Anticardiolipin antibodies in patients with primary immunodeficiency diseases

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SUMMARY The presence of antibodies to cardiolipin was determined (by an ELISA) in 143 patients with primary immunodeficiency diseases. Thirty (21%) had raised anticardiolipin antibody levels compared with only three in 98 age matched controls. The highest prevalence of this autoantibody was found in the Wiskott-Aldrich syndrome. Patients with selective IgA deficiency also showed a high prevalence of this autoantibody (32%), while patients with severe defects in antibody production showed a low prevalence or did not have such autoantibodies. This study provides further evidence of the association between autoimmune phenomena and primary immunodeficiency diseases.

Key word: autoimmunity.

Primary immunodeficiency diseases (ID) have been associated with an increased prevalence of autoimmune disorders and multiple positive laboratory tests for autoantibodies.^{1–3} Regulatory defects in the immune response, increased susceptibility to viral and bacterial infections, and unusual antigenic stimulation could underlie these associations.

Recently, a lot of attention has been focused on antiphospholipid antibodies, mainly anticardiolipin antibodies (ACLAs). The presence of ACLAs has been found associated with venous or arterial thrombosis, or both, thrombocytopenia, and recurrent fetal loss in patients with systemic lupus erythematosus and other autoimmune diseases.^{4–7} Furthermore, several investigators have found ACLAs in other conditions in which thrombosis, as well as vascular and neurological features, is prominent.^{8–9} The presence of ACLAs has also been reported in patients with various manifestations of early onset occlusive vascular disease¹⁰ and in young survivors of myocardial infarction.¹¹

Because of the frequent occurrence of autoimmune manifestations in patients with ID we have evaluated the occurrence of ACLAs, measured by an enzyme linked immunosorbent assay (ELISA), in 143 patients with ID.

Patients and methods

PATIENTS

We studied 230 serum samples from 143 subjects diagnosed as having ID according to the 1986 report of the WHO scientific group on primary immunodeficiency diseases.¹² The age of the patients varied between 6 months and 47 years, 95 of them were male and 48 female. Table 1 summarises the diagnoses of the patients. Most of the patients with selective IgA deficiency had been referred for treatment of an underlying medical problem.

AUTOANTIBODY ASSAYS

Antinuclear, anti-smooth muscle, antimitochondrial, antireticulin, and anti-native DNA (nDNA) antibodies were measured by an indirect immunofluorescence technique.¹³ Sections of rat liver, kidney, and stomach were used for 'tissue' autoantibody determination, and *Crithidia luciliae* for anti-nDNA autoantibodies. Autoantibodies to thyroglobulin and thyroid microsomal antigens were assayed with a haemagglutination test kit (Ames, Miles, Laboratories Inc, Indiana, USA). Rheumatoid factor was determined by nephelometry (ICS, Beckman Instruments Inc, Brea, California, USA).

ANTICARDIOLIPIN ASSAY

IgG and IgM anticardiolipin levels were measured using the ELISA technique reported by Gharavi *et al*.

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al¹⁴ with slight modifications. Briefly, each well of polyvinylchloride plates (Dynatech Laboratories, Alexandria, Virginia, USA) was first coated with 50 µl of cardiolipin (Sigma Ltd, Poole, UK) (50 µg/ml in ethanol) by evaporation overnight at 4°C. Plates were washed with phosphate buffered saline (PBS; 0.14 M NaCl, 0.01 M sodium phosphate pH 7.2), and were then blocked by 90 minutes' incubation at room temperature with 75 µl/well of 10% calf serum (CS) in PBS (PBS/CS) to prevent non-specific binding of immunoglobulins to the well surface. After three washes with PBS 50 µl samples of controls and sera to be tested, diluted 1/50 in PBS/CS, were added to each well and the plates incubated for two hours at room temperature. After three washes with PBS 50 µl of alkaline PBS/CS diluted 1/1000 was added to each well, and the plates were incubated for 90 minutes at room temperature. After washing seven times 50 µl of *p*-nitrophenyl phosphate (1 mg/ml) (Sigma) in 1 M diethanolamine buffer pH 9.8 was added to each well, and the plates were incubated at 37°C for 30 minutes. The reaction was stopped with 100 µl of 3 M NaOH, and the optical absorbance was read at 405 nm with a Titertek Multiskan (Flow Laboratories, Irvine, UK). An antibody response curve with an ACLA positive serum as well as appropriate controls were included in each plate to assess the accuracy of the assay. Results were expressed as a binding index (BI) calculated from optical density (OD) values at 405 nm as follows:

$$BI = \frac{OD(\text{test sample}) - OD(\text{blank})}{OD(\text{negative pool}) - OD(\text{blank})}$$

An ACLA result was taken to be positive when

the BI was more than 3SD above the mean of 98 normal controls (mean controls+3SD=2.8BI).

Results

Thirty of the 143 patients studied showed significantly increased levels of ACLAs (Table 1). The prevalence of this autoantibody was higher in the population with ID (21%) than in 98 age matched controls, of whom only three were positive (BI=3.3, 3.1, 3.8). There was no relation between the age of the patient and ACLA positivity.

The highest prevalence of this autoantibody was found in the patients with Wiskott-Aldrich syndrome (4/5). Two of the seven patients with ataxia-telangiectasia and two of the five patients with Di George syndrome were also ACLA positive. Patients with X linked agammaglobulinaemia, common variable immunodeficiency, and severe combined immunodeficiency showed a low prevalence or did not have such antibodies. In contrast, patients with selective IgA deficiency proved to have a high prevalence of ACLAs (32%). Seven of the 50 IgA deficient patients also had IgG2 deficiency (more than 2SD below the mean values for their age). Two of these seven patients were ACLA positive. The ACLA prevalence in this group was similar to that found in the whole IgA deficient population.

In 27/30 patients with ACLAs the isotype was only of the IgG class. In the only positive patient suffering from immunoglobulin deficiency with increased IgM the autoantibody was only of the IgM isotype. Two other patients had ACLAs of the IgM class—namely, one patient with Di George syndrome and one with selective IgA deficiency, both of these also had ACLAs of the IgG class.

Table 1 Prevalence of anticardiolipin antibodies in various primary immunodeficiency disorders

Diagnosis	Number of cases	Binding index			Total number of positives	Per cent positive
		3-5	5-10	Over 10		
X linked agammaglobulinaemia	15	—	—	1	1	6.6
Immunoglobulin deficiency with increased IgM	6	—	—	1	1	16.6
Selective IgA deficiency	50	4	10	2	16	32
Selective IgM deficiency	3	—	—	1	1	33.3
Transient hypogammaglobulinaemia of infancy	3	—	—	—	0	0
Common variable immunodeficiency	29	—	1	1	2	6.9
Severe combined immunodeficiency	11	—	—	—	0	0
Wiskott-Aldrich syndrome	5	—	3	1	4	80
Ataxia-telangiectasia	7	—	1	1	2	28.5
Di George syndrome	5	—	1	1	2	40
Chronic mucocutaneous candidiasis	4	—	—	1	1	25
Hyper IgE syndrome	5	—	—	—	0	0
Total	143	4	16	10	30	21

Except for four patients, no association was found between the presence of anti-tissue antibodies, anti-nDNA antibodies, antithyroid antibodies, rheumatoid factor, and the presence of ACLAs. These four patients comprised two with Wiskott-Aldrich syndrome who were rheumatoid factor positive, one with common variable immunodeficiency with high titres of IgG and IgE anti-IgA antibodies, and one patient with rheumatoid arthritis and IgA deficiency who showed low titres of antinuclear, anti-extractable nuclear antigens, and anti-smooth muscle autoantibodies, but was rheumatoid factor negative. No other patients with autoantibodies against nucleus, smooth-muscle, thyroid, nDNA, reticulin, or rheumatoid factor were found to have ACLAs. The ACLAs in patients with rheumatoid factor did not disappear when the serum samples were previously incubated for 30 minutes at 37°C with IgG coated latex particles, (Rapitex-RF, Behring) and the rheumatoid factor activity was removed.

From some of these patients more than one serum sample could be studied. In most of them the ACLAs persisted over the years, but in a few, especially in one patient with Wiskott-Aldrich syndrome and one with immunoglobulin deficiency with increased IgM, positive values were found in only one of the several serum samples studied. The ACLA positivity could not be related to other analytical or clinical findings.

Table 2 summarises the clinical features of our patients with selective IgA deficiency. ACLA posi-

tivity was mainly associated with recurrent upper respiratory infections, recurrent diarrhoea, and rheumatoid arthritis.

Discussion

We have observed a high prevalence of ACLAs (21%) in the patients with ID studied. This prevalence is similar to the total prevalence (18%) of other autoantibodies (antinuclear, anti-smooth muscle, anti-nDNA, antithyroid, and rheumatoid factor) studied by our group in a similar population.³

As expected, most of the ACLA positive patients suffered from diseases in which antibody production is only partially impaired. The prevalence of this autoantibody is lower in those conditions in which the disease is characterised by a severe defect in antibody production (severe combined immunodeficiency, X linked agammaglobulinaemia, and common variable immunodeficiency).

The prevalence of ACLAs in our IgA deficient patients is comparable with that of antibodies against nDNA, IgM, or native collagen previously described in this condition.¹⁻¹⁵ Various mechanisms, not mutually exclusive, could underly the high prevalence of autoantibodies in selective IgA deficiency. In a series of IgA deficient patients we were able to show a relation between immunoregulatory T cell abnormalities—namely, lack of T cell suppression, and autoimmune phenomena.¹⁰ Moreover, it has been suggested that these autoantibodies are present not merely owing to a defect in the control of antibody production.¹⁵ Owing to the lack of secretory IgA, environmental antigens that have a tendency to induce a systemic immune response against cross reactive autoantigens may be absorbed from mucous surfaces. Evidence for increased absorption of food proteins in IgA deficiency is given by Cunningham-Rundles *et al.*¹¹ In accord with this most of our IgA deficient ACLA positive patients suffered from recurrent upper respiratory infections or recurrent diarrhoea, five of them being diagnosed as having gluten associated enteropathy.

The highest prevalence of ACLA positivity was found among the patients with Wiskott-Aldrich syndrome. Such a finding may be germane to the well known occurrence of autoimmune phenomena in these patients, such as autoimmune haemolytic anaemia,¹⁸ autoimmune thrombocytopenia,¹⁹ autoantibodies to a B cell subset,²⁰ etc. Moreover, paraproteins, usually of a transient nature, have been found in these patients.²¹ Immunoregulatory T cell alterations may account for both abnormalities.²² As children with this syndrome are

Table 2 Clinical features of patients with selective IgA deficiency

Clinical feature	Number of patients studied	Anticardiolipin antibodies	
		IgG	IgM
Asymptomatic	3	—	—
Recurrent upper respiratory infections	19	7	—
Chronic osteomyelitis	1	—	—
Recurrent diarrhoea	7	5	1
Asthma	2	—	—
Spondylarthritis	2	—	—
Rheumatoid arthritis	4	3	—
Progressive systemic sclerosis	2	—	—
Vasculitis	2	—	—
Autoimmune haemolytic anaemia	2	1	—
Alopecia	2	—	—
Vitiligo	1	—	—
Raynaud syndrome	1	—	—
Recurrent parotitis	1	—	—
Nephrosis	1	—	—

thrombocytopenic at birth this has led to the assumption that the thrombocytopenia could not have been acquired. The frequent association of ACLAs with thrombocytopenia,²³ however, and the raised levels of platelet bound IgG found in several patients affected by the Wiskott-Aldrich syndrome,¹⁹ suggest a superimposed autoimmune thrombocytopenia in some of these patients.

Two of the five patients with Di George syndrome were found to be ACLA positive. Both patients reconstituted their immune function—one after a thymus implant and the other after prolonged thymopoietin treatment. In one of these patients an autoimmune haemolytic anaemia developed simultaneously with the ACLA positive test. As in the cases reported by Ammann *et al.*,² these findings suggest that under certain circumstances the recovery of a previously depressed T cell function could lead to immunoregulatory abnormalities and result in autoantibody formation.

High levels of ACLAs are associated with pathological conditions such as thrombosis or thrombocytopenia, or both. This kind of pathology is uncommon in patients with primary immunodeficiency diseases. During our 15 year experience in the management of these patients we have only recorded two cases of thromboembolic disease occurring in patients with hyper IgE syndrome, neither of whom had ACLAs.

Long term follow up of patients with ACLAs will be required to determine the transience or persistence as well as the significance of the presence of this autoantibody in the absence of autoimmune disease.

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