Low serum C4 concentrations: an inherited predisposition to insulin dependent diabetes?

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Abstract

Twenty two out of 86 insulin dependent diabetics had serum C4 concentrations below the normal range. None of 41 non-insulin dependent diabetics tested had low concentrations. Low C4 values were seen in insulin dependent diabetes irrespective of the duration of the disease and did not appear to correlate with complement activation. There was a close correlation in C4 values between identical cotwins, even when only one was diabetic.

These results suggest that a low serum C4 concentration is an inherited phenomenon and may predispose towards the development of insulin dependent diabetes.

Introduction

Complement is required for the mediation of immune processes that result in destruction of pancreatic islet cells. An antibody directed against the surface of islet cells is found in insulin dependent diabetes and, in the presence of complement, destroys islet cells in vitro.[1] Another antibody that appears in most patients with newly diagnosed insulin dependent diabetes fixes complement in vitro.[2] Possibly complement fixation by antibodies in vivo mediates destruction of B cells.

Rare phenotypes of C4 appear to have an increased frequency in insulin dependent diabetes.[3 4] These phenotypes might act as a genetic marker for the disease[4] or alternatively possession of particular phenotypes may affect the functional activity of C4 and directly predispose towards the development of this type of diabetes.

To study the role of the complement system in insulin dependent diabetes we measured the serum complement factors C3 and C4 in insulin dependent and non-insulin dependent diabetics. C3 is the central component of the complement cascade belonging to both the classical and alternative pathways. C4 is exclusive to the classical pathway. We assessed complement activation in vivo by measuring C3d (a product of C3 activation).[5] We also looked for possible activators of the classical pathway, such as islet cell antibodies (both conventional and complement fixing) and circulating immune complexes. To assess the genetic influence on the complement system we tested a series of identical twins both concordant and discordant for the insulin dependent form of the disease.

Patients and methods

Group 1 comprised 86 patients with insulin dependent diabetes. Of these, 14 (8 male, 6 female; age range 2-37 years, mean 19·6) had been diagnosed within one year and 72 (32 male, 40 female; age range 8-77 years, mean 29·7) had had the disease for 2-53 years (mean 12·5 years).

Group 2 comprised 41 non-insulin dependent diabetics (none receiving insulin). Sixteen were men and 25 women, mean age was 62·4 years (range 39-80), and mean duration of their disease was 9·2 years (range 2-49). All were studied as outpatients.

Twins—Thirty two pairs of monozygotic twins were studied. Of these, 16 were concordant (both diabetic) and 16 discordant (only one twin diabetic) for insulin dependent diabetes. All the unaffected twins in the discordant pairs had had normal oral glucose tolerance test results and at the time of study had normal random blood glucose and HbA_1 concentrations. Each discordant pair had been discordant for at least five years.

Serum and plasma (taken into edetic acid (EDTA)) were obtained from each patient and stored at −70°C. Concentrations of C3 and C4 were measured by laser nephelometry using specific nephelometric grade antisera (Behring). The normal range for C3 is 0·55-1·2 g/l and for C4 0·2-0·6 g/l. The C3d concentration was measured as follows. Plasma in edetic acid was brought to 11% final concentration of polyethylene glycol 6000 and centrifuged at 1500 *g* for 30 minutes at 4°C. This precipitated native C3 and C3b, leaving C3d in the supernatant. The concentration of C3d was then measured by laser nephelometry using C3d antiserum (Dako, Mercia-Brocades Ltd). Results were expressed in mg/dl using a C3d standard (Behring). Among 32 normal healthy control subjects the mean plasma C3d concentration was 0·5 mg/dl (SD 0·1). Conventional cytoplasmic islet cell antibody and complement fixing islet cell antibodies were determined according to Bottazzo *et al*[2] with a minor modification for complement fixing islet cell antibodies. This consisted in allowing the in vitro complement fixation step to occur at 37°C rather than 20°C.

Circulating immune complexes were measured by C1q binding assay.[6]

Statistical analyses were with Student's *t* test, the χ^2 test with Yates's correction, Fisher's exact test, and coefficient of correlation.

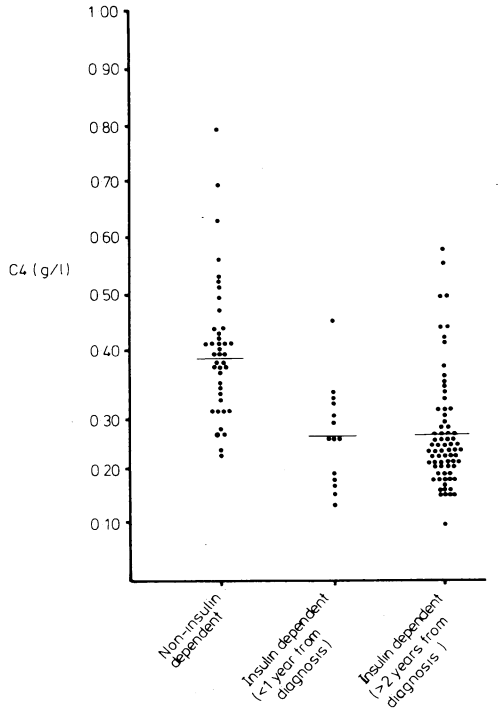

FIG 1—Serum C4 values in insulin dependent and non-insulin dependent diabetes.

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Results

Serum C4 concentrations were significantly lower in the insulin dependent diabetics (group 1) than in the non-insulin dependent diabetics ($t=5{\cdot}2$; $p<0{\cdot}001$) (fig 1). In the insulin dependent group there was no difference in concentrations between those with newly diagnosed disease and those whose disease had been diagnosed two to 53 years before ($t=0{\cdot}32$; $p>0{\cdot}05$).

Twenty two of the 86 patients in group 1 (25·6%) had serum C4 values below the lower limit of the normal range. The prevalence of low values was not related to the duration of diabetes, being as common in those diagnosed within one year (5/14) as in those with disease of longer duration (17/72—$\chi^2=0{\cdot}38$; NS). There was no difference ($t=1{\cdot}24$) in mean age between insulin dependent diabetics with low values (23·8 ± SEM 2·7 years) and those with normal values (28·5 ± SEM 1·9 years). This excluded an influence of age on C4 values, and in the remaining analyses the results for the insulin dependent diabetics were therefore combined.

The presence of islet cell antibodies (both conventional and complement fixing) was more frequent in the insulin dependent than non-insulin dependent diabetics ($p<0{\cdot}001$). There was no significant difference, however, in the frequency of circulating immune complexes or raised C3d or low serum C3 concentrations (table I).

TABLE II—*Correlation between markers of complement activation and low C4 values in insulin dependent diabetics*

	Cytoplasmic islet cell antibody	Complement fixing islet cell antibodies	Circulating immune complexes	Low C3	Increased C3d
Low C4	18/22 (82%)	10/22 (45%)	4/12 (33%)	2/22 (9%)	2/14 (14%)
Normal C4	37/64 (58%)	19/64 (30%)	9/49 (18%)	5/63 (8%)	10/41 (24%)
Total tested	86	86	61	85	55
p*	0·0267	0·0834	0·1549	0·329	0·233

* Values of p calculated with Fisher's exact probability test, where correlation is significant when $p<0{\cdot}05$.

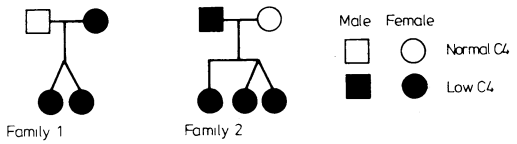

FIG 3—Distribution of low C4 values in two families.

TABLE I—*Comparison between insulin dependent and non-insulin dependent diabetics and possible markers of complement activation*

	Low C3	Increased C3d	Circulating immune complexes	Cytoplasmic islet cell antibody	Complement fixing islet cell antibodies
Insulin dependent	7/85 (8%)	12/55 (22%)	12/61 (20%)	55/86 (64%)	30/86 (35%)
Non-insulin dependent	0/41	3/23 (13%)	12/32 (38%)	5/41 (12%)	1/41 (2%)
	NS	NS	NS	$\chi^2=27{\cdot}62$; $p<0{\cdot}001$	$\chi^2=14{\cdot}12$; $p<0{\cdot}001$

NS = Not significant.

Table II shows the correlation between low serum C4, raised C3d (>3 SD), and low serum C3 concentrations and the presence of cytoplasmic islet cell antibody and circulating immune complexes in the insulin dependent group. Conventional islet cell antibody was the only factor that showed a significant correlation with low serum C4 values, being present in 18 subjects of the 22 with low values.

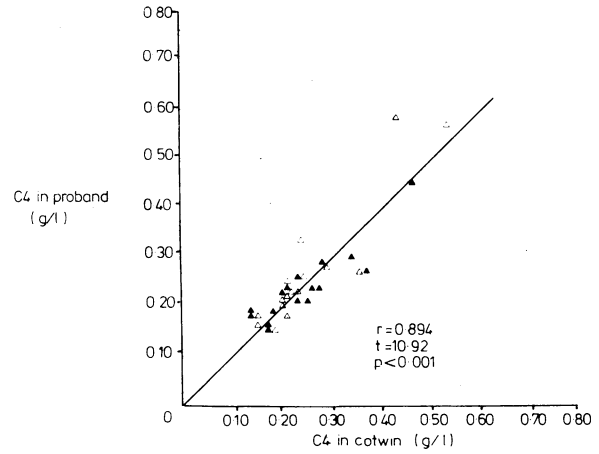

FIG 2—Correlation in serum C4 values between cotwins. Proband refers to diabetic in discordant pairs and first diagnosed in concordant. Closed symbols refer to discordance (16 pairs), open symbols to concordance (16 pairs).

Figure 2 shows the serum C4 concentrations in the 32 pairs of identical twins. There was a close correlation in values between the cotwins of each pair, whether they were concordant or discordant for insulin dependent diabetes ($r=0{\cdot}894$; $p<0{\cdot}001$). In five discordant pairs both twins had low C4 concentrations, indicating that low values may occur in non-diabetics. In each of the two families tested one parent as well as the twins had low serum concentrations of C4 (fig 3).

Discussion

Low serum C4 concentrations were found in 22 out of 86 insulin dependent diabetics but in none of 41 patients with non-insulin dependent disease. Low C4 values might therefore be associated with either the presence of or the genetic predisposition to insulin dependent diabetes.

Reduced C4 values might result from either increased a complement consumption or reduced synthesis or a combination of the two. C4 consumption usually occurs by activation of the classical pathway, triggered by a reaction between an antigen and a complement fixing antibody. Activation of the classical pathway also includes C3, leading to the formation of split products such as C3d and reducing the serum concentration of the native protein.

The insulin dependent diabetics with low C4 values (with the exception of one patient with newly diagnosed diabetes in whom a low value was associated with the presence of complement fixing islet cell antibodies and low C3 and raised C3d concentrations) did not show any evidence of complement activation. Of the possible activators of the classical pathway, only the presence of conventional islet cell antibody correlated with low C4 concentrations, but this might have been due merely to a common association with this type of diabetes.[7] A low serum C4 concentration was as frequent in those with longstanding disease as in those newly diagnosed. Furthermore, we were unable to detect any evidence of complement activation. Thus the low C4 values did not appear to be due to consumption.

The concentrations in identical twins suggested that reduced C4 may be an inherited phenomenon. There was a close correlation between cotwins in each pair, which was true whether both or only one was diabetic. Low serum concentrations of C4 were seen in the affected and unaffected twins of five discordant pairs, showing that the reduction was not due to the presence of insulin dependent disease.

These unaffected cotwins may have been developing diabetes, though we think that this is unlikely because (*a*) only pairs that had been discordant for at least five years were included, and most concordant twin pairs become concordant within a short

period[8]; and (*b*) none of the subjects had any chemical evidence of diabetes. Hence the low values in identical twins were more likely to be a consequence of a shared genetic factor.

Further support for the low concentrations of C4 being inherited came from the study in the families of two pairs of twins, where in each case one parent also had low values.

Serum C4 is coded for by two separate gene loci,[9] each with a variable number of alleles resulting in a polymorphic system with the possibility of many different phenotypes.[10] Some of these phenotypes contain null alleles which might result in deficient expression of the gene, giving rise to lower serum concentrations of the protein.[10] Neutralisation of viruses by human sera low in C4 is impaired,[11] and in an animal model a low C4 value was associated with an altered immune response.[12] Thus a low serum C4 concentration could directly predispose towards the development of insulin dependent diabetes.

The genes coding for C4 are located within the HLA region[12 13] on the sixth chromosome, and there is linkage disequilibrium between alleles at the C4 locus and DR antigens.[12 13] In populations of insulin dependent diabetics the genes of C4 that contain null alleles are in linkage with the antigens DR3 and DR4. Hence the low concentrations of C4 as found in this series may have only an indirect association with insulin dependent diabetes, the primary association being with the DR locus.[14]

Even if the primary association is with the DR locus, however, the contribution of specific genes for C4 inherited with DR antigens in a particular haplotype could result in subjects at greater risk.[14] Further studies of serum C4 in relation to its function and HLA and C4 phenotypes both in insulin dependent diabetics and in non-diabetics will show whether the low serum C4 concentration does or does not confer a primary association with insulin dependent diabetes.

We thank Dr D A Pyke for allowing us to study the twins and for his critical comments on the manuscript, Dr B Stagg (Behring), for donating the C3d standard, and Mrs A Spink for secretarial help.

NB-A is sponsored by University Kebangsaan, Malaysia, and CJ is and AHB was sponsored by the Medical Research Council.

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(*Accepted 27 January 1983*)

ONE HUNDRED YEARS AGO The morning bath holds a well-established place among domestic arrangements. Cold water is usually and properly preferred for this purpose by those who can bear it. Apart from its cleansing property, which is valuable as promoting excretion from the skin, and so relieving deeper excretory organs, especially the kidneys, we can, by means of cold water, apply a degree of cold to the surface of the body which, when suitably regulated, is both salutary and agreeable. Such a shock essentially constitutes a check upon the peripheral blood-vessels, causing their contraction and a concentration of the blood contained in them on the heart and internal organs. Associated with this, and practically inseparable from it, is nervous shock, of which the sensation of cold is the indication. This vascular repression, however, has its natural and unfailing counterpoise in the reaction by which a healthy heart, stimulated by the temporary and unwonted resistance, redoubles its action to overcome it, and succeeds in flushing anew the surface-channels of the circulation. We may thus regard the action of cold, in moderation, on the muscle of the heart, as a means of seasonably exercising this most important of our vital organs, and thus of maintaining its tone. It is clear, from the above remarks, that we do not oppose the practice of morning bathing, but rather approve it. But a caution must accompany every general statement, and this is no exception. There are those whom a cold bath injures, instead of invigorating. The readiest test of benefit is the glow of free surface-circulation, or at least the absence of any decided sense of chill after immersion. Some do not experience this. Among these are the subjects of heart-weakness, arising from whatever cause; it may be consequent on organic disease of the heart, on old gout or rheumatism, or on overwork and underfeeding, in which case it is a part of a general debility. Again, there is in some a tendency to engorgement of one or other deep-seated organ with blood, a kidney, the liver, etc. This is commonly the result of a previous inflammatory attack, or of visceral disease at the time existing. Surface-cold aggravates the congestive tendency. Obviously, therefore, such persons, if they bathe, ought to use tepid water; and, in renal disorders, this method is often advantageous. In slight cases, cold is not injurious if the ablutions be expeditiously gone through, and restricted to periods of summer weather. The aged should avoid cold-baths, and commonly do. Infants, if ordinary despatch is used in bathing and in dressing, have no reason to fear them. Their powers of reaction are excellent. We will not say that children suffer seriously from the maternal dread which warms their morning tubful; but they lose somewhat by the want of that salutary exercise of the circulatory organs which we have mentioned. In the absence of actual disease, debility, if present in a decided degree, contraindicates sudden cold affusion. We say decided, because there are lesser degrees of atony which are the better for it. These are not far below the level of health, and retain a fair measure of latent energy capable of development. All persons in health and of average strength may use a cold bath daily, in summer at least. In winter, we have mornings of extreme frost, which try the strongest constitution. Only the strongest are likely to benefit by a plunge on these occasions. Fortunately, with us they are rather the exception than the rule; and, for the most part, individuals of moderate powers and free from disease may carry the cleanly practice of summer through the winter months. A word on the bath itself. Those who take to it should begin in summer, not winter, and so become gradually accustomed to its lowest temperature. No one should linger over it; three or four minutes are ample. After immersion, the body should be quickly and well dried and rubbed before dressing. Light gymnastic, dumb-bell, or club-exercise, may occupy the next few minutes, the clothes being partly on if the weather be cold, and breakfast, or a cup of warm tea or coffee, should shortly follow, to prevent chilling. (*British Medical Journal*, 1883; ii:687.)