CLINICAL RESEARCH

Low plasma C4 concentrations: association with microangiopathy in insulin dependent diabetes

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Abstract

Plasma C4 concentrations were measured in insulin dependent diabetics with and without microangiopathy and in controls. The diabetics had significantly lower C4 values than controls ($p < 0.001$), and patients with insulin dependent diabetes and microangiopathy had lower values than those without this complication ($p < 0.001$). There was a 7.1-fold increase in the prevalence of complications in the diabetics with low C4 values. Of 41 diabetics whose rate of albumin excretion was measured, 13 had increased rates and 11 of these had low C4 concentrations.

Low plasma C4 concentration in insulin dependent diabetes is strongly associated with microvascular disease and may identify diabetics with a particular propensity to develop this complication.

Introduction

Although the development of diabetic microangiopathy appears to be strongly related to poor long term metabolic control,^{1,2} many patients remain relatively free of complications despite

inadequate control while others develop problems within a few years after diagnosis. Probably genetic factors also influence the predisposition to complications.^{3,4} Controversy surrounds the possible influence of the HLA system,^{5,6} but support for an immunological basis for microangiopathy comes from studies showing raised values of circulating immune complexes⁷ and insulin binding capacity⁸ in retinopathy and linear deposition of IgG on the glomerular basement membrane in nephropathy.⁹

Recently about one quarter of insulin dependent diabetics have been shown to have low plasma concentrations of the fourth component of complement (C4) irrespective of age and duration of disease.^{10,11} This might be due to either complement consumption or phenotypic variation, the latter having been described.¹² There is a close correlation in C4 values between identical twins, even when discordant for diabetes, which suggests that a low C4 value is inherited.¹¹ Plasma C4 is coded for by two separate loci,¹³ each with several variants resulting in a polymorphic system with the possibility of many different phenotypes.¹⁴ In some people there is deficient expression of the gene, giving rise to low plasma concentrations of the protein.¹³ We have studied the relation of plasma C4 concentrations in an unselected group of insulin dependent diabetics and their non-diabetic relatives to the prevalence of microangiopathy.

Subjects and methods

Group 1 comprised 97 insulin dependent diabetics presenting consecutively at a diabetic clinic. Ages ranged from 16 to 54 years (mean 29 (SD 11)) and durations of diabetes from two to 38 years (mean 12 (SD 8)). All subjects had been diagnosed before 30 years of age, were prone to ketosis, and had been taking insulin since diagnosis. Seventy two subjects had no evidence of microangiopathy and eight had mild background retinopathy but no proteinuria on clinic stick testing. Seventeen subjects had severe microvascular complications: 11 had proliferative retinopathy alone, three proliferative retinopathy and persistent proteinuria, and three background retinopathy (haemorrhages and exudates) and persistent proteinuria.

Group 2 comprised 47 non-diabetics and 10 non-insulin dependent diabetics, including one with proliferative retinopathy. The non-diabetics were selected consecutively from a general medical outpatient clinic and none had evidence of autoimmune disease or a family history of diabetes. Since there was no statistically significant difference in C4 concentrations between the non-diabetics and non-

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insulin dependent diabetics (confirmed with larger numbers elsewhere)¹¹ they were analysed as a single group. Their ages ranged from 15 to 57 years (mean 37 (SD 12)). In addition, a further 10 non-insulin dependent diabetics with severe complications (same criteria as in group 1) were studied.

Group 3 (relatives)—The first 14 of the 26 insulin dependent diabetics with low C4 concentrations were selected for family studies. Twelve of these subjects gave names of close relatives who lived locally and who were not diabetic. Thirty three first degree relatives and three first cousins were studied. None had clinical evidence of autoimmune disease.

Blood samples were taken into edetic acid and the plasma stored at -70°C . C4 concentrations were measured by radial immunodiffusion (assay precision 6%, Serotec Ltd, Oxford). Repeat samples were obtained for most of the insulin dependent diabetics and the mean concentration used.

The following two groups were also selected for further studies. (a) Eight randomly selected insulin dependent diabetics with low C4 values (including three with severe complications) and six insulin dependent diabetics (plus one non-insulin dependent diabetic) with normal C4 values were studied for evidence of complement activation. CH_{50} and functional C4 tests were carried out as well as C2 radial immunodiffusion assays.¹⁵ (b) Forty one of the 97 insulin dependent diabetics (duration of diabetes five years or more) also had a two hour resting urine collection for albumin excretion carried out on two separate occasions five weeks apart, each subject drinking 500 ml water over the first hour. Albumin excretion was measured by single radial immunodiffusion.¹⁶ Mean value in an age matched, non-diabetic control group was 8.1 (SD 6.3) $\mu\text{g}/\text{min}$, and the precision (interassay coefficient of variation) 6%. Values greater than 2 SDs above the mean were taken as abnormal.

The data were analysed using two non-parametric statistical tests. The following comparisons were made: (1) all insulin dependent diabetics (with and without complications) *v* controls; (2) insulin dependent diabetics with complications *v* controls; (3) insulin dependent diabetics without complications *v* controls; (4) insulin dependent diabetics with complications *v* insulin dependent diabetics without complications.

The Kolmogorov-Smirnov test was applied to see if there was a difference in the distribution of plasma C4 concentrations of each group. The Mann-Whitney U test was then applied to establish whether the difference was in the median values of each group. A *t* test was done to see if an observed correlation coefficient was significant for activation studies.

Relative risk (RR) for microangiopathy¹⁷ was calculated using the formula $\text{RR} = (a/b/c)$, where *a* represents the number of insulin dependent diabetics with complications and low C4 values, *b* represents the number of insulin dependent diabetics with complications but normal C4 values, *c* represents the number of insulin dependent diabetics without complications but with low C4 values, and *d* represents the number of insulin dependent diabetics without complications and with normal C4 values.

Results

There was a highly significant difference in distribution and median values of C4 concentrations among the various groups (figure; table I).

Given that the groups had significantly different median values of plasma C4 and no control subject had a value below 0.24 g/l, concentrations of 0.23 g/l or less were defined as low. Overall, of 26 subjects with low C4 values, 14 (54%) had microvascular disease, 10 with severe and four with mild complications. Two subjects with severe and two with

mild complications had been diagnosed only within the past eight years. The relative risk of subjects with low C4 concentrations developing severe microangiopathy was 7.1 and of developing any microangiopathy 6.3. Twelve of the 72 insulin dependent diabetics without complications had low C4 concentrations, but nine of them had been diagnosed only within the past seven years and were therefore unlikely to have developed evidence of microangiopathy. Three out of five tested in this group of 12 in whom albuminuria was measured had increased excretion rates (range 21 – 80 $\mu\text{g}/\text{min}$), including one patient with a duration of diabetes of 29 years. Of the 41 insulin dependent diabetics in whom albuminuria was measured, 13 had increased excretion rates (range 21 – 190 $\mu\text{g}/\text{min}$; mean 51 (SEM 13) $\mu\text{g}/\text{min}$); of these, 11 also had low plasma C4 concentrations.

None of the 11 non-insulin dependent diabetics with severe complications had low C4 values (figure).

Plasma C4 concentrations in various groups of insulin dependent (IDD), non-insulin dependent (NIDD), and control subjects. Bars are median values.

Of the 36 non-diabetic relatives of insulin dependent diabetics with plasma C4 concentrations of 0.23 g/l or less, 9 (25%) also had low values—four out of 15 parents, three siblings, one child, and one first cousin.

Table II shows the interrelations of the various complement components measured. The results indicate that low C4 concentrations as measured immunologically correlated with functional C4 values. Overall complement function as measured by CH_{50} correlated with C2, C3, and C4 concentrations.

Discussion

This study confirms other reports that about a quarter of insulin dependent diabetics have low plasma C4 concentrations^{10 11} and, in addition, identifies a strong association with microangiopathy. Patients with microangiopathy had a different distribution of C4 values not only from controls but also from insulin dependent diabetics without such complications. The

TABLE I—Comparison of distribution and medians of plasma C4 concentrations

	Level of significance*	
	Kolmogorov-Smirnov test	Mann-Whitney U test
All insulin dependent diabetics <i>v</i> controls	$p < 0.005$	$p < 0.001$
Patients with insulin dependent diabetes and microangiopathy <i>v</i> controls	$p < 0.001$	$p < 0.001$
Patients with uncomplicated insulin dependent diabetes <i>v</i> controls	$p < 0.1$	$p < 0.001$
Patients with insulin dependent diabetes and microangiopathy <i>v</i> patients with uncomplicated insulin dependent diabetes	$p < 0.025$	$p < 0.001$

*Kolmogorov-Smirnov test describes difference in distribution between groups and Mann-Whitney U test difference in medians between groups.

association with low C4 value was further emphasised by the fact that many insulin dependent diabetics with a low C4 value but without clinical evidence of complications already showed an increased rate of albumin excretion, which is a predictor for diabetic nephropathy.¹⁸ Most subjects with a low C4 value and no microangiopathy had been diagnosed only within the past seven years and would not normally have been expected to develop complications until later, and half of those tested already showed increased albumin excretion.

The explanation for this association is not clear, but one possibility is the relation of the C4 gene to the major histocompatibility complex. Insulin dependent diabetes is associated with various HLA haplotypes including HLA-B8, B15, DR3, and DR4, and genes controlling C4 are on the same chromosome

TABLE 11—Interrelation of complement test results in 14 diabetic patients

		R	p
C2 v	C3	0.77	<0.001
C2 v	C4	0.47	NS
C2 v	C4f*	0.56	<0.05
C2 v	CH ₅₀	0.74	<0.01
C3 v	C4	0.48	NS
C3 v	C4f	0.6	<0.02
C3 v	CH ₅₀	0.76	<0.001
C4 v	C4f	0.92	<0.001
C4 v	CH ₅₀	0.79	<0.001
C4f v	CH ₅₀	0.81	<0.001

NS = Not significant.

*f = Functional assay.

as the HLA genes.¹⁹ There have been isolated reports of an association between HLA-B8 and nephropathy²⁰ and between HLA-B15²¹ and DR4²² and severe retinopathy, although others have found no association at all.^{5 6 23-26} Indeed, recent reviews conclude that there is little evidence to implicate the HLA complex in the aetiology of microangiopathy.^{6 27}

Another possibility is that the C4 gene products might be implicated in the pathogenesis of microangiopathy. Deficient expression of C4 might lead to failure of clearance of immune complexes resulting in local damage to blood vessel walls.⁷ The C4 produced might also be functionally defective or be consumed. Our studies of the correlation relations between the individual complement proteins suggest activation, but the results are not conclusive. More data and determination of phenotypes should allow this to be assessed.

Studies of identical twins¹¹ and our own family data suggest that a low C4 concentration in insulin dependent diabetes might be inherited. This implies that a low C4 value is due to abnormal phenotypic expression. The relation between C4 phenotypes and microangiopathy is being evaluated. Since seven subjects with severe complications (and all of the non-insulin dependent diabetics with complications) had normal C4 values other factors must also play a part.

We conclude that a low plasma C4 concentration is significantly associated with diabetic microangiopathy and might be a predictor of a subgroup of insulin dependent diabetics who have a particular propensity to develop severe microvascular disease.

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ONE HUNDRED YEARS AGO

A very regrettable case of assault by an infuriated husband, acting under a wholly groundless impression of grievance, on a medical practitioner who had attended the assailant's wife in her confinement, and afterwards received a present from her, again calls attention to the extremely perilous position in which medical men are often unavoidably placed. In this instance the present, a ring, appears to have been sent anonymously, and the recipient had no notion from whom it came. It was, however, it seems, sent without the husband's knowledge, and he not only resented the fact, but, after demanding the return of the ring (which was very properly sent back when demanded), waylaid and assaulted the medical man. It was inevitable that the case should come into court, and Dr. Howell of St. John's Wood has, to the entire satisfaction of the magistrate by whom the matter was heard, vindicated his character, and cleared his reputation from a serious charge. There was abundant evidence of misconception on the part of the husband, and a great presumption of actual delusion on the part of the wife. The disagreeable business is well ended; but, while it passes out of memory, let us again urge, with all the earnestness of which we are capable, the need and importance—vital importance often—of avoiding friendly relations with female patients. We are strongly of opinion that the more strictly and exclusively professional the intercourse of medical men and female patients, the better for all parties. We would even go so far as to insist on the prudence of observing even punctilious etiquette, and the restriction of all intercourse to purely medical directions and remarks, so far as may be compatible with the requirements of perfect courtesy. It is impossible to be too careful, and difficult to be careful enough. (*British Medical Journal* 1884;ii:1026.)