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HLA and Complement Allotypes in Type 1 (Insulin-Dependent) Diabetes

J. McCluskey[1], V. J. McCann[2], P. H. Kay[1], P. J. Zilko[1], F. T. Christiansen[1], G. J. O'Neill[3] and R. L. Dawkins[1]

[1]Department of Clinical Immunology, Royal Perth Hospital and Queen Elizabeth II Medical Centre,
[2]Diabetic Unit, Royal Perth Hospital, Perth, Western Australia and [3]Department of Pathology, Columbia University, New York, USA

Summary. A group of patients with Type 1 (insulin-dependent) diabetes mellitus was investigated for HLA-A, B and DR antigens as well as C4 and factor B polymorphism. A significant excess of DR3/DR4 heterozygotes was observed (27% versus 17% by Hardy-Weinberg expectation). The factor B allele BfF1 was present in 13% of patients with Type 1 diabetes (gene frequency of 0.08 versus 0.01 in control subjects). A rare C4B allele, C4B2.9, was found in 18% of patients with Type 1 diabetes ($n=63$) compared with 1.1% of control subjects ($n=176$). Total C4 deficiency at the C4A locus (C4AQ0,0) was present in 10% of patients with Type 1 diabetes compared with 0% of control subjects. Examination of HLA, C4 and Bf phenotypes in patients with Type 1 diabetes suggested that three high risk supratypes, HLA-A1 B8 BfS C4AQ0 C4B1 DR3; HLA-B18 BfF1 C4A3 C4BQ0 DR3; HLA-A2 CW3 BW62 BfS C4A3 C4B2.9 DR4 are markers for susceptibility alleles.

Key words: HLA, complement allotypes, genetics, diabetes.

Immunogenetic influences conferring susceptibility to Type 1 (insulin-dependent) diabetes are well documented [1]. Increased frequencies of HLA-B8, B15, B18 and B40 have been reported. The primary associations appear to be with DR3 and DR4 with a disproportionate number of DR3/DR4 heterozygotes suggesting interaction between alleles on opposing chromosomes. The likelihood that such susceptibility alleles do exist within the major histocompatibility complex is supported by associations with other genetic markers on chromosome 6 including properdin factor BfF1 [2, 3], the C2 allotype C2B [4] and altered phenotype frequencies of the red cell enzyme glyoxalase [5]. The C4 loci are also located within HLA and are sufficiently polymorphic to help in the characterisation of the putative susceptibility loci involved in the pathogenesis of Type 1 diabetes.

Recently we began an examination of those patients with potentially important supratypes (or combinations of alleles). The supratype marked by HLA-B15 (62) and DR4 was of particular interest because of its occurrence in rheumatoid arthritis as well as Type 1 diabetes [6].

More recently, Bertrams et al. [7] identified a rare C4B allotype, designated C4B4, in 26.5% of 223 patients with Type 1 diabetes but only 7% of 385 healthy control subjects. We now report that this allotype is identical to that we have designated C4B3 [6] and subsequently C4B2.9 [8] and confirm that the B15, C4B2.9, DR4 supratype is increased in frequency in Type 1 diabetes as well as rheumatoid arthritis. Furthermore, we have identified two other high risk supratypes which include B8 and B18.

Materials and Methods

A group of 82 unrelated patients with Type 1 diabetes mellitus, randomly selected from the diabetic clinic of the Royal Perth Hospital, Western Australia was studied. Type 1 diabetes was defined by a tendency to ketosis, non-obesity and absolute insulin requirement within at least 2 years of onset. The onset of diabetes was before the age of 40 years in 64 patients and after the age of 40 years in the other 18. Healthy unrelated control subjects were selected from the West Australian town of Busselton. Both patients and control subjects were of European ancestry.

HLA typing was performed using sera well characterised against cells typed in previous International Workshops. HLA-ABC typing was performed using the standard microlymphocytotoxicity method. DR typing was performed by a microcytotoxicity method using a nylon wool separated B cell enriched population [9].

Three complement components (properdin factor B (Bf), C2 and C4) are encoded by genes in the HLA complex. Current mapping data place these genes as a cluster between HLA-B and D/DR. Bf is controlled by codominant genes at a single locus, with two common alleles, F and S, and two rare alleles, F1 and S1. C4 is controlled by codominant genes at two distinct loci, Chido (C4B) and Rodgers (C4A). At the two loci many structural alleles and two quite frequent null alleles (C4AQ0 and C4BQ0) have been found. The polymorphisms of the complement components have been identified using immunoelectrophoretic techniques.

Table 1. HLA-DR antigen frequencies in Type 1 diabetes and control subjects

HLA-DR	Type 1 diabetes ($n=64$) (%)	Control subjects ($n=544$). (%)
DR2	3[a]	33[a]
DR3	58[a]	30[a]
DR4	42	33
DR3+4	27[a]	6[a]

[a] $p<0.001$

Table 2. Properdin factor B gene frequencies in Type 1 diabetes and control subjects

Allotype	Type 1 diabetes (onset under 40 years) ($n=64$) (%)	Type 1 diabetes (onset over 40 years) ($n=18$) (%)	Control subjects ($n=200$) (%)
S	81	78	77
F	9	14	20
S1	2	0	2
F1	8[a]	8[b]	1[a, b]

[a] $p<0.001$; [b] $p<0.01$

Table 3. C4 A and B phenotype frequencies in Type 1 diabetes and control subjects

Allotype	Type 1 diabetes ($n=63$) (%)	Control subjects ($n=176$) (%)
C4AQ0*	10[a]	0[a]
C4A3	79	80
C4A4	24	18
C4B1	80	88
C4B2	30	20
C4B2.9	18[a]	1.1[a]

[a] $p<0.001$; *only homozygous null phenotype is shown

Properdin factor B typing was performed on serum samples subjected to high voltage electrophoresis on agarose gel medium followed by immunofixation with antiserum to factor B [10]. C4 typing was performed using the immunofixation electrophoresis system of Awdeh and Alper [11] and the glycine-barbital buffer system described by O'Neill et al. [12]. Briefly EDTA plasma samples were frozen to −70 °C within 1–2 h of collection. Before electrophoresis, samples were treated overnight at 4 °C with neuraminidase from Clostridium perfringens (Sigma, St. Louis, Missouri, USA). The treated plasma was subjected to high voltage electrophoresis until an HbS1 marker migrated 7 cm. The gel was then overlaid with anti-C4 (Atlantic/Scarborough, Maine, USA), pressed, washed and stained with Coomassie brilliant blue.

Characteristic bands were identified corresponding to the gene products of the two loci C4A and C4B. Standards were included on each run and assignments made by two independent observers. On occasions the bands of the C4A and C4B loci may overlap. The C4B locus products have greater haemolytic activity than those of the C4A locus. Therefore a haemolytic overlay technique was used to differentiate overlapping bands. Allotypes were numberically designated C4A1–7 and C4B1–7 using the notation of Awdeh and Alper [11]. An unusual C4B variant, designated by us as C4B2.9, was identified. Exchange of sera has confirmed that this variant is identical to the C4B4 variant described by Bertrams et al. [7]. At the Fourth International Workshop for the Genetics of Complement held at Boston July 1982 this variant was distinguished from the C4B3 variant described by Awdeh et al. and associated with 21 hydroxylase deficiency [13].

Allele frequencies in the disease groups were compared to those in the control groups using the χ^2 test with Yates correction. In general it was not possible to assign genotypes and haplotypes as in family studies. The term 'supratype' is used to describe a particular combination of alleles at different loci. In previous studies we have shown that these supratypes are generally, if not always, inherited from one parent but we prefer to use 'haplotype' only when a family study has given unequivocal results. For convenience the three supratypes referred to here are abbreviated as follows: HLA-A2, CW3 BW62, BfS, C4A3, C4B2.9 DR4 (62/4-S-3-2.9); A1, CW7, B8, BfS, C4AQ0, C4B1, DR3 (8/3-S-0-1); B18, BfF1, C4A3, C4BQ0 DR3 (18/3-F1-3-0). These supratypes were assigned whenever all of the relevant complement alleles were represented in the phenotype together with at least one of the B or DR alleles. The only difficulty arose in the case of null alleles at the C4 loci. If there is a complete blank at one locus, homozygous deficiency may be assumed. If two allotypes are identified it can be assumed that null alleles cannot be present. If only one allotype (a) is demonstrated the genotype could be a, a; a, o or a, x where x is an unidentified allele. Distinction between a,a and a,o can often be achieved by comparing the density of the C4A and C4B bands.

Results

As previously reported, significant increases in HLA-B15, B18 and B40 in Type 1 diabetes were found. HLA-DR antigens were examined in 64 patients with Type 1 diabetes and the results are shown in Table 1. The previously documented increased frequencies of HLA-DR3 and DR4 and decreased frequency of HLA-DR2 in Type 1 diabetes compared with control subjects are again noted. When DR antigen combinations are studied the observed frequency of DR3, DR4 heterozygotes in Type 1 diabetes is more than predicted for Hardy-Weinberg equilibrium (27% versus 17%) and nearly five times that observed in the control population (27% versus 6%, $p<0.001$). Properdin factor B allotype frequencies in Type 1 diabetes are shown in Table 2. The unusual allotype BfF1 was present at a gene frequency of 8.0% in Type 1 diabetes compared with 1% in control subjects and the frequency of BfF1 was similar in those with onset greater than and less than 40 years (Table 2). Two BfF1 homozygous patients were identified both of onset less than 40 years. Among the 11 patients with a BfF1 phenotype, nine were HLA-B18 positive and blanks were present in the remaining two patients. HLA-DR3 was present in all nine of the BfF1 positive patients who were DR typed. These findings are consistent with the presence of a 'diabetogenic' supratype marked by B18, BfF1 and DR3.

The frequencies of C4A and C4B phenotypes in Type 1 diabetes versus control subjects are shown in Table 3. The most salient finding is the presence of the rare C4B allotype designated by us as C4B2.9 and found in 18% of Type 1 diabetic patients versus 1.1% of control subjects ($p<0.001$). The variant C4B2.9 migrates electrophoretically slightly cathodal to the C4B3 found in association with 21 hydroxylase deficiency and HLA-BW47.

62/4-S-3-2.9 Supratype

When HLA and Bf were examined in the 11 C4B2.9-positive diabetic patients, all were found to carry BfS. Eight were B15 (BW62) positive and seven were DR4 positive with DR blanks in some of the remaining cases. Accordingly 10 of these 11 patients had the 62/4-S-3-2.9 supratype as defined above.

8/3-S-0-1 Supratype

From Table 3, it can also be seen that there is a significant increase in the frequency of apparent C4AQ0 homozygotes in Type 1 diabetes (10% versus 0%, $p < 0.001$). HLA phenotypes of all six C4AQ0,0 patients with Type 1 diabetes included HLA-A1, B8, C4B1, BfS, DR3 and three of the six appeared to be homozygous at all loci. These findings suggest the HLA-A1, B8, DR3 supratype found in Type 1 diabetes can now be further defined by including C4AQ0, C4B1 and BfS.

18/3-F1-3-0 Supratype

Examination of two presumed BfF1 homozygous patients with Type 1 diabetes disclosed the phenotype B18, C4A3, C4BQ0, BfF1, DR3 in both cases. In addition the other BfF1 positive patients with Type 1 diabetes were B18, C4A3, DR3 and apparently C4BQ0. This suggests that a third supratype 18/3-F1-3-0, is also important.

Discussion

Previously recognised HLA associations with Type 1 diabetes are again confirmed. In particular, the frequency of DR3, DR4 heterozygotes in Type 1 diabetic patients is nearly five times that found in control subjects ($p < 0.01$) and significantly increased even for Hardy-Weinberg expectations within Type 1 diabetes. This agrees with other reports and suggests that susceptibility to Type 1 diabetes involves interaction of major histocompatibility region loci on opposing chromosomes. By helping to map this region, complement markers may help determine whether any such interaction involves identical or differing alleles on opposing chromosomes. Complement allotyping of the C4 and Bf loci has allowed the definition of three supratypes associated with Type 1 diabetes, namely 8/3-S-0-1, 62/4-S-3-2.9 and 18/3-F1-3-0. It seems probable that these supratypes are in linkage with a putative disease locus. Current genetic models of the inheritance of Type 1 diabetes usually assume that susceptibility alleles at the putative disease locus are identical. However, there is evidence that supratypes may have differing genetic effects in relation to disease pattern. The 18/3-F1-3-0 supratype may have an association with younger age of onset [14, 15] though this is not found in all studies [4] and is not obvious from our data. On the other hand, the supratype 62/4-S-3-2.9 may be associated with high titre anti-insulin antibodies [16] and low prevalence of islet cell antibodies [17] while 8/3-S-0-1 probably correlates with low titre anti-insulin [16] and persistent anti-islet cell antibodies [17]. Similarly associations with other diseases and each supratype are distinct. Systematic lupus erythematosus is associated with the supratype 8/3-S-0-1 [18] while idiopathic membranous nephropathy is associated with 18/3-F1-3-0 [19]. In a recent study of a large group of patients with rheumatoid arthritis we have demonstrated the 62/4-S-3-2.9 supratype in approximately 10% of patients compared with 1% of control subjects [6, 8]. Family studies in rheumatoid arthritis indicate 62/4-S-3-2.9 is a haplotype [20]. These findings may explain the observation of an increased prevalence of rheumatoid arthritis in relatives of patients with Type 1 diabetes and vice versa [21].

Thus, apparently differing clinical and immunological associations of the supratypes found in Type 1 diabetes and their overlap with separate immunopathic diseases suggests that linkage with identical disease susceptibility alleles cannot be assumed. Furthermore, examination of the full phenotypes of our patients reveals one or more of the defined supratypes in two-thirds of cases. Indeed, the observed excess of DR3, DR4 heterozygotes largely reflects assortment of these supratypes. Identical susceptibility alleles on all supratypes would not predict an excess of DR3, DR4 heterozygotes over DR3 or DR4 homozygosity as has been documented [22]. Thus the data are most consistent with interaction of different diabetogenic susceptibility alleles associated with discrete supratypes of the major histocompatibility complex.

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Professor R. L. Dawkins
Department of Clinical Immunology
Royal Perth Hospital
Wellington Street
Perth, Western Australia 6000