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Genetic and immunological characteristics of Type I diabetes mellitus in an Indo-Aryan population

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Abstract

Aims/hypothesis. Our aim was to characterise the genetic and immunological features associated with Type I (insulin-dependent) diabetes mellitus in a cohort of Indo-Aryan children resident in the United Kingdom.
Methods. Children with Type I diabetes ($n = 53$), unaffected first-degree relatives ($n = 146$) and unrelated healthy control children ($n = 54$) were typed for alleles of the *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* genes. Islet cell antibodies and antibodies to glutamic acid decarboxylase, protein tyrosine phosphatase-2 (IA-2ic) and insulin were measured in the diabetic and control children.
Results. The DRB1*03.DQA1*05.DQB1*02 haplotype was positively associated with the disease, occurring in 78% of diabetic children compared with 22.6% of healthy children ($p_c < 2.4 \times 10^{-5}$). In simplex families, this haplotype was transmitted more frequently to the diabetic children than to their unaffected siblings ($p < 1 \times 10^{-4}$). The DRB1*04.DQA1*03.DQB1*0302 haplotype was also transmitted preferentially to the diabetic probands ($p < 0.025$) but was not associated with disease in the case control study. Islet-related autoantibodies were detected in 89.6% of diabetic patients compared with 11.8% of control children ($p < 1 \times 10^{-6}$). Although protein tyrosine phosphatase-2 autoantibodies were detected more frequently among DRB1*04-positive diabetic patients compared with patients lacking this allele, the overall frequency of these autoantibodies was lower than observed in Europid diabetic subjects. This could reflect the absence of a disease association with DRB1*04 in the Indo-Aryan cohort.
Conclusion/interpretation. Type I diabetes in our Indo-Aryan cohort is similar to the disease observed in Anglo-Europeans but has important immunogenetic differences. The low frequency of protein tyrosine phosphatase-2 autoantibodies among the Indo-Aryan diabetic children could have important implications for the design of future strategies for disease prediction in this population. [Diabetologia (2000) 43: 450–456]

Keywords Type I diabetes, Indo-Aryan, immunogenetics, *HLA* genes, islet-related autoantibodies.

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Abbreviations: ICA, Islet cell antibodies; IA-2ic, intracellular fragment of protein tyrosine phosphatase-2; cDNA, complementary DNA; JDF, Juvenile Diabetes Foundation; RR, relative risk; TDT, transmission disequilibrium test.

Type I (insulin-dependent) diabetes results from the progressive autoimmune destruction of pancreatic beta cells [1]. The autoimmune aetiology is signified in Europid populations by a strong association with alleles of the *HLA* class II histocompatibility genes, particularly those carried on the *DR3.DQ2* and *DR4.DQ8* haplotypes [2], and the presence of circulating antibodies specific for islet antigens including glutamic acid decarboxylase (GAD), the intracellular fragment of protein tyrosine phosphatase-2 (IA-2ic) and insulin [3, 4].

Autoimmune Type I diabetes in childhood is rare in the Asian population of India [5, 6] and is also uncommon among migrant Asian Indian populations in the United Kingdom [7, 8]. The Indian community resident in Birmingham in the United Kingdom is mainly of Indo-Aryan stock and originates predominantly from the Punjab (a region spanning north-east Pakistan to north-west India). The genetic and immunological features associated with Type I diabetes in Indo-Aryans are poorly defined. Previous genetic studies of this population have shown a strong disease association with the HLA-DR3 antigen and a weaker, but inconsistent, association with HLA-DR4 [9–11]. The disease risk conferred by the *HLA-DQ* alleles has not yet been investigated in this population. Islet cell antibodies (ICA) have been reported to be less frequent among Indo-Aryan diabetic patients than among those of Europid origin [6, 10–13] but the frequency of autoantibodies to GAD, IA-2ic and insulin in the Indo-Aryan population is yet to be determined. The purpose of our study, therefore, was to further analyse the genetic and immunological markers associated with Type I diabetes in a cohort of Indo-Aryan children resident in Birmingham in the United Kingdom. The aim was to establish whether the disease in this group has similar immunogenetic characteristics to those observed in the indigenous Anglo-European population.

Subjects and methods

Subjects. Children with Type I diabetes ($n = 53$) were recruited from four paediatric diabetes clinics in Birmingham. The patients were unrelated and of Indo-Aryan ethnic origin (both parents originated from Pakistan or the Indian Punjab). All the patients developed diabetes before the age of 16 years and were dependent on insulin from the time of diagnosis. Approximately 50 % of the patients presented with diabetic ketoacidosis and the remaining subjects had hyperglycaemia combined with ketonuria. The cohort consisted of 37 girls and 16 boys and constitutes 93 % of the paediatric Indo-Aryan Type I diabetic population of Birmingham. The mean age of disease onset was 7.1 ± 4.6 years (range 2 months to 15 years) and the mean disease duration at the time of recruitment was 3.4 ± 3 years (range 1 month to 12 years). Unaffected first-degree relatives ($n = 146$, parents or siblings or both of 45 index cases) and 54 healthy control children were also investigated. The control subjects were matched with the diabetic cohort for age at recruitment, sex ratio, ethnic origin and area of residence. The study was approved by the local ethics committee and written informed consent was obtained from all participants.

HLA typing. Genomic DNA was isolated from peripheral blood samples using a Nucleon kit (Scotlab, Strathclyde, Scotland). Alleles of the *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* genes were determined for each of the study subjects using the polymerase chain reaction (PCR) with 48 sequence-specific primer pairs [14]. The PCR products were separated by agarose gel electrophoresis and could be seen under ultraviolet light. The *HLA* type of each subject was determined from the pattern of allele-specific amplicons.

Detection of islet-related autoantibodies

Serum samples, taken from the diabetic and control children at the time of recruitment, were analysed for the presence of islet-related autoantibodies.

Cytoplasmic islet cell antibodies (ICA). These were detected by indirect immunofluorescence on unfixed cryostat sections of blood group O human pancreas, as described previously [15]. Quantification was achieved by assaying serial dilutions of each test serum in parallel with a standard serum, calibrated against an international reference sample of 80 Juvenile Diabetes Foundation (JDF) units. A titre greater than 5 JDF units was considered to be positive. The sensitivity of the assay was 78 % and the specificity was 98 %.

Glutamic acid decarboxylase (GAD65) antibodies. These were assayed by immunoprecipitation of radiolabelled human recombinant GAD65, as described previously [16]. Human islet GAD65 complementary DNA (cDNA) was transcribed and translated in vitro in the presence of [^{35}S]-methionine. The radiolabelled tracer was incubated overnight with the test serum samples and immune complexes were precipitated with protein A Sepharose (Pharmacia Biotech, Uppsala, Sweden) and quantified by scintillation counting. Antibody titres were expressed as units based on the percentage of radioactivity precipitated by the test serum compared with that precipitated by a reference serum. A titre greater than 13 U was considered positive. The sensitivity of the assay was 77 % and the specificity was 98 %.

IA-2ic antibodies. These were detected by immunoprecipitation of in vitro labelled human recombinant IA-2ic, as described for GAD antibodies above. Serum samples were regarded as positive if the antibody titre exceeded 13 U. The sensitivity of the assay was 73 % and the specificity was 94 %.

Insulin antibodies. These were measured using a micro-radiobinding assay (sensitivity 69 %, specificity 98 %), as described previously [17]. Test serum samples were incubated with ^{125}I-labelled human insulin, with and without an excess of unlabelled insulin to correct for non-specific binding. Immune complexes were precipitated with protein A Sepharose and protein G Sepharose (Pharmacia Biotech) and counted for radioactivity. The results were converted into arbitrary units by the use of a standard curve. A titre greater than 4 U was considered positive.

The laboratories in London and Milan, which conducted the antibody assays, have consistently performed well in various proficiency standardisation programmes for ICA determination and, that in Milan, in the combined islet-related autoantibody workshop [18].

Statistical analysis. The association between Type I diabetes and the *HLA* loci was tested using the chi-squared test or Fisher's exact probability test where appropriate. Corrected p values (p_c) were obtained by multiplying each p value by the number of haplotypes/genotypes analysed. Relative risk (RR) values were calculated for markers which differed significantly in frequency between the diabetic and control groups ($p_c < 0.05$) using the method of Wolf, with Haldane's modification for

Table 1. Distribution of *DR/DQ* haplotypes among diabetic and control subjects

DRB1.DQA1.DQB1 haplotype	Type I diabetic patients (%) $n = 50$	Control subjects (%) $n = 53$
*01.*0101.*05	1 (2.0)	4 (7.5)
*1501.*0102.*05	0 (0)	5 (9.4)[a]
*1501.*0102.*0601	0 (0)	6 (11.3)[b]
*1501.*0102.*0602	1 (2.0)	3 (5.7)
*1501.*0103.*0601	1 (2.0)	5 (9.4)
*1502.*0103.*0601	0 (0)	7 (13.2)[c]
*03.*05.*02	39 (78.0)	12 (22.6)[d]
*04.*03.*0301	1 (2.0)	2 (3.8)
*04.*03.*0302	10 (20.0)	7 (13.2)
*11.*05.*0301	3 (6.0)	14 (26.4)[e]
*13.*0103.*0603	5 (10.0)	3 (5.7)
*13.*0102.*0604	2 (4.0)	2 (3.8)
*14.*0101.*05	6 (12.0)	5 (9.4)
*07.*02.*02	3 (6.0)	11 (20.8)[f]
*10.*0101.*05	5 (10.0)	3 (5.7)

Number (%) of diabetic and control subjects with each haplotype. Only haplotypes observed in more than one subject are shown. [a] $p = 0.033$, p_c = NS; [b] $p = 0.016$, p_c = NS; [c] $p = 0.008$, p_c = NS; [d] $p < 1 \times 10^{-6}$, $p_c < 2.4 \times 10^{-5}$, RR = 11.40 [4.65–27.94]; [e] $p < 0.01$, p_c = NS; [f] $p < 0.05$, p_c = NS

Table 2. Frequency of DRB1*04 subtypes among *DR4.DQ8*-positive diabetic and control subjects

DRB1*04 allele	*DR4.DQ8*-positive diabetic patients (%) $n = 10$	*DR4.DQ8*-positive control subjects (%) $n = 7$
*0401	2 (20.0)	0 (0)
*0402	3 (30.0)	2 (28.6)
*0403	2 (20.0)	4 (57.1)
*0404	1 (10.0)	1 (14.3)
*0405	2 (20.0)	0 (0)
*0407	0 (0)	1 (14.3)

Number (%) of diabetic and control subjects with each allele. p = NS for all comparisons. Overall X^2 for allele distribution in diabetic and control subjects = 5.72 (5 df), p = NS

small numbers. Each RR value is quoted with a 95% confidence interval (95% CI) [19]. Transmission of *HLA* susceptibility markers in the Indo-Aryan families was investigated using the transmission disequilibrium test (TDT) [20]. The frequency of islet-related autoantibodies among the diabetic and control subjects was compared using the chi-squared test and Fisher's exact test. These tests were also used to identify associations between autoantibody status and *HLA* type. The distribution of age-of-onset and disease duration data between groups was analysed using the Kolmogorov-Smirnov test.

Results

The DRB1*03.DQA1*05.DQB1*02 (*DR3.DQ2*) haplotype was significantly positively associated with Type I diabetes in the Indo-Aryan cohort ($p_c < 2.4 \times 10^{-5}$, RR = 11.40 [4.65–27.94]) (Table 1). In contrast, five haplotypes were decreased in frequency among the diabetic patients compared with the control subjects (p_c = NS) (Table 1).

Table 3. Distribution of *DR* genotypes among diabetic and control subjects

DR genotype	Type I diabetic patients (%) $n = 50$	Control subjects (%) $n = 53$
*03/*03	19 (38.0)	2 (3.8)[a]
*03/*04	6 (12.0)	0 (0)[b]
*03/*10	4 (8.0)	0 (0)
*03/*13	5 (10.0)	1 (1.9)
*03/*14	3 (6.0)	2 (3.8)
*03/*15	1 (2.0)	3 (5.7)
*04/*11	1 (2.0)	2 (3.8)
*04/*15	0 (0)	5 (9.4)[c]
*07/*15	0 (0)	9 (17.0)[d]
*11/*15	1 (2.0)	5 (9.4)

Number (%) of diabetic and control subjects with each genotype. Only genotypes occurring in more than two subjects are shown (total number of genotypes = 36) NB. All subjects with DRB1*03/*04 genotype carried the *DR4.DQ8* haplotype [a] $p < 1 \times 10^{-4}$, $p_c < 3.6 \times 10^{-3}$, RR = 12.75 [3.51–46.37]; [b] $p = 0.011$, p_c = NS; [c] $p = 0.033$, p_c = NS; [d] $p = 1.77 \times 10^{-3}$, p_c = NS

The DRB1*04.DQA1*03.DQB1*0302 (*DR4.DQ8*) haplotype was not associated with susceptibility to Type I diabetes in our study group (Table 1). Among the *DR4.DQ8*-positive subjects, 7 out of 10 (70%) diabetic patients carried DRB1*04 alleles associated with a high risk of diabetes (DRB1*0401, *0402 and *0405) compared with 2 out of 7 (28.6%) control subjects (p = NS) (Table 2). In contrast, the low-risk DRB1*0403 allele was present in 2 out of 10 (20%) diabetic subjects compared with 4 out of 7 (57.1%) control subjects (p = NS).

The homozygous genotype, DRB1*03/*03, was significantly more frequent among the diabetic patients than among the control group (Table 3). The DRB1*03/*04 genotype was also increased in frequency among the diabetic subjects, whereas DRB1*04/*15 and DRB1*07/*15 were less frequent among the diabetic patients than among the control group (p_c = NS in all three cases).

The transmission disequilibrium test (TDT) was used to analyse the *HLA* haplotype data from 39 complete simplex families. These families consisted of two unaffected parents, the diabetic proband and between one and six unaffected siblings (mean 2). The *DR3.DQ2* and *DR4.DQ8* haplotypes were transmitted from the unaffected parents to the diabetic offspring more frequently than to the unaffected offspring ($p < 1 \times 10^{-4}$ and $p < 0.025$ respectively) (Table 4). In contrast, the DRB1*1502.DQA1*0103.DQB1*0601 haplotype was transmitted preferentially to the unaffected siblings (Table 4). No other haplotypes had transmission distortion (data not shown).

Islet-related autoantibodies were measured in 48 of the diabetic children and 51 of the healthy control

Table 4. Transmission of the *DR/DQ* haplotypes to diabetic and non-diabetic offspring in simplex families

Haplotype	Offspring	Transmitted haplotypes	Non-transmitted haplotypes
DRB1*03.DQA1 *05.DQB1*02			
	Diabetic	45	5
	Unaffected	34	29
DRB1*04.DQA1 *03.DQB1*0302			
	Diabetic	8	4
	Unaffected	7	18
DRB1*1502.DQA1 *0103.DQB1*0601			
	Diabetic	0	8
	Unaffected	4	5

Comparison of haplotype distribution to affected and unaffected offspring; DRB1*03.DQA1*05.DQB1*02, $X^2 = 17.21$, $p < 1 \times 10^{-4}$; DRB1*04.DQA1*03.DQB1*0302, $X^2 = 5.03$, $p < 0.025$; DRB1*1502.DQA1*0103.DQB1*0601, $X^2 = 4.65$, $p < 0.05$

Table 5. Frequency of islet-related antibodies among diabetic and control subjects

Antibody	Type I diabetic patients (%) $n = 48$	Control subjects (%) $n = 51$
ICA	17 (35.4)	2 (3.9)[a]
GAD	27 (56.3)	2 (3.9)[b]
IA-2ic	8 (16.7)	2 (3.9)[c]
Insulin	41 (85.4)	2 (3.9)[d]
ICA + GAD	1 (2.1)	1 (2.0)
ICA + IA-2ic	0 (0)	0 (0)
ICA + insulin	1 (2.1)	0 (0)
GAD + IA-2ic	0 (0)	1 (2.0)
GAD + insulin	11 (22.9)	0 (0)[e]
IA-2ic + insulin	0 (0)	0 (0)
ICA + GAD + IA-2ic	1 (2.1)	0 (0)
ICA + GAD + insulin	8 (16.7)	0 (0)[f]
ICA + IA-2ic + insulin	1 (2.1)	0 (0)
GAD + IA-2ic + insulin	1 (2.1)	0 (0)
ICA + GAD + IA-2ic + insulin	5 (10.4)	0 (0)[g]

Number (%) of diabetic and control subjects with each antibody: [a]$p < 1 \times 10^{-4}$; [b]$p < 1 \times 10^{-6}$; [c]$p < 0.05$; [d]$p < 1 \times 10^{-6}$; [e]$p = 1.8 \times 10^{-4}$; [f]$p = 2.2 \times 10^{-3}$; [g]$p = 0.02$

children (Table 5); one or more of these markers was found in 43 (89.6%) of the diabetic patients compared with 6 (11.8%) of the control subjects ($p < 1 \times 10^{-6}$). Of the diabetic patients, 14 (29.2%) were positive for a single antibody, 13 (27.1%) possessed two, 11 (22.9%) had three antibodies and all four antibodies were detected in 5 (10.4%) children. In the control group, four children (7.8%) were positive for a single autoantibody and two subjects (3.9%) possessed two autoantibodies.

Positivity for ICA was shown to decline with increasing disease duration, from 55.6% among patients who developed diabetes less than 1 year before antibody measurement (short duration, $n = 18$) to 18.2% among those with disease duration longer than 6 years ($n = 11$) ($p < 0.01$ using the Kolmogorov-Smirnov test). A similar, but smaller, decline was observed for GAD antibodies (from 55.6% to 36.4%) ($p < 0.01$). Antibodies to IA-2ic were found with similar frequencies among patients recruited within 1 year of disease onset (16.7%) and those with longer disease duration (18.2%). Among the subjects with short disease duration, there was no association between age of onset and the presence of antibodies to insulin ($p > 0.1$).

The *DR4.DQ8* haplotype was significantly more frequent among the children positive for IA-2ic antibodies than among those negative for this humoral marker (57.1% vs 10.5% respectively, $p = 0.026$). There was also a trend towards an association between this haplotype and ICA; 26.7% ICA-positive subjects carried the *DR4.DQ8* haplotype compared with 13.3% ICA-negative patients (p = NS). The *DR3.DQ2* haplotype was increased in frequency among patients positive for GAD autoantibodies (84.0% vs 65.0% among GAD-negative subjects) and among subjects with insulin antibodies (78.9% vs 57.1% among subjects lacking insulin antibodies) (p = NS in both cases).

Discussion

A high-resolution genomic typing technique was used to investigate the role of *HLA-DR* and *HLA-DQ* alleles as susceptibility determinants for Type I diabetes in an Indo-Aryan population. The DRB1*03. DQA1*05.DQB1*02 (*DR3.DQ2*) haplotype was associated with a statistically significantly increased risk of the disease in our cohort. Homozygosity for DRB1*03 was also positively associated with diabetes. These observations are consistent with the findings of earlier studies of Indo-Aryan populations which showed a strong disease association with the DR3 antigen [9–11]. The relative risk value for DRB1*03 in our study (RR = 11.40) is of similar magnitude to that reported for DR3 in an indigenous cohort in India (RR = 10.52) [11]. Furthermore, the frequency of the DRB1*03 allele in our control group (22.6%) is similar to that observed for DR3 in the indigenous healthy Indian cohort (25.7%), suggesting that the subjects in our study are genetically representative of the Indo-Aryan population of north India. The RR values for DRB1*03 (RR = 11.40) and the DRB1*03/*03 genotype (RR = 12.75) in our Indian cohort are higher than those observed in an Anglo-European study group resident in Birmingham (RR = 4.14 and RR = 5.71, respectively; unpublished observation).

Heterogeneity tests, however, suggest that these differences are not statistically significant (DRB1*03 alone; $X^2 = 3.37$, p = NS: DRB1*03/*03; $X^2 = 0.81$, p = NS). This could be because the number of subjects in the Indo-Aryan cohort was too small to show statistical significance for a moderate increase in relative risk (two to threefold). Alternatively, it could indicate that the risk conferred by DRB1*03 is similar in both ethnic groups.

The association between Type I diabetes and the *DR3.DQ2* haplotype in our Indo-Aryan cohort was further confirmed by the analysis of simplex families, which showed that this haplotype was transmitted significantly more frequently to the diabetic offspring of unaffected parents than to the non-diabetic siblings.

The DRB1*04.DQA1*03.DQB1*0302 (*DR4.DQ8*) haplotype did not differ significantly in frequency between the diabetic and control subjects in our study. This finding is consistent with a study of Indo-Aryans from the New Delhi area of India which reported no disease association with DR4 [11]. In contrast, a study of a cohort of Indo-Aryans resident in the United Kingdom showed a weak positive disease association with this antigen [9]. The discrepant observations between different studies could largely be attributable to the small number of subjects investigated. Although *DR4.DQ8* was not significantly associated with disease in our case control study, we cannot exclude the possibility that this marker has a minor influence on disease risk. It is important to note that the majority of diabetic subjects positive for this haplotype carried *DRB1* alleles associated with a high risk of Type I diabetes in Europid populations (DRB1*0401, *0402 and *0405), whereas the control subjects predominantly carried alleles associated with a lower risk of disease (DRB1*0403, *0404 and *0407) [21]. Furthermore, heterozygosity for DRB1*03/*04 was increased in frequency among the diabetic patients compared with the control subjects and the DRB1*03/*04 genotype was associated with a higher relative risk compared with DRB1*03 alone (15.63 vs 11.40, respectively; p = NS). The strongest evidence to support a role for *DR4.DQ8* in disease susceptibility was, however, provided by the family study which showed that this haplotype was transmitted preferentially to the diabetic offspring. In summary, the *DR4.DQ8* haplotype appears to be a minor susceptibility marker for Type I diabetes in our Indo-Aryan cohort and a larger study may help to define its role in this ethnic group.

Protection from Type I diabetes has been associated with *DR2* in both Europid and Indo-Aryan populations [2, 9, 11]. In the former ethnic group, this protective influence is largely attributed to the DRB1*1501.DQA1*0102.DQB1*0602 (*DR15.DQ6*) haplotype. This haplotype, however, was not a major protective marker in our Indo-Aryan cohort, as it was rare in both diabetic and control groups. In our case control study, five haplotypes were associated with a decreased risk of disease. The TDT showed that the DRB1*1502.DQA1*0103.DQB1*0601 haplotype was negatively associated with diabetes in the simplex families.

Islet cell antibodies (ICA) were detected in 35 % of our Indo-Aryan diabetic children. This is similar to the ICA frequencies reported previously in Indo-Aryans with established diabetes [6, 10–13]. The apparently low prevalence of ICA in the present study is largely due to the time lag between disease onset and serum sampling for antibody measurement. As in Europids with Type I diabetes, the highest prevalence of ICA in our Indo-Aryan cohort was observed in diabetic patients sampled within 1 month of disease onset (67 % vs approximately 80–90 % in Europids) [3, 22]. Beyond this period, antibody positivity declined with increasing disease duration, as reported previously in both Europid and Indo-Aryan populations [12]. The presence of ICA at disease onset is associated with *HLA-DR4* and *HLA-DQ8* in Europid populations [22, 23]. In our Indo-Aryan cohort there was a trend towards an association between ICA positivity and the *DR4.DQ8* haplotype, although this was not statistically significant. This could be due to the time of serum sampling in our study, as many of the subjects might have lost ICA positivity before antibody analysis. Thus the association with *DR4.DQ8* could possibly be observed at diagnosis but decrease in statistical significance with time as the ICA concentrations decline.

Autoimmunity to GAD in the Indo-Aryan cohort had similar characteristics to those reported previously in Europid diabetic patients. Antibodies to GAD were present at similar frequencies in both ethnic groups (56 % in the Indo-Aryan cohort compared with 50–80 % in newly diagnosed Europids [3, 4, 22]) and showed a similar time course for persistence in the circulation. Autoimmunity to GAD is associated with *HLA-DR3* and *HLA-DQ2* in Europid populations [23–25]. In our Indo-Aryan cohort we observed a trend towards a positive association between GAD antibody status and the *DR3.DQ2* haplotype, but this was not statistically significant. This could reflect the young age of disease onset in our patients as the association between GAD antibodies and *HLA* genes has been reported to be weak among patients diagnosed below the age of 15 years [25].

Autoantibodies to IA-2ic were a poor marker for Type I diabetes in the Indo-Aryan cohort, occurring in only 17 % of our diabetic patients (compared with 50–80 % of Europid diabetic patients at disease onset [4, 22, 24]). This low frequency could not be attributed to the time lag between disease onset and antibody measurement, as the per cent of positivity for IA-2ic antibodies was found to be similarly low among patients sampled within 1 year of diagnosis and those

with disease duration in excess of 6 years. The IA-2ic antibodies were shown to occur most frequently among diabetic patients positive for the *DR4.DQ8* haplotype. This is consistent with the findings in Europid populations [22, 24], which have suggested that the DRB1*0401 allele could have a more important role in IA-2ic antibody formation than *DQ8* [22]. We were, however, unable to confirm this in our Indo-Aryan diabetic cohort due to the small number of IA-2ic-positive subjects in this group. The close relation between antibody status and *HLA* type suggests that the low frequency of IA-2ic antibodies among Indo-Aryan diabetic patients reflects the absence of a disease association with DRB1*04 in this population.

The frequency of antibodies to insulin in our Indo-Aryan diabetic cohort increased statistically significantly with increasing disease duration, suggesting that they were predominantly a consequence of insulin therapy rather than an autoimmune response to endogenous insulin. Further support for this possibility was provided by two observations: residual endogenous insulin production was found in only 9 of the 41 insulin antibody-positive patients (data not shown) and 5 of the 7 diabetic children who lacked insulin antibodies had received insulin therapy for less than 8 months. It is not clear why such a high proportion of diabetic patients develop antibodies to human insulin.

Islet-related antibodies were detected in 11.8% of the Indo-Aryan control children. Although this percentage seems rather high, it is important to note that the majority of these subjects (four out of six) were positive for a single autoantibody and only two children possessed two antibodies. These data are similar to the frequency of islet antibodies reported in healthy schoolchildren of Anglo-European origin (9.4%) [26].

Our study shows that Type I diabetes in a cohort of Indo-Aryans resident in the United Kingdom is similar to the disease seen in Anglo-Europeans, but exhibits important genetic and immunological differences. The *HLA-DR3.DQ2* haplotype is the major disease susceptibility marker in the Indo-Aryan group, with *DR4. DQ8* apparently contributing less to disease risk. This contrasts with the Anglo-European population in which DRB1*04 confers the greatest risk of disease and DRB1*03 has a less important role [2]. The two ethnic groups also differ in the *HLA* markers which confer protection against diabetes. The most important immunological difference between the two groups is the low frequency of autoantibodies to IA-2ic in the Indo-Aryan diabetic patients. This might have important implications for disease prediction in this population. It has been suggested that Europids at high risk of Type I diabetes can be identified by screening for a combination of autoantibodies to GAD and IA-2ic [26]. In the Indo-Aryan population however, this approach is, unlikely to be of greater predictive value than screening for GAD antibodies or ICA or both.

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