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Immunoglobulin Levels, Immunodeficiency and HLA in Type 1 (Insulin-Dependent) Diabetes Mellitus

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Summary. In 129 patients with Type 1 (insulin-dependent) diabetes mellitus, 100 healthy control subjects and 91 non-diabetic first degree relatives of Type 1 patients, we investigated variation in serum IgA, IgG and IgM concentrations with sex and HLA-B phenotype. Two patients with onset before the age of 15 years were completely IgA-deficient. One additional patient was completely IgG-deficient. Excluding these three cases, diabetic patients had serum IgA and IgM concentrations comparable to control subjects. IgG levels of patients were, however, significantly lower than those of control subjects (11.66 versus 12.69 g/l, $p = 0.003$). Non-diabetic first degree relatives of patients had IgG levels intermediate between those of diabetic patients and control subjects. There was some indication that IgA concentrations were lower in the 53 patients with HLA-B8 (1.91 versus 2.21 g/l, $p = 0.038$). No association was found between IgM or IgG levels and HLA phenotypes.

Key words: Types 1 and 2 diabetes, immunoglobulin A, G and M, HLA-type, family study.

There is considerable epidemiological [1] and experimental [2] evidence that Type 1 (insulin-dependent) diabetes mellitus is an immune-mediated disorder which may be precipitated by viral infections. The increased susceptibility of diabetic patients to infection is well recognized [3]. Studies of serum immunoglobulins in diabetes are relevant to this susceptibility to infection, and may elucidate mechanisms of liability to diabetes.

Of the numerous investigations of the levels of the major immunoglobulin subclasses in diabetic patients [4–13], many have not distinguished Type 2 (non-insulin-dependent) from Type 1 diabetes, which may account at least in part for the inconsistent results obtained. Several case reports have appeared [5, 14–21] of Type 1 diabetes associated with complete IgA deficiency, and IgA deficiency is relatively common in Type 1 diabetic patients [22].

Variations in immunoglobulin levels in patients and the relationship of this variation to HLA phenotype have not been systematically studied; the occurrence of deficiencies of immunoglobulins other than IgA has not been investigated.

Subjects and Methods

We investigated serum IgA, IgG and IgM concentration in 129 consecutive patients with Type 1 diabetes, 91 non-diabetic first degree relatives of 36 probands (29 of whom formed part of the consecutive series) and 100 healthy control subjects. The characteristics of the diabetic patients are summarized in Table 1. At least two relatives of each proband were studied; in none did 2-h post-prandial blood glucose levels show any evidence of diabetes or impaired glucose tolerance.

Typing for 52 HLA-A, B, C antigens was done on lymphocytes isolated by gradient flotation on Hypaque-Ficoll, using a complement-dependent cytotoxicity method [23]. Some patients were HLA-DR typed, using standard techniques on isolated B-lymphocytes [24] and local sera identifying 7 DR-antigens [25].

Serum immunoglobulin A, G and M levels were measured using radial immunodiffusion methods [26] (HC-Partigen Immunodiffusion Plates, Behring Diagnostic, Marburg). The coefficients of variation ranged between 2 and 6.5% for different immunoglobulins. When very low levels of IgA and IgG were observed with these plates, sera were restudied using low-concentration plates (LC-Partigen Immunodiffusion Plates).

Table 1. Characteristics of the 129 patients studied

	Patients	*Control subjects*
Males : Females	53 : 76	51 : 49
HLA-B8 (%)	43	25
HLA-B15 (%)	28	8
Age (years)[a]	20.20 ± 11.15 (8–51)	20.96 ± 10.38 (10–50)
Diabetes:		
Age at onset (years)	12.3 ± 5.8 (0.3 –34)	
Duration (years)	7.3 ± 10.2 (0.75–28)	
Insulin required (Units · kg body weight^{-1} · day^{-1})	0.64 ± 0.22 (0.34– 1.6)	

[a] Results expressed as mean ± SD, range in parentheses

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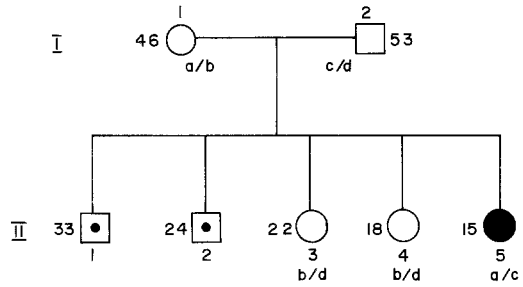

Fig. 1. A family with aggregation of IgA deficiency
HLA haplotypes: a = A2, B8 (w6) Cw-, DR3, Bf^S, GLO^1
b = A28, B35 (w6) Cw4, DR4, Bf^F, GLO^1
c = Aw32, B14, Cw-, DR7, Bf^S, GLO^2
d = Aw31, B14, Cw-, DR1, Bf^S, GLO^1

Serum immunoglobulins (g/l):		*IgA*	*IgG*	*IgM*
	I 1	1.37	7.75	1.35
	2	0	19.50	1.15
	II 3	0.55	8.50	2.30
	4	1.64	10.00	1.90
	5	0.30	6.75	1.35

Roman numerals signify generations; Arabic numbers to left of symbols the ages of the individuals. ● – Type 1 diabetes; ⊡ – Not typed.

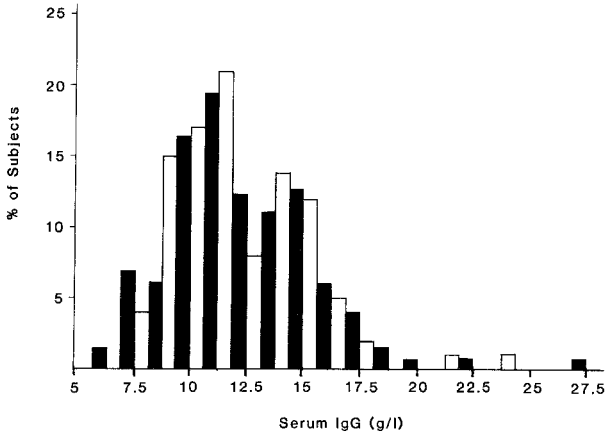

Fig. 2. Distribution of IgG levels ■ = patients, □ = controls subjects

Analysis of variance [27] was used to evaluate the significance of differences in Ig levels among patients, first degree relatives and control subjects, and to evaluate Ig levels among patients in relation to possession of B8 and B15. Analysis of variance is appropriately applied only to normally distributed data, whereas in these data, as is generally found [28], serum immunoglobulins show skewed distributions. Natural logarithms of observed IgG and IgM values were therefore analysed. The natural logarithm transformation over-corrected for the skewness of IgA distributions; square-root transformed values were appropriately normal, and were analyzed instead. (Complete analysis of variance tables are available from the authors on request).

Results

Two patients were completely IgA-deficient and a third had a serum IgA level of 0.30 g/l (Table 2). One IgA-deficient patient had been adopted, and family studies could not be pursued. The parents and four siblings of the second patient were studied; none had IgA deficiency. The father of the patient with serum IgA of 0.30 g/l was completely IgA deficient but was not diabetic (Fig. 1).

We found one patient with complete IgG deficiency (Table 2). Judging from the onset of sinopulmonary infections in her early teens, she was immunodeficient by that age.

Table 3 presents the mean ± SD of the Ig values of the patients, control subjects and unaffected first degree relatives. IgA levels were unrelated to disease status or sex; IgM levels were higher in females than in males, as is generally found [28]. IgG levels were significantly lower in patients than in controls ($p = 0.003$). As may be seen in Figure 2, this depression of IgG levels in non-immunodeficient patients results from an excess of patients with low values compared with the control distribution. There is an excess of extremely low values, that is, values beyond −1.96 SD from the mean of the control distribution (8.03 g/l, very similar to the 8.00 g/l cut-off specified by the manufacturers of the assay kits). None of the 100 control subjects but 13 of 126 diabetic patients and 10 of 90 first degree relatives had values of 8.00 g/l or below ($p < 0.01$ for identical IgG distributions in control subjects, patients and relatives).

The comparison of the 35 diabetic probands, their unaffected first degree relatives and the control subjects (Table 3) indicates a familial – not necessarily genetic – pattern, the relatives being intermediate between diabetics and controls with respect to IgG levels ($p = 0.004$).

IgA levels were somewhat lower in patients with HLA-B8 than in those without ($p = 0.038$, not significant in view of the number of significance tests done). IgG showed no variation with sex or B8, and IgM no variation with B8. No association was found between HLA B15 and immunoglobulin levels in diabetic subjects.

Discussion

We encountered two IgA-deficient Type 1 diabetic patients among the 82 screened whose age of onset was below 15 years. This is consistent with the report by Smith et al. [22] of an incidence of selective IgA deficiency of 1/40 among juvenile onset Type 1 patients, some 12 times that in the general population. IgA deficiency may, at least in a proportion of patients, be due to immunoregulatory T cell defects [29, 30]. The incidence of autoaggression in IgA deficient persons is increased [31, 32].

It is of interest that IgA deficiency is itself associated with HLA-B8 [19, 33] and appears to segregate in family studies with the haplotype HLA-A1, B8 [34]. Combined published data (including one of twin brothers [20]) show 12 of 14 Type 1 diabetic IgA-deficient patients to be B8-positive, a prevalence greater than that in Type 1 diabetic patients or IgA-deficient individuals (45% in 33). Possibly the two disorders are related by immunoregulatory genes mapping in the HLA region.

Table 2. Patients with absent or very low immunoglobulin concentrations

Patient no.	Age at diagnosis (years)	HLA	Serum immunoglobulin concentration (g/l)		
			A	G	M
1	14	A3, 28; B7, 12; Cw-, –; DR2, 4	1.80	0	0.85
2	8	A1, 2; B8, 16; Cw-, –; DR3, –	0	20.73	0.62
3	4	A1, –; B8, –; Cw-, –; DR3, –	0	22.20	1.09
4	8	A2, w32; B8, 14; Cw-, –; DR3, 7	0.30	6.75	1.35

Incidence of complete IgA deficiency is 1/40 in Type 1 diabetic patients diagnosed before age 15 years. The incidence of IgA deficiency remains unchanged (11/447) when combined with the series of Smith et al. [22]. Patients were studied 5–7 years after diagnosis. Patients 2 and 4 were re-studied 2 years later and had Ig levels identical to those reported here

Table 3. Immunoglobulin concentrations for diabetic patients, unaffected first degree relatives and control subjects[a]

	Immunoglobulin concentrations (g/l)		
	IgA	IgG	IgM
All patients ($n = 126$)	2.08 ± 0.84	11.66 ± 3.24[b, c]	1.54 ± 0.79
Male ($n = 51$)	1.96 ± 0.78	11.12 ± 3.50	1.27 ± 0.55
Female ($n = 75$)	2.17 ± 0.88[d]	12.02 ± 3.02	1.73 ± 0.87
B8 negative ($n = 73$)	2.21 ± 0.89[d]	11.54 ± 3.30	1.53 ± 0.77
B8 positive ($n = 53$)	1.91 ± 0.75	11.81 ± 3.19	1.55 ± 0.82
Probands ($n = 35$)	1.97 ± 0.72	10.83 ± 2.62	1.61 ± 0.74
Male ($n = 16$)	1.98 ± 0.62	10.87 ± 3.02	1.34 ± 0.48
Female ($n = 19$)	1.97 ± 0.81	10.80 ± 2.33	1.84 ± 0.85
First degree relatives ($n = 90$)	2.22 ± 0.93	12.16 ± 3.12[c]	1.79 ± 1.01
Male ($n = 37$)	2.14 ± 0.99	11.88 ± 3.17	1.41 ± 0.76
Female ($n = 53$)	2.27 ± 0.90	12.36 ± 3.10	2.05 ± 1.09
Controls ($n = 100$)	2.07 ± 0.84	12.69 ± 2.94[b, c]	1.56 ± 0.62
Male ($n = 51$)	2.13 ± 0.89	12.73 ± 3.09	1.52 ± 0.59
Female ($n = 49$)	2.01 ± 0.79	12.64 ± 2.80	1.60 ± 0.67

[a] Persons with zero IgA or IgG values are excluded. Results expressed as mean ± SD. [b] $p = 0.003$; [c] $p = 0.004$; [d] $p = 0.038$

We found one patient with selective IgG deficiency, apparently the first description of this association. No patients with Type 1 diabetes were encountered in a review of 50 patients with common variable immunodeficiency [35]. It is premature to conclude that selective IgG deficiency is increased in Type 1 diabetes; the only previous survey [22] did not examine IgG or IgM levels.

We found no overall decrease in serum IgA levels in Type 1 diabetic patients who were not completely IgA-deficient. Patients with HLA-B8 had IgA levels non-significantly lower than those of patients without this antigen; if confirmed, this finding would indicate that quantitative reductions as well as absence of IgA are associated with B8.

IgG levels in Type 1 diabetic patients were about 8% lower than in control subjects. First-degree non-diabetic relatives of diabetic probands were intermediate between patients and control subjects in IgG levels, excluding the possibility that IgG abnormalities in patients are secondary to carbohydrate intolerance.

Hypergammaglobulinaemia G has generally been associated with diabetes requiring insulin or hypoglycaemic drugs. This may be partly attributable to a rising trend of IgG levels seen above middle age [28]. Lower IgG levels have previously been noted in Type 1 than in Type 2 diabetic patients [4], and in reports in which individual results are provided a proportion of Type 1 diabetic patients are found to have low IgG and IgM levels [10]. Two studies have commented on abnormally low serum IgG and IgM levels among diabetics requiring insulin [8, 13]. An excess of Type 1 diabetic patients with extremely low IgG levels was found in the present study.

Patients with subnormal IgG and IgM levels often fail to show humoral responses to specific antigens [36]. It is therefore of interest that Ludwig et al. [11] showed that well-controlled patients with Type 1 diabetes were unable to mount antibody responses against common bacterial antigens. This lack of specific responses could not be related to HLA phenotype or to abnormalities of immunoglobulin levels.

Type 1 diabetes is probably an immune-mediated disease triggered by viral infections [2], and the impairment of immune responsiveness seems not to be secondary to the disease [11]. It is therefore possible that suboptimal immune responses may allow infectious agents to gain access to and disrupt immunoregulatory mechanisms, and that aberrant immune response may be a substantial component of genetic liability to Type 1 diabetes. Further quantitative and qualitative studies of the humoral immune response in patients and their families are warranted.

More detailed information concerning the data may be obtained from the authors upon request.

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