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Anticardiolipin antibodies: occurrence in Behçet's syndrome

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SUMMARY Anticardiolipin antibodies have recently been described in association with arterial and venous thrombosis, and with neurological symptoms, in connective tissue diseases. In a study of 70 patients with Behçet's syndrome 13 patients had these antibodies. Of these 13 patients eight had a history of either retinal vascular pathology, cerebral infarction, or thrombophlebitis. The association of retinal vascular disease and the presence of anticardiolipin antibodies was statistically significant.

Key words: retinal vascular disease, cerebral infarction, thrombosis.

We have recently described a sensitive solid-phase radioimmunoassay for the detection of antibodies against the phospholipid cardiolipin.[1] Initial studies from this unit have shown that such antibodies have been associated with vascular disease, thrombosis, and cerebral infarction.[1 2] These antibodies are members of a group which include antibodies directed against cephalin and sphingomyelin.

In a study of patients with systemic lupus erythematosus Harris *et al.*[1] showed the presence of anticardiolipin antibodies in eight of nine patients with cerebral infarction and 24 of 27 patients with arterial or venous thromboses. Anticardiolipin antibodies have been described in a patient with Degos's disease[3], a condition characterised by thrombosis and vasculopathy of the neurological system, gastrointestinal tract, and the skin. They have also been shown in a patient with acute Guillain-Barré syndrome as a complication of a connective tissue disease.[4]

One condition in which thrombosis and vascular and neurological features are prominent is Behçet's syndrome. The classical triad of recurrent orogenital ulceration and uveitis[5] is increasingly being recognised as part of a multisystem disorder which may affect the retina and vascular and nervous systems. Its aetiology remains obscure, though genetic, viral, toxic, and allergic factors have been implicated.[6] Serological markers have been unhelpful in the diagnosis and management of Behçet's syndrome.

In view of the encouraging results of earlier studies from this unit in Behçet's syndrome[1 7] and the similarity of syndromes previously described in association with these antibodies we undertook a larger study of 70 patients with Behçet's syndrome to assess the frequency of anticardiolipin antibodies.

Materials and methods

Seventy patients with Behçet's syndrome were studied. Forty originated from Italy, 19 from the United Kingdom, 10 from Middle Eastern countries, and one from the West Indies. Sixty-eight patients fulfilled the criteria of O'Duffy[8] or the criteria of definite Behçet's syndrome of Mason and Barnes.[9] The remaining two patients had orogenital ulceration only. Patients were not known to possess antinuclear antibodies.

Case records were reviewed for clinical symptoms with particular reference to thrombotic and neurological complications. All patients had slit-lamp examination of their eyes, and the diagnosis of

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Table 1 *Comparison of clinical features of patients with normal and elevated anticardiolipin antibodies*

Clinical feature	*Raised anticardiolipin antibody*		*Normal cardiolipin antibody*	
	n=13	*% in group*	*n=57*	*% in group*
Oral ulcers	12	92	55	96
Genital ulcers	10	77	48	84
Uveitis	6	46	42	74
Retinal vascular disease	7	54	13	23*
Erythema nodosum	1	7	21	37*
Other skin diseases	7	54	17	30
Thrombophlebitis	2	15	13	22
Arthritis	11	87	40	70
Chest disease	2	15	4	7
Cardiac disease	1	7	1	2
Gastrointestinal symptoms	2	15	11	19
Epididymitis	—	—	2	3·5
Vascular	1	7	3	5
Neurological disease	4	31	14	25

* 0·05> p> 0·02.

retinal vascular disease was made by fluorescein angiography.

Anticardiolipin antibody levels of immunoglobulin classes G and M were determined by the modified solid phase radioimmunoassay method described by Harris *et al.*[1] Cardiolipin in ethanol was absorbed to the surface of polyvinyl microtitre plates by evaporation under nitrogen. The plates were blocked with 1·5% BSA/0·1% gelatin solution for two hours at room temperature and were washed once with the BSA/gelatin solution. Aliquots of 100 µl of patient sera appropriately diluted in BSA/gelatin solution were added in duplicate and the plates incubated for four hours. After incubation samples were aspirated from the wells and the plates washed twice with 0·3% solution of gelatin in phosphate buffered saline (PBS) and once with 1% BSA in PBS. Aliquots of 100 µl of ^{125}I labelled affinity-purified rabbit antihuman IgG or IgM in 1% BSA/PBS were then added to each well and incubated overnight at room temperature. The labelled antibody was then aspirated and the plates washed three times in 1% BSA in PBS. The plates were dried and the wells cut out and counted. Each plate included 12 normal controls and two positive controls. An elevated anticardiolipin antibody level was taken as 3·0 standard deviations above the mean of normal controls.

Table 2 *Clinical features of 13 patients showing IgM or IgG anticardiolipin antibodies*

Patient	*SD* above mean*	*Principal clinical features*
1	13·6	Retinal vein occlusion, retinal vasculitis, cerebral infarction
2	13·0	Retinal vasculitis
3	6·8	—
4	5·3	Thrombophlebitis, deep vein thrombosis
5	4·4	—
6	4·2	Retinal vasculitis
7	3·8	—
8	3·7	Retinal vasculitis, thrombophlebitis
9	3·7	Retinal vasculitis
10	3·4	—
11	3·1	—
12	3·0	Retinal vasculitis, epilepsy
13	3·0	Retinal vasculitis

* SD=standard deviation.

Results

Thirteen of the 70 patients tested showed significant levels of anticardiolipin antibodies. Seven patients had IgG antibody, three had both IgG and IgM, and three had IgM antibodies only.

The group possessing the antibody was compared with the group without the antibody (Table 1). The incidence of major symptoms such as orogenital ulceration, uveitis, and arthritis were not significantly different. Of the 20 patients with retinal vascular disease seven showed the antibodies, and the difference was statistically significant by the χ^2 test ($p<0·05$). Erythema nodosum had a negative correlation with the presence of the antibody ($p<0·05$).

Patients possessing the antibodies were ranked and their clinical associations tabulated (Table 2). Eight patients had features previously associated with the presence of anticardiolipin antibodies.

Discussion

Anticardiolipin antibodies appear to belong to a subgroup of antiphospholipid antibodies that include the lupus anticoagulant. Several studies have shown a relationship between the lupus anticoagulant and venous and arterial thrombosis.[2 10–12] More recently work in our laboratory has demonstrated a strong correlation between anticardiolipin antibodies, the lupus anticoagulant, and thrombosis in patients with SLE and 'lupus-like' disorders.[1] These antibodies have also been shown to cross-react with the brain phospholipids, cephalin and sphingomyelin.[13] This suggests that these antibodies

may make up a subpopulation of antibodies directed against central nervous system tissue which have already been demonstrated in cerebral lupus[14–19] and other diseases including Behçet's syndrome.[20–24] Inaba and Aoyama have demonstrated antibodies to complex-lipid determinants of brain tissue membranes in Behçet's syndrome.[25] We have demonstrated anticardiolipin antibodies in four patients with central nervous system disease (one patient with cerebral infarction, two with meningoencephalitis, and one with epilepsy) and seven patients with retinal vascular pathology. Of the 70 patients reported in this study 13 had elevated anticardiolipin antibodies. A significant association has been demonstrated in retinal vascular disease, though there appears to be a negative correlation with another form of vasculopathy, erythema nodosum. The two patients with the highest anticardiolipin antibodies showed evidence of retinal vasculitis, retinal vein occlusion, and cerebral infarction in one and retinal vasculitis in the other.

It is probable that Behçet's syndrome represents a number of pathological processes forming a symptom complex. Our observations of anticardiolipin antibodies in this syndrome may therefore have pathogenetic implications in a subgroup of patients with Behcet's syndrome.

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