,9,10] of HLA type with the presence of islet-cell antibodies have been reported. Similar conflicting patterns exist for seasonality,[11–14] viral titers at the time of IDDM onset,[1,15] and the development of insulin antibodies during its course.[16,17]

As part of these discrepancies arise from studying small, unrepresentative populations, the current study was designed to evaluate the relationship of the presenting characteristics of newly diagnosed children with IDDM to specific HLA types in a large consecutive series of cases. This case series has been previously shown to be comparable to a community-based registry of childhood-onset cases of IDDM.[18]

MATERIALS AND METHODS

A consecutive admission series of 200 newly diagnosed IDDM patients seen at the Children's Hospital of Pittsburgh (CHP) was identified during the 3.5 yr between 4 February 1979 and 11 August 1982. A case was defined as any CHP admission who met the following criteria: (1) was <18 yr old; (2) was admitted to CHP within 1 wk of receiving a primary diagnosis of IDDM, either at CHP or another health facility; and (3) was discharged from CHP on insulin treatment.

This series of patients was compared with the Allegheny County population-based registry of IDDM.[18] The study patients included 64% of all newly diagnosed IDDM cases under the age of 18 yr in the county over the study period in

From the Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania (M.S.E., D.K.W., T.J.O., R.E.L., D.E.C., L.H.K.); the Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania (B.S.R.); the Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania (R.W.A.); the Division of Endocrinology and Metabolism, Department of Pediatrics/School of Medicine, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania (A.L.D., D.J.B.); the Department of Psychiatry, W.P.I.C., University of Pittsburgh, Pittsburgh, Pennsylvania (D.K.W.); and the Department of Internal Medicine, University of Texas Health Science Center, Dallas, Texas (D.E.C.).

Address reprint requests to Dr. T. J. Orchard, A530, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, 130 DeSoto Street, Pittsburgh, Pennsylvania 15261.

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1979 and 1980. Data for 1981 and 1982 for the county are not as yet available.

The presenting characteristics of each subject were evaluated using the patient's hospital chart, which was abstracted using standardized coding sheets by three medical chart abstractors. Periodic duplicate record reviews were performed to determine interobserver variation. For symptom histories, laboratory test results, and clinical variables such as weight, the agreement rate was >95%. The chart reviewers were unaware of the HLA-DR types of the patients. Family history of diabetes was collected separately by questionnaires and reviewed with the family by trained interviewers.

Laboratory measures, such as serum glucose, immunoglobulin and beta-hydroxybutyrate (BOH) concentrations, plasma C-peptide, and urine ketones were determined on samples obtained before insulin administration. In addition, in 95% of the cases, a serum sample was obtained within the first 2 wk of their CHP admission for the purpose of determining the titer of circulating antibodies to Coxsackie-B virus serotypes 1–6 (CBV). These titers were determined using an immunofluorescent technique similar to that described by Henle and Henle.[19]

Glycosylated hemoglobin was measured on whole blood using Quik-Sep microcolumn (Isolab Inc., Akron, Ohio) in a water bath at 22.5±0.5°F.[20,21] The normal range for this method is 4.9–7.8%. Beta-hydroxybutyrate was measured in serum after protein precipitation using an enzymatic microfluorometric technique.[22] C-peptide was measured by radioimmunoassay in unextracted plasma containing Trasylol (aprotinin) using tracer and antiserum M^{1230} obtained from Novo Industri, Copenhagen, Denmark.[23]

Lymphocytes from heparinized blood specimens were employed to determine HLA-DR types according to the method of Van Rood and Van Leeuwan.[24]

If an item of data was not available, such as a laboratory result or information as to the presence or absence of a symptom, then that variable was coded as missing. Thus, a symptom was not assumed to be absent from the patient's clinical history unless it was specified as such in the chart. Only variables for which information was available on at least 75% of the cases were considered in the following analyses.

Data analyses. For the analyses, patients were initially divided into four mutually exclusive groups according to their HLA-DR phenotype, that is, those with both HLA-DR3 and HLA-DR4 antigens (DR3/4), those with a HLA-DR3 but not a HLA-DR4 antigen (DR3/x), those with a HLA-DR4 but not a HLA-DR3 antigen (DR4/x), or those with neither a HLA-DR3 nor HLA-DR4 antigen (DRx/x). For dichotomous variables, a 2 × 4 contingency table was created and their cell frequencies were tested using the chi-square test for nonrandom distribution among the HLA-DR types. For continuous variables, an analysis of variance was used to test for group effects. For most biochemical variables, a strong relationship existed with age, so an analysis of covariance was employed to statistically control for age.

The association of the presenting features of IDDM with the presence or absence of a particular HLA-DR antigen was also assessed. For these purposes, the subjects were classified according to whether or not they possessed a HLA-DR4 antigen (i.e., DR4+ versus DR4−). Subjects were also classified according to their HLA-DR3 antigen status (i.e., DR3+ versus DR3−). For dichotomous variables, 2 × 2 contingency tables were used for chi-square tests of independence. Odds ratios and their confidence intervals were calculated according to Haldane.[25] For continuous variables, unpaired *t*-tests were used to test for group differences, or ANOVA with age as a covariate was used.

The means of several variables were found in univariate analyses to differ between the HLA groups (see RESULTS). To determine whether a multivariate function could be found that adequately discriminated individuals in these groups, a stepwise discriminant analysis was used. The selection of variables to be used in this analysis was dependent on the completeness of reporting (i.e., ≥80% of cases with some information on that variable), its univariate association, and its biologic plausibility. To test the significance of the discriminant classification, a chi-square test was used. For these analyses, the significant threshold of the F-to-enter was set at P = 0.15 and the F-to-remove at P = 0.30. The prior probabilities for the two groups were set to the overall probabilities of DR types for the white subjects, i.e., DR4+ = 0.66. Homogeneity of the covariance matrices was evaluated with the B- or M-statistic.[26]

All of the data analyses were performed using the SPSS statistical package.[27]

The seasonality associated with HLA types was evaluated in two ways using subjects who were diagnosed in the first three complete years of the study. They were classified into four groups according to the month of disease onset. The groups were December through February, March through May, June through August, and September through November. Chi-square statistics for the appropriate 4 × 4 and 2 × 4 tables were calculated. The other method of analysis was a nonparametric procedure described by Weinberg et al.[28] The procedure compares two populations using an approximation to the permutation distribution of a rank-based statistic. This statistic was used to compare the DR4+ cases with the DR4− cases.

RESULTS

Among the 200 cases, 183 were Caucasian and 17 were Black. HLA-DR types were determined on 95% of the cases (Table 1). Eighty-nine percent of the cases possessed either a HLA-DR3 or HLA-DR4 antigen. There was a significant increase in the frequency of the HLA-DR3 antigen among

TABLE 1
HLA-DR status and race

	DR3x	DR4x	DR3/4	DRx/x
Whites (N = 172)	23% (39)	36% (62)	30% (52)	11% (19)
Blacks (N = 17)	53% (9)	12% (2)	29% (5)	6% (1)

	Whites	Blacks
Odds ratios*		
DR3/x vs. DRx/x	6.3 (3.2, 12.2)	16.0 (2.1, 121.6)
DR4/x vs. DRx/x	8.0 (4.2, 14.7)	11.7 (0.9, 154.7)
DR3/4 vs. DRx/x	43.8 (15.5, 103.3)	62.3 (3.1, >1000)

*The 95% confidence interval is given in parentheses.

TABLE 2
Association of various onset characteristics with HLA-DR (N = 172)

Variable	HLA-DR classification				Significance
	DR3/x	DR4/x	DR3/4	DRx/x	
Demographic					
Sample size	39	62	52	19	
Sex (% female)	31%	66%	52%	53%	$P < 0.05$
Age at diagnosis (yr)	8.8	8.7	7.7	9.6	NS
Family history of IDDM*	39%	28%	31%	38%	NS
Family history of NIDDM*	34%	35%	18%	54%	NS
Clinical					
Impaired consciousness*	3%	25%	16%	17%	$P < 0.05$
Dehydration (≥5% weight gain over 5 days)	22%	46%	34%	21%	$P < 0.05$
Laboratory					
Blood glucose (mg/dl)	406	462	495	463	NS
Hemoglobin A_1 (%)	13.7	14.7	14.0	14.1	NS
Discharge insulin dose (U/kg)	0.78	0.89	0.91	0.86	NS
C-peptide (pmol/ml)	0.10	0.11	0.10	0.10	NS
Blood pH	7.33	7.28	7.30	7.34	NS
Plasma bicarbonate (≤18 meq/L)*	54%	74%	62%	43%	NS
Urine ketones (% positive)	69%	90%	92%	78%	$P < 0.02$
Serum BOH (μmol/L)	1272	1612	1392	370	NS
Immunoglobulin G (mg/dl)	848	775	835	999	$P < 0.02$
Viral studies					
History of recent viral infection*	42%	63%	61%	27%	$P < 0.05$
Coxsackie-B antibodies†					
Criteria A (% positive)	10%	24%	22%	12%	NS
Criteria B (% positive)	21%	34%	35%	12%	$P < 0.05$

*Percent of patients with this characteristic for each HLA-DR classification.
†Coxsackie-B virus antibody titers: criteria A, IgM >1:4 for any serotype B1–B6; and criteria B, IgM >1:4 or IgG >1:32 for any serotype B1–B6.

Blacks ($P < 0.02$), and a similar increased frequency of the HLA-DR4 antigen among Caucasians ($P < 0.04$). However, using race-specific reference population data from the Eighth Histocompatibility Workshop,[13] and the DRx/x cases as the appropriate comparison group,[27] the DR3/x and DR4/x groups did not have significantly different odds ratios in either race (Table 1). The odds ratio for the DR3/4 cases was significantly different from 1.0 and from both of the DR3/x and DR4/x odds ratios among the Caucasians, but because of the small sample size, a significant increase could not be verified among the Blacks.

Since race was associated with HLA-DR status and there were only a small number of Black subjects, the subsequent analyses were limited to the 172 Caucasian cases who were HLA-DR typed. Table 2 gives the distribution of the variables among the four HLA-DR subgroups of Caucasian cases. Table 3 gives the distribution of the same variables among the cases grouped according to whether or not they possess the DR4 antigen, DR4+ and DR4−.

As can be observed in Table 2, HLA-DR type was not related to age or reported positive family history of diabetes. It should be noted that 54% of DRx/x cases reported a first- or second-degree family member with NIDDM (non-insulin-dependent diabetes mellitus) as compared with 29% of the remainder. This yielded an odds ratio of 2.8 (not significant). The season of diagnosis for the 158 HLA-DR–typed cases during the three full years (first 36 mo) was not significantly associated with the HLA-DR type using either the contingency table analyses or nonparametric statistics (see MATERIALS AND METHODS, data not shown).

A significant association, however, was identified between the subjects' sex and HLA-DR type (Tables 2 and 3). The HLA-DR3 antigen was significantly more common among males and the HLA-DR4 antigen was more common among females (odds ratio = 2.4, $P < 0.01$).

When the frequencies of reported recent illnesses and symptoms, such as polydipsia, polyuria, fatigue, fever, or recent weight loss, were evaluated with respect to HLA-DR type, the only significant difference was for reports of "viral-like" infection (Tables 2 and 3). The frequency of reported recent viral-like infections, primarily upper respiratory tract, was significantly increased in DR4+ cases (odds ratio = 2.6, $P = 0.02$). This association was significant for males (68% versus 34%, odds ratio = 4.1, $P < 0.01$), but not for females (58% versus 46%, odds ratio = 1.6, $P > 0.2$). DR4+ patients were more likely to have an impaired level of consciousness at admission (odds ratio = 3.2, $P = 0.03$). DR4+ cases were also more likely to be dehydrated on admission, as indicated by a weight gain of at least 5% after rehydration between day 0 and day 5 (odds ratio = 2.3, $P = 0.02$).

Although DR4+ patients had somewhat higher mean values for serum glucose, BOH, hemoglobin A_1, lower blood pH (on admission), and higher mean insulin dose at hospital discharge, these differences were not significant at the 0.05 level. Neither day 0 nor day 5 plasma C-peptide levels varied by HLA-DR type. A significant difference was observed, however, for the proportion of cases who presented with ketonuria, 91% of the DR4+ cases as compared with 72% of the DR4− cases (odds ratio = 3.7, $P < 0.001$). Plasma bicarbonate concentrations on admission were also lower among the DR4+ cases ($P < 0.05$).

TABLE 3
Association of various onset characteristics with HLA-DR antigens

Variable	HLA-DR classification		Significance
	DR4+	DR4−	
Demographic			
Sample size	114	58	
Sex (% female)	60%	38%	$P < 0.005$
Age at diagnosis (yr)	8.23	9.04	NS
Family history of IDDM*	29%	39%	NS
Clinical			
Impaired consciousness*	22%	8%	$P < 0.03$
Dehydration (5% weight gain)*	41%	22%	$P < 0.02$
Laboratory			
Blood glucose (mg/dl)	477	424	NS
Hemoglobin A_1 (%)	14.4	13.8	NS
Discharge insulin dose (U/kg)	0.90	0.80	NS
C-peptide (pmol/ml)	0.10	0.10	NS
Blood pH	7.29	7.33	NS
Plasma bicarbonate (<18 meq/L)*	68%	51%	$P < 0.05$
Urine ketones (% positive)	91%	72%	$P < 0.01$
Serum BOH (μmol/L)	1516	1007	NS
Immunoglobulin G (mg/dl)	803	896	NS
Viral studies			
Recent viral infection*	62%	37%	$P < 0.02$
Coxsackie-B antibodies†			
Criteria A	23%	11%	$P = 0.06$
Criteria B	35%	18%	$P < 0.05$

*Percent of patients presenting with this characteristic for each subgroup.
†Coxsackie-B virus antibody titers: criteria A, IgM >1:4 for any serotype B1–B6; and criteria B, IgM >1:4 or IgG >1:32 for any serotype B1–B6.

The serum immunoglobulin concentrations were also compared by HLA-DR type. While mean concentrations of IgA and IgM did not differ by HLA-DR type (data not shown), those cases who possessed either or both HLA-DR3 or HLA-DR4 antigens had significantly lower total serum IgG immunoglobulin concentration compared with the DRx/x cases whose levels were close to the concentrations expected for non-ill children of similar age and sex (Table 2). This finding persisted after transforming the data with a square root function to normalize the distribution and using age as a covariate. Higher mean concentration among DRx/x cases compared with the rest of the cases persisted after stratifying by the extent of dehydration (i.e., < 5%, 5%+); however, the sample sizes in the strata were extremely small, and the significance level was only $P = 0.13$.

A significant difference between HLA-DR types was noted for sero-positivity to Coxsackie-B virus. As demonstrated in Table 3, a higher proportion (35%) of DR4+ cases had elevated CBV IgG titers (≥ 1:32) and/or CBV IgM antibody titers (criteria B) than did DR4− cases (odds ratio = 2.3, $P = 0.03$). The majority of this difference seemed to be due to differences in CBV IgM titer (criteria A, odds ratio = 3.45, $P = 0.06$), thereby suggesting recent infection. These sero-positivity findings by HLA-DR type were consistent over the 3 yr of the study analyzed on an annual basis (data not shown).

The DR4+ cases, therefore, were associated with an increased history of recent viral-like infections, increased clinical and laboratory evidence of a more severe onset, and increased antibody titers of Coxsackie-B viruses. Although there is a potential problem of some significant differences arising purely by chance because of the multiple comparisons made, we feel that this is unlikely to be the case. There were 18 comparisons made for these analyses. If the variables had been independent, the probability of six significant findings at $P < 0.05$ would be < 0.0002. However, these variables were incompletely correlated in a nonsystematic fashion. Therefore, the Bonferroni correction for multiple, correlated tests is not strictly appropriate for these data either. What emerges from the comparisons shown in Table 3 is several comparisons, each individually significant at $P < 0.05$, that all agree with the interpretation that DR4+ individuals have a more severe illness at presentation, associated with recent viral infections.

Because of the association between DR type and sex, sex-specific analyses were performed to examine whether the above significant findings reflect primarily DR or sex differences. Though the sample sizes are reduced, DR4 cases had a severer presentation in both males and females; for example, ketonuria was more frequent in those with DR4 than those without DR4 both in males (86% versus 72%) and in females (94% versus 71%), the female difference reaching significance ($P < 0.02$). The viral differences by DR4, however, showed a different pattern with the DR4 effect being limited to males. For example, the percent of patients with raised Coxsackie-B viral titers suggestive of recent infection (criteria B) was 33% for DR4 males but only 8% for non-DR4 males ($P < 0.01$), in contrast to the females in whom 34% of DR4 patients were positive and 32% of non-DR4 females were negative.

Since none of the variables alone completely differentiates the DR4+ cases from the DR4− cases, a multivariate approach (discriminant analysis) was used to identify the best linear function of these presentation characteristics that

would discriminate the DR4+ and DR4− cases. Five variables were chosen for this analysis, all of which showed significant mean differences in univariate analyses by DR4 status and were available on over 80% of the cases. The variables chosen were sex, age, serum IgG, and the log base 2, of the CBV-specific IgG and IgM titers. From among these variables, only sex, serum IgG levels, and CBV-IgM titers made significant contributions to the discriminant function ($N = 133$, $X^2 = 17.5$, $P < 0.0006$). There was no significant heterogeneity of the covariance matrix, so the classification procedure did not use these matrices. The sensitivity of the predicted group membership for DR4+ was 81%; however, only 67% of the subjects were correctly classified because of the poor discrimination of the DR4− group.

DISCUSSION

The pattern that emerges from these analyses is that the DR4+ cases are, in general, more severely ill at presentation, more frequently have a history suggesting viral infection, and more often have raised antibody titers against Coxsackie-B viruses. That these features relate to DR4 is supported by the findings that when all four HLA-DR groups are compared, the HLA-DR4/x and DR3/4 groups tend to be similar in their presenting picture. The DRx/x cases appear to differ from both DR3+ and DR4+ cases in some regards, such as serum immunoglobulin concentrations, history of recent infection, and increased family history for NIDDM. In other regards, the DRx/x cases are similar to either those with DR3+ or DR4+ (e.g., dehydration). However, the relatively small numbers of these DRx/x cases preclude any definitive statements as to their presentation characteristics. The lack of an association between age and HLA type probably reflects the limitation in our study to childhood-onset cases.

The more severe presentation in the DR4+ cases is evident from both clinical and laboratory assessments. Furthermore, even the parents' report of symptoms differentiates between these cases; more "viral-like" illnesses were reported for DR4+ cases. This observation by the parents is consistent with our findings of more frequent elevated CBV antibodies, particularly IgM levels, indicative of recent infection.

The association between the DR4+ cases and indications of viral-like infections before onset is particularly interesting in light of the hypothesized association of DR4+ diabetes and a viral etiology as contrasted with the autoimmune etiology hypothesized to be important in DR3+ diabetes.[1] However, we have also evaluated the presence of circulating islet-cell antibodies at diagnosis and found no association with HLA-DR type in the same series of patients.[29] Therefore, this does not give support to an autoimmune form of IDDM associated with DR3 in our population. Furthermore, it should be noted that in another study, a history of congenital rubella and diabetes is more strongly associated with DR3 than DR4.[30] This contrasts with the hypothesized viral association with DR4.[1]

In our study, heterozygous DR3/4 cases were more similar to the DR4/x cases on most variables, including the viral-related variables. This may indicate that the DR4+ patients, regardless of other histocompatibility antigens, are more closely related to a viral-related "form." However, this association appears to be limited to males, as we have previously reported for CBV antibodies.[31] This could reflect either a lower exposure rate to these viruses among DR4− males, or an inability to produce antibodies to the CB viruses. This latter possibility could be tested by viral isolation/culture studies. It may be concluded, in view of all these findings, that a clear demarcation of DR3 being autoimmune and DR4 viral is unlikely, although viral infection would appear to be more important in the final stages of DR4 than DR3 cases. Different viruses may also be important for different DR types (e.g., Rubella/DR3, Coxsackie-B/DR4).

Although the reason for the more severe clinical presentation of the DR4+ cases may well relate to the etiologic process itself, an alternative explanation may be a longer delay in recognition of the disorder on the part of the parents. Parents of DR4+ cases reported a nonsignificant longer duration of symptoms before admission (data not shown). Perhaps, because of the recent viral-like infection, the parents did not recognize the symptoms as anything other than a protracted recovery period for their child.

The association of sex with the HLA-DR antigen, which we have previously reported,[31,32] is intriguing. Girls had a severer presentation than boys; however, after stratification by sex and DR type, it was apparent that the DR4+ cases were more severely affected than the DR4− within each sex.

In summary, the presenting characteristics of patients with IDDM indicate that DR4+ cases have a more severe presentation as assessed by clinical measures (consciousness, dehydration), laboratory assessments (bicarbonate, ketonuria), history of a recent viral-like infection, and elevation of antibody titers to Coxsackie-B viruses. Finally, they are more likely to be girls. Although the role of recent infection in the etiology of IDDM cannot be fully addressed by studies such as this, these findings strongly suggest that DR4+ cases may have different pathogenetic mechanisms, or at least that the final pathway takes a different course, and is more likely to be associated with viral infection. The DR4+ individuals may have an altered immune response due to a gene in linkage disequilibrium with the HLA-DR4 antigen. This gene may, or may not, be the hypothesized diabetogenic gene that is indicated by linkage studies of IDDM patients. It is further possible that DR3 and DR4 cases differ in the type of virus or viruses that may be involved in the pathogenetic process leading to IDDM, and that non-Coxsackie viral infections (not tested for in this study) may play a bigger role in DR3 cases. To determine whether the immune response varies according to a gene in linkage disequilibrium with HLA-DR3 or DR4, nondiabetic HLA-identical or haplo-identical siblings of diabetic cases could be given an immune challenge and their response monitored. Such a study is currently in progress.

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REFERENCES

[1] Rotter, J. I., and Rimoin, D. L.: Heterogeneity in diabetes mellitus. Diabetes 1978; 27:599–608.

[2] Irvine, W. J., McCallum, C. J., Gray, R. S., et al.: Pancreatic islet-cell antibodies in diabetes mellitus correlated with the duration and type of dia-

betes, coexistent autoimmune disease, and HLA type. Diabetes 1977; 26:138–47.

[3] Cudworth, A. G., and Woodrow, J. C.: HLA system and diabetes mellitus. Diabetes 1975; 24:345–49.

[4] Schernthaner, G., Ludwig, H., and Mayr, W. R.: Juvenile diabetes mellitus: HLA antigen frequencies dependent on the age of onset of the disease. J. Immunogenet. 1976; 3:117–21.

[5] Barbosa, J., Bach, F. H., and Rich, S. S.: Genetic heterogeneity of diabetes and HLA. Clin. Genet. 1982; 21:25–32.

[6] Farid, N. R., Sampson, L., Noel, P., Barhard, J. M., Davis, A. J., and Hillman, D. A.: HLA-D-related (DRW) antigens and juvenile diabetes mellitus. Diabetes 1979; 28:552–57.

[7] Platz, P., Jakobsen, B. K., Morling, N., et al.: HLA-D and -DR antigens in genetic analysis of insulin-dependent diabetes mellitus. Diabetologia 1981; 21:108–15.

[8] Morris, P. J., Irvine, W. J., Gray, R. S., et al.: HLA and pancreatic islet-cell antibodies in diabetes. Lancet 1976; 2:652–53.

[9] Landrum, R., Walker, G., Cudworth, A. G., Woodrow, J. C., and Gamble, D. R.: HLA-linked genes and islet-cell antibodies in diabetes mellitus. Br. Med. J. 1976; 1:1565–67.

[10] Ginsberg-Fellner, F., Dobersen, M., Witt, M., et al.: HLA antigens, cytoplasmic islet-cell antibodies and carbohydrate tolerance in families of children with insulin-dependent diabetes mellitus. Diabetes 1982; 31:292–98.

[11] Rolles, C. J., Rayner, P. H. W., and MacKintosh, P.: Aetiology of juvenile diabetes. Lancet 1975; 2:220.

[12] Ilieni, M. T., Pellegris, G., DelGuercio, M. J., et al.: HLA antigen in diabetic children. Diabetes 1977; 26:870–73.

[13] Svejgaard, A., Platz, P., and Ryder, L. P.: Insulin-dependent diabetes mellitus. *In* Histocompatibility Testing 1980. Teraski, P. I., Ed. Los Angeles, UCLA Press, 1980:638–56.

[14] Weinberg, C. R., Dorman, T. L., Hansen, J. A., Raglue, P. K., and Palmer, J. P.: HLA-related heterogeneity in seasonal patterns of diagnosis in type I (insulin-dependent) diabetes. Diabetologia 1984; 26:199–202.

[15] West, R., Colle, E., Belmonte, M. M., et al.: Prospective study of insulin-dependent diabetes mellitus. Diabetes 1981; 30:584–89.

[16] Ludvigsson, J., Safwenberg, J., and Heding, L. G.: HLA types, C-peptide and insulin antibodies in juvenile diabetes. Diabetologia 1977; 13:13–17.

[17] Schernthaner, G., Ludwig, H., and Mayr, W. R.: Immunoglobulin G-insulin antibodies and immune region-associated alloantigens in insulin-dependent diabetes mellitus. J. Clin. Exp. Med. 1979; 48:403–407.

[18] LaPorte, R. E., Fishbein, H. A., Drash, A. L., et al.: The Pittsburgh insulin-dependent diabetes mellitus (IDDM) registry: the incidence of insulin-dependent diabetes mellitus in Allegheny County, Pennsylvania. Diabetes 1981; 30:279–84.

[19] Henle, W., and Henle, G. E.: Epstein-Barr virus specifies diagnostic tests in infectious mononucleosis. Hum. Pathol. 1979; 5:551–65.

[20] Daneman, D., Tsalikian, E., Hengstenberg, F., Becker, D. J., and Drash, A. L.: Glycosylated hemoglobin in children with insulin-dependent diabetes mellitus. Diabetologia 1980; 19:423–26.

[21] Daneman, D., Wolfson, D. H., Becker, D. J., and Drash, A. L.: Factors affecting glycosylated hemoglobin values in children with insulin-dependent diabetes. J. Pediatr. 1981; 99:847–53.

[22] Lowry, O. H., and Passonneau, J. V.: A Flexible System of Enzymatic Analysis. New York, Academic Press, 1972.

[23] Heding, L. G.: Radioimmunological determination of human C-peptide in serum. Diabetologia 1975; 11:541–48.

[24] Van Rood, J. J., and Van Leeuwan, A.: Simultaneous detection of two cell populations by two-color fluorescence and application to the recognition of β-cell determinants. Nature 1976; 262:795–97.

[25] Haldane, J. B. S.: The estimation and significance of the logarithm of a ratio of frequencies. Ann. Hum. Genet. 1956; 2:309–11.

[26] Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K., and Bent, D. H.: SPSS: Statistical Package for the Social Sciences. New York, McGraw-Hill, 1975.

[27] Curie-Cohen, M.: HLA antigens and susceptibility to juvenile diabetes: do additive relative risks imply genetic heterogeneity? Tissue Antigens 1981; 17:136–48.

[28] Weinberg, C. R., Dorman, T. L., Hansen, J. A., Raglue, P. K., and Palmer, J. P.: HLA-related heterogeneity in seasonal patterns of diagnosis in type I (insulin-dependent) diabetes. Diabetes 1984; 26:199–202.

[29] Rabin, B. S., Cavender, D. E., and Orchard, T. J.: No evidence for heterogeneity of IDDM based on presence of islet-cell cytoplasmic antibody (ICA) at diagnosis. Abstract. Diabetes 1984; 33 (Suppl. 1):82A.

[30] Rubinstein, P., Walker, M. E., Fedun, B., Witt, M. E., Cooper, L. Z., and Ginsberg-Fellner, F.: The HLA system in congenital rubella patients with and without diabetes. Diabetes 1982; 31:1088–91.

[31] Orchard, T. J., Kuller, L. H., Eberhardt, M., et al.: Approaches to the environmental etiologies of insulin-dependent diabetes mellitus. *In* Advances in Diabetes Epidemiology. Eschwege, E., Ed. Amsterdam, Elsevier/North Holland, 1982:41–48.

[32] Cavender, D. E., Wagener, D. K., Rabin, B. S., Becker, D. J., Orchard, T. J., Eberhardt, M. S., LaPorte, R. E., Drash, A. L., and Kuller, L. H.: The Pittsburgh insulin-dependent diabetes mellitus (IDDM) study: HLA antigens and haplotypes as risk factors for the development of IDDM in IDDM patients and their siblings. J. Chronic Dis. 1984; 37:555–68.