The HLA System in Congenital Rubella Patients With and Without Diabetes

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SUMMARY

The HLA antigens of 173 patients with the congenital rubella syndrome (CR) are reported. Twenty-one of these patients are also clinically diabetic, and among them the frequencies of the HLA antigens DR2 and DR3 are significantly lower and higher, respectively, than in CR patients without diabetes or in controls. These data suggest that the genes that control susceptibility to type I or insulin-dependent diabetes mellitus are necessary for the development of glucose intolerance in CR patients. DIABETES *31*:1088–1091, December 1982.

The genetics of type I, insulin-dependent diabetes mellitus (IDDM) has been clearly separated from that of type II, non-insulin-dependent diabetes mellitus (NIDDM) largely because of the association between HLA antigens and IDDM but not NIDDM.[1,2]

Linkage between an IDDM-susceptibility locus and HLA has also been demonstrated;[3,4,5] the form of inheritance cannot be directly inferred from the data, however, because the susceptibility phenotypes cannot be identified in the absence of disease[4] and the penetrance of genotype(s) is incomplete.[6] Environmental factors are needed to "trigger" the state of genetic susceptibility into one of clinical disease, as shown by the lack of concordance in identical twin studies.[7,8] It is thus reasonable to assume that the frequency, extent, and severity of exposure to such environmental "trigger(s)" may determine the penetrance of the genotype conferring susceptibility. Under extreme conditions, the penetrance might be expected to approach 100%. Insulin-dependent diabetes develops, for example, in a substantial proportion of children with the congenital rubella syndrome (CR).[9] If it could also be shown that diabetes develops only in CR patients who have the same genetic susceptibility factors that underlie idiopathic IDDM we might obtain a more direct and less penetrance-dependent estimate of the frequency of the IDDM-susceptibility gene. Formal genetic analyses would obviously be precluded by the difference in rubella-virus exposure of the different pregnancies in a given family but a new and independently obtained estimate of the susceptibility gene frequency would be of enormous importance for both the evaluation of different genetic models and the computation of risks for relatives of IDDM patients. Previous studies have shown that the HLA-B8 antigen is associated with diabetes in CR patients.[9] This antigen is known to associate with idiopathic IDDM, which does suggest the possible existence of a common genetic background for insulin dependence. We report here the HLA-A-B-C, and -DR antigen frequencies of 173 children with stigmata of CR, 21 of whom are clinically diabetic.

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Received for publication 1 June 1982.

MATERIAL AND METHODS

PATIENTS

The 173 patients in this study are either being followed at the Developmental Disabilities Center of St. Luke's-Roosevelt Hospital or at the Pediatric Diabetes Clinic of Mount Sinai Hospital in New York City. Their mean age is 14 yr and the range is from 6 mo to 24 yr. They all have stigmata of the congenital rubella syndrome, which include different combinations of mental retardation, microcephaly, deafness, cataracts, congenital heart disease, and chorioretinopathy. Further diagnosis of CR was based on one or more of three criteria: (1) isolation of rubella virus from the throat, urine, and/or cerebrospinal fluid of the newborn; (2) serologic confirmation by detection of rubella-specific IgM hemagglutination-inhibition (HI) antibody in serum from newborns or persistence of rubella-specific IgG HI antibody on serial specimens beyond the first year of life; and (3) presence of clinical stigmata *and* detection of rubella-specific HI antibody levels compatible with congenital rubella in speci-

mens obtained at times that do not permit us to rule out postnatal infection. In more than half of all cases the diagnosis was established on the basis of criterion (1) or (2) (16% and 41%, respectively). No significant differences in the frequency of diagnostic criteria were observed for children who are currently diabetic (19% and 29%). All the patients in this sample have had periodic testing for blood and urinary glucose, and for hemoglobin A_{1c} (and for total glycohemoglobin when hemoglobins S, C, or F were seen), and all have had at least one determination of oral glucose tolerance. Twenty-one patients are clinically diabetic according to the criteria of the National Diabetes Data Group.[10] Sixteen are insulin-dependent (eight of each sex) and have had at least one episode of ketoacidosis; the other five evidence low and progressively decreasing insulin responses to oral glucose. Their insulin levels during a 3-h glucose tolerance test were at least two standard errors below the mean for normal children of the same age. The analysis of the HLA data has been done without differentiating patients on this basis.

CONTROLS

Two control samples have been HLA typed to provide "normal" frequencies of HLA antigens. Control 1 is a sample of 136 individuals, the ethnic backgrounds of whom are very similar to those of our patients, examined at Mount Sinai Hospital during studies on gestational diabetes; control 2 is a sample of 231 normal, random, unrelated New York Caucasians.

HLA TYPING

Reagents. One hundred eighty mono- or oligospecific reagents able to recognize 65 HLA antigens were used. Because of the sample size, however, the "splits" of antigens A9 and A10, B5, B12, B15, Bw16, B17, Bw21, Bw22, and Bw40 have not been analyzed separately even though they have been determined. The sera used in these typings have been evaluated on a panel typed with the Eighth International Histocompatibility Workshop serum set.[11]

Techniques. HLA-A, -B, and -C antigens were tested with the contrast fluorescence test;[12] HLA-DR with the two-color fluorescence test.[13]

STATISTICAL ANALYSIS

All comparisons have been investigated for significance using Fisher's exact text.

RESULTS AND DISCUSSION

The HLA phenotypes and the frequencies of HLA antigens in CR patients with and without diabetes are given in Tables 1 and 2, respectively, the latter including the HLA frequencies in both control samples.

The frequencies for CR patients without diabetes and for all CR patients taken together do not differ markedly from those in control sample 1. Because the two control samples differ in a few antigens, mostly of the HLA-A and -B series, control 1, made up of patients of a similar racial background to those in the CR sample, has been used for comparative purposes. Control sample 2 is included to allow for the further evaluation of any differences, a necessary step owing to the clear genetic heterogeneity within each of these samples. Table 3 (top) summarizes the significant differences

TABLE 1
HLA phenotypes of CR patients with diabetes

	HLA loci		
Patients	A	B	DR
J.C.	10	18	3,w6
L.C.	3,w32	8,13	3,4
L.J.	2	5,15	1,4
N.M.	2,26	w38,w39	4,8
R.R.	2	14,w50	3,7
N.I.	2	w40,18	1,7
M.E.	2,w31	5,7	4,5
B.W.	28,w30	12,17	3,7
H.C.	26,w30	w22,w53	3,5
D.I.	9	w21,w44	5,7
D.J.	1,w24	8*	3*
F.V.	2,3	w40,w49	4,5
McG.R.	1,3	w40,8	3,4
R.Z.	w23,28	8,18	7,8
D.J.M.	25,w33	w39,14	1,3
B.A.	1,26	8,w38	5,3
D'D.S.	1	8	3
W.R.	3	—	7,w6
G.L.	2,28	15,17	3,4
M.D.	2	w53	7,w6
R.C.	9,w30	37,w39	4,w10

* Family-proven homozygote.

($P \leq 0.05$) in antigen frequencies between control sample 1 and the two CR patient samples either together or separately. For the A and B series, the most interesting difference refers to Bw35, which is decreased in the CR patients. This reduction in frequency is significant for the group as a whole ($P = 0.022$) and, especially, for CR patients with diabetes, among whom Bw35 is absent ($P = 0.017$). The lower frequency of Bw35 should be confirmed in an independent study, since ours is the first sample in which it has been observed, but is interesting because Bw35 associates positively with other diseases of possibly viral etiology.[14–16]

Deviations in the frequencies of DR2 and DR3 and to a lesser extent (Table 2) of DR4, on the other hand, are restricted to the group of CR patients with diabetes and are the same as those encountered in patients with idiopathic IDDM. As in IDDM, the frequency of B8 is elevated together with, though not as much as, that of DR3. DR2 is absent in CR patients with diabetes. The frequency of DR4, an antigen associated with IDDM in most Caucasian samples, is also elevated in our CR diabetic patients (38% versus 23% in control 1), but the difference is not as yet significant. We conclude that the frequencies of the IDDM-associated DR antigens in the CR patients with diabetes are significantly different from those in CR patients without diabetes and in each of the two control samples. Furthermore, since the frequencies of DR2, DR3, and DR4 are almost the same in both control samples, it is unlikely that these associations are due to differences caused by racial stratification.

DR3 and the B8, DR3 haplotype associate with a number of diseases other than IDDM and, hence, the positive association between DR3 and diabetes in CR patients is not by itself proof of identity in genetic backgrounds with the conventional form of IDDM. There may also be some clinical differences between these forms of diabetes, particularly in the abruptness of onset. It is remarkable, therefore, that the distinctive feature of the HLA-IDDM association, namely, the

TABLE 2
HLA antigens (%) in CR patients with and without diabetes and in controls

Antigen	CR patients			Control 1*	Control 2*
	Without diabetes (N = 152)	With diabetes (N = 21)	All (N = 173)	(N = 136)	(N = 231)
A					
1	19.7	19	19.7	13.2	25.1
2	36.8	38	37.0	35.3	38.5
3	18.4	24	19.1	21.3	20.0
9	25.0	19	24.3	25.7	20.3
10	13.2	24	14.5	10.3	12.1
11	4.0	0	3.5	2.2	11.3
w19†	3.3	0	2.9	0	
28	11.2	14	11.6	16.2	8.2
w29	8.6	0	7.5	11.0	7.8
w30	13.8	14	13.9	18.4	5.2
w31	5.3	0	4.6	3.7	5.2
w32	4.0	10	4.6	7.4	8.2
w33	6.6	5	6.4	3.7	3.5
w34	2.0	0	1.7	3.7	0.5
B					
5	18.4	10	17.3	11.0	11.3
7	13.8	5	12.7	13.2	18.2
8	13.8	29	15.6	8.8	16.4
12	25.7	10	23.7	33.8	21.6
13	3.3	5	3.5	2.2	6.1
14	7.9	10	8.1	8.8	7.4
15	12.5	10	12.1	13.2	13.0
w16	7.9	24	9.8	8.8	10.0
17	12.5	10	12.1	16.2	7.4
18	6.6	14	7.5	3.7	10.8
w21	5.3	14	6.4	5.2	6.1
w22	9.2	5	8.7	7.4	3.9
27	4.6	0	4.0	0.7	9.1
w35	11.8	0	10.4	19.1	18.0
37	2.0	5	2.3	2.2	5.0
w40	9.2	14	10.4	3.7	17.0
w53	6.6	10	6.9	7.4	1.7
DR	(N = 142)	(N = 21)	(N = 163)		
1	19.0	14	18.4	18.4	10.0
2	25.4	0	22.1	25.0	25.1
3	24.7	52	28.2	21.3	20.3
4	28.2	38	29.4	22.7	23.4
5	24.0	19	23.3	24.3	23.4
w6	21.1	14	20.3	11.0	14.7
7	19.0	33	20.9	24.3	20.0
w8	0.7	10	1.8	10.3	3.5
w9	1.4	5	1.8	3.7	ND‡
w10	3.5	5	3.7	2.9	ND‡

* See text.
† Splits not determined in five CR patients without diabetes.
‡ ND is not determined.

combination of increased frequency of DR3 and *decreased* frequency of DR2[17] (thus far found solely in IDDM) holds true for CR patients with diabetes. The additional increase of the frequency of DR4 in IDDM[17] is also apparent, though not in itself significant. This unique combination of deviations is unlikely to result from chance or from a different type of genetic background in diabetic CR patients, suggesting that insulin-dependent diabetes may indeed affect only those CR patients who carry the common IDDM-susceptibility genes. Since the prevalence of insulin-dependent diabetes is so much higher among CR patients than in the general population it may be assumed that the difference is caused by either an increase of the penetrance of the susceptibility genotype(s) or of the number of phenocopies or by both. An important increase in the number of phenocopies (i.e., disease without genetic susceptibility) should have resulted in a substantial reduction of the strength of the HLA associations. It is, thus, likely that the severe intrauterine infection with a beta-cell cytopathic virus[18,19] may have pushed the penetrance of the common IDDM-susceptibility genotype toward 100%. On the basis of this preliminary interpretation, the minimal frequency (further patients may become diabetic in the future) of the HLA-associated IDDM-susceptibility gene(s) may be calculated directly for both the "dominant" and "recessive" hypotheses. The prevalence (21/173 or 0.1214) would be equal to $p^2 + 2pq$, or to p^2, respectively, for dominant or recessive IDDM-susceptibility genes of frequency p. Thus, minimally, p (dominant) would equal 0.0627, and p (recessive) would equal 0.3484, which are close to the gene frequencies that best fit the International

TABLE 3
Significant associations between HLA and CR with and without diabetes

	Antigen	Control (N = 136)	CR (all) (N = 173)	P*	CR without (N = 152)	P	CR with (N = 21)	P*
Comparison with control sample 1	B8	12 (8.8)†	—	—	—	—	6 (29)	0.018
	B12	46 (33.8)	—	—	—	—	2 (10)	0.017
	Bw35	26 (19.1) (N = 136)	18 (10) (N = 163)	0.022	— (N = 142)	—	0 (0) (N = 21)	0.017
	DR2	34 (25.0)	—	—	—	—	0 (0)	0.0039
	DR3	29 (21.3)	—	—	—	—	11 (52)	0.0042

	Antigen	CR with (N = 21)	CR without (N = 142)	P*
CR with diabetes versus CR without diabetes	DR2	0 (0)	36 (25.4)	0.0035
	DR3	11 (52)	35 (24.7)	0.011

* Fisher's exact test.
† (%).

Workshop's family data, assuming simple, one-locus genetic models.[17] The gene frequency expected under an intermediate inheritance model[20] should be close to that under a dominant model if the penetrance for the heterozygotes is very high, as postulated for CR patients. The dominant model, however, has been already rejected for any gene frequency on the grounds that affected sib-pairs too often share both HLA alleles.[17]

One of the main reasons for the difficulty in choosing between the various possible genetic models for IDDM is our inability to estimate disease gene frequency independently of the penetrance. The example of CR as a trigger suggests that the penetrance of IDDM as a polyfactorial disease may depend in each individual case on the severity of the environmental exposure. This probably explains the differences between the penetrances calculated for the second affected child in a diseased sibship and for the population as a whole.[21]

ACKNOWLEDGMENTS

We are thankful to Alice Varipapa for excellent secretarial help. This work was supported, in part, by NIH grant AM 19631 and by a grant from the American Diabetes Association.

REFERENCES

[1] Singal, D. P., and Blajchman, M. A.: Histocompatibility (HLA) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes *22*:429–32, 1973.

[2] Nerup, J., Platz, P., Ortved-Anderson, O., Christy, M., et al.: HLA antigens and diabetes mellitus. Br. Med. J. *2*:133–35, 1975.

[3] Falk, C. T., Suciu-Foca, N., and Rubinstein, P.: Localization of the gene(s) for juvenile diabetes mellitus (JDM) to the HLA region of chromosome 6. Winnipeg Conference (1977), 4th International Workshop on Human Gene Mapping. Cytogenet. Cell Genet. *22*:298–300, 1978.

[4] Thomson, G., and Bodmer, W. F.: The genetic analysis of HLA and disease associations. *In* HLA and Disease. Dausset, J., and Svejgaard, A., Eds. Copenhagen, Munksgaard, 1977, pp. 84–93.

[5] Barbosa, J., King, R., Noreen, H., and Yunis, E. J.: The histocompatibility system in juvenile, insulin-dependent diabetic multiplex kindreds. J. Clin. Invest. *60*:989–98, 1977.

[6] Steinberg, A. G.: Heredity in diabetes mellitus. Diabetes *10*:269, 1961.

[7] Tattersall, R. B., and Pyke, D. A.: Diabetes in identical twins. Lancet *2*:1120–24, 1972.

[8] Cahill, G. F., Jr.: Current concepts of diabetic complications with emphasis on hereditary factors: a brief review. *In* Genetic Analysis of Common Diseases: Applications to Predictive Factors in Coronary Heart Disease. Sing, C. F., and Skolnick, M., Ed. New York, Alan R. Liss, 1979, pp. 113–25.

[9] Menser, M. A., Forrest, J. M., Honeyman, M. D., and Burgess, J. A.: Diabetes, HL-A antigens and congenital rubella. Lancet *2*:1508–1509, 1974.

[10] National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes *28*: 1039–57, 1979.

[11] Terasaki, P. I. (Editor): Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, 1980.

[12] Martel, J. L., Jaramillo, S., Allen, F. H., Jr., and Rubinstein, P.: Serology for automated cytotoxicity assays. Contrast fluorescence test. Vox Sang. *27*:13, 1974.

[13] van Rood, J. J., van Leeuwen, A., and Ploem, J. A.: Simultaneous detection of two cell populations by two-colour fluorescence and its application to the recognition of B-cell determinants. Nature *262*:795–97, 1976.

[14] Nyulassy, S., Hnilica, P., and Stefanovic, J.: The HLA system and subacute thyroiditis. Tissue Antigens *6*:105, 1975.

[15] Jenkins, H., and Farid, N. R.: Subacute thyroiditis-like syndromes. Relation to HLA. Tissue Antigens *13*:167, 1979.

[16] Dupont, B., Good, R. A., Hauptmann, G., Schreuder, I., and Seligmann, M.: Immunopathology, immunodeficiencies and complement deficiencies. *In* HLA and Disease. Dausset, J., and Svejgaard, A., Eds. Copenhagen, Munksgaard, 1977, pp. 233–48.

[17] Svejgaard, A., Platz, R., and Ryder, L. P.: Insulin-dependent diabetes mellitus (Joint Report). *In* Histocompatibility Testing 1980. Terasaki, P. I., Ed. UCLA Tissue Typing Laboratory, Los Angeles, 1980, pp. 638–56.

[18] Bunnel, C. E., and Monif, G. R. G.: Interstitial pancreatitis in the congenital rubella syndrome. J. Pediatr. *80*:465–66, 1972.

[19] Naeye, R. L., and Blanc, W.: Pathogenesis of congenital rubella. JAMA *194*:109–15, 1965.

[20] Spielman, R. S., Baker, L., and Zmijewski, C. M.: Gene dosage and susceptibility to insulin-dependent diabetes. Ann. Hum. Genet. *44*:135–50, 1980.

[21] Rubinstein, P., Ginsberg-Fellner, F., and Falk, C.: 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.