HLA Antigens in Diabetic Children

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SUMMARY

Ninety insulin-dependent diabetic children were HLA-typed in order to elucidate the role played by HLA complex-linked genes in the pathogenesis of diabetes mellitus of childhood. HLA-Aw30 and HLA-Bw35 were significantly increased and decreased, respectively, in the diabetic group as compared with controls. In relation to age at onset of diabetes, HLA-B8 was significantly increased in the 0-5-year group.

By dividing the patients according to the season at onset of the disease, only HLA-Aw30 in the October-January group reached the level of significance (P=0.05). DIABETES *26*:870-73, September, 1977.

Hereditary factors seem to play a significant role in the etiology of the maturity-onset type of diabetes mellitus of childhood (MODY),[1] while the importance is doubtful in the insulin-dependent juvenile-onset diabetes (JOD).[2,3] Some authors have reported a statistically increased frequency of HLA-B8 and/or HLA-Bw15, while others were unable to find a correlation between HLA antigens and JOD.[4-8] We remind the reader that linkage disequilibrium in the *HLA* locus is a possible mechanism to explain the association between the serologic antigens and a clinical disease.

Furthermore, it has been suggested that some HLA antigens may interact with Coxsackie B4 or other viruses injurious to pancreatic islet cells,[9] so that predisposed children may develop clinical diabetes within a few weeks or months after the infection.[10] In the present study, HLA typing was performed in diabetic children and adolescents in an attempt to cast further light on the pathogenesis of this disease.

Presented in part at the Symposium on HLA and Disease, Paris, June 1976.

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Accepted for publication April 29, 1977.

PATIENTS AND METHODS

Ninety insulin-dependent diabetic children (46 boys and 44 girls) aged 2-20 years (mean 10.7 years) were studied. All had acute onset of the disease between 11 months and 16 years of age (mean seven years and one month), and its duration varied from a few days to ten years and 6 months (mean three years and six months); 52 per cent of the diabetic children had their first clinical symptoms of the disease within the months of October through January. The control population was 488 blood donors of both sexes living in the same geographic area as the diabetic patients.

HLA antigens were identified with the use of a panel of 83 selected antisera defining 27 specificities in the microlymphocytotoxicity test. The antisera were supplied by Biotest (Serum Institut GmbH, Frankfurt/Main), Behringwerke (Marburg-Lahn), and Dr. G. B. Ferrara (Laboratorio di Immunoematologia, Ospedale Civile di Massa, Italy). The cytotoxicity testing was performed in duplicate by a modification of the Terasaki micromethod.[11]

A statistical analysis of the results of the HLA typing was performed according to Woolff's method.[12] The P values were also corrected by multiplying P by the number of antigens tested, as suggested by Svejgaard.[13] In the following paragraph, the results obtained with and without such correction are described.

RESULTS

Table 1 shows that in diabetes mellitus, the frequency of HLA-Aw30 and HLA-B8 is statistically

TABLE 1

Frequency of HLA antigens in 90 diabetic children and 488 controls

Antigens	Controls	Diabetic children				
	(n)	n	R.R.*	Chi-square	P	P × A†
A1	122	16				
A2	215	49				
A3	132	16				
A9	132	32				
A10	63	10				
A11	54	3	0.277	4.503	0.0310¶	0.84
A28	34	5				
A29	24	6				
Aw30	34	16	2.887	10.444	0.0012//	0.03//
Aw31	6‡	7				
Aw32	54	7				
Aw33	9	0				
B5	102	11				
B7	63	8				
B8	61	21	2.130	7.075	0.0075//	0.20
B12	83	18				
B13	29	7				
Bw40	31	4				
Bw35	133§	15	0.375	10.458	0.0012//	0.03¶
B14	31	4				
Bw15	37	4				
Bw16	54	10				
Bw17	38	3				
B18	61	18				
Bw21	40	8				
Bw22	5	0				
B27	18	2				

*Relative risk.
†Number of antigens tested = A.
‡90 tested.

§383 tested.
¶P<0.05.
//P<0.01.

increased and that of HLA-A11 and HLA-Bw35 decreased, as compared with that of controls. Following multiplication of P by the number of antigens tested, the significance of HLA-A11 and HLA-B8 is nil.

The diabetic patients were divided according to their age at onset of the disease (0-5, 5-10, and 10-16 years) and to the season of the start of symptoms (October-January and February-September).

Table 2, which reports only the statistically significant differences in HLA typing, shows that other antigens, besides those aforementioned, may be associated with diabetes in children. The frequency of HLA-Aw30 and HLA-B8 is significantly increased and that of HLA-Bw35 decreased, as compared with that of controls, when the disease occurs before the fifth year of age. In those in whom the disease occurs at 5-10 years, the incidence of the antigens HLA-A2, HLA-Aw30, and HLA-B18 is significantly increased and HLA-Bw35 decreased as compared with controls, while no significant differences are observed in the group in which diabetes starts between 10 and 16 years of age.

Following multiplication of P values by the number of antigens tested, only HLA-B8 in the 0-5-year age group remains significant. When the seasonal onset of the syptoms of JOD is considered (table 3), the frequency of HLA-A2, HLA-Aw30, and HLA-B8 is significantly increased and that of HLA-A11 decreased within the October-January group. In the February-September group the frequency of antigens HLA-A29 and HLA-B12 is significantly increased. In both seasonal groups the antigen HLA-Bw35 is significantly

TABLE 2

Frequency of HLA antigens in diabetic children in relation to age at onset of the disease and controls

Antigens	Controls	Diabetic children														
		0-5 years					5-10 years					10-16 years				
	n=488	n=28	R.R.*	Chi-square	P	P×A†	n=34	R.R.	Chi-square	P	P×A	n=26	R.R.	Chi-square	P	P×A
A2	215	15					23	2.65	6.681	0.01§	0.27	11				
Aw30	34	6	3.64	6.853	0.0087¶	0.230	6	2.86	4.492	0.03§	0.86	3				
B8	61	10	3.88	10.582	0.0010¶	0.027§	4					7				
Bw35	133†	2	0.14	6.799	0.0090¶	0.240	6	0.40	3.865	0.05§	1.37	7				
Bw15	37	0					3					1				
B18	61	5					9	2.52	5.029	0.02§	0.67	4				

*Relative risk.
†number of antigens tested = A.
‡383 tested.

§P<0.05
¶P<0.01.

TABLE 3

Frequency of HLA antigens in diabetic children in relation to season at onset of the disease and controls

Antigens	Controls	Diabetic children									
		October-January					February-September				
	n=488	n=42	R.R.*	Chi-square	P	P×A†	n=38	R.R.	Chi-square	P	P×A
A2	215	26	2.06	4.081	0.0275§	0.740	20				
A11	54	0		4.296	0.0365§	0.990	3				
A29	24	1					5	2.93	4.213	0.0380§	1.03
Aw30	34	8	3.14	8.487	0.0035	0.095	5				
B8	61	11	2.48	5.833	0.0157	0.420	9				
B12	83	5					12	2.25	4.835	0.0275§	0.74
Bw35	133‡	6	0.31	6.540	0.0110	0.300	6	0.36	5.192	0.0220§	0.59
Bw15	37	2					1				

*Relative risk.
†Number of antigens tested = A.
‡383 tested.

§P≤0.05.
P<0.02.
P<0.01.

rarer than in controls.

Although none of these antigens remains significant following the usual correction, HLA-Aw30 reaches the level of significance (P=0.05) in the October-January group.

DISCUSSION

Many diseases have been associated with different HLA histocompatibility antigens. The significant increase of HLA-B8 and HLA-Bw15, which has been frequently,[4-6] though not always,[7,8] observed in nonobese insulin-dependent juvenile diabetic patients, suggests that genetic factors may have a role in the pathogenesis of the disease.

However, when Cudworth and Woodrow[6] combined all the data available from the literature on this matter, there was evidence that the relative risk of insulin-dependent JOD is doubled not only for HLA-B8 but also for HLA-Bw15-positive individuals; these authors found that HLA-Bw15 had a relatively low incidence in the diabetic patients whose age at onset of the symptoms was 0-5 or 11-16 years.[6] Our data support a definite association of HLA-Aw30 but not of HLA-B8 and HLA-Bw15 with diabetes mellitus in childhood.

As regards the negative association of HLA-Bw35 with JOD, a similar finding has been reported for antigen HLA-B7. Indeed, even a minor frequency of some antigens may increase the risk of contracting diabetes.[14] The importance, if any, of HLA-Bw35 as a "protection marker" against the development of JOD must be determined by performing a survey of families with high incidence of the disease.

According to Rolles et al.,[10] children predisposed to the disease because of their HLA asset may be susceptible to Coxsackie B4 virus, which initiates pancreatic damage and causes clinical diabetes a few weeks or months after the infection.[10]

These authors observed that the highest incidence of the onset of diabetes takes place in the 81 per cent of HLA-B8 positive children within the October-February period. They point out that the peak incidence of the Coxsackie B4 virus infection occurs from August to December—i.e., in a period of the year almost matching the onset of the diabetic symptoms. In our experience, the antigen HLA-B8, which is commonly associated with JOD, occurs in most 0-5-year-old children whose symptoms begin within the October-January interval. However HLA-B8 is significantly increased following Woolff's test, but not after multiplication of P by the number of the antigens tested. We point out that the amount of patients within each seasonal group is scanty and therefore the statistical results may be questionable. Although included in this report, these results must be considered as preliminary data and as a useful hint to other researchers.

It is possible that the association of diseases with HLA antigens merely reflects a high degree of linkage disequilibrium between the corresponding HLA genes

and other, yet unknown, genes that are directly active in the pathogenesis of the disease.

The HLA region contains about 2,000 cistrons between HLA-A and HLA-B loci, and only a very small number of them are known. Furthermore, there might be a similar number of cistrons between the locus HLA-B and HLA-D. The function of all these genes is little known, but there is curcumstantial evidence that at least some of them govern the specific immune responses towards various antigens. Although the pathogenesis of JOD is unknown, autoimmune phenomena are frequently demonstrated.[15,16] The production of anti-pancreatic islet cell autoantibodies may be under the control of an Ir gene within the HLA region, closely linked to HLA-B8 and HLA-Bw15 and, probably, to other HLA antigens, as demonstrated in this study by us, or even to HLA-D locus antigens, as suggested by Thomsen et al.[17] If this is so, we are tempted to speculate that genetically predisposed children are easily affected by Coxsackie B4 or other pancreas-damaging viruses, which may prime the production of autoantibodies by a formerly quiescent Ir-gene.

Studies are in progress in this direction with the long-term view of identifying the JOD at-risk population and recognizing the precipitating agent.

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