Age-Related Association of Insulin-Dependent Diabetes Mellitus with BfF1 and the HLA-B18, BfF1 Haplotype

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Summary. Five hundred and ninety-nine patients with insulin-dependent (Type 1) diabetes mellitus were typed for Bf (factor B) polymorphism, and 318 of them for HLA-A, B and C antigens. Bf and HLA antigen frequencies were compared with those in 536 normal controls. A significant positive association between Type 1 diabetes and the rare factor B variant BfF1 was found ($p < 10^{-3}$), but this was present only in patients aged under 16 years at onset of the disease ($p < 0.005$). There was a strongly positive linkage disequilibrium between BfF1 and HLA-B18 in the diabetic patients. This, too was especially pronounced in the juvenile onset cases, in whom it was significantly stronger than in controls ($p < 10^{-3}$). The known positive associations between Type 1 diabetes and HLA-B8, -B15 and -Cw3 were confirmed.

Key words: Insulin-dependent (Type 1) diabetes, HLA-B18, BfF1, linkage disequilibrium

Insulin-dependent (Type 1) diabetes mellitus shows a significant positive association not only with HLA-Dw3/DR3 and HLA-Dw4/DR4 [1, 2] but also with BfF1, a rare gene product of the properdin factor B system which is linked to HLA [3, 4]. In this study a group of patients with Type 1 diabetes mellitus was typed for HLA-A, B, C antigens and Bf phenotypes. The HLA and Bf associations with Type 1 diabetes and the linkage disequilibrium between HLA-B18 and BfF1 were investigated in relation to age at onset of disease.

Material and Methods

599 Caucasoid patients with Type 1 diabetes mellitus were typed for Bf polymorphism and 318 of them also for the HLA-A, B, C antigens.

The following HLA-antigens could be defined, using the standard micro-lymphocytotoxicity test [5]: HLA-A1, A2, A3, Aw23, Aw24, A25, A26, A28, A29, Aw30, Aw31, Aw32, Aw33; B5, B7, B8, B13, B14, B18, B27, Bw35, B37, Bw38, Bw39, Bw40, Bw41, Bw44, Bw45, Bw47, Bw49, Bw50, Bw53, Bw55, Bw56, Bw57, Bw58, Bw60, Bw61, Bw62, Bw63, Cw1, Cw2, Cw3, Cw4, Cw5. Bf polymorphism (BfS, F, S0.7, F1) was analysed by thin layer high voltage agarose electrophoresis and subsequent immunofixation with Bf antisera (Behring-Werke) as described by Alper et al. [3] and Reuter and Hoffman [7]. All BfF1 and BfS0.7 results were corroborated by typing relatives.

Table 1. HLA and Bf phenotype frequencies, BfF1, B18 haplotype frequency (hf) and its gametic association (delta value) in patients with Type 1 diabetes and normal controls. (If there is no association between two genes at two linked loci, they are in *linkage equilibrium*, and the frequency of their occurrence together (= *haplotype frequency*) must be the product of the gene frequencies. If the two genes are positively or negatively associated, they are in *linkage disequilibrium*, the haplotype frequency deviates from the expected gene product and the deviation is expressed by the so-called *linkage disequilibrium parameter* or *delta value*.)

	Patients	Controls ^a	<i>p</i> value ^b
HLA	<i>n</i> = 318	<i>n</i> = 536	
–B7	57 (18%)	118 (22%)	NS
–B8	119 (37%)	89 (18%)	$< 10^{-5}$
–B15	79 (25%)	69 (13%)	$< 10^{-5}$
–B18	48 (15%)	62 (12%)	NS
–Cw3	97 (31%)	117 (22%)	< 0.01
Bf	<i>n</i> = 599	<i>n</i> = 536	
F	156 (26%)	143 (27%)	NS
S	554 (93%)	513 (96%)	< 0.05
F1	36 (6%)	10 (2%)	$= 10^{-3}$
S0.7	18 (3%)	11 (2%)	NS
hf (BfF1, B18):	0.0270	0.0044	
Delta value (BfF1, B18):			
Absolute:	0.0244	0.0038	
Relative:	0.8250	0.4368	$= 3 \times 10^{-5}$

^a Bertrams and Baur [6]

^b uncorrected

NS = not significant

Table 2. Age relationship of HLA and Bf frequencies and BfF1, B18 linkage disequilibrium (delta value) in patients with Type 1 diabetes mellitus and normal control subjects

	No of patients (%)			No of controls ^a (%)	Significance		
Age at onset of disease (years)	a	b	c	d	Comparison between		<i>p</i>
	(0–10) (<i>n</i> = 128)	(11–15) (<i>n</i> = 136)	(16–29) (<i>n</i> = 54)	(<i>n</i> = 536)	Group	Group	
HLA–B7	15 (12%)	32 (24%)	10 (19%)	118 (22%)	a	b + c	< 0.05
–B8	42 (33%)	56 (41%)	21 (39%)	99 (18%)			
–B15	41 (32%)	29 (21%)	9 (17%)	69 (13%)	a	b + c	< 0.025
–B18	19 (15%)	20 (15%)	8 (15%)	62 (12%)			
–Cw3	47 (37%)	37 (27%)	13 (24%)	117 (22%)			
BfF1	10 (8%)	9 (7%)	1 (2%)	10 (2%)	a	d	< 0.005
					b	d	< 0.005
Delta value (BfF1, B18):							
Absolute:	0.0325	0.0231	0.0086	0.0038			
Relative:	0.8839	0.7424	0.1002	0.4368	a	d	< 10 ^{–4}
					b	d	< 10 ^{–3}

^a Bertrams and Baur [6]

Bf and HLA phenotype frequencies of the patients were compared with corresponding control values [6] by chi-square tests with Yates' correction. HLA-Bf haplotype frequencies and linkage disequilibrium parameters (delta, *D*) were calculated by standard methods [8–10]. Relative delta values of HLA-B18, BfF1 in Type 1 diabetic patients and controls were computed according to Dausset et al. [11] and compared for significance by 2 × 2 × 2 contingency tables as given in detail elsewhere [12].

Results

As expected HLA-B8, –B15 and –Cw3 antigen frequencies were significantly increased in the patient group. However, minor differences in frequencies of B7 (decreased) and B18 (increased) were not statistically significant (Table 1).

One Bf gene product showed a major deviation: the rare variant BfF1 was significantly increased (6.0% versus 1.9%, *p* = 10^{–3}). The frequency of BfS was slightly decreased (92.5% versus 95.7%, *p* < 0.05), although an increase might have been expected in view of the positive linkage disequilibrium with HLA-B8 reported elsewhere.

Of the 20 BfF1-positive patients, 17 were also positive for B18, indicating a strong association between the B18 and BfF1 genes (*d* = 0.0244). In normal controls the gametic association of B18 and BfF1 is much weaker (*d* = 0.0038) [6]. An increased B18, BfF1 haplotype frequency was also found in the diabetics (0.0270 versus 0.0044 in controls). For comparison of the absolute delta values the relative delta values have been calculated. The difference between the Type 1 diabetic patients and normal controls was highly significant (*p* = 3 × 10^{–5}).

The data were also analysed in relation to the age of the patients at the time of onset of diabetes and

compared with the findings in controls. As shown in Table 2, a significantly increased frequency of BfF1 was found in the patients under 11 years of age (7.8%, *p* < 0.005) and in those from 11 to 15 years old (6.6%, *p* < 0.005) but not in the age group 16 to 29 years (1.9%, *p* > 0.05). The strength of the gametic association between B18 and BfF1 genes was also age related. In comparison to the normal delta value between BfF1 and B18 (*d* = 0.0038), significantly increased linkage disequilibria were found only in the two groups of patients aged ≤ 15 years (0–10 years: *d* = 0.8839, *p* < 10^{–4}; 11–15 years: *d* = 0.7424, *p* < 10^{–3}), while patients older than 15 years showed only a very weak positive gametic association of BfF1 and B18 (*d* = 0.1002) which is even weaker than in normal controls (*d* = 0.4368). The HLA antigen frequencies were also analysed in relation to the age of the patients at the onset of diabetes (Table 2). The frequency of B18 was almost identical in all three age groups and only slightly increased relative to controls. The youngest patients (0–10 years) showed the highest frequency of B15 and the lowest of B7, these differences from older patients being statistically significant (Table 2).

Discussion

There are three significant findings of this study: the increased frequency of the rare properdin factor B allele BfF1, the very strong linkage disequilibrium between BfF1 and the B18 genes, and the age relationship of the BfF1 association with diabetes as well as of the strength of this gametic association.

Increased BfF1 frequency in Type 1 diabetic patients was first reported independently by Bertrams et al. [12] and by Alper (Third Workshop on the Genetics of the Complement System 1979; see also [13]), and was confirmed by others [14–18]. The great differences in the strength of the association reported by different authors can be explained by its strong age relationship [15, 18, 19].

Another result of our study is the pronounced increase of B15 in the youngest patients (0–10 years) and the trend of a decreasing frequency of B15 in patients with later manifestation of Type 1 diabetes (11–15 and 16–29 years), as also shown by Dausset [20] and Kirk et al. [15]. The frequency of B7 was very low in the youngest age group (0–10 years). Thus Type 1 diabetes mellitus may develop early in life predominantly in BfF1- and B15-positive and B7- negative individuals.

The most striking finding of this study is the marked linkage disequilibrium between BfF1 and B18 genes in Type 1 diabetes ($d = 0.0244$), which is significantly stronger than in normal controls ($d = 0.0038$).

This very strong linkage disequilibrium is also age related and could be demonstrated only in children who were under 16 years of age at the onset of the disease.

Thus we have been able to demonstrate another HLA linkage group indicating increased susceptibility to Type 1 diabetes, namely BfF1, B18, in addition to the well known 'high risk' gene complexes HLA-A1, B8, Dw3/DR3 and HLA-A2, Cw3, B15, Dw4/DR4.

As shown by the Type 1 diabetes study of the last Histocompatibility Workshop [21] the BfF1 and B18 genes are also linked to HLA-DR3. Further studies should investigate whether the increase of BfF1 in Type 1 diabetes is secondary to the increase of HLA-DR3 or whether it stands for functional involvement of this rare complement variant in the pathogenesis of the disease.

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