Properdin Factor B(Bf) Allele Bf^{F1} Specifies an HLA-B18 Diabetogenic Haplotype

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SUMMARY

We found the rare properdin factor B(Bf) variant F_1 to be present in 11% of 72 patients suffering from insulin-dependent diabetes (IDDM) compared with 2% among 150 normal controls. Bf^{F1} thus confers a relative risk for IDDM of 5.55. All eight patients and three controls who were Bf^{F1} positive were also HLA-B18 positive, reflecting the strong linkage disequilibrium between these two factors. We suggest that Bf^{F1} marks a 'diabetogenic' B18-bearing HLA haplotype. Studies of unselected families with one or more affected members suggest that the B18, Bf^{F1} does not necessarily segregate with IDDM phenotype. This study provides further evidence for the genetic heterogeneity of IDDM.

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The description of associations of HLA antigens[1,2] with insulin-dependent diabetes mellitus (IDDM) has reawakened a great interest in the genetics of this variety of the diabetes syndrome. These association studies have uncovered the heterogeneity of the disorder;[3,4] this heterogeneity becomes even more remarkable when differences in season of onset,[2,4] presence or absence of anti-islet cell antibodies[5] or of antibodies against therapeutic insulin,[6] and the progression of late complications are considered.[7] Two modes of inheritance have been forwarded by different workers: recessive[8,9] and 'overdominant';[2,10] however, polygenic inheritance cannot be excluded.[11] Attempts at linkage of a putative diabetogenic gene in the HLA region are fraught with problems—mainly of the necessary selection of multiplex families for such studies and the apparently high rate of nonpenetrance.[8,9] The relatively low expressivity probably reflects the fact that several genes as well as environmental factors are at play.[11]

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In most Caucasian populations studied, an increased incidence of HLA-B8, -B15, -B18, -D(R)w3, and -D(R)w4 was found whereas -B7 and -D(R)w2 appeared to be protective against IDDM.[2,4,10,12] The D(DR) locus antigens show stronger association with IDDM than the B antigens with which they are in linkage disequilibrium i.e. DRw3 versus B8.[2,4,10] In contrast with the many studies of the HLA antigens per se, limited investigations of the association of gene products of loci that map within the major human histocompatibility complex (MHC), e.g. properdin factor B(Bf),[13,14] second and fourth complement (C_2 and C_4) components[15] with IDDM were performed. In this report we investigate the genetic polymorphism of Bf in a group of patients with IDDM and relate it to their HLA phenotypes in an attempt to confirm whether this is indeed a useful genetic marker for the disease,[16–18] quite independent from HLA.

MATERIALS AND METHODS

Seventy-two patients suffering from IDDM and 150 controls were studied for HLA antigens and Bf allotypes. We also studied 16 consecutive families in which one or more members had IDDM; in two of these families, the Bf^{F1} allele was identified.

HLA antigens were determined by microcytotoxicity methods,[19,20] whereas Bf alleles were identified by high voltage gel electrophoresis followed by immunofixation.[21] The polymorphisms of C_2 and glyoxalase I (GLO) were determined by previously described methods.[22,23]

Relative risk and estimates of mean risk based on pooled data were calculated as described by Svejgaard et al.[24,25]

RESULTS

Eight patients with IDDM (11.1%) were found to be Bf^{F1} positive compared with 2% of controls. Seven of these eight patients were phenotypically F_1S whereas one was F_1S_1; two of three controls were F_1S and the third, F_1S_1. The Bf gene frequencies for the IDDM group and the control group are shown in Table 1. The χ_2^2, comparing gene frequencies of these two populations, was highly significant (12.968, $P<0.001$). In view of the small numbers in the expected Bf^{F1}

TABLE 1
Bf phenotypes and gene frequencies in controls and IDDM groups

	No. tested	Bf Phenotypes							Gene Frequencies			
		SS	FS	FF	SS_1	FS_1	F_1S	F_1S_1	Bf^S	Bf^F	Bf^{F_1}	Bf^{S_1}
Controls	150	102	38	3	2	2	2	1	0.82	0.153	0.01	0.0167
IDDM	72	49	14	—	1	—	7	1	0.833	0.0972	0.056	0.0139

The expected frequency of the Bf alleles was calculated from these gene frequencies in controls as they apply to the number of patients studied, 72. Because the expected numbers in the S_1 and F_1 cells were small, χ_2^2 for the combinations of S_1 and F_1 was performed. This results in a χ_2^2 of 10.01 ($P < 0.005$), with F_1 making the major contribution to this difference.

and Bf^{S_1} categories, these were combined. The difference in Bf^{F_1} and Bf^{S_1} made the major contribution to that χ^2 value (10.01). In both the controls and patients, Bf phenotypes conformed to that expected for Hardy-Weinberg equilibrium ($P > 0.2$ and $P > 0.3$, respectively).

The relative risk calculated for Bf^{F_1} amounted to 5.553 ($\chi^2 = 6.1136$, $P < 0.01$). Table 2 shows similar calculations made for the results of Raum et al.,[16] Bertram et al.,[17] and Kirk and his co-workers.[18] The estimated mean relative risk for the pooled data is 5.233 (95% confidence limits = 3.33 to 8.20), with no apparent significant heterogeneity between four sources of data.

We then examined the HLA antigens with which Bf^{F_1} was associated. Eleven diabetics were B18 positive; of these, eight were Bf^{F_1} positive. By comparison, 14 controls were B18 positive, only three of whom were also positive for Bf^{F_1}. We encountered no patients or controls in whom Bf^{F_1} was associated with a B antigen other than B18. Table 3 details the HLA phenotypes of the Bf^{F_1} positive patients. In only two of the eight patients was family data available for us to be certain that Bf^{F_1} B18 segregated on the same haplotype.

We previously found that HLA B18 confers[4] a moderate relative risk (2.7) for the susceptibility to IDDM, which contrasts with a value of 5.55 for Bf^{F_1}. To establish statistical comparisons between risks contributed by each factor, we tested the presence and the absence of one of the two factors (B18 and Bf^{F_1}) in patients and controls with and without the other antigen.[24] It is quite clear that (Table 4), when the influence of Bf^{F_1} was neutralized, B18 had little effect on the susceptibility to the disease. In contrast, when the influence of B18 was neutralized, it was clear that the effect of Bf^{F_1} on the susceptibility was completely restricted to B18 + ve individuals. The fact that no patients or controls were B18 − ve Bf^{F_1} + ve attests to the strong linkage disequilibrium between these two alleles.

Figure 1 shows two families studied in whom a family member was found to be positive for Bf^{F_1}. The diabetic offspring in the first family was not included in the series of 72 patients in view of the difference in the ascertainment methods. Apart from positivity for B18 and Bf^{F_1}, the components of the haplotypes in the two families were quite distinct. In one family, the diabetic child inherited the B18, Bf^{F_1} and DRw3 haplotype; in the second family, the B18 Bf^{F_1} was not transmitted to the diabetic child.

TABLE 2
Combined estimate of the relative risk (x) of IDDM in Bf^{F_1}-positive versus Bf^{F_1}-negative individuals

	x	y	w	wy	wy^2
Present study (n = 72)	5.553	1.7144	2.08005	3.5660	6.1136
Raum et al.[16] (n = 106)	15.043	2.1711	3.8797	8.4232	18.2877
Bertram et al.[17] (n = 448)	3.373*	1.216	7.0771	8.60573	10.4648
Kirk et al.[18] (n = 239)	6.171	1.820	5.948	10.823	19.694
			18.985	31.418	54.560

If a = number of patients positive for Bf^{F_1} when b = number negative and c = number of controls positive for Bf^{F_1} when d = number negative, then relative risk (x) = $\frac{(2a + 1)(2d + 1)}{(2b + 1)(2b + 1)}$ (Haldane's modification), $\log_e$ of x = y, and variance of y is $\frac{a + b}{a \times b} + \frac{c + d}{c \times d}$; 1/variance of y = w.

The significance of y for individual studies is defined by calculating χ^2 for each study (= wy^2) with one degree of freedom. χ^2 values (or wy^2) for individual studies are summed to give total χ^2 (= Σwy^2), with degrees of freedom equaling the number of studies combined. The pooled χ^2 tests the significance of the deviation of the mean value of x from unity, = $(\Sigma wy)^2/\Sigma w$, and has one degree of freedom.

Heterogeneity of χ^2 tests consists of departure of individual values of x from the overall mean and = $\Sigma wy^2 - \frac{(\Sigma wy)^2}{\Sigma w}$, with degrees of freedom one less than the number of studies being combined.

The weighted estimated mean value of x = nat antilog $\Sigma wy/\Sigma w$ and its SE = nat antilog of $\sqrt{1/\Sigma w}$. 95% confidence limits of x = nat antilog $\Sigma wy/\Sigma w \pm (1.96 \times \sqrt{1/\Sigma w})$. *In this table*, combined mean estimate of x = 5.233, with pooled $\chi_3^2 = 51.99$ ($P < 0.0005$), 95% confidence limits of x = 3.33 − 8.20 and χ_2^2 heterogeneity = 2.567, $P > 0.30$.

* Because they reported only gene frequencies, this estimate was made with the assumption that all Bf^{F_1}-positive patients and controls were heterozygous.

DISCUSSION

Until recently, only passing mentions were made to Bf alleles in IDDM or else MHC data of families with the disorder

TABLE 3
Phenotypes of the eight B18 Bf^{F_1}-positive patients

HLA −	A	B	C	DRw	Bf
# 1	2,_	17, 18, w4, 6	_,_	3,7	F_1S
# 2	2, 11	18, w21	ND	3,7	F_1S_1
# 3	2, w30	15, 18 w6	3,_	4,5	F_1S
# 4	2, w26	18, 40 w6	3,_	4,_	F_1S
# 5	11, 28	13, 18 w4, 6	2,5	5,7	F_1S
# 6	2, w32	18, 40	ND	ND	F_1S
# 7	1, w30	8, 18	ND	ND	F_1S
# 8	1, 9	8, 18	ND	ND	F_1S

ND = Not done.
Haplotypes associated with B18/Bf^{F_1} in controls were A28, B18; Aw30, B18; and Aw30, B18.

TABLE 4
Association of Bf^{F1} and HLA B18 with insulin-dependent diabetes mellitus

		IDDM	Controls	χ^2	χ_2^2 sig.	χ_2^2 hetero
Bf^{F1} +ve	B18 +	8	3*	0.00		
	B18 −	0	0			
Bf^{F1} −ve	B18 +	3	11	0.2009		
	B18 −	61	136			
Totals				0.2009	0.7647 ($P > 0.60$)	0.6025 ($P > 0.70$)
B18 +	Bf^{F1} +	8	3	4.6616		
	Bf^{F1} −	3	11			
B18 −	Bf^{F1} +	0	0	0		
	Bf^{F1} −	61	136			
Totals				4.6616	6.4674 ($P < 0.025$)	0.6778 ($P > 0.60$)

To determine primacy of association, we examined the relation of Bf^{F1} with IDDM after dividing the patients and controls into B18+ and B18− subgroups (upper panel) and that of HLA B18 with IDDM after dividing the patients and controls into Bf^{F1} + and Bf^{F1} − subgroups (lower panel).

* Haldane's modification for small numbers and reference 24 were used to calculate relative risk (x).

were detailed without comment.[26–28] Thus, Cudworth[26] noted that Bf^{S} is increased in IDDM, whereas a perusal of Rubenstein's[27,28] data indicates an increased incidence of the rare allele Bf^{F1} in IDDM families. More recently, Raum et al.[16] reported that Bf^{F1} was found in 22.6% of patients with IDDM compared with 1.9% of controls. On the basis of these findings as well as family data, they postulated a diabetogenic gene linked to Bf and in strong linkage disequilibrium with Bf^{F1}. Unfortunately, these authors have not detailed their HLA data; this would have been of particular interest, as their high Bf^{F1} gene frequency is probably explicable by the high proportion of Bostonians of Mediterranean extraction and, therefore, with high HLA B18 frequency. Independently, Bertrams et al.[17] reported a significant increase in the gene frequency of Bf^{F1} in IDDM patients compared with controls; moreover, this difference became much more marked when the incidence of the haplotype B18, Bf^{F1} was compared. Although Kirk et al.[18] typed their patients for HLA, they only reported a negative association between Bf^{F1}, Bf^{S1}, and BF^{F} with HLA B8; they did comment on the association of the former allele with B18.

The present study concurs with the previous reports,[16–18] in showing that Bf^{F1} is increased in IDDM. We found 11% of these patients to be positive for Bf^{F1} compared with a figure of 22.6% in Boston, 11.7% in Melbourne, and 6.0% in the German study (control frequency of Bf^{F1} was about 1.9% in all four studies). No significant heterogeneity was found between these three sets of data. Our findings agree with those of Bertrams et al.[17] in that a greater increase of B18 Bf^{F1} haplotype was observed—in fact, all eight Bf^{F1}-positive individuals were positive for B18. The greater degree of haplotypic association observed by these authors probably reflects the increase in B18 and Bf^{F1} in the IDDM group compared with controls. In two of four of these studies,[17,*] no significant increase in Bf^{S1} was observed in patients suffering from IDDM, whereas Kirk et al.[18] commented on such an increase. Bf^{S1} is in linkage disequilibrium with HLA B12 and B21.[21] Altogether, these findings from populations with different ethnic backgrounds suggest that the increase in the gene frequency of Bf alleles among patients suffering from IDDM reflects the MHC haplotypes in linkage disequilibrium. In this study, six of the eight patients who were positive for B18 Bf^{F1} were of English or Irish origin and two were of French background.

The nature of the association of Bf^{F1} with IDDM then becomes a matter for interpretation: is this Bf allele primarily associated with IDDM or is it secondary to the increase of B18 in IDDM? The statistical treatment of the data (Table 4) shows that Bf^{F1} was more strongly associated with IDDM than was B18, the apparent association of the latter antigen being due to its strong linkage disequilibrium with Bf^{F1}. Bf^{F1} has been described to maintain strong linkage disequilibrium with B18 in several populations.[21,29 32] The question then becomes: is there a diabetogenic gene close to the Bf locus, which appears to be very close indeed to the HLA-B locus,[14,15] or does Bf^{F1} mark specific B18-bearing haplotypes that are particularly diabetogenic? The statistical demonstration of the apparent primacy of Bf^{F1} does not answer this question. However, the fact that Bf^{F1} rarely occurs in B18-negative haplotypes supports our contention that B18 Bf^{F1} haplotype is specifically diabetogenic, as has been shown for B8, DRw3 and B15, DRw4 haplotypes.[4] This would not be surprising, in view of preliminary evidence from this laboratory, suggesting that unusual HLA haplotypic arrangements are encountered in autoimmune endocrine diseases.[33,34] This includes distortion of C_2 alleles/HLA-B association in Graves' disease[33] and the concurrence of B8 with Bf^{F} in polyglandular autoimmune syndromes comprising Graves' disease.[34]

This finding of an association of Bf^{F1} with IDDM raises an issue as to the mechanism of its action. Bf is crucial to the activation of the alternate complement pathway.[13 15,34] To our knowledge, there has been no indication that specific Bf allelic gene products are biologically more effective than

* The data reported here.

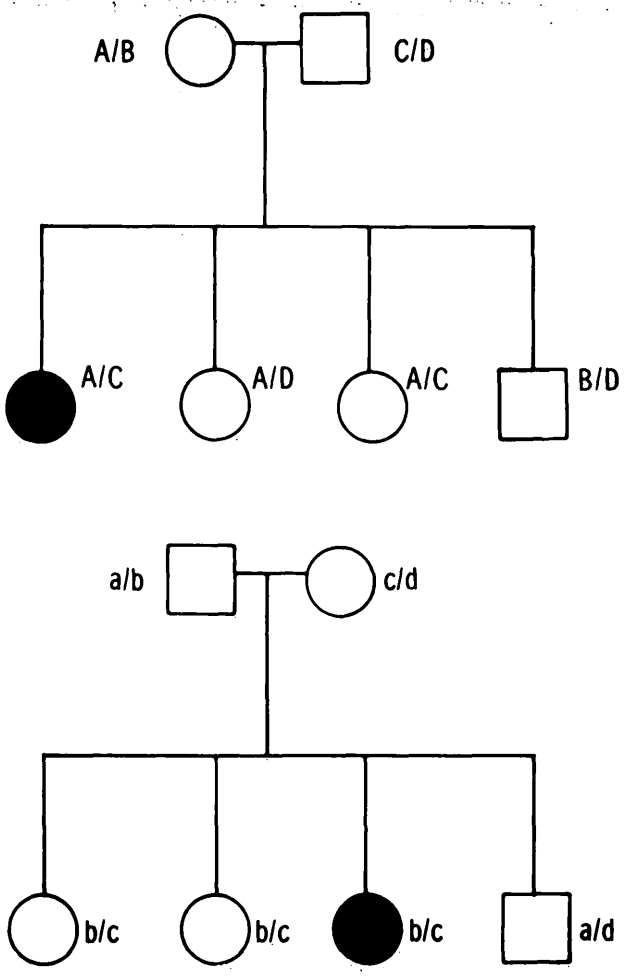

FIG. 1. The families with Bf^{F1} identified: ○ female, □ male, and ● IDDM.

Haplotypes are as follows:
A = A30, B18, Cw-, DRw3, Bf^{F1}, C_2^1, GLO^1
B = A2, Bw38, Cw-, DRw7, Bf^S, C_2^1, GLO^1
C = A24, B35, Cw4, DRw5, Bf^S, C_2^1, GLO^2
D = A3, B7, Cw-, DRw2, Bf^S, C_2^1, GLO^1

a = A2, B18, Cw-, DRw-, Bf^{F1}, GLO^2
b = A3, B7, Cw-, DRw4, Bf^S, GLO^2
c = A2, B40, Cw2, DRw3, Bf^S, GLO^2
d = A2, B40, Cw3, DRw4, Bf^S, GLO^2

others nor that certain HLA antigens affect the level of Bf biologic activity.

HLA-B18 is the antigen most frequently associated with C_2 deficiency;[36] however, the haplotype active in that disorder comprises D(R)w2, an antigen rarely encountered in IDDM.[4,10,12] Indeed, its presence in the genotype appears to protect against IDDM.[4,10] In this study we did not find any DRw2-positive individuals among the B18 Bf^{F1} patients. Therefore, if B18 is required to determine serum Bf level, a haplotypic arrangement other than B18/DRw2 may be necessary.

Our results in the two families in which one parent was positive for B18 Bf^{F1} serve to remind us that this haplotype is not a necessary accompaniment to the IDDM phenotype in such families; in only one of the two families did Bf^{F1} B18 pass on to an affected family member. Thus, one such family was encountered among 16 consecutive simplex or multiplex families studied, which compares well with an incidence of Bf^{F1} positivity of 11% among the population of juvenile diabetics studied. It may be speculated that the dissociation between B18 Bf^{F1} and IDDM in the second family was likely related to the fact that haplotypes b/c (Figure 1) ensured an HLA phenotype (DRw3/DRw4 heterozygote) associated with an enhanced susceptibility to IDDM.[4]

Our findings that, in family 1, the diabetic child was B18 and Bf^{F1} as well as DRw3 positive led us to examine HLA DRw phenotypes of the B18 Bf^{F1} patients to see whether this diabetogenic haplotype may be further specified by the associated DRw allele. Our data are limited in that respect; however, it would be important to examine the haplotypic arrangement of B18, Bf^{F1}, DRw alleles in data from larger series of patients to answer this question. In a preliminary report by Deschamp et al.,[37] the finding that the Bf^{F1} is a component of the haplotype Aw30, Cw5, B18, Bf^{F1}, DRw3 observed in significantly higher frequency and linkage disequilibrium, at least in French IDDM patients, is informative in this context. Our results in this and previous studies[4] do not conflict with the proposals by Raum et al.[16] for a recessive or dominant diabetogenic gene linked to Bf^{F1} and which is incompletely penetrant. Sibpair studies, which show that some two-thirds of such pairs are HLA identical as well as the increased risk attached to DRw3/DRw4 heterozygosity, suggest that, for the susceptibility to IDDM to be expressed, both parental haplotypes may be necessary,[2-4,10,26] only one of which will be B18, Bf^{F1} (in view of the rarity of this allele), and that in only 11% of patients. It is apparent that the possible HLA phenotypes of diabetic patients are numerous indeed. Taken with the additive influence of certain HLA alleles on disease susceptibility, the difference in seasonal variation, presence and persistence of anti-B cell antibodies, and reported association with retinopathy[2-4] further underscores the heterogeneity of the genetic basis of IDDM.

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REFERENCES

[1] Nerup, J., Platz, P., Ortved Anderson, O., Christy, M., Lyngsøe, J., Poulsen, J. E., Ryder, L. P., Staub Nielsen, L., Thomsen, M., and Svejgaard, A.: HLA antigens and diabetes mellitus. Lancet 2:864-66, 1974.

[2] Nerup, J., Cathelineau, C. R., Seignalet, J., and Thomsen, N.: HLA and endocrine diseases. *In* HLA and Disease. Dausset, J., and Svejgaard, A., Eds. Copenhagen, Munksgaard, 1977, pp. 46-71.

[3] Rotter, J. I., and Rimoin, D. L.: Heterogeneity in diabetes mellitus—update 1978. Evidence for further genetic heterogeneity within juvenile-onset insulin-dependent diabetes mellitus. Diabetes 27:599-608, 1978.

[4] Farid, N. R., Sampson, L., Noel, P., Barnard, J. M., Davis, A. J., and Hillman, D. A.: HLA-D-related (DRw) antigens in juvenile diabetes mellitus. Diabetes 28:552-57, 1979.

[5] Irvine, W. J., McCallum, C. J., Gray, R. S., Campbell, C. J., Duncan, L. J. P., Farquhar, J. W., Vaughan, H., and Morris, P. J.: Pancreatic islet-cell antibodies in diabetes mellitus correlated with the duration and type of diabetes, coexistent autoimmune disease, and HLA type. Diabetes 26:138-47, 1977.

[6] Bertrams, J., Janse, F. K., Gruneklee, D., Reis, H. E., Drost, H., Gries,

F. A., and Kuwert, E.: HLA antigens and immunoresponsiveness to insulin in insulin-dependent diabetes mellitus. Tissue Antigens *8*:13–19, 1976.

[7] Barbosa, J., Noreen, H., Emme, L., Goetz, F., Simmons, R., de Leiva, A., Najarian, J., and Yunis, E. J.: Histocompatibility (HLA) antigens in diabetic microangiopathy. Tissue Antigens *7*:233–37, 1976.

[8] Rubinstein, P., Suciu-Foca, N., and Nicholson, J. F.: Genetics of juvenile diabetes mellitus. A recessive gene closely linked to HLA D and with 50 percent penetrance. N. Engl. J. Med. *297*:1036–40, 1977.

[9] Barbosa, J., King, R., Noreen, H., and Yunis, E. J.: The histocompatibility (HLA) system in juvenile insulin dependent diabetic multiplex kindreds. J. Clin. Invest. *60*:989–98, 1977.

[10] Thomson, M., Platz, P., Christy, M., Nerup, J., Ryder, L. P., and Svejgaard, A.: HLA-D-associated resistance and susceptibility to insulin-dependent diabetes mellitus. Transplant. Proc. *11*:1307–08, 1979.

[11] Neel, J. V.: The genetics of juvenile-onset-type diabetes mellitus (Editorial). N. Engl. J. Med. *297*:1062–63, 1977.

[12] Ilonen, J., Herva, E., Tillikainen, A., Åkerbolm, H. K., Loivukangas, T., and Kouvalainen, K.: HLA Dw2 as a marker of resistance against juvenile diabetes mellitus. Tissue Antigens *11*:144–46, 1978.

[13] Alper, C. A., Boenisch, T., and Watson, L.: Genetic polymorphism in human glycine-rich beta-glycoprotein. J. Exp. Med. *135*:68–80, 1972.

[14] Allen, F. H., Jr.: Linkage of HL-A and GBG. Vox Sang. *27*:382–84, 1974.

[15] Lachman, P. J., and Hobart, M. J.: Complement genetics in relation to HLA. Br. Med. Bull. *34*:247–52, 1978.

[16] Raum, D., Alper, A. C., Stein, R., and Gabbay, K. H.: Genetic marker for insulin dependent diabetes mellitus. Lancet *1*:1208–10, 1979.

[17] Bertrams, J., Baur, M. P., Grüneklee, D., and Gries, F. A.: Association of Bf^F1, HLA-B18 and insulin-dependent diabetes mellitus. Lancet *2*:98,1979.

[18] Kirk, R. L., Theophilus, J., Whitehouse, S., Court, J., and Zimmet, P.: Genetic susceptibility to diabetes mellitus: the distribution of properdin factor B(Bf) and glyoxalase (GLO) phenotypes. Diabetes *28*:949–51, 1979.

[19] Mittal, K. K., Mickey, M. R., Singal, D. P., and Terasaki, P. I.: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. Transplantation *6*:913–27, 1968.

[20] Farid, N. R., Sampson, L., Noel, E. P., Barnard, J. M., Mandeville, R., Larsen, B., Marshall, W. H., and Carter, N. D.: A study of human leukocytes D locus related antigens in Graves' disease. J. Clin. Invest. *63*:108–13, 1979.

[21] Larsen, B., Arnason, A., Barnard, J. M., Buehler, S. K., Edwards, J. H., and Marshall, W. H.: Bf types of HLA haplotyped individuals in an isolated Newfoundland population. Tissue Antigens *10*:403–09, 1977.

[22] Meo, T., Atkinson, J., Bernoco, M., Bernoco, D., and Cepellini, R.: Mapping of HLA locus controlling C2 structural variants and linkage disequilibrium between C_2^2 and Bw15. Eur. J. Immunol. 6:916–19, 1976.

[23] Parr, W., Bagster, I., and Welch, S. G.: Human red cell glyoxalase I polymorphism. Biochem. Genet. *15*:109–33, 1977.

[24] Svejgaard, A., Jersild, C., Staub-Nielsen, L., and Bodmer, W. F.: HLA antigens and disease. Statistical and genetical considerations. Tissue Antigens *4*:95–105, 1974.

[25] Svejgaard, A., and Ryder, L. P.: Associations between HLA and disease. Notes on methodology and a report from the HLA and disease registry. *In* HLA and Disease. Dausset, J., and Svejgaard, A., Eds. Copenhagen, Munksgaard, 1977, pp. 46–71.

[26] Cudworth, A. G.: Type I diabetes mellitus. Diabetologia *14*:281–91, 1978.

[27] Rubinstein, P., Suciu-Foca, N., Nicholson, J. F., Fotino, M., Molinaro, A., Harisiadis, L., Hardy, M. A., Reemstma, K., and Allen, F. H., Jr.: The HLA system in the families of patients with juvenile diabetes mellitus. J. Exp. Med. *143*:1277–82, 1976.

[28] Suciu-Foca, N., and Rubinstein, P.: Complete HLA and Bf typing in families: II Bf and the structure of the HLA region. Transplant. Proc. 9 (Suppl. 1):83–86, 1977.

[29] Rittner, C., Grosse-Wilde, H., Rittner, B., Metzel, B., Scholz, S., Lorenz, H., and Albert, E. D.: Linkage group HL-A-MLC-Bf (properdin factor B). Humangenetik *27*:173–83, 1975.

[30] Bijnen, B. B., Schreuder, I., Khan, P. M., Allen, F. H., Giles, C. M., Lost, W. R. T., Volkers, W. S., and van Rood, J. J.: Linkage relationship of the loci of the major histocompatibility complex in families with a recombination in the HLA region. J. Immunogenet. *3*:171–83, 1976.

[31] Marcelli-Barge, A., Benjam, A., Poirier, J. C., Nunez-Roldan, A., and Dausset, J.: Observation of an HLA-Cw5, B18, Bf^F1 linkage disequilibrium in women. Tissue Antigens *10*:228, 1977.

[32] Arnason, A.: Personal communication, 1977.

[33] Noel, E. P., Sampson, L., Pepper, B. M., and Farid, N. R.: Polymorphism of the second component of complement (C2) in Graves' disease. Hum. Hered. (in press) 1980.

[34] Farid, N. R., Larsen, B., Payne, R., Noel, E. P., and Sampson, L.: Polyglandular autoimmune disease and HLA. Tissue Antigens (in press) 1980.

[35] Fearon, D. T., Austen, K. F., and Ruddy, S.: Properdin factor D: characteristics of its active site and isolation of the precursor form. J. Exp. Med. *139*:355–66, 1974.

[36] Day, N. K., L'Esperance, P., Good, R. A., Michael, A. F., Hansen, J. A., Dupont, B., and Jersild, C.: Hereditary C2 deficiency: genetic studies and association with the HL-A system. J. Exp. Med. *141*:1464–69, 1975.

[37] Deschamps, L., Lestradet, H., Marcelli-Barge, A., Benajam, A., Busson, M., Hors, J., and Dausset, J.: Properdin factor B alleles as markers for insulin-dependent diabetes. Lancet *2*:793, 1979.