Anticardiolipin Antibodies in Patients with Behçet's Disease

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Anticardiolipin antibodies(ACA) were assayed by an enzyme-linked immunosorbent assay(ELISA) in 68 patients with Behçet's disease. Twenty seven(39.7%) patients showed levels of ACA five standard deviations above the value of the control group. The frequency of ACA isotype IgM was found to be significantly increased in these patients. However, ACA was not found to have a significant association with clinical activity, thrombosis, positive Venereal Disease Research Laboratory(VDRL) test or antinuclear antibodies(ANA).

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Behçet's disease is a multisystemic disorder with involvement of veins and arteries of all sizes, causing significant morbidity and mortality.[1] The etiology of the vessel involvement has not been explained. Nevertheless, there is significant evidence suggesting that endothelial cell injury modulated by enhanced neutrophil activity,[2] increased release of neutrophil-derived oxygen intermediates,[3] and production of anticardiolipin antibodies(ACA) play a pathogenic role in the development of the vascular complications of Behçet's syndrome.[4] To date, there is no direct in-vivo evidence for ACA being pathogenic. However, there is in-vitro evidence, some of it conflicting, that ACA may indeed play a part in the disease process.[4,5] Antiphospholipid antibodies including ACA, lupus anticoagulant, or anti-Venereal Disease Research Laboratory(VDRL) antibodies are a group of circulating autoantibodies seen primarily in patients with systemic lupus erythematosus(SLE), other autoimmune diseases, and a variety of seemingly unrelated diseases.[5] These antibodies can be detected various tests such as enzyme-linked immunosorbent assay (ELISA) and radioimmunoassays.[6,7] ELISA is currently considered the method of choice due to its higher sensitivity and specificity.[8,9] The present study was designed to determine serum levels of ACA in our Behçet's disease patients by ELISA in relation to clinical activity, clinical type, thrombosis, positive VDRL test and anti-

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nuclear antibodies.

MATERIALS AND METHODS

Materials

Sixty-eight patients(30 males, 38 females) with Behçet's disease, who were enrolled in the Behçet's Disease Speciality Clinic of Severance Hospital, were studied(Table 1). Each met the diagnostic criteria for Behçet's disease proposed by Shimizu et al.[10] or Lehner and Barnes.[11] Serum samples obtained from each patiet were tested for the presence of anticardiolipin antibodies, antinuclear antibodies, VDRL test, serum immunoelectrophoresis, C_3 and C_4. Control blood samples were obtained from 20 healthy blood donors.

Classification of the Patients

The patients were classified by the following three methods:

1. Classification by Shimizu et al.[10]
   1) The complete type, which has all four clinical manifestations, including oral ulcers, genital ulcers, skin and ocular lesions.
   2) The incomplete type, which presents with three of the clinical manifestations, or ocular lesions together with another lesion.
   3) The suspected type, which exhibits two of the clinical manifestations.
   4) A possible type, which has only one of the clinical manifestation.
2. Classification by Lehner and Barnes[11]
   1) The mucocutaneous type involves oral and genital ulcers with or without skin lesions.
   2) An arthritic type with joint involvement has some or all of the mucocutaneous manifestations.
   3) A neurologic type with brain involvement demonstrates some or all of the lesions found in the mucocutaneous and arthritic types.
   4) The ocular type with uveitis shows some or all of the mucocutaneous, arthritic and neurologic manifestations.
3. Classification according to clinical activity[12]

The clinical symptoms at the beginning of this study included oral ulcer, genital ulcer, skin manifestations such as an erythema nodosum-like lesion or an erythema multiforma-like lesion, eye involvement, arthritis, thrombophlebitis and neurologic manifestations. One point was given for each clinical symptom, and the sum was defined as the number of clinical activities.

Table 1. Patient characteristics

Clincal type	No.(%) of patients			Mean age (Year)
	Male	Female	Total	
By Shimizu et al.[10]				
Complete	9	6	15(22)	31.0
Incomplete	13	19	32(47)	37.2
Suspected	3	9	12(18)	32.3
Possible	5	4	9(13)	39.7
By Lehner and Barnes[11]				
Mucocutaneous	11	18	29(43)	37.1
Arthritic	3	9	12(18)	37.3
Neurological	1	0	1(1)	30.0
Ocular	15	11	26(38)	32.5
Total	30	38	68(100)	35.3

ELISA of Anticardiolipin Antibodies

The ACA levels in the patient's serum were measured according to Biswas et al.[13] Briefly, one hundred microliters(ul) of a 100ug/mℓ solution of bovine heart cardiolipin(Sigma, St. Louis, MO) in absolute ethanol was placed into each well of a flat-bottomed microtiter plate. One hundred ul of absolute ethanol was then placed into each of the next remaining wells. The plates were then placed in a desiccator and left overnight in the dark at room temperature. The following day, the dry plates were washed five times with phosphate-buffered saline (PBS). One hundred microliters of Dulbecco's PBS(D-PBS), containing 10% heat-inactivated fetal calf serum(FCS), was added to each well, and plates were incubated for one hour at room temperature followed by washing with PBS. The sera to be screened for ACA were diluted in D-PBS with 10% FCS to 1:20 for the ACA-IgG or 1:40 for the ACA-IgM test. One hundered microliters of test or standard serum dilution was placed in the coated and control wells of the microliter plates. The screening and quantitative plates were incubated at room temperature for two hours and then washed. One hundred microliters of the appropriate dilution of alkalin phosphatase-conjugated goat antihuman IgM(1:200) or goat antihuman IgM(1:100) was added, and the plated were incubated at room temperature for one hour, followed by washing. One hundred microliters of freshly prepared substrate(1mg/mℓ of p-nitro-phenyl phosphate in diethanolamine buffered, pH 9.8, Sigma) was added, and plates were incubated. The reaction was terminated with 3M NaOH, and the absorbents were read at 405nm in ELISA reader.

Statistical Analysis

Positivity of ACA in our Behçet's disease patients was determined by ELISA in relation to clinical activity, clinical type, thrombosis, positive VDRL test and antinuclear antibodies. ACA level was considered positive if they exceed 5.0 standard deviations above the mean of the normal population. Positive ACA levels were those that exceed 0.245 optical density(O.D.) in IgG and 0.347 O.D. in IgM. The statistical significance of the results was evaluated by the chi-square method.

RESULTS

Anticardiolipin antibodies in Behçet's disease

The results of these measurements are given in Table 2. Higher than normal anticardiolipin antibody titer was found with high frequency($p<0.05$); particularly significant was the high frequency with which IgM isotype was found($p<0.01$). There was no significant difference in IgG isotype titer compared to a normal healthy person. There was no significant difference between the anticardiolipin antibody in regard to age(Table 3).

Table 2. Number of patients with anticardiolipin antibodies(ACA) in Behçet's disease

	Patients(%) (n=68)	Controls(%) (n=20)	p value
IgG &/or M ACA isotype	27(40)	2(10)	$p<0.05$
IgM ACA isotype	18(26)	0(0)	$p<0.01$
IgG ACA isotype	10(15)	2(10)	NS

NS : Statistically not significant

Relation between clinical type and anticardiolipin antibodies

According to Shimizu's classification,[10] the percentage of anticardiolipin antibody decreased in the following order; complete type, incomplete type, suspected type, and with no antibody found in the possible type(Table 4). According to Lehner's classification,[11] the percentage of anticardiolipin antibody decreased in the following order; neurological type, mucocutaneous type, ocular type and arthritic type (Table 5).

Relation between clinical activity and anticardiolipin antibodies

The score of clinical activities found at the time of the test was more than 2 in the case of anticardiolipin antibody positives(Table 6).

Table 3. Number of patients with anticardiolipin antibodies(ACA) in Behçet's disease by age

Age(yr)range	IgG ACA(%)	IgM ACA(%)	IgG &/or M ACA(%)
10−19	0/ 3(0)	0/ 3(0)	0/ 3(0)
20−29	2/13(15)	2/13(15)	4/13(31)
30−39	6/33(18)	11/33(33)	16/33(48)
40−49	1/13(8)	4/13(31)	5/13(38)
50−59	1/ 4(25)	1/ 4(25)	2/ 4(50)
60−69	0/ 2(0)	0/ 2(0)	0/ 2(0)
Total	10/68(15)	18/68(26)	27/68(40)

Table 4. Number of patients with anticardiolipin antibodies(ACA) in Behçet's disease according to classification by Shimizu et al.

Clinical type	IgG ACA(%)	IgM ACA(%)	IgG &/or M ACA(%)[a]
Complete	6/15(40)	4/15(27)	9/15(60)
Incomplete	3/32(9)	12/32(38)	15/32(47)
Suspected	1/13(8)	2/13(15)	3/13(23)
Possible	0/ 8(0)	0/ 8(0)	0/ 8(0)
Total	10/68(15)	18/68(26)	27/68(40)

[a]$p<0.05$

Table 5. Number of patients with anticardiolipin antibodies(ACA) in Behçet's disease according to classification by Lehner and Barnes.

Clinical type	IgG ACA(%)	IgM ACA(%)	IgG &/or M ACA(%)
Mucocutaneous	4/29(14)	22/29(26)	25/29(86)
Arthritic	0/12(0)	9/12(75)	9/12(75)
Neurological	0/ 1(0)	1/1(100)	1/1(100)
Ocular	7/26(27)	19/26(73)	20/26(77)
Total	11/68(16)	51/68(75)	55/68(81)

Table 6. Number of patients with anticardiolipin antibodies(ACA) in Behçet's disease according to clinical activities

Score	IgG ACA(%)	IgM ACA(%)	IgG &/or M ACA(%)
1	0/ 8(0)	0/ 8(0)	0/ 8(0)
2	1/ 8(13)	3/ 8(38)	4/ 8(50)
3	1/19(5)	4/19(21)	5/19(26)
4	5/20(25)	6/20(30)	11/20(55)
5	3/13(23)	5/13(38)	7/13(54)
Total	10/68(15)	18/68(26)	27/68(40)

Relation between thrombosis and anticardiolipin antibodies

Intravascular thrombosis was found in 11% of the patients with positive anticardiolipin antibody, and the isotype was always IgM(Table 7).

Table 7. Frequency of thrombosis in Behçet's disease with positive anticardiolipin antibodies(ACA)

		Thrombosis positive(%)
IgG & or IgM ACA	positive	3/27(11)
	negative	7/41(17)
IgG isotype	positive	0/10(0)
	negative	2/58(3)
IgM isotype	positive	3/18(17)
	negative	8/50(16)

Relation between laboratory findings and anticardiolipin antibodies

The results of the antinuclear antibody test were documented in only one patient who was negative for ACA. VDRL test, serum immunoelectrophoresis, C_3 and C_4 were all negative or within normal limits.

DISCUSSION

Since the etiology of Behçet's disease remains an enigma, there has been no unifying hypothesis to explain the underlying pathogenisis for the abnormalities observed in this syndrome. However, recently there has been significant evidence suggesting that production of anticardiolipin antibody may play a pathogenic role in the development of the vascular complication of Behçet's disease.[4] In our study, we found about forty percents of Behçet's disease patients were ACA positive, and the presence of ACA was not influenced by age, clinical activity, or the clinical type as for Lehner and Barnes classification.[11] All patients with only one symptom had oral ulcers, and under the Shimizu et al.[10] classification, were classified as "possible type". Because all "possible type" patients by classification by Shimizu et al.[10] had no ACA, the number of clinical symptoms found at the time of the positive anticardiolipin test was two or more. Pereira et al.[14] reported IgG isotype was detected mainly in Behçet's disease patients with "ocular type", according to the classification by Lehner and Barnes[11](30 %). A high incidence of ACA isotype IgG was also found in our study(27%). The presence of antiphospholipid antibodies(including antibodies to cardiolipin, lupus anticoagulants, and false-positive serologic tests for syphilis) in systemic lupus erythematosus(SLE) is associated with a syndrome characterized by thrombosis, recurrent abortion, and thrombocytopenia.[15,16,17] We now use the term 'Antiphospholipid syndrome' to characterize a complex of clinical and pathologic findings mediating a group of antibodies formed against a family of antiphospholipids.[15,18,19]

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Antiphospholipid antibodies associated with autoimmune disease are oligonal or polygonal, most often IgG or IgM, and rarely IgA or a combination of these.[20] Our results showed a higher incidence of positive ACA than the most recent reports in the literature(Fig. 1) except the data of Bergman et al.,[21] in which 50%(13 in 26 patients) of Behçet's disease patients had positive ACA. In accordance with a report by Bergman et al.[21], we also found that only the frequency of positive ACA IgM isotype was found tc be significantly increased in Behçet's disease patients.

One of the key features in antiphospholipid syndrome is that thrombosis may occur anywhere in the vascular tree. Although thrombosis is not infrequent in patients with Behçet's disease, its relationship with antiphospholipid antibodies is unclear. So, it is not certain whether Behçet's disease is also an antiphospholipid antibody syndrome. Hull et al.[4] found an association betwwn increased ACA levels and retinal vascular disease in a group of Behçet's disease patients. But, Efthimiou et al.[23] claimed that there was no association between serum levels of ACA and thrombotic complication. Hamza and Meyer[24] did not find increased levels of ACA isotype IgG in patients with

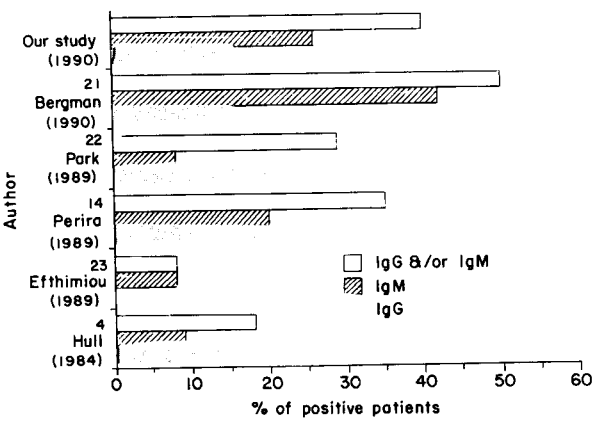

Fig. 1. Frequency of positive anticardiolipin antibodies in Behçet's syndrome in the literature

Behçet's disease, most of whom had vascular complications. This result suggests that Behçet's disease may not be included in the 'Antiphospholipid syndrome'.[23,25] At present, there is no clear evidence that the antiphospholipid antibodies play a causative role in the thrombosis, but because of the association between thrombosis and this class of antibodies, various mechanisms have been proposed.[26] Proposed mechanisms include inhibition of prostacyclin release from vascular endothelium,[27,28] inhibition of fibrolytic activity,[27,29] inhibition of prekallikrein activity,[27,29] inhibition of antithrombin Ⅲ activity,[27,29] inhibition of protein C activation directly,[30] inhibition of thrombomodulin with subsequent inhibition of protein C activation,[31] and binding of phospholipids in platelet membrane and endothelial cell membranes, that may lead to platelet aggregation and/or endothelial damage.[25] Bang et al.[32] reported ultrastructual findings associated with overpro-ductive proliferation of the endothelial cells leading to obliteration of the vascular lumen and suggested they may be involved in thrombogenesis in Behçet's disease patients. Kansu et al.[1] also reported endotheliai cell dysfuction could play a significant role in the thrombotic vascular complication by investigating the biosysnthesis of prostacyclin by vascular endothelium and thromboxane B2 levels in acute Behçet's disease patients. As yet, there is no clear evidence that antiphosphoilipid antibodies play a causative role in damaging the endothelial cell in patients with Behçet's disease, and it is also not certain whether the presence of ACA(as one of antiphospholipid antibodies) in Behçet's disease patients may have a role in the pathogenesis of Behçet's disease or may be only an epiphenomenon. So further studies are needed in order to clarify whether ACA, including the other antiphospholipid antibodies, contributes to the pathogenesis of Behçet's disease.

REFERENCES

1. Kansu E, Sivri B, Sahin G, et al : *Endothelial cell dysfunction in Behçet's disease. The 5th International Behçet's disease conference. Rochester.* 1989.(Abstract)
2. Sobel JD, Haim S, Obedenau N, et al : *Polymorphonuclear leukocyte function in Behcet's disease, J Clin Pathol* 30:250−253, 1977.
3. Niwa Y, Mijake S, Sakane T, et al : *Autooxidative damage in Behçet's disease: Endothelial cell damage following the elevated oxygen radicals generated by stimulated neutrophils. Clin Exp Immunol* 49:247−255, 1982.
4. Hull RG, Harris EN, Gharavi AE, et al : *Anticardiolipin antibodies: Occurrence in Behçet's syndrome. Ann Rheum Dis* 43:746−748, 1984.
5. Sontheimer RD : *The anticardiolipin syndrome: A new way to slice an old pie, or a new pie to slice? Arch Dermatol* 123:590−595, 1987.
6. Loizou S, MaCrea JD, Rudge AC, Reynolds R, Boyle CC, Harris EN : *Measurement of anticardiolipin antibodies by an enzyme-linked immunosorbent assay(ELISA): Standardization and quantitation of results. Clin Exp Immunol* 62: 738−745, 1985.
7. Harris EN, Gharavi AE, Boey ML, et al : *Anticardiolipin antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet* 2:1211−1214, 1983.
8. Lenzi R, Rand JH, Spiera H : *Anticardiolipin antibodies in pregnant patients with systemic lupus erythematosus. N Engl J Med* 314:1392, 1986.
9. Lockshin MD, Druzin ML, Goei S, et al : *Antibody to cardiolipin in pregnant patients with systemic lupus erythematosus(letter to editor). N Engl J Med* 314:1392, 1986.
10. Shimizu T, Inaba G, Hashimoto T : *Diagnostic criteria and their problems of the Behçet's disease. Intern Med* 33:278−282, 1974.
11. Lehner T, Barnes CG : *Criteria for diagnosis and classification of Behçet's syndrome. Lehner T, Barnes CG(eds), Academic press Inc. London,* 1979, pp 1−9.
12. Lee ES, Lee YD, Bang D, Lee SH, Lee S : *A study of interleukin-2 activity in Behçet's syndrome. Ann Dermatol (In press)* 3:5−11, 1991.
13. Biswas RM, Cronin ME, Bowerman DL, et al : A solid phase enzyme immunoassay (ELISA) for the detection and quantitation of anticardiolipin antibody. Am J Clin Pathol 90: 591−596, 1988.
14. Pereira RM, Gonçalves CR, Bueno C, Meirelles ES, Cossermelli W, Oliveira RM : *Anticardiolipin antibodies in Behçet's syndrome: A predictor of more severe disease. Clin Rheumatol* 8: 289−291, 1989.
15. Smith KJ, Skeleton HG, James WD, et al : *Cutaneous histologic findings in 'Antiphospholipid syndrome'. Arch Dermatol* 126:1176−1183, 1990.
16. Koike T, Sueishi H, Tomioka, et al : *Antiphospholipid antibodies and biological false positive serological test for syphilis in patients with systemic lupus erythematosus. Clin Exp Immunol* 56:193−199, 1984.
17. Boey ML, Colaco GB, Gharavi AE, et al : *Thrombosis in systemic lupus erythematosus: Striking association with the presence of circulation lupus anticoagulant. Br Med J* 287:1021−1023, 1983.
18. Grob JJ, Bonerandi JJ : *Cutaneous manifestations associated with the presence of the lupus anticoagulant. J Am Acad Dermatol* 15:211−219, 1986.
19. Mackworth-Young CG : *Antiphospholipid antibodies: More than just a disease marker? Immunol Today* 11:60−65, 1990.
20. Harris EN, Phil M : *Solid-phase anticardiolipin test revisited. Am J Med.* 85:599−601, 1988.
21. Bergman R, Lorber M, Lerner M, Brik R, Friedman-Birnbaum R : *Anticardiolipin antibodies in Behçet's disease. J Dermatol* 17:164−167, 1990.
22. Park DJ, Hong OJ, Kim SH, Park SY, Kim HY, Kim DJ : *Anticardiolipin antibody measure*

by ELISA in Behçet's disease. Kor J Int Med 37: 204−209, 1989.

23. Efthimiou J, Harris EN, Hughes GRV : *Negative anticardiolipin antibodies and vascular complications in Behçet's syndrome. Ann Rheum Dis* 44:725−726, 1985.
24. Hamza M, Meyer O : *Anticardiolipin antibodies in Behçet's disease. Press Med* 15:1281, 1986.
25. Hughes GRV, Harris EN, Gharavi AE : *The anticardiolipin syndrome. J Rheumatol* 13:486−489, 1986.
26. Levine SR, Kim S, Deegan MJ, Welch KMA : *Ischemic stroke associated with anticardiolipin antibodies. Stroke* 18:1101−1106, 1987.
27. Harris EN, Gharavi AE, Hughes GRV : *Antiphospholipid antibodies. Clin Rheum Dis* 11: 591−609, 1985.
28. Carreras LO, Defreyn G, Machin SJ : *Arterial thrombosis, intrauterine death and lupus anticoagulant: Detection of immunoglobulin interfering with prostacyclin formation. Lancet* 1:244−246, 1981.
29. Vermylen J, Blockmans D, Spitz B, Deckmyn H : *Thrombosis and immune disoredrs. Clin Haematol* 15:393−412, 1986.
30. Cariou R, Tobellem G, Soria C, Caen J : *Inhibition of protein C activation by endothelial cells in the presence of lupus anticoagulant. N Engl J Med* 314:1193−1194, 1984.
31. Comp PC, DeBault LE, Esmon NL, Esmon CT : *Human thrombomodulin is inhibited by IgG from two patients with nonspecific anticoagulants. Blood* 62:299, 1983(suppl 1).
32. Bang D, Honma T, Saito T, et al : *Ultrastructure of vascular changes in cutaneous manifestations of Behçet's disease. Acta Dermatol Venereol(Stockh)* 68:33−40, 1988.