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Valvular deposition of antiphospholipid antibodies in the antiphospholipid syndrome: A clue to the origin of the disease

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ABSTRACT

In this report we present an unusual case of a 45-year-old female patient with systemic lupus erythematosus (SLE) who was hospitalized for mitral valve replacement. In her childhood she presented with mitral stenosis and chorea on which grounds a preliminary diagnosis of rheumatic fever was established. After a quiescent period lasting two decades her disease erupted with mitral stenosis, thromboembolic phenomena, and nephritis. Due to severe malfunctioning of her mitral valve, the patient eventually underwent mitral valve replacement. The antibodies involved in the pathogenesis of our patient's valvular disease were studied by immunohistochemical analysis, applying rabbit polyclonal anti-human IgG and IgM anti-human C3c and anti-idiotypes to a mouse monoclonal naturally occurring polyspecific human monoclonal anticardiolipin antibody termed S2.9, and to the 16/6 Id which defines a common Id on anti-DNA antibodies in patients with SLE. Immunoperoxidase staining using an anti-idiotype mAb to anticardiolipin antibodies demonstrated the deposition of these anti-bodies in the subendothelial layer of the valve. We believe that anti-phospholipid syndrome (APS) with SLE was the initial and primary disease in this patient. These findings clearly indicate that APS must be considered in the differential diagnosis of rheumatic fever, particularly in young female patients who present with mitral stenosis and chorea.

Introduction

Valvular disease is a common and clinically important cardiac finding in patients with systemic lupus erythematosus (SLE) (1-5). It appears that since the introduction of corticosteroid therapy valvular involvement has become more prevalent among patients with SLE due to their increased longevity (6). In the case report presented here the distinction between childhood lupus and rheumatic fever turned out to be a rather complex issue, that was solved only 30 years following the disease onset.

Case report

A 45-year-old female patient with SLE and severe mitral stenosis with moderate regurgitation was admitted to the hospital for mitral valve replacement. At the age of ten she was found to have a mitral stenosis which was succeeded by recurrent bouts of chorea athetosis. The findings were ascribed to rheumatic fever and the patient was placed on a prophylactic antibiotic therapy until the age of 18.

At the age of 33 she was hospitalized due to an acute confusional state, sensory aphasia and retrograde amnesia. Her physical examination was significant for the presence of systolic and diastolic murmurs that were auscultated best on the apex. An increased sedimentation rate of 80 mm/hr; low complement levels (C3 40 mg/dl, C4 9 mg/dl); and high titers of antinuclear antibodies (ANA), a lupus anticoagulant, and anti-DNA antibodies (Farr assay - 52%) were observed. A brain CT scan revealed a left parieto-temporal infarct, and an echocardiogram demonstrated mild mitral stenosis without a co-existing thrombus. Anticoagulant therapy and corticosteroids were started and gradual improvement was seen.

Following a quiescent period lasting one decade the patient was again hospitalized due to a high-grade fever with stabbing left pleuritic chest pain and dyspnea. A pericardial friction rub was noticed on her left precordium. An echocardiogram disclosed small pericardial and pleural effusions together with marked leukopenia, mild anemia and high circulating concentrations of ANA. Anti-DNA antibodies (58%) and low complement concentrations confirmed the diagnosis of lupus exacerbation with pericarditis. This episode resolved rapidly following corticosteroid therapy. The patient was re-admitted 6 months later due to hematuria and abdominal tenderness. An abdominal CT scan demonstrated an enlarged and multi-infarcted spleen. Mild proteinuria and a nephritic sediment suggested renal infliction; hence pulse therapy with cyclophosphamide and corticosteroids was initiated.

Eight months later the patient arrived in a comatose state with a left hemiparesis.

On brain CT scan a new right parieto-occipital infarct was identified. An echocardiogram showed a mitral valve gradient of 26 mmHg, a mitral valve orifice smaller than 1 cm^2 and a non-functional subvalvular apparatus. A test was positive for lupus anticoagulant; however, no concomitant anticardiolipin antibodies were found. Improvement was attained by the administration of intravenous heparin, cyclophosphamide and methylprednisolone pulse therapy. After the patient's neurological status had improved, she was referred for mitral valve replacement.

Materials and methods

Heart valves and control tissue specimens

The valves of the patient and two other controls were fixed in 10% neutral buffered formaldehyde solution (formalin) and embedded in paraffin. As a positive control we used a mitral valve that was excised during autopsy from a 44-year-old female patient with APS who suffered from Libman-Sacks endocarditis, and who was reported in a previous study to have anti-phospholipid antibodies deposited in the subendothelium (6). As negative controls we employed mitral valves originating from a healthy 18-year-old male who committed suicide and from a 60-year-old patient with rheumatic heart disease involving the mitral valve.

Antibodies and reagents

The primary antibodies used in the immunohistochemical stainings were: rabbit polyclonal anti-human IgG and IgM anti-human C3c (DAKO, Dakopatts, Denmark), mouse monoclonal anti-idiotypic antibodies; an anti-H3 Id MAb named S2.9 and anti-16/6 Id MAb and normal mouse IgG as controls (Sigma Chemical Co., St. Louis, MO, USA). The S2.9 anti-Id MAb was originally produced by Sutjita *et al.* (7) upon the immunization of mice with H3, a naturally occurring polyspecific human monoclonal anticardiolipin antibody. The anti-16/6 Id MAb was produced as described earlier (8). This idiotype defines a common Id on anti-DNA antibodies in patients with SLE and other autoimmune diseases (9, 10).

Immunohistochemical analysis

The valves were cut into 4 μm thick sections, placed on poly-L-lysine coated slides, and stained with hematoxylin and eosin. The specimens were examined for the presence of antibodies by the labeled streptavidin biotin method (immunoperoxidase staining).

The sections were first deparaffinized in xylene and rehydrated with decreasing concentrations of ethanol. For the immunoperoxidase staining, the slides were incubated with 3% H_2O_2 for 5 minutes at room temperature (RT) to quench the endogenous peroxidase activity, followed by 5 minutes of incubation in distilled water. The slides were then placed in Coplin jars with 10 mM citrate buffer (pH 6.0) and heated twice for 5 minutes in a microwave oven (Jetpoint MX 246, 2.45 GHz) at a maximum power of 630 W, exchanging citrate buffer between the heatings. After heating, the jars were left to cool for 10 minutes at room temperature (RT). The slides were rinsed for 5 minutes in tris-buffered saline (TBS; 0.05m Tris/HCL, 0.1 M NaCl, pH 7.6) containing 0.1% bovine serum albumin (BSA) and 0.05% Tween 20 (BSA-TBS-Tween). To reduce background signals, a 15-minute incubation period with 10% non-immune goat serum followed by a 30-minute incubation period with CASS block (Zymed Laboratories Inc., San Francisco, CA) were performed at RT. After blocking, the various primary antibodies diluted in 1% BSA-TBS, (biotinylated second antibody) and streptavidin peroxidase conjugate (kit produced by Zymed Laboratories Inc., San Francisco, CA) were sequentially added for 30 minutes at RT. The slides were rinsed for 5 minutes in BSA-TBS-Tween between steps. Antibody binding was visualized with 3-amino-9-ethylcarbazole (Sigma). Finally, the slides were counterstained with Mayer's hamatoxylin and mounted using Glycergel (DAKO, Carpinteria, CA).

Results

Pathological findings

On macroscopic examination the mitral valve of the patient with SLE was found to be deformed. Microscopic examination following hematoxylin and eosin staining revealed diffuse fibrosis and calcification (Fig. 1). In the subendothelium foci of chronic inflammation mainly composed of lymphocytes and a few plasma cells were observed.

Immunohistochemical findings

Positive staining for human immunoglobulins and the complement C3c component, with a well-demarcated pattern in the affected valve tissue section, was observed both in our SLE patient and in the disease control with APS. A continuous subendothelial ribbon-like layer

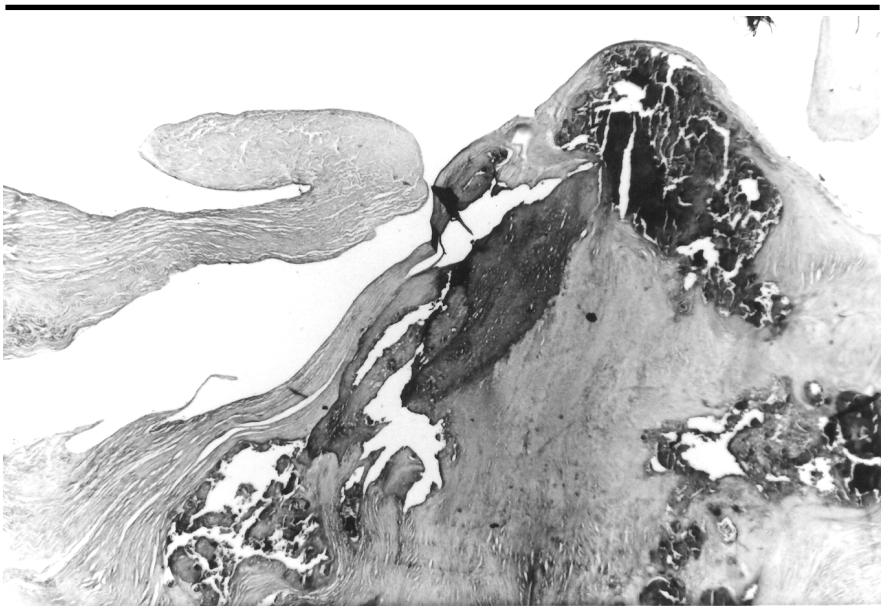
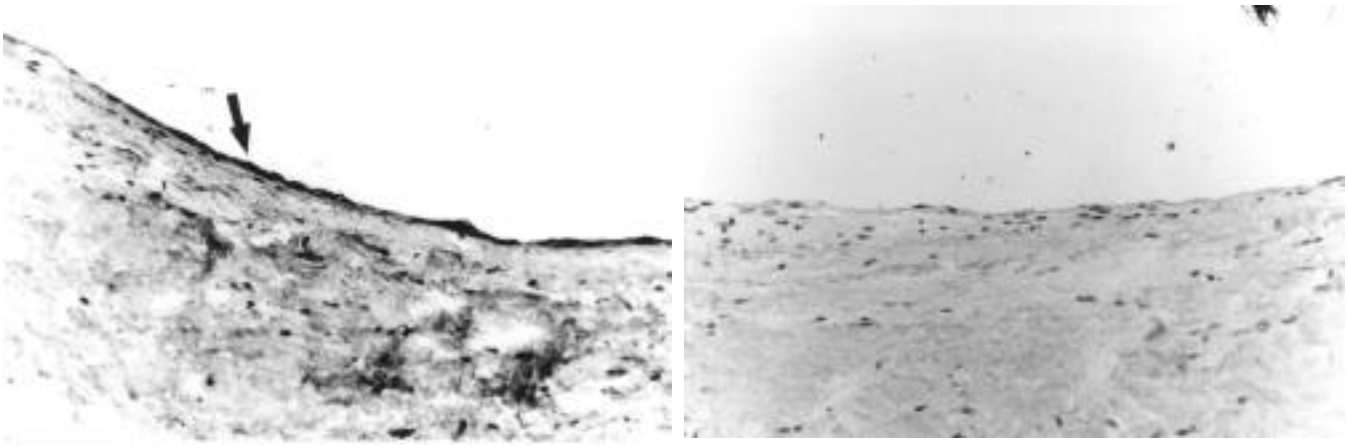


Fig. 1. Tissue section of the SLE patient's mitral valve. Massive fibrosis and calcification typical of an end stage inflammatory process can be seen (HE x 10).



(a) **(b)**

Fig. 2. Subendothelial immunoperoxidase staining for S2.9 in: (**a**) the valve of the patient with lupus and APS (black arrow) (x 40); (b) the valve of the normal control (x 40).

was identified along the surface of the valve leaflets and cusps. Intensive staining was obtained mainly for the IgG isotype and for the C3c complement component. The strongest positive staining was achieved by the S2.9 anti-Id MAb (anti-idiotype to an anticardiolipin antibody) (Fig. 2), while anti-16/6 Id MAb at the same concentration and normal mouse IgG yielded weak, non-significant staining results.

No staining was obtained when anti-human serum albumin was employed as the primary antibody. No analogous trend was seen in the tissue specimens originating from the valves of the normal control and from the patient with rheumatic heart disease (Fig. 3).

Discussion

We described here a 45-year-old female with APS and SLE who had multi-systemic manifestations. Her mitral disease and chorea had commenced in childhood and were ascribed to rheumatic fever. Could both have represented the initial manifestations of SLE in this patient ?

In a survey of 106 children with SLE, only 13% had central nervous system involvement (1). Chorea in SLE is particularly uncommon and has been reported in less than 2% of patients. However, chorea often occurs as the presenting sign of childhood lupus, rendering the differentiation between lupus chorea and Sydenham's chorea impossible (2). Moreover, chorea is one of the neurological manifestations that is most often associated with APS, together with transverse myelopathy, atypical migraines, epilepsy and amaurosis fugax (11,12).

In contrast to chorea, cardiac involvement has been more frequently reported in childhood lupus. Reports going back 40 years state that "heart lesions develop in nearly all patients [with SLE] at some time during the course of their disease when life is prolonged by modern antibiotic and corticosteroid therapy" (3). Fish *et al.* (4) reported cardiac involvement in 31% of a series of pediatric SLE patients, all whom were treated with long-term corticosteroids. The true incidence of cardiac involvement in children with SLE has not, however, been completely elucidated.

Recently, Roldan *et al.* (5) performed transesophageal echocardiograms in 69 adult patients with SLE. Valvular abnormalities were observed in 61% of the patients, vegetations in 43%, valvular regurgitation in 25% and pure valvular stenosis in 4%.

Non-bacterial verrucous endocarditis or Libman-Sacks endocarditis is one of the typical cardiac lesions associated with SLE and occurs in up to 50% of patients (7). The lesions vary in size from 0.1 to 4 mm and may appear singly or in clumps, but rarely do these lesions bear any hemodynamic significance (3). In contrast to the pre-steroid era, when Libman-Sacks endocarditis could occur in any one of the cardiac valves, nowadays there is a preponderance of left-sided lesions affecting the mitral valve in particular (13). Bulkley and Roberts (14) postulated that corticosteroid therapy facilitates a 'healing' process in which fibrosis and calcification replace the classical histological findings of fibrin clumps, focal necrosis and mononuclear infiltrates. This can be ascribed either to the direct effect of the drug on the inflamed valve or to the extended longevity of patients and to the older age at which they undergo valve replacement.

Similar to previous reports, neither corticosteroid nor cyclophosphamide treatment prevented the progression of the valvular disease in our patient, and she eventually required replacement surgery. However, Nesher *at al.* (15) recently reported on 4 patients with APS who developed severe mitral regurgitation with significant leaflet thickening that dramatically improved following corticosteroid therapy. After the failure of other symptomatic measures, prednisolone halted the leaflet inflammation, leading to a decrease in the size of the leaflets and to the improvement of the patients' hemodynamic indexes.

It has been postulated that in APS the anti-phospholipid antibodies directly cause valvular or endothelial injury unrelated to the clinical severity of the disease (16-20). Recently, Ziporen *et al.* (6) presented immunohistochemical studies conducted on heart valve specimens derived from patients with APS. Positive staining for human immunoglobulins and for the C1q, C3c and C4 complement components were seen as a subendothelial ribbon-like layer along the surface of the leaflets and cusps. The strong-

est positive staining was obtained with the S2.9 anti-Id MAb (anti-idiotype to cardiolipin), while the anti-16/6 Id MAb (anti-idiotype to DNA) at the same concentration did not attain a significant level of staining. Our failure to detect elevated titers of antiphospholipid antibodies does not preclude their possible contribution to the inflammation leading to valvular degeneration. Fluctuating titers of serum antiphospholipid antibodies have been previously described (21). We believe that the binding of antiphospholipid antibodies to valvular tissue reflects their existence more accurately than episodic blood sampling.

In this paper we show that the deposition of antiphospholipid antibodies takes place in SLE patients with secondary antiphospholipid syndrome. We suggest that this process initiates an inflammatory process that recruits the complement system, leading to a cascade that ends in the development of Libman-Sacks endocarditis. The patient in this case required mitral valve replacement after years of active disease that caused the native valve to become stenotic, fibrotic and calcified. We believe that this disease process explains the lack of active inflammation in the valve specimen studied. The deposition of anticardiolipin antibodies on the mitral valve resembles the deposition of immune complexes in the dermo-epidermal junction or in the kidney basement membrane in patients with lupus (22). We believe that all of these processes may reflect a single underlying immune mechanism by which autoantibodies inflict tissue damage.

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