The Bf System in Diabetes – Gene Interaction or Linkage Disequilibrium?

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Summary. HLA and Bf genotypes have been determined in 75 Type 1 (insulin-dependent) diabetic probands ascertained from two family studies. The Bf associations were similar in those families with two affected children compared with those families with one affected child. The strong association of B18 with the rare allele BfF1 was confirmed but, with rare exception, the gene encoding for BfF1 only occurred on chromosomes coding for both B18 and CW5. These findings are similar to the strong allelic association between these specificities found more frequently in Southern Europe and Spain, but no HLA-A locus association was found in the present study. All HLA-B8 positive subjects and 15 out of 16 BW62 positive probands possessed BfS. In contrast to other reports, we could not find any evidence to support the idea that BfF1 is associated with a younger age of onset of diabetes. It is concluded that there is no specific susceptibility gene for Type 1 diabetes linked to the Bf locus and that the Bf associations can be explained purely on the basis of the known allelic association, or linkage disequilibrium, within the HLA system.

Key words: Type 1 (insulin-dependent) diabetes, HLA, enzyme precursors, properdin factor B, genetics, linkage.

There have been considerable claims concerning properdin or factor B (Bf) polymorphism in Type 1 (insulin-dependent) diabetes, and in particular whether the rare allele BfF1 is in strong linkage disequilibrium with a specific disease susceptibility gene [1]. The concept put forward was that this gene is separate from the putative disease susceptibility gene or genes in linkage disequilibrium with HLA-DW3 (DR3) and -DW4 (DR4) which form the primary HLA association with the disease, the corresponding HLA -B, -C, and -A locus antigens being secondarily associated due to linkage disequilibrium within the HLA system [2–4].

It has also been argued that BfF1 specifies an HLA-B18 diabetogenic haplotype [5] and that the presence of BfF1 is associated with a younger age of onset of Type 1 diabetes [6–8]. Thus, it has been claimed that HLA-B18-BfF1 reflects genetic heterogeneity within Type 1 diabetes. In addition, it has been suggested that the delta value for HLA-B18 and BfF1 is higher in Type 1 diabetics than in healthy subjects [7, 9].

However, it has been pointed out that the frequency of BfF1 depends on the ethnic background of the population studied. This is particularly relevant to people who are indigenous to Southern Europe or who have migrated from this area. In these populations, BfF1 is relatively more common and is a component of the HLA-DR3-B18-CW5-AW30 haplotype [10, 11]. Apart from the latter study in Basques [11], none of the other reports concerning BfF1 in Type 1 diabetic subjects includes information relating to HLA-CW5.

Subjects and Methods

The following groups of Type 1 diabetic patients and their families (parents and siblings) were investigated with particular reference to the factor B(Bf) polymorphism.

1. Fifty-two out of a total of 155 Caucasoid families belonging to the Barts-Windsor family study were Bf genotyped. This family study consists of all families resident in East Berkshire, UK, which contain a classical Type 1 diabetic child and at least one unaffected sibling. The mean age at onset of diabetes in the 155 probands was 8 years. All families have been HLA-A, B, C genotyped. Many of the families who were Bf genotyped were selected for the presence of haplotypes containing HLA-B18.

2. Twenty-three out of a total of 184 Caucasoid families with two or more Type 1 diabetic siblings ascertained from the British Diabetic Association Register 1972–78 [12] were also genotyped. These families were located throughout the UK and the majority had been HLA-A, B, C genotyped [13], including all those in the present study. The mean age at onset of diabetes in the probands was 8 years, and again there was a biased selection of families for Bf typing on the basis of informative HLA-haplotypes containing HLA-B18.

Table 1. BfF1 in relation to HLA-B18 and CW5

HLA, BfF1	Genotypes	Phenotypes	
	150 haplotypes from Type 1 diabetic probands	46 Type 1 diabetics with proliferative retinopathy	19 Type 1 diabetics without complications
B18+, CW5+, F1+	21 ^a	4	3
B18+, CW5-, F1+	0	0	0
B18-, CW5+, F1+	0	1	0
B18-, CW5-, F1+	2	0	0
B18+, CW5+, F1-	1	0	0
B18+, CW5-, F1-	10	3	0
B18-, CW5+, F1-	6	3	3
B18-, CW5-, F1-	110	35	13

BfF1 genotype results according to the presence or absence of B18 and CW5 in the same haplotypes. Combined data from two family studies: 75 unrelated Type 1 diabetic probands.

BfF1 phenotype results showing the association with B18 and CW5 in two groups of long-standing Type 1 diabetics with and without retinopathy.

^a One child is BfF1-B18-CW5 homozygous

3. In addition, 46 randomly selected, long-standing Type 1 diabetic patients (mean duration of diabetes 25 years) with severe proliferative retinopathy and 19 patients matched for age at onset and duration of diabetes with no evidence of diabetic complications were HLA and Bf phenotyped.

The HLA typing of all the patients was performed by a modified microlymphocytotoxicity method [14] using well characterised antisera against all the officially recognised HLA-A, B, C specificities (1980 WHO Nomenclature Committee).

Bf typing was carried out by thin layer agarose gel electrophoresis of serum using veronal buffers of pH 8.6 and immunofixation with antiserum specific for properdin factor B (Atlantic Antibodies, Westbrook, Maine, USA) as described by Alper et al. [15].

Results

Unequivocal HLA and Bf genotypes were obtained in 47 families belonging to the Barts-Windsor family study; Bf genotypes were deduced in four families where only one parent was typed and in one family where both parents possessed the same HLA-B and -C locus antigens. In 47 probands, HLA and Bf were unequivocally established and in five probands they were deduced, making a total of 104 haplotypes available for analysis.

In 21 multiplex Type 1 diabetic families, genotyping for HLA and Bf was unequivocal. Bf genotypes were deduced in two families. In one family, only one parent was typed and in the other family, one parent was homozygous. The 46 haplotypes of the oldest diabetic child in each family were used for analysis. No recombinations between Bf and HLA-B were observed in either family study. There were four Type 1 diabetic parents in the Barts-Windsor family study and three Type 1 diabetic parents in the multiplex study.

Analysis of the HLA Association with BfF1

In the diabetic probands, there were 17 B18 positive haplotypes in the Barts-Windsor family study and 15 positive haplotypes in the multiplex study. Of these, 11 in each group were also positive for CW5. In the probands from the Barts-Windsor family study, all the B18 and CW5 haplotypes possessed BfF1 and 10 out of 11 of the B18 and CW5 haplotypes in the multiplex study were BfF1 positive. In addition, there were two other BfF1 positive haplotypes, both of which were not associated with B18 or CW5.

We have already shown that the HLA characteristics of these two family studies are very similar [13]. Because the Bf association is also similar in these two family studies, the data for the probands have therefore been combined (Table 1). Thus, this study demonstrates that the gene encoding for BfF1 occurs almost exclusively on chromosomes coding for B18 and CW5. Furthermore, the same phenomenon was observed in the random long-standing diabetic patients who were HLA and Bf phenotyped (Table 1). Eight of these (12%) were BfF1 positive, five in the group with retinopathy and three in the group without complications (11% and 16% respectively). All eight BfF1 positive individuals were CW5 positive and seven also possessed HLA-B18.

No strong association with any particular A-locus antigen was found with the BfF1-B18-CW5 haplotype. This is in contrast to studies in Southern Europe [11] where there is strong linkage disequilibrium with AW30 or A25. The absence of any HLA-A locus association in the present study probably reflects the different ethnic background of the families studied.

BfF1 and Age of Onset of Diabetes

There were two Type 1 diabetic parents who possessed the BfF1-B18-CW5 haplotype; the age of onset of diabetes was 36 and 39 years respectively. The mean age of onset ($\pm$ SD) of diabetes in the 22 BfF1 positive probands was 10.2 ± 3.9 years which was not significantly different from the age of onset in those children who possessed different Bf alleles (8.5 ± 4.8 years). The age of onset in the BfF1 positive random long-standing Type 1 diabetics was 18.1 ± 10.6 years which was similar to the BfF1 negative cases (18.4 ± 9.5 years). We conclude therefore that the presence of BfF1 is not associated with a younger age of onset of Type 1 diabetes.

Analysis of the HLA Association with Other Bf Alleles

Although the present study was primarily designed to investigate the BfF1 association with HLA and susceptibility to Type 1 diabetes, certain conclusions can

Table 2. Data based on 110 BfS, 17 BfF and 23 BfF1 positive haplotypes found in 75 Type 1 diabetic probands

	S1-Axis ^a		S2-Axis ^a				
	B8	B18	B15		B40		B7
			BW62	BW63	BW60	BW61	
BfS	35 (100%)	8 (25%)	15 (94%)	0	6 (100%)	1 (100%)	5 (62%)
BfF	0	3 (9%)	1 (6%)	0	0	0	3 (38%)
BfF1	0	21 (66%)	0	0	0	0	0
BfS1	0	0	0	0	0	0	0

^a S1 and S2 refers to the two common haplotype associations with Type 1 diabetes [3]. Only the HLA-B antigens relevant to Type 1 diabetes are listed.

The percentage of haplotypes positive for certain B-locus antigens and particular Bf alleles are shown in parentheses.

also be drawn relating to the other Bf alleles, even though the families were highly selected for B18 and BfF1. The association of the four different Bf alleles with certain B-locus antigens in 75 Type 1 diabetic probands is shown in Table 2. All the B8 positive probands possessed BfS in the same haplotype, emphasising the strong linkage disequilibrium between these two specificities. It is of interest that the majority of B18 positive, CW5 negative subjects possessed BfS. Only one individual possessed the very rare allele BfS1, which was associated with BW50.

Discussion

We have analysed the association of the relatively rare allele of the complement factor B polymorphism (BfF1) in relation to HLA loci determinants and the relevance of this to the age of onset of Type 1 diabetes. These studies were performed by Bf genotyping families who were particularly selected for the presence of HLA haplotypes containing B18. In addition, we investigated the Bf polymorphism in two groups of long-standing Type 1 diabetic patients matched for the presence or absence of retinopathy. These studies have provided evidence for the first time in a Northern European population that BfF1 is, with rare exception, found only in haplotypes containing both B18 and CW5. In contrast, B18 without CW5 is not associated with BfF1. Furthermore, the frequency of the BfF1-B18-CW5 haplotype in the probands was similar in families with one diabetic child and in families with two or more affected children, confirming that there is no difference in HLA susceptibility between 'sporadic' cases and those families with multiple affected siblings [13].

It is generally agreed that the Bf system is determined by one locus with four alleles, and that the Bf locus lies between HLA-B and -D. The HLA-C locus is also very close to HLA-B, but on the other side of it. Therefore it follows that in the BfF1-B18-CW5 haplotype, there is very strong allelic association between the genes which determine these specificities, reflect-

ing the close proximity of the relevant loci and the very low frequency of recombinations between them. Linkage disequilibrium between certain alleles at the HLA-B and Bf loci has been reported in several populations [16, 17], and the occurrence of BfF1 as a component of the B18-CW5-AW30 haplotype in Southern Europe has already been mentioned [11]. No strong HLA-A locus association with BfF1-B18-CW5 was found in the present study.

The strongest (or primary) HLA association found with Type 1 diabetes to date is with HLA-DR3 and DR4 [2, 3]. It is most likely that the BfF1-B18-CW5 haplotype is secondarily associated with Type 1 diabetes because of linkage disequilibrium with HLA-DR3, and therefore the most likely conclusion based on these associations is that there is a major gene in linkage disequilibrium with DR3, which is controlling part of the susceptibility to the disease. A very strong association between DR3 and BfF1 has been described recently in both normal subjects and patients with renal disease including idiopathic membranous glomerulonephritis [18]. It was concluded from this study that the Bf locus may be more closely linked to HLA-DR than to HLA-B. Furthermore it was shown that BfF1 and B18 are invariably associated with DR3. We can confirm these observations in our diabetic families. Of those so far HLA-DR typed, all those with the BfF1-B18-CW5 haplotype were DR3, whereas those with B18 without CW5 (which were BfF or BfS) were DR3 negative.

A similar phenomenon can be seen in relation to B8 and BfS. All of the haplotypes positive for B8 had the BfS allele. It is well established that there is a strong association between B8 and DR3. Our findings are identical with those of Arnason et al. [16], who also found a 100% allelic association between B8 and BfS. These observations confirm the original reported increase of BfS in Type 1 diabetes [19: NB. BfF1 typing was not performed in that study].

An alternative explanation for these haplotypes is the influence of natural selection on propagating a group of genes which is in strong linkage disequilibrium. These gene combinations might influence specif-

ic immune response interactions with environmental factors (e.g. viruses) which are capable of initiating structural damage to particular cell membranes. Thus, separate genes closely linked to the HLA loci, including the Bf locus, might each contribute to the overall susceptibility. If the DR3-BfF1-B18-CW5 group of genes influences a particular pathogenetic feature of Type 1 diabetes, then it might be possible to find a correlation between this gene combination and some clinical facet of the disease, e.g. age of onset. It is apparent from our ongoing prospective studies of Type 1 diabetic families that the development of clinical disease in HLA genetically susceptible siblings is a relatively slow process, with a long prodromal period of existing islet cell antibodies [20] and other immunological abnormalities [21]. Thus, the actual onset of clinical symptoms is probably a chance phenomenon in many cases, dependent on multiple non-specific environmental, metabolic and hormonal influences. Therefore heterogeneity based on clinical phenomena, such as age of onset, is relatively crude and imprecise. In the present study, we have not been able to confirm the reported association between BfF1 and a young age of onset of diabetes, nor have we found any association between DR3 or B8 and age of onset [22]. Although it cannot be excluded that multiple HLA-linked genes may be operating to influence the susceptibility to certain diseases, such as Type 1 diabetes and psoriasis [23], linkage disequilibrium within the HLA complex remains the most likely explanation for these multicomponent haplotype associations.

We are led to conclude that there is no evidence to support the idea that there is a separate susceptibility gene for Type 1 diabetes in strong linkage with the Bf locus. In contrast, HLA-DR3 and DR4 continue to have the strongest associations with the disease and if evidence for heterogeneity is to be found it is most likely to emanate from studies involving the DR system.

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