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Anti-cardiolipin antibodies and circulating immune complexes in type 1 diabetes mellitus: increased prevalence and relation to vascular complications

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SUMMARY

Anti-cardiolipin antibodies, oxidatively modified low-density lipoproteins (oxLDL) and circulating immune complexes are humoral factors that have been linked to vascular damage. To analyse their possible role in the vascular complications in type 1 diabetes mellitus, we investigated patients with and without vascular complications (retinopathy, nephropathy, polyneuropathy, foot ulcers). The patients were matched for age, sex and duration of diabetes. The patients were also compared with 102 healthy individuals. Anti-cardiolipin antibodies of IgG and IgA type were more common in patients compared with healthy individuals. There was no difference between patients with and without vascular complications. There was no increased prevalence of IgM anti-cardiolipin antibodies, but the levels of these antibodies were higher in patients with vascular complications compared with patients without complications and controls. Eighty-three percent of patients had circulating immune complexes in comparison with 5% of healthy individuals. Such complexes were more common in patients with complications. Both the prevalence and the levels of immune complexes were higher in patients with null alleles of complement factor C4. Patients with vascular complications had higher prevalence of C4A than of C4B null alleles. Anti-cardiolipin antibodies were present in higher relative concentrations in immune complex form than in serum in all six patients analysed. There was no increased prevalence of antibodies against oxidatively modified LDL in the patients. The higher prevalence and levels of anti-cardiolipin antibodies and circulating immune complexes in patients with vascular complications suggests that these humoral factors might be involved in the vascular complications of type 1 diabetes mellitus.

Keywords anticardiolipin antibodies immune complexes diabetes mellitus

INTRODUCTION

Diabetes has been shown to be an independent risk factor for the development of atherosclerosis and its complications. The pathophysiological process is incompletely understood. Humoral factors associated with accelerated atherosclerosis are anti-cardiolipin antibodies (aCL), other negatively charged phospholipids, antibodies against oxidatively modified low-density lipoproteins (oxLDL) and circulating immune complexes (CIC). aCL have been found in association with systemic lupus erythematosus (SLE) [1], thrombovascular disease [2], thrombocytopenic purpura [3] and myocardial infarction (MI) [4]. Patients with type 2 diabetes (NIDDM) have a higher prevalence of antiphospholipid (aCL and/or phosphatidylserine) antibodies and the highest prevalence is observed in patients with macrovascular complications [5]. The role of aCL in type 1 diabetes mellitus has not been studied.

Antibodies against oxidatively modified LDL (oxLDL) have been associated with the development of atherosclerosis [6], MI [7] and peripheral vascular disease [8]. In NIDDM there was no association between these antibodies and development of atherosclerosis [9], but their role in type 1 diabetes has not been investigated.

CIC cause inflammation of the vessel wall and accelerate atherosclerosis both in experimental animal models and in humans [10]. Complement factor 4 (C4) plays a central role in the solubilization and clearance of CIC [11]. C4 is encoded at two highly polymorphic loci, C4A and C4B. One of the two C4A or C4B genes may be null (deficient or non-expressed), termed as C4A*Q0 or C4B*Q0. Null alleles are associated with immune complex diseases such as SLE and have been implicated in immune complex disease associated with premature MI [12].

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Patients with anti-phospholipid syndrome (APLS) have a high prevalence of CIC containing these antibodies [13].

The purpose of this study was to investigate the possible contribution of the humoral factors aCL, anti-oxLDL antibodies and CIC to the vascular complications in type 1 diabetes mellitus.

PATIENTS AND METHODS

Patients

Sera of 38 patients, 20 male and 18 female, with type 1 diabetes mellitus (c-peptide negative, early onset, ketosis-prone, insulin therapy required at diagnosis) (mean age 46·8 years, range 29–80 years) treated at the Department of Endocrinology and Diabetology at the Karolinska Hospital, were studied. The patients had been selected from a group of patients who were observed for 7 years. Half of the patients had developed vascular complications at time of study. The groups were matched with regard to age, sex and duration of disease. Criteria for exclusion were co-existent autoimmune disorders, recent infective diseases and age > 80 years. Vascular complications were defined by microangiopathy (presence of retinopathy assessed by routine ophthalmological examination performed by an ophthalmologist, microaneurysms with exudate and/or bleeding), nephropathy defined as stable microalbuminuria of > 35 mg/24 h), peripheral polyneuropathy assessed by sensation test, tendon reflexes and vibration test with tuning fork. Six patients had foot ulcers. Nineteen patients without vascular complications had a mean diabetes duration of 18 years, and 19 patients with vascular complications had a mean diabetes duration of 20 years. Blood was collected in the morning after overnight fasting. All patients had given their informed consent to the study.

CIC and C4 allotypes were determined in the 38 patients and in an additional 14 type 1 diabetic patients with vascular complications defined as above. The study was approved by the local ethics committee.

Control population

The control group was composed of 102 age- and sex-matched healthy individuals. Among them, 69 controls were randomly selected from a population-based register from the Stockholm County and 33 controls were randomly selected from healthy blood donors. Of the 102 healthy controls, 56 were males and 46 were females (mean age 47·8 years, range 16–72 years). Individuals with autoimmune diseases were excluded.

Routine laboratory evaluations

The haematological investigations, acute-phase parameters and serum biochemistry were done at the Department of Clinical Chemistry, Karolinska Hospital. The analysis of complement factors C3, C4 and C3d and immunoglobulins was carried out in the Department of Clinical Immunology, Karolinska Hospital.

Autoantibodies

Rheumatoid factor, autoantibodies against nuclear antigens, granulocytes, proteinase 3, myeloperoxidase, smooth muscle and mitochondria were analysed in the Department of Clinical Immunology, Karolinska Hospital.

Determination of aCL

aCL were measured by ELISA, as described [14,15]. Both sera and immunoglobulins from isolated immune complexes were tested using this method.

Determination of antibodies against oxLDL

This was performed by ELISA as described [16].

Quantitative determination of CIC

This was performed by density gradient centrifugation and gel filtration as described [13].

Isolation of CIC by precipitation

This was performed as described [17].

Determination of C4 allotypes

This was performed as described [18].

Statistical evaluation

The Mann–Whitney *U*-test was used for comparing data. Z statistics were used for comparison between proportions of two groups.

RESULTS

General investigations

Patients with signs of vascular complications had higher urinary albumin excretion ($P < 0{\cdot}05$), higher ASAT ($P < 0{\cdot}005$), lower C4 ($P < 0{\cdot}05$) and higher IgE ($P < 0{\cdot}05$) values than patients without such complications.

Anti-cardiolipin antibodies

The levels of aCL in all patients, with and without vascular complications, and controls are reported in Fig. 1. The levels of

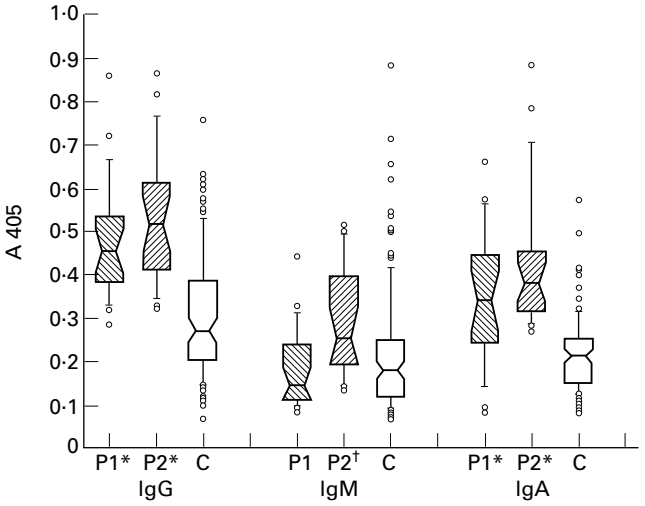

Fig. 1. Box plot showing the levels of anti-cardiolipin antibodies in patients without vascular complications (P1), patients with vascular complications (P2) and healthy controls (C). The levels are expressed as optical density (OD) values. The lower, mid and upper horizontal lines of the boxes represent 25th, 50th, and 75th percentiles, respectively; the vertical lines extend from the 10th to the 90th percentile. *Statistically different from controls ($P < 0{\cdot}001$); †statistically different from patients without complications (P1) and controls (C) ($P < 0{\cdot}005$).

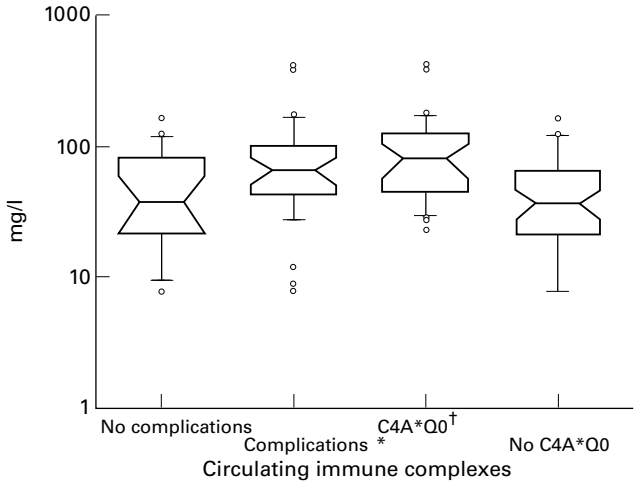

Fig. 2. Box plot showing the levels of circulating immune complexes in patients with no vascular complications, patients with vascular complications, patients with C4A*Q0 and patients with no C4A*Q0. The lower, mid and upper horizontal lines of the boxes represent 25th, 50th, and 75th percentiles, respectively; the vertical lines extend from the 10th to the 90th percentile. *Statistically different from patients with no complications ($P < 0{\cdot}05$); †statistically different from patients with no C4A*Q0 ($P < 0{\cdot}005$).

IgG and IgA aCL were higher in both patient groups than in the control group ($P < 0{\cdot}001$). There was no difference in patients with and without complications. The levels of IgM aCL were higher in patients with vascular complications than in patients without complications or controls ($P < 0{\cdot}005$).

The prevalence of IgG aCL was 21% (8/38) in all patients and 4·9% (5/102) in controls ($P < 0{\cdot}005$). The prevalence in patients with complications was 31·5% (6/19) ($P < 0{\cdot}001$ compared with controls) and in patients without complications was 10·5% (2/19) ($P > 0{\cdot}05$ compared with controls). There was no significant difference between patients with and without complications.

The prevalence of IgA aCL was 44·7% (17/38) in all patients and 6·8% (7/102) in controls ($P < 0{\cdot}0001$). The prevalence in patients with complications was 47·3% (9/19) and in patients without complications was 42·1% (8/19) ($P > 0{\cdot}0001$ compared with controls). There was no difference between patients with and without complications.

The prevalence of IgM aCL did not differ between patients and controls.

Antibodies against oxLDL

There was no increased prevalence or levels of antibodies against oxLDL in both patient groups with and without vascular complications when compared with controls.

*Circulating immune complexes and C4A*Q0*

The concentrations of CIC are shown in Fig. 2. Eighty-three percent of patients had CIC in comparison with 5% of the controls. The prevalence in patients with complications was 90·9% and in those without complications was 68·4% ($P < 0{\cdot}05$). Patients with complications also had higher concentrations of CIC than those without complications (median concentration 67 mg/*l* *versus* 38 mg/*l*, $P < 0{\cdot}05$).

Table 1. Relative comparisons of circulating immune complex (CIC)–anti-cardiolipin antibody (aCL) concentrations with serum–aCL concentrations. The comparisons were made using the amounts of immunoglobulins that gave the same absorbance value in the ELISA determination of aCL. The reactivity for the serum–aCL was set to 1·0 [13]

Patient	IgG	IgM	IgA
1	2	8	42
2	4	8	79
3	8	12	61
4	2	25	130
5	7	5	61
6	5	3	163

Of patients with vascular complications, 16/33 had C4A*Q0 compared with 10/19 patients without complications. Patients with complications more often had C4A*Q0 than C4B*Q0 (16/33 *versus* 5/33, $P < 0{\cdot}01$). The prevalence of CIC in patients with C4A*Q0 was higher than in patients with no C4A*Q0 (96·1% *versus* 69·2%, $P < 0{\cdot}05$), and there was also a difference in the concentrations of CIC (median concentration 81·5 mg/*l* *versus* 37 mg/*l*, $P < 0{\cdot}005$) (Fig. 2).

All the six patients tested had higher reactivity against cardiolipin in CIC than in serum. This difference was most pronounced for IgA aCL (Table 1). The binding curves of non-complexed antibodies in serum and antibodies derived from CIC are shown in Fig. 3a–c. The slope of the binding curve was steeper and the maximal binding was obtained using lower concentrations of antibodies in the case of CIC-derived aCL compared with serum aCL. This could indicate that aCL derived from CIC might be concentrated in immune complex form or they might have a higher affinity than those derived from serum.

DISCUSSION

The humoral factors analysed in this study have all been reported to be associated with vasculitis and also with atherosclerosis and its complications [4,7,8,10,12,15]. In this study, we have tried to analyse their association with microangiopathy/atherosclerotic complications in type 1 diabetes. There are few reports on aCL in association with diabetes mellitus. In one study, the overall frequency of anti-phospholipid antibodies in NIDDM was 51% and the highest prevalence was observed in patients with macrovascular complications (86%). The predominant isotypes of the antibodies were IgG and IgA [5]. In another report, low titres of aCL were observed in a mixed patient with type 1 and NIDDM [19]. Our results show aCL of both IgG and IgA type with increased prevalence in patients and with no difference in patients with and without vascular complications. Regarding aCL of IgM type, there was a higher level in patients with vascular complications only.

What initiates the formation of aCL is unknown. The higher levels of IgM might indicate an ongoing and thus a more active immune/inflammatory reaction against cardiolipin in patients with vascular complications. IgA antibodies are usually initiated at the mucosal level and IgA aCL are common after certain gastrointestinal and respiratory infections, possibly because of the frequent occurrence of cardiolipin on certain microorganisms [20]. The

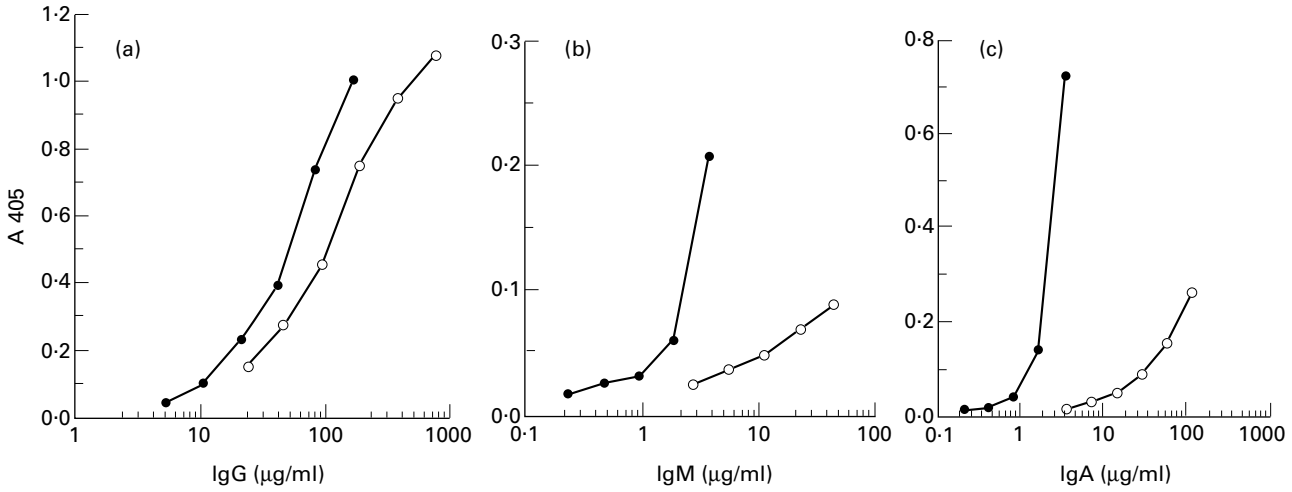

Fig. 3. (a–c) Binding curves showing binding to cardiolipin by immunoglobulins from circulating immune complexes (●) and from serum (○) in patient 1.

inducing event for these serum antibodies in diabetes might be an infection on the mucosal surface.

The mechanism of action of aCL is also poorly understood. These antibodies can bind to the phospholipids of endothelial cells [21] and to the phospholipid epitopes on platelet membranes [3], causing platelet activation and aggregation. A plasma cofactor named β_2-glycoprotein I (β_2-GPI) is required for most antibody–phospholipid interactions [22] and β_2-GPI-dependent aCL induce hypercoagulation or thrombus by blocking the inhibitory effect of β_2-GPI on the activation of factor X [23].

CIC are clearly related to both acute and chronic vascular damage and elevated levels are present in both NIDDM and in type 1 diabetes mellitus [24,25]. An increased prevalence of IgA and/or IgG immune complexes was found in patients, more frequently in patients with microvascular complications [26,27]. In our study, using a quantitative method for the estimation of CIC, we found an increased prevalence in all patients and an even higher one in patients with vascular complications. CIC may induce platelet activation and aggregation and also activate the complement system via the classical pathway [28–30]. Our patients with vascular complications had lower C4 levels, suggesting such an activation of the complement cascade. The higher IgE levels in these patients is another, although non-specific, indication of inflammatory vascular damage, since higher IgE levels are common in patients with certain vasculitis.

The complement C4 is of major importance for the clearance of immune complex from circulation, and C4A is four times more efficient than C4B [31]. The relation between C4A*Q0 and prevalence and concentration of CIC in our study reflects the importance of C4A for CIC clearance. Moreover, the higher prevalence of C4A*Q0 in patients with vascular complications further supports the critical role of C4A and suggests that patients with C4A*Q0 are specially predisposed to immune complex-induced vascular complications.

Of interest was the relative concentration of aCL in CIC. Such a concentration was also present in our earlier study [13] and this presence of anti-cardiolipin reactivity in complexes might endow them with special pathogenic potential, for example with regard to binding to membrane phospholipids in addition to the Fc binding.

There are conflicting reports on the association of autoantibodies against oxLDL with atherosclerotic vascular disease in NIDDM [9,32], and no such studies have been reported in type 1 diabetes. In the present study, there was no increased prevalence or levels of anti-oxLDL antibodies in patients.

In conclusion, the higher prevalence and levels of aCL and CIC in type 1 diabetes mellitus patients with vascular complications point towards a possible role of these humoral factors in the pathogenesis and/or progression of vascular complications in this disease.

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