Thyrogastric autoimmunity and MHC associated alleles at the C4 locus in patients with Type 1 (insulin-dependent) diabetes

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Summary. HLA antigens, complement allotypes, insulin antibodies and thyrogastric autoantibodies were determined in 69 patients with Type 1 (insulin-dependent) diabetes defined by a tendency to ketosis, non-obesity and insulin requirement within 2 years of diagnosis. Analysis of HLA and C4 allotypes suggested that Type 1 diabetes was associated with only certain DR3- and DR4-containing supratypes. Low antibody response to insulin was associated with all HLA-DR3, being present in 89% of those with DR3 compared with 48% of those without. Thyrogastric autoantibodies were associated with a null allele at the C4A locus, usually with HLA-B8-C4AQ0-C4B1-BfS-DR3. These results indicate that, un-

like Type 1 diabetes, low insulin antibody response was associated with all HLA-DR3. Thyrogastric autoantibodies, on the other hand, were associated with a null allele at the C4A locus. It is probable that while interaction between certain HLA-DR3- and -DR4-containing supratypes is important in conferring susceptibility to Type 1 diabetes, other manifestations of autoimmunity are associated with supratypes containing C4AQ0, and in particular the diabetogenic supertype HLA-B8-C4AQ0-C4B1-BfS-DR3.

Key words: Type 1 diabetes, MHC allotype, C4 allotype, heterogeneity, thyrogastric antibodies, insulin antibody.

The association between certain HLA antigens and Type 1 diabetes is well documented [1]. Increased frequencies of HLA-B8, -B15, -B18 and -B40 have been reported but it has been suggested that the primary association is with DR3 and DR4, with a disproportionate number of DR3/DR4 heterozygotes indicating interaction between alleles on paired chromosomes. The likelihood that such susceptibility alleles do exist within the MHC is supported by associations with other genetic markers on chromosome 6, including properdin factor BfF1 [2–3], the C2 allotype C2B [4] and the rare C4B allotype C4B2·9 [5]. The recognition of complement allotypes has permitted refined definition of 'diabetogenic' or possible high risk 'haplotypes' in Type 1 diabetes and indicates that the risk is increased with only certain DR3- and DR4-containing 'haplotypes' or 'supratypes'. The term 'supertype' refers to a combination of alleles recognised from the phenotype.

Three supratypes determining susceptibility to Type 1 diabetes have been identified: B8-C4AQ0-C4B1-BfS-DR3, B18-C4A3-C4BQ0-BfF1-DR3 and B15-C4A3-C4B2·9-BfS-DR4 [6]. These appear to have different clinical associations and disease overlap. The B8-DR3 supertype is associated with low-titre anti-insulin antibodies and persistent islet-cell antibody, and

is increased in other autoimmune diseases. The B15-DR4 supertype is associated with rheumatoid arthritis and the B18-DR3 with idiopathic membranous nephropathy.

In the present study, insulin antibody response and thyrogastric autoimmunity were examined in relation to MHC supratypes in a group of patients with Type 1 diabetes.

Subjects and methods

Subjects

HLA antigens, complement allotypes, insulin antibodies and thyrogastric autoantibodies were determined in 69 unrelated Type 1 diabetic patients defined by a tendency to ketosis, non-obesity and insulin requirement within 2 years of diagnosis.

Methods

HLA typing was performed by the standard microlymphocytotoxicity method on routine laboratory trays [7]. Properdin factor B typing was performed on serum samples subjected to high voltage electrophoresis on agarose gel medium followed by immunofixation and antiserum to factor B [8]. C4 typing was performed using a discontinuous Tris-glycine-barbital buffer system described by O'Neill et al. [9].

Briefly, EDTA plasma samples were frozen to -70°C within 1–2 h of collection, and before electrophoresis were treated overnight at 4°C with neuraminidase from *Clostridium perfringens* (Sigma) [10]. 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), pressed, washed and stained with Coomassie Brilliant Blue.

Characteristic bands were identified, corresponding to the gene products of the two closely-linked loci C4A and C4B. Because the protein products of the two loci sometimes overlap electrophoretically, several standards were included on each run and assignments were made by two independent observers. Where A and B locus bands overlapped a haemolytic overlay was performed. Allotypes were numerically designated C4A₁₋₇ and C4B₁₋₇ in the notation of Awdeh and Alper [10]. Thyroid microsomal antibodies were detected by haemagglutination (Wellcome; cut off $1/1600$) and parietal cell antibodies by immunofluorescence (cut off $1/125$). Insulin antibodies were measured by a radioimmunoassay (Amersham International) with a cut off of $1/160$. Statistical analysis was carried out using χ^2 test with Yates correction.

Results

Insulin antibodies were present in titres of 1/160 or more in 17 patients (25%). The majority of patients were treated with monocomponent insulins but most had received other insulins in the past. Low antibody response to insulin was associated with HLA-DR3 being present in 34 (89%) of those with DR3 compared with 11 (8%) of those without DR3 ($p < 0.01$). In eight patients DR3 was not excluded. All 10 patients with the B18-C4A3-C4BQ0-BfF1-DR3 supratype, 21 patients (88%) with the B8-C4AQ0-C4B1-BfS-DR3 supratype and six patients (86%) with other DR3 supratypes had a low antibody response to insulin. Insulin antibody response did not appear to be related to the presence or absence of HLA-DR4, low response being equally frequent in patients with DR3/DR4 or DR3 alone.

Thyrogastric autoantibodies were present in 16 patients and were associated with supratypes which included a null allele at the C4A locus, usually HLA-B8-C4AQ0-C4B1-BfS-DR3. Significant titres of antibody were present in 11 (41%) of 27 patients with the C4AQ0 allele compared with 2 (93%) of 23 patients in whom this allele was not present ($p < 0.01$). In the remaining 19 patients the presence of C4AQ0 could not be excluded and thyrogastric antibodies were present in three (16%) of these patients. Five patients were homozygous for C4AQ0, but the prevalence of thyrogastric antibodies was similar in this group to that in heterozygotes. The prevalence of autoantibodies was similar in those with B8-DR3 supratypes and in those with other C4AQ0 supratypes.

Discussion

Unlike Type 1 diabetes itself, which is associated with only certain DR3-containing supratypes, namely

B8-C4AQ0-C4B1-BfS-DR3 and B18-C4A3-C4BQ0-BfF1-DR3, a low insulin antibody response was associated with all DR3. Low insulin antibody response did not appear influenced by the presence or absence of DR4 and was equally frequent in patients with DR3 regardless of the presence or absence of DR4. These results would suggest, therefore, that either DR3 itself or genes closely linked to it play a part in determining antibody response to insulin.

Thyrogastric autoantibodies, on the other hand, were associated with supratypes containing a null allele at the C4A locus, usually B8-C4AQ0-C4B1-BfS-DR3. This supratype is also associated with other autoimmune diseases including systemic lupus erythematosus and myasthenia gravis and is increased in Graves' disease and atrophic thyroiditis. It is probable that while interaction between supratypes is important in conferring susceptibility to Type 1 diabetes, other manifestations of autoimmunity are determined by the B8-C4AQ0-C4B1-BfS-DR3 supratype.

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