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HLA-antigens and some autoimmune features of juvenile diabetes mellitus

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A group of 67 juvenile insulin dependent diabetic patients and their 167 healthy first degree blood relatives were HLA-typed. In the patients the frequency of HLA—A9 and B8 antigens was significantly increased as compared to healthy controls, while in the family members only the presence of HLA—B8 was significantly increased. All diabetics carrying HLA—B8 antigen had frequently higher ^{125}I-insulin-antibody complex levels than those lacking the antigen.

Prevalence of some autoantibodies to human thyroglobulin, microsomal thyroid and antigastric mucosa antigen were investigated and compared to healthy controls. Increased antibody titres were more frequent in diabetics and their blood relatives than in the healthy controls, and more frequent in those carrying the HLA—B8 antigen than in those lacking it.

Recent studies of juvenile insulin dependent diabetes mellitus (JIDDM) have emphasized the pathogenetic importance of hereditary-genetic factors and of autoimmune features. Association with JIDDM of HLA antigens B8 [7, 13, 34], Bw15 [10, 13, 39, 40] and to a less extent of B18 [10, 13] has been reported and it was postulated that a gene (or genes) linked to and in disequilibrium with loci B and D of the HLA complex has some role in the disease [10, 24, 33, 38].

Some indirect evidence of an abnormal immune mechanism in diabetes mellitus has been offered by its histologic demonstration in JIDDM [18, 20], as well as of a certain clinical and serological association between diabetes and organ-specific autoimmunity [14, 21, 22, 25, 27, 34]. It is well known that cell-mediated immunity may play a decisive role leading to beta cell destruction directly or through an autoimmune mechanism. Circulating islet-cell autoantibodies [8, 22, 25, 28, 36] and other organ-specific autoantibodies were demonstrated in insulin dependent patients with and without associated autoimmune disorders.

Taking into consideration the above facts, the purposes of the present investigation were,

1. to study the distribution of HLA antigens in JIDDM and in the first degree blood relatives of juvenile diabetic patients;

2. to look for correlations between the onset of the disease and some frequently occurring HLA antigens;

3. to compare the presence of HLA-B8, the most frequently occurring HLA antigen, with the daily required insulin dose, and to try to find some correlation between the HLA-B8 antigen and the insulin binding capacity of the plasma (^{125}I-insulin-antibody complex);

4. to study the prevalence in diabetics and their first degree blood relatives of some humoral organ-specific autoantibodies i.e. circulating antibodies to thyroid microsomal antigen, human thyroglobulin and antigastric mucosa antigen;

5. to compare the presence of these autoantibodies with the prevalence of HLA-B8 antigen.

Material

Subjects

Patient group: 67 unrelated insulin dependent diabetics, 23 boys and 44 girls aged 1.5 to 28 (mean, 9.6) years, were studied. All of them had acute onset disease between 16 months and 18 years (mean, 10.1 years) and its duration varied from 2 months to 18 years (mean, 7.3 years). The patients had been treated since the onset of the disease with commercial mixed insulin.

Healthy family member group consisted of 164 healthy first degree blood relatives of the above diabetics. Among them there were 110 parents, 34 siblings and 20 offsprings of young diabetic mothers. None had any symptom or laboratory data pointing to some endocrine or metabolic disorder.

Control group: 450 healthy blood donors of both sexes living in the same geographic area as the two other groups.

Autoantibody studies

Patient group: the above 67 insulin treated JIDDM patients.

Healthy family member group: 105 healthy first degree blood relatives of the diabetics, except offsprings.

Control group: 1100 healthy control subjects with a female-to-male ratio of 2 : 1, with no endocrine or metabolic disturbances.

Methods

HLA-typing

All subjects were HLA-typed for 27 antigens of the A and B segregant series using NIH lymphocytotoxicity microtest.

Autoantibody tests

Human thyroglobulin antibodies were detected by the haemagglutination technique according to Boyden [9] and Takeda [44].

Circulating microsomal antibodies to thyroid antigen were prepared and measured by the method of Amino et al. [1].

Antibodies to antigastric mucosa were detected as recommended by DeDuve and Grant [14]. Patients with an anti-thyroglobulin, microsomal and antigastric mucosa titre of 32 or more were considered positive.

^{125}I-insulin-antibody complex determination was carried out by Sephadex-G 100 gel filtration according to Chao et al. [11, 46] using ^{125}I-labelled Amersham insulin.

Statistical analysis was performed according to Woolff [47] and by the chi square test. P values were corrected by multiplying P by the number of antigens tested [7, 43].

Results

Table I shows that in JIDDM the most frequent HLA antigens were A9 and B8, but after correction according to the number of antigens tested, only the presence of HLA-B8 has increased significantly ($P < 0.027$). In the diabetics the frequency of HLA B7 antigen was less than in the controls (1.1% *vs.* 19.8%), and the presence of HLA-B18 decreased, while that of HLA B15 was as much as in the controls.

In Fig. 1. the data of patients and family studies show that the frequency of HLA-B8 increased significantly as compared to the controls (31.8% *vs.* 18.8%, corrected $P < 0.027$), while the incidence of HLA B18 was decreased. There were no significant differences in the presence of HLA-B15, HLA-B35, and HLA-Bw40 between healthy blood relatives and controls. (At the time of the control studies only these antigens could be defined.)

As to the correlation between HLA antigens and the patient's age, no relationship could be established (Fig. 2.)

As to the presence of the HLA-B8 antigen and the daily required insulin dose, no difference could be demonstrated, and there was no difference in the amount of ^{125}I-insulin-antibody complex between the groups. All diabetics carrying the HLA-B8 antigen had significantly more often ^{125}I-

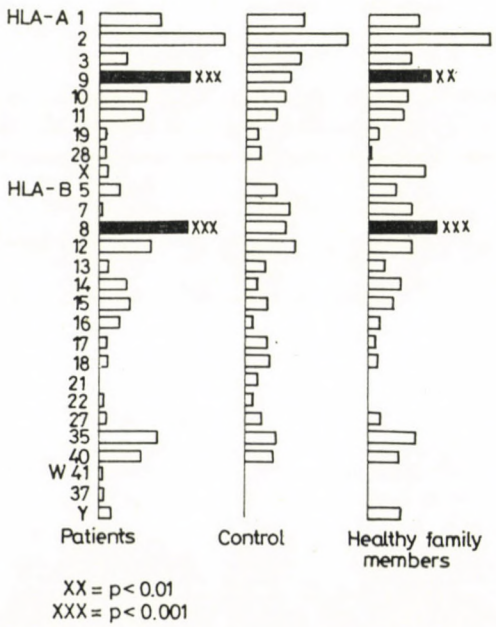

Fig. 1. Percentile distribution of HLA antigens in diabetic patients, healthy family members and healthy controls.

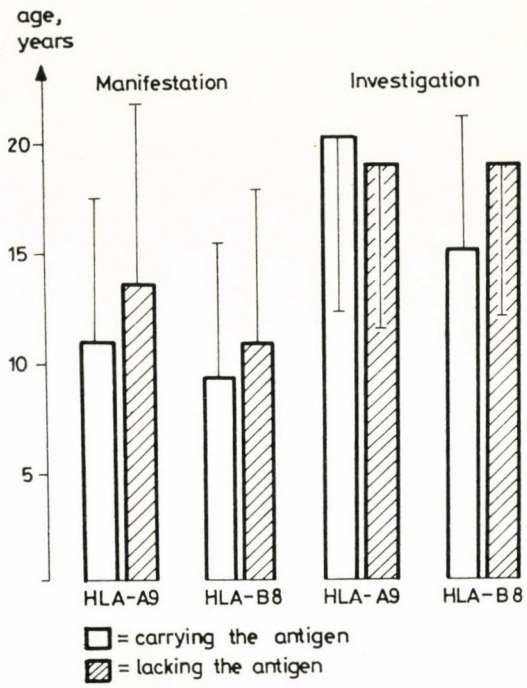

FIG. 2. Average age of 67 diabetic patients with JIDDM in the period of manifestation and investigation.

TABLE I

Most frequently occurring HLA antigens in 67 patients with JIDDM

Antigens	Patients		Control		p <	RR
	No.	per cent	No.	per cent		
HLA—A9	25	37.6	94	20.8	0.005	2.25
HLA—B8	25	37.6	85	18.8	0.001	2.38

TABLE II

Correlation between HLA—B8 antigen and daily required insulin doses and ^{125}I-insulin-antibody-complex in 67 diabetic patients

Antigens	Daily requ. Insulin U/kg bw.		^{125}I-Insulin-antibody-complex in percentage					p<
	Mean SD	p	Mean SD	p	0—19	20—39	>40	
HLA—B8 pos. N = 25	0.90 ± 0.31	ns.	44.13 ± 11.64	ns.	0	6	19	0.02
HLA—B8 neg. N = 42	0.98 ± 0.41		40.47 ± 16.79		0	21	21	—

insulin-antibody complex than those lacking the antigen (Table II).

Increased antibody titres were more frequent in the diabetics than in the healthy controls; the differences were highly significant with reference to all antigens tested. In the healthy first degree relatives the results were similar but less expressed (Table III).

Increased antibody titres to antimicrosomal and antigastric antigen were detected significantly more frequently in diabetics carrying the antigen in question than in those lacking it, except for the human thyroglobulin antibody titre. Among the healthy family members, increased antibody titres to human thyroglobulin and antimicrosomal antigen were significantly more frequent in the subjects carrying HLA-B8 antigen than in those lacking it, but no difference was found as regards antigastric mucosa antibody titres (Table IV).

TABLE III

Presence of different antibodies in diabetics, their healthy family members, and in healthy controls

Groups	Human Thyroblogulin antibody titre			Antimicrosomal antibody titre			Antigastric antibody titre		
	1—16	≥32	per cent	1—16	≥32	per cent	1—16	≥32	per cent
Patients	41	26***	38.8	38	29***	43.3	39	28***	41.8
Healthy family members	93	12*	11.4	94	11**	10.5	95	10**	9.5
Controls	1035	65	5.9	470	20	4.1	245	5	1.9

* $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$

TABLE IV

Correlation between HLA—B8 antigen and different antibody titres in diabetics and in their healthy family members

Groups		Human Thyroglobulin antibody titre			Antimicrosomal antibody titre			Antigastric antibody titre		
		1—16	≥32	per cent	1—16	≥32	per cent	1—16	≥32	per cent
Patients	HLA—B8 pos.	12	13	52	7	18	72	7	18	72
	—B8 neg.	29	13	30.9	30	12	23.1	30	12	23
	χ^2 values		2.923			11.953			11.953	
	p values		ns.			<0.001			<0.001	
Healthy family members	HLA—B8 pos.	15	6	28.5	22	9	29	5	28	16.1
	—B8 neg.	76	5	6.1	72	2	2.7	5	68	6.8
	χ^2 values		8.358			16.149			1.612	
	p values		<0.01			<0.001			ns.	

Discussion

Among Caucasian persons, HLA-B8 and B15 are more common in JIDDM than in controls [2, 10, 13, 30, 35, 39]. The frequency of HLA-B8 antigen was reported also in Graves disease [19, 41] and in idiopathic Addison disease [45]. It was suggested that an HLA-B8-associated immune response gene may be the common denominator in the development of endocrine autoimmunity. HLA-B8 and other, i.e. B15, B18 and other antigens on C [39] and D [34] locus may be situated close to some major pathogenic genes which may predispose to JIDDM on the sixth chromosome [5, 40, 42, 43]. There are data in the literature [13, 34, 35] that HLA-B8 and Dw3 and Dw4 antigens of the HLA system do not necessarily facilitate the development of JIDDM but there are genes in linkage disequilibrium with the mentioned ones enhancing or facilitating immune responses.

In contrast with data in the literature [13, 20, 35], no increased frequency of HLA-B15 was found, and the B18 antigen was less frequent in our patients than in the controls. The function of HLA-A, B, and D locus antigens is not well-known; it, is however, evident that at least some of them govern the specific immune responses to various antigens. Autoimmune phenomena were frequently demonstrated in JIDDM in our material as well as in other studies [8, 21, 22, 29, 36]. The production of antipancreatic islet-cell, antigastric and antithyroid autoantibodies may be under the control of an Ir gene within the HLA region, closely linked to HLA-B8, D [31] and probably to other antigens, too [45]. A third SD determinant antigen HLA-B7 may also be significantly less frequent and on this basis the existence of two different patterns of immune reactivity ("immune personality") has been suggested [6]; one form may be associated with the presence of HLA-B8 and the absence of HLA-B7, while the other form may be related to HLA-B15. These data have been interpreted to mean that B7 is associated with a genetic protective factor for diabetes and the HLA-B8 associated form may be the result of an autoimmune reaction. An abnormal humoral and cell-mediated immune function was observed in many JIDDM patients [4, 5, 6, 15, 16, 21, 23, 25, 27, 32, 36]. Persons carrying the B8 antigen carry humoral islet cell antibodies more frequently than those lacking it [22] and our data were similar in this respect. Some authors [30] could only demonstrate a slight correlation between islet-cell antibodies and HLA-B8 antigen, while others [35] reported on their frequent occurrence in subjects having the antigen in question.

Our results are in agreement with the findings in autoimmune disease [41] and autoimmune endocrinopathies of autoantibodies to thyroid and gastric antigens. Autoimmunity against these antigens was statistically significantly more frequent in the HLA-B8 positive subjects both among

the diabetics and their family members as compared to B8 negative individuals.

A tendency was demonstrated toward a correlation between HLA-B8 antigen and high titre neutralizing antibodies to Coxsackie B4 virus [17] and seasonal variations in the manifestation of JIDDM in B8 positive subjects [12, 37]. HLA-B8, B15, Dw3 and Dw4 antigens can be associated with immune response genes controlling the development of cell-mediated immunity to infectious agents which may destroy pancreatic islet cells directly or by triggering the autoimmune reaction [17, 35, 37, 39].

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