

Low serum haemolytic function of the fourth complement component (C4) in insulin dependent diabetes

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SUMMARY Low serum concentrations of the fourth component of complement (C4) are found in insulin dependent diabetes, and may be important in the aetiology of the disease. To ascertain whether function of C4 is also impaired both its haemolytic activity and its concentration were measured in 34 insulin dependent diabetics, 15 non-insulin dependent diabetics, 20 healthy subjects, and 12 pairs of monozygotic twins discordant for insulin dependent diabetes. C4 function was measured by a radial immune haemolytic assay, and C4 concentration by laser nephelometry. Both measurements were significantly lower in insulin dependent diabetics (C4 function: median 47%, range 4-100%; C4 concentration: 0.22 g/l, 0.10-0.38 g/l) than in non-insulin dependent diabetics (67%, 33-138%, $p < 0.01$; 0.27 g/l, 0.16-0.50 g/l, $p < 0.02$) and controls (74%, 33-138%, $p < 0.01$; 0.27 g/l, 0.18-0.40 g/l, $p < 0.03$). C4 function and concentration were lower in both diabetic (48%, 12-100%; 0.17 g/l, 0.08-0.31 g/l) and non-diabetic twins (47%, 12-100%; 0.17 g/l, 0.07-0.36 g/l) than controls ($p < 0.01$; $p < 0.01$). Thirteen (38%) of the insulin dependent diabetics had a reduction in either C4 function or concentration, but in only five were both features reduced. Values of function and concentration were strongly correlated in both diabetic and non-diabetic twins ($r = 0.95$, $p < 0.001$; $r = 0.92$, $p < 0.001$). These results show defects in C4 function and concentration in insulin dependent diabetes, which—being present in the non-diabetic co-twin of diabetics—may represent a genetic predisposition to the disease.

Low serum concentrations of the fourth component of complement (C4) are found in about a quarter of insulin dependent diabetics.¹⁻⁴ Concentrations of C4 do not, however, entirely account for its function as an essential component of complement induced cell lysis. The inherited structural polymorphism in C4 protein includes several allelic variants^{5,6} that differ widely in their haemolytic potency.⁷ In addition, post-translational events such as glycosylation could impair the haemolytic activity of C4 in diabetes.⁸

We wondered whether the function as well as the concentrations of C4 were affected in insulin dependent diabetics, and we therefore investigated simultaneously C4 concentrations and function, as measured by its ability to lyse red cells, in patients with insulin dependent diabetes. To assess whether C4 function could be genetically influenced we also studied identical twin pairs discordant for insulin

dependent diabetes (one twin diabetic, the other non-diabetic).

Subjects and methods

We studied 34 patients with insulin dependent diabetes (17 men, mean age (SD) 34 (8) years; 12 had been diagnosed within one year (mean 3 months), 22 had been diagnosed three or more years previously (mean 18)), 15 patients with non-insulin dependent diabetes (seven men, mean age (SD) 61 (17) years), 20 healthy subjects (11 men, mean age (SD) 34 (9) years), and 12 pairs of monozygotic twins (six men, mean age (SD) 22 (15) years), who had been discordant for insulin dependent diabetes for more than one year (mean 9 years).⁹ Serum samples were obtained from each subject and stored at -70°C .

MEASUREMENT OF C4 HAEMOLYTIC FUNCTION
C4 haemolytic function was measured with a radial

immune haemolytic assay.¹⁰ Five millilitres of a 5% suspension of sheep red blood cells (SRBC) (Tissue Culture Services, Slough, Berkshire, United Kingdom) in complement fixing diluent (CFD) (Oxoid, Basingstoke, Hampshire, United Kingdom) were incubated with 10 µl of rabbit antisheep haemolysin (Flow Laboratories, Rickmansworth, Hertfordshire, United Kingdom) for 30 minutes at 37°C. After washing, 5 ml of a 5% suspension of these sensitised SRBC were mixed with an equal volume of C4 deficient guinea pig serum (Porcellus Animal Breeding, Heathfield, Sussex, United Kingdom) and added to 40 ml of a 1.5% solution in CFD of low gelling temperature agarose (FMC Corporation, Rockland, Maine, United States of America) at 45°C. The final mixture was then poured into plates to obtain a 1.5 mm thickness. After gelification, 2.5 mm wells were punched out and 10 µl of serum were added. After incubation overnight at 4°C and thereafter for 60 minutes at 37°C, the diameter of lysis around each well was measured in a photographic enlarger, squared, and then read against a standard curve obtained by double diluting a reference serum five times. The top

point of the standard curve (undiluted reference serum) was arbitrarily set at 100%; thus values of C4 function from the unknowns were expressed as percentage haemolysis.

MEASUREMENT OF C4 CONCENTRATION

C4 concentration was measured by laser nephelometry using a specific nephelometric grade antiserum (Behring Diagnostics, Hounslow, Middlesex, United Kingdom), following the manufacturer's instructions. Results were given in g/l.

Statistical analysis was by the Wilcoxon rank sum test and Spearman's rank correlation.

Results

Serum C4 haemolytic function was significantly lower in insulin dependent diabetics (median 47%, range 4–100%) than in non-insulin dependent diabetics (67%, 33–138%, $p < 0.01$) and controls (74%, 33–138%, $p < 0.01$), in whom it was similar. No significant difference was observed between newly diagnosed and long standing insulin dependent diabetics. In the twins C4 function was lower in both the diabetic (48%, 12–100%) and the non-diabetic (47%, 12–100%) co-twins when compared with controls ($p < 0.01$) (figure).

Median serum C4 concentration was significantly decreased in insulin dependent diabetics (0.22 g/l, 0.10–0.38 g/l) compared with non-insulin dependent diabetics (0.27 g/l, 0.16–0.50 g/l, $p < 0.03$) and controls (0.27 g/l, 0.18–0.40 g/l, $p < 0.03$), in whom it was similar. In the twins both the diabetic (0.17 g/l, 0.08–0.31 g/l) and the non-diabetic (0.17 g/l, 0.07–0.36 g/l) co-twins had lower concentrations than controls ($p < 0.01$).

In all the groups of subjects investigated there were significant positive correlations between C4 function and C4 concentration (insulin dependent diabetics: $r = 0.64$; non-insulin dependent diabetics: $r = 0.67$; controls: $r = 0.64$; diabetic twins: $r = 0.83$; non-diabetic twins $r = 0.74$; in each case $p < 0.01$).

Eleven (33%) of the insulin dependent diabetics had C4 haemolytic function below the normal range and seven (21%) had reduced C4 concentrations. Overall, 13 (38%) of the insulin dependent diabetics showed a reduction in C4 function or concentration; six (18%) had reduced function but normal concentration, two had reduced concentration but normal function, and in five both were reduced.

Diabetic and non-diabetic twins of a pair had similar values for both C4 haemolytic function ($r = 0.95$, $p < 0.001$) and C4 concentration ($r = 0.92$, $p < 0.001$).

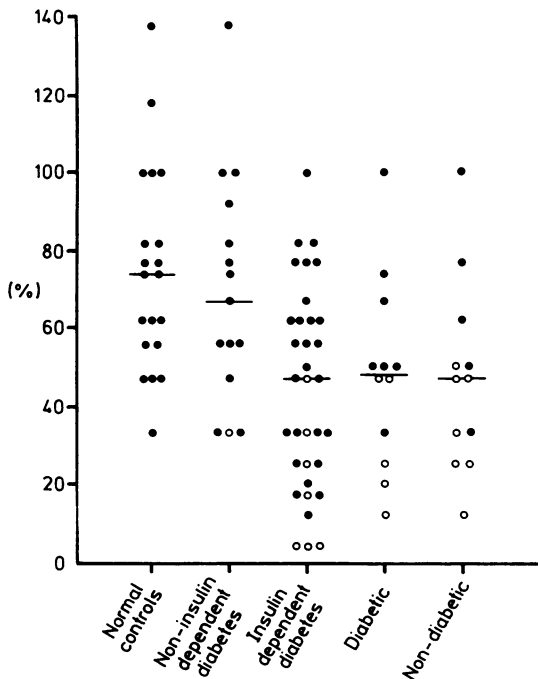


Figure 1 Serum C4 haemolytic function in normal controls, patients with non-insulin dependent diabetes, insulin dependent diabetes, and diabetic and non-diabetic twins. Horizontal bars represent the median values. ●/○ = normal/low concentration.

Discussion

This study shows that serum C4 haemolytic function is reduced in 33% of patients with insulin dependent diabetes and that a decrease of either C4 function or concentration is present in 38%.

Impaired C4 function could result from the metabolic disturbance of diabetes or be due to low concentrations of the C4 molecule. Non-enzymatic glycosylation occurs in diabetes to a sufficient extent to change the function of proteins such as haemoglobin.⁸ Non-enzymatic glycosylation or the metabolic disturbance of diabetes do not, however, seem to affect the haemolytic activity of C4, as C4 function was normal in the non-insulin dependent diabetic patients. In addition, C4 function was reduced in identical co-twins, whether or not they were diabetic. Low concentrations of the C4 molecule could derive from immune consumption or defective production. Immune consumption of C4 is unlikely to result in impaired C4 function in insulin dependent diabetes, as we have found normal concentrations of the activation fragment C4d in 10 diabetic patients,¹¹ three of whom had impaired C4 function. C4 function was strongly correlated in diabetic and non-diabetic identical co-twins, so it is probable that C4 function is genetically determined in insulin dependent diabetes and is derived from an inherited defect.

C4 function was correlated with C4 concentration; thus the reduced synthesis of C4, which both twin and family studies have indicated to be inherited in insulin dependent diabetes,¹ is an important factor in determining low C4 function in this disease. Nevertheless, six (18%) insulin dependent diabetics had reduced function but normal concentration of C4, indicating that in insulin dependent diabetes concentration is not the only factor on which C4 function depends. The discrepancy between function and concentration could be explained by the extensive polymorphism of the C4 molecule.^{5,6} As different C4 allotypes code for variants with different degrees of haemolytic potency,⁷ it is possible that an allelic variant could account for reduced function in spite of a normal concentration.

A genetically determined defect of C4, either in function or concentration, could predispose to the development of insulin dependent diabetes. Susceptibility to diabetes is associated with certain HLA haplotypes,¹² including critical class I, II, and III alleles. Class III genes code for complement factors including C4.⁶ Three extended haplotypes commonly found in patients with insulin dependent diabetes are B8-BfS-C2C-C4AQ0-C4B1-DR3, B15-BfS-C2C-C4A3-C4B3-DR4, and B18-BfF1-C2C-C4A3-C4BQ0-DR3.¹³ These haplotypes not only include C4 silent alleles (C4AQ0 and C4BQ0)¹⁴ responsible for low C4 concentrations,¹⁵ but also contain C4A3, which codes

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for a C4 molecule with poor haemolytic activity.⁷

The association between defective C4 and insulin dependent diabetes could be due to the allotypes coding for defective C4 being in linkage disequilibrium with susceptibility genes, HLA-DR3, and -DR4, or a deficiency of C4 could directly predispose to the disease by impairing the efficiency of the complement system. C4 has a key role in virus neutralisation,¹⁶ and its deficiency might enable a virus to initiate the process leading to insulin dependent diabetes.

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