HLA Antigens in Japanese Patients with Diabetes Mellitus

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SUMMARY

Lymphocytes of 84 Japanese patients with diabetes mellitus and 150 normal controls were tested for HLA antigens by the microcytotoxicity test. HLA-B12 was found in 37.5 per cent of 32 patients with juvenile-onset diabetes mellitus, 13.5 per cent of 52 patients with late-onset diabetes, and 9.3 per cent of 150 normal controls. In the insulin-dependent juvenile form, HLA-B12 was associated with the disease more frequently and at a significant level ($p<0.0005$). In Japanese patients with juvenile-onset insulin-dependent diabetes mellitus the association is with HLA-B12, not with HLA-B8 and BW15, as in Caucasian patients. There was no significant increase of HLA-B12 in patients with insulin-independent diabetes mellitus. DIABETES*26*:736-39, August, 1977.

In recent years the close association of genes controlling the immune response (Ir-genes) with a variety of antigens of the major histocompatibility has been well established.[1-3] The susceptibility of laboratory animals to experimentally induced diseases such as thyroiditis[4] or encephalomyelitis[5] is also under control of Ir-genes. These studies have led to an extensive search for an analogous association in the human being.[2,3] In a series of investigations, close associations between HLA antigens and a number of diseases have been clarified. Such an association was found mainly in those diseases in which immunologic mechanisms are active in their pathogenesis.

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In the case of acute-onset juvenile diabetes mellitus the circumstantial evidence for a viral etiologic role has been accumulated.[6,21] This diabetogenic effect is under strong genetic influences. Furthermore, autoimmune antipancreatic cell-mediated immunity has been demonstrated in juvenile-onset diabetes.[7-9] Among Caucasians, a definite positive association of acute-onset juvenile diabetes with HLA-B8 and HLA-Bw15 has been reported recently.[10,11] Since racial differences in linkage disequilibrium have been observed,[12] the present study was designed to study the relationship between HLA phenotypes and juvenile-onset diabetes mellitus in Japanese populations.

MATERIAL AND METHODS

Eighty-four unrelated Japanese patients with diabetes mellitus and 150 normal controls living in the same geographic area were HLA-typed by the microlymphocyte-cytotoxicity test.[13] In 32 individuals, diabetes had been diagnosed before the age of 30 (juvenile-onset diabetes) and 52 patients had late-onset diabetes. The numbers of patients with insulin-dependent and insulin-independent diabetes are shown in table 1. None had received special drugs that might affect lymphocyte viability or cell surface properties.

Twenty-six specificities, including 11 locus A and 15 locus B antigens, were determined by use of the sera obtained from the Serum Bank of National Institute of Health (Bethesda, Maryland) and Behring-Berke tissue-typing laboratories. The following HLA antigens were tested: HLA-A1, A2, A3, A9, A10, A11, A28, A29, Aw30, Aw31, and Aw32 in the locus A and HLA-B5, B7, B8, B12, B13, B14, B18, B27, Bw15, Bw16, Bw17, Bw22, Bw35, and Bw40. Statistical significance was evaluated by χ^2 test.[3]

TABLE 1

Frequencies of HLA antigens in normal subjects, juvenile-onset diabetes, and late-onset diabetes mellitus

HLA Antigen		Control (N=150)		Juvenile-onset diabetes mellitus: Total (N=32)		Juvenile-onset diabetes mellitus: Insulin-dependent (N=29)		Juvenile-onset diabetes mellitus: Insulin-independent (N=3)		Late-onset diabetes mellitus: Total (N=52)		Late-onset diabetes mellitus: Insulin-dependent (N=30)		Late-onset diabetes mellitus: Insulin-independent (N=22)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Locus A	A 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A 2	76	50.7	18	56.2	17	58.6	1	33.3	21	40.3	12	40.0	9	40.9
	A 3	2	1.3	3	5.7	3	10.3	0	0	0	0	0	0	0	0
	A 9	91	60.7	17	53.1	15	51.7	2	66.6	30	57.7	17	56.6	13	59.0
	A10	38	25.3	0	0	0	0	0	0	5	9.6	4	13.3	1	4.5
	A11	31	20.7	2	6.2	2	6.8	0	0	9	17.3	5	16.6	4	18.2
	A28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Aw29	0	0	1	3.1	1	3.4	0	0	0	0	0	0	0	0
	Aw30	21	14.0	2	6.2	1	3.4	1	33.3	5	9.6	4	13.3	1	4.5
	Aw31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Aw32	0	0	2	6.2	2	6.8	0	0	3	5.8	2	6.6	1	4.5
Locus B	B 5	58	38.7	7	21.8	5	17.2	2	66.0	21	40.3	10	33.3	11	50.0
	B 7	18	12.0	3	9.3	2	6.8	1	33.3	5	9.6	4	13.3	1	4.5
	B 8	3	2.0	0	0	0	0	0	0	0	0	0	0	0	0
	B12	14	9.3	12	37.5	11	37.9	1	33.3	7	13.5	5	16.6	2	9.0
	B13	5	3.3	2	6.2	1	3.4	1	33.3	1	1.9	0	0	1	4.5
	B14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B17	0	0	0	0	0	0	0	0	1	1.9	0	0	1	4.5
	B27	1	0.7	1	3.1	1	3.4	0	0	0	0	0	0	0	0
	Bw15	10	6.7	2	6.2	2	6.8	0	0	1	1.9	1	3.3	0	0
	Bw16	5	3.3	5	15.6	4	13.7	1	33.3	8	15.3	5	16.6	3	13.6
	Bw18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Bw21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Bw22	37	24.7	7	21.8	7	29.1	0	0	17	32.7	13	43.3	4	18.2
	Bw35	20	13.3	5	15.6	4	13.7	1	33.3	9	17.3	5	16.6	4	18.2
	Bw40	60	40.0	8	25.0	8	27.5	0	0	17	32.7	10	33.3	7	31.8

RESULTS

The frequencies of HLA antigens in the two groups of diabetic patients and in controls are shown in table 1. HLA-B12 was found in 37.5 per cent of 32 juvenile-onset diabetics as against 13.5 per cent of 52 patients with late-onset diabetes and 9.3 per cent of 150 normal controls. The differences between the former and the latter two groups were significant ($p<0.0005$). In the juvenile-onset group, only three patients out of 32 were insulin-independent. This number is too small for consideration. HLA-B12, however, was also significantly increased in the insulin-dependent group. In the late-onset group, no significant increase of HLA-B12 was observed even in those who were insulin-dependent.

A decreased incidence of HLA-A10 in both the juvenile-onset ($\chi^2=7.81$) and late-onset ($\chi^2=2.92$) and of A11 in the juvenile-onset group ($\chi^2=2.78$) was observed, but statistically these tendencies were not significant.

HLA-Bw16 was increased both in juvenile-onset ($\chi^2=6.40$) and in late-onset group ($\chi^2=7.53$), but statistically these tendencies were not significant.

DISCUSSION

There are at least two distinct patterns of diabetes mellitus—(1) an insulin-dependent, juvenile-onset form and (2) a late-onset, usually insulin-independent. The insulin-dependent, juvenile-onset diabetes is characterized by early onset, nonobesity, lymphocytic infiltration of the islets of Langerhans, reduction of the functioning beta-cell mass, and anti-pancreatic–cell-mediated immunity.[8,9,14] The evidence suggests a probable autoimmune mechanism in its pathogenesis.

In Caucasians, HLA antigens B8 and Bw15 are more common in insulin-dependent diabetes mellitus than in controls. The frequency of HLA-B8 was also reported to be increased in Graves' disease[15] and idiopathic Addison's disease[16] in Caucasians. It has consequently been suggested that HLA-B8–associated immune-response genes may be the common denominator for the development of endocrine autoimmunity. In Japanese populations, however, HLA-B8 is one of the rare phenotypes of HLA antigens. We found a statistically significant association of Graves' disease not with HLA-B8 but with Bw35.[17] The same

results have been reported by Grumet et al.[18] in studying Japanese patients with Graves' disease.

Our present study demonstrates the significantly increased phenotype frequency of HLA-B12 in insulin-dependent juvenile-onset diabetes mellitus. It is possible that HLA-B8 and B12 are situated close[19,20] to some major pathogenetic genes, which may be predisposed to juvenile-onset diabetes, on the sixth chromosome. The data appear to show that HLA-B8 is not itself necessary to the development of juvenile diabetes mellitus but, rather, there is a gene in linkage disequilibrium with HLA-B8. This follows directly from the lack of association of diabetes with HLA-B8 in Japanese and also from the fact that HLA-B8 is found in only about half of Caucasian juvenile diabetes patients.

Our study is of interest in showing that in a different race a postulated immune-response gene may be linked to a different HLA specificity in locus A and locus B. Another possibility is that the association between HLA antigens in diabetes and other endocrinopathies associated with autoimmunity is not due to an abnormal immune-response gene linked to HLA genes and specific for given diseases but to the fact that HLA-B8 and HLA-B12 may be in linkage disequilibrium with a gene that enhances or facilitates immune responses generally. However, if a viral connection with an etiology of juvenile diabetes is

TABLE 2

Statistical significance of frequencies of HLA antigens in Japanese patients with diabetes mellitus

		HLA Antigens							
		A10	A11	Aw30	B5	B12	Bw15	Bw16	Bw22
Normal control (N=150)	Number	38	31	21	58	14	10	5	37
Juvenile-onset diabetes									
Total	Number	0	2	2	7	12	2	5	7
(N=32)	chi square	7.81	2.78	1.37	1.67	11.13†	0.001>	6.40	0.07
	p value	0.01–0.005	0.10–0.05			0.005–0.001		0.025–0.01	
	Corrected p value *	0.26–0.13	2.6–1.3			0.13–0.026		0.65–0.026	
Insulin-dependent	Number	0	2	1	5	11	2	4	7
(N=29)	chi square	7.10	2.29	2.09	2.63	14.68†	0.001>	4.72	0.002
	p value	0.01–0.005	0.50–0.10			0.001–−0.0005		0.05–0.025	
	Corrected p value	0.26–1.3				0.026–0.013		1.3–0.65	
Late-onset diabetes									
Total	Number	5	9	5	21	7	1	8	17
(N=52)	chi square	2.92	0.26	2.92	0.02	0.56	1.54	7.53	0.71
	p value	0.10–0.05		0.10–0.05				0.01–0.005	
	Corrected p value	2.6–1.3		2.6–1.3				0.26–0.13	
Insulin-dependent	Number	4	5	4	10	5	1	5	13
(N=30)	chi square	1.33	0.17	0.001>	0.14	1.10	0.43	7.00	2.24
	p value							0.01–0.005	0.50–0.01
	Corrected p value							0.26–0.13	13–2.6
Insulin-independent	Number	1	4	1	11	2	0	3	4
(N=22)	chi square	3.42	0.05	1.26	0.41	0.43	1.45	3.90	0.28
	p value	0.10–0.05						0.05–0.025	
	Corrected p value	2.6–1.3						1.3–0.65	

*Corrected p value was calculated by multiplying p value by number of antigens tested.
†Statistically significant.

valid,[21] HLA-B12 can be associated with immune-response genes controlling the development of cell-mediated immunity to infectious agents, which in turn may destroy pancreatic islet cells, thus causing insulin-dependent diabetes.

Our present study suggests that there are genetically at least two different types of diabetes. The juvenile form of diabetes appears to be associated with histocompatibility antigens. Late-onset diabetes is shown by these data to be genetically different from juvenile diabetes, and therefore this is further evidence for heterogeneity of diabetes within the Japanese population, as has been demonstrated in the Caucasian population.

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