Immunopathogenesis of Libman-Sacks endocarditis

Assessment by light and immunofluorescent microscopy in two patients

R. F. SHAPIRO, C. N. GAMBLE, K. B. WIESNER, J. J. CASTLES, A. W. WOLF, E. J. HURLEY, AND A. F. SALEL

From the Sections of Rheumatology and Cardiology, Department of Internal Medicine, and the Section of Thoracic Surgery, Department of Surgery, University of California, Davis; and Department of Pathology, Sutter Memorial Hospital, Sacramento, California

SUMMARY The possible contribution of immunological mechanisms in the development of Libman-Sacks endocarditis was studied in 2 patients with systemic lupus erythematosus who underwent aortic valve replacement. Sections of verrucous lesions, stained with haematoxylin and eosin, showed three apparently distinct zones: an outer exudative zone of fibrin, nuclear debris, and haematoxylin-stained bodies; a middle organizing zone of proliferating capillaries and fibroblasts and an inner zone of neovascularization which showed distinct, thin-walled junctional vessels. The striking finding was the apparently selective deposition of immunoglobulins and complement identified by direct immunofluorescence, within the walls of the small junctional vessels of the zone of neovascularization. We suggest that the observed immune deposits are immune complexes and that circulating immune complexes may play a critical role in the growth and proliferation of the verrucous lesion.

Libman-Sacks endocarditis, which can be distinguished pathologically from both rheumatic and bacterial endocardial disease, is a feature of systemic lupus erythematosus (SLE) and occurs in nearly 50% of all autopsied cases (Libman and Sacks, 1924; Gross, 1940; Klemperer *et al.*, 1941; Harvey *et al.*, 1954; Brigden *et al.*, 1960; Bulkley and Roberts, 1975). The vegetations can be found on any valve and are often multivalvular; however, they occur most frequently on the mitral leaflets (Gross, 1940; Klemperer *et al.*, 1941; Bulkley and Roberts, 1975).

The typical valvular and mural endocardial lesions, which are verrucous in character, can be single or conglomerate in the form of mulberry-like clusters. When present on the semilunar valves the vegetations are found most often on the ventricular surface close to, but not interrupting, the line of closure. Even when the valvular vegetations become prominent, valvular distortion is minimal. As a result, clinically recognizable valvular dysfunction is uncommon and only rarely is there a haemodynamically significant valvular lesion (Shearn and Pirofsky,

1952; Bernhard *et al.*, 1969; Shulman and Christian, 1969; Myerowitz *et al.*, 1974; Paget *et al.*, 1975).

The histopathological features of the verrucae are characteristic (Libman and Sacks, 1924; Gross, 1940; Klemperer *et al.*, 1941; Bulkley and Roberts, 1975), and include haematoxylin-stained bodies which were first described in the valvular lesions of SLE (Gross, 1940). While there is extensive evidence that immune complex mediated tissue injury accounts for much of the organ pathology in SLE, evidence is lacking for an immunological mechanism as the basis of the Libman-Sacks lesion. We studied the histopathology, by light and immunofluorescent microscope, of Libman-Sacks verrucae on aortic valves resected from 2 patients with SLE who required valvular replacement because of symptomatic aortic insufficiency.

Case reports

CASE 1

A 47-year-old retired Air Force sergeant was admitted in April 1974 for aortic valve surgery. He had been well until 1963 when investigation of chest pain and fever led to a diagnosis of pericarditis

which rapidly resolved. In 1968, on admission for a history of visual hallucinations, extensive assessment showed positive LE cells, positive VDRL (FTA negative), thrombocytopenia, proteinuria, and reduced creatinine clearance. Percutaneous renal biopsy showed mesangial hypercellularity and basement membrane thickening in a portion of the glomeruli observed by light microscopy. The central nervous system disturbance responded rapidly to phenothiazines, and no other therapy was given.

A raised blood pressure of 190/110 mmHg and a murmur of aortic insufficiency were first noted in 1970. In July 1973, after two generalized seizures, lumbar puncture showed a normal opening pressure and clear fluid with no cells; protein 0.71 g/l; glucose 47 mg/100 ml (2.61 mmol/l) with a serum glucose of 80 mg/100 ml (4.44 mmol/l). Electroencephalogram and brain scan were normal. During the next several months he experienced increasing fatigue and exertional dyspnoea. Progressive cardiomegaly was evident on a roentgenogram of the chest, and electrocardiogram showed left ventricular hypertrophy. In March 1974, cardiac catheterization was performed. A dilated aortic root, enlargement and hypertrophy of the left ventricle, aortic reflux, and calcification at the apex of the left ventricle were noted and the patient was hospitalized for aortic valve replacement.

On admission he specifically denied a history of rheumatic fever. Physical examination showed normal vital signs. Cardiac examination showed a broad apical impulse from the midclavicular line to the axilla in the fifth intercostal space and a grade IV/VI systolic ejection murmur accompanied by a loud diastolic blow heard along the lower left border of the sternum. Laboratory results showed haemoglobin 15 g/dl, haematocrit 45%, leucocyte count $3800/\text{mm}^3$ ($3.8 \times 10^9/\text{l}$), platelet count $90\,000/\text{mm}^3$ ($90 \times 10^9/\text{l}$), and reticulocyte count 1.2%. Westergren ESR was 57 mm/h. Both direct and indirect Coombs's tests were strongly positive, and the serum haptoglobin level was 2.0 g/l. Antinuclear antibody was positive at a titre of 1:160 with a homogeneous pattern of staining, and differential immunoglobulin determination showed that this antibody belonged to the IgG and IgM classes but not to the IgA class. ^3H -DNA binding was 25.2% (normal <6.1%). A quantitative serum C3 (B_1C) was 0.93 g/l (normal 0.8–1.2 g/l), and C4 was 0.16 g/l (normal 0.2–0.5 g/l). Serum immunoglobulins were raised and included IgG 24 g/l, IgM 4.6 g/l, and IgA 6.1 g/l. Renal function tests showed serum blood urea nitrogen 42 mg/100 ml (14.99 mmol/l), serum creatinine 1.9 mg/100 ml (168 $\mu\text{mol/l}$), creatinine clearance 42 ml/min, and 24-hour urinary protein 735 mg.

Roentgenogram of the chest showed cardiomegaly and an electrocardiogram was consistent with marked left ventricular hypertrophy. A biopsy specimen taken from a sun-exposed area of skin was stained with fluorescein-conjugated antisera and showed granular staining at the level of the dermo-epidermal junction in sections stained with anti-IgG and anti-IgM; sections stained with anti-IgA and the remainder of the fluorescein-conjugated antisera (C3, C4, C3PA, fibrinogen, albumin, and IgD) failed to show the presence of any specific staining in any area.

Open heart surgery was performed in April 1974. Findings included enlargement of the aorta to a diameter of 5.5 cm, approximately 2 cm above a normal annulus. The noncoronary and right cusps of the aortic valve were prolapsed, and friable non-calcified verrucous vegetations were present on the undersurface (flow side) (Fig. 1). The mitral valve, as viewed from the aortic root, was normal. The aortic valve was removed, the annulus sized, and a no. 29 Bjork-Shirley aortic prosthesis was inserted. The patient tolerated the procedure well and returned home 2 weeks later.

CASE 2

A 45-year-old housewife was admitted in February 1975 for aortic valve replacement. She had been in excellent health until 1969, when she presented with lethargy, and a diastolic murmur was noted. Fatigue increased, and in September 1971, she was admitted with complaints of chest pain, orthopnoea, paroxysmal nocturnal dyspnoea, and ankle swelling. Examination showed a large pericardial effusion; pericardicentesis was performed and she improved. In November 1971, symptoms recurred and a murmur of aortic insufficiency as well as a pericardial effusion were detected on examination. Cardiac catheterization showed severe aortic regurgitation. She was readmitted in January 1975, because of progressive fatigue and exertional dyspnoea. Cardiac catheterization showed gross aortic regurgitation and a raised left ventricular end diastolic pressure which increased markedly with exercise. Left ventricular angiography showed a dilated aortic root and normal contractility without evidence of mitral regurgitation. Coronary angiography was normal. Echocardiography showed a small posterior pericardial effusion. She was admitted for valvular replacement.

Additional history included a description of grand mal seizures, bizarre behaviour, malar rash, pleurisy, and renal insufficiency, but no history of rheumatic fever. Examination showed an emotionally unstable woman with a blood pressure of 160/70 mmHg and an erythematous malar rash. Cardiac examination

Fig. 1 Case 1. Photograph of aortic valve taken during surgery (left) and artist's drawing showing granular verrucous lesions.

showed lateral displacement of the point of maximal impulse 4 cm to the left of the midclavicular line, a soft first heart sound, an accentuated pulmonic component of the second heart sound, an audible third sound, and a II/VI diastolic blow at the apex. She was not disorientated but had impaired memory and stammering speech.

Leucocyte count was $4500/\text{mm}^3$ ($4.5 \times 10^9/\text{l}$), haemoglobin 12 g/dl, and direct Coombs's reaction positive. Platelet count was $140\,000/\text{mm}^3$ ($140 \times 10^9/\text{l}$). Westergren ESR was 45 mm/h. Urinalysis gave a 4+ test for protein with a normal sediment. VDRL test was nonreactive. Serum antinuclear antibody determination was positive at a titre of 1:20 with a homogeneous pattern of staining, and differential immunoglobulin determination showed that this antibody belonged to 3 classes (IgG, IgM, and IgA). ^3H -DNA binding was 24.5%. Quantitative serum C3 (B_1C) was 0.87 g/l (normal 0.8–1.2 g/l), and C4 0.12 g/l (normal 0.2–0.5 g/l). Serum immunoglobulin concentrations: IgG 14.8 g/l, IgM 2.05 g/l, and IgA 1.2 g/l; serum blood urea nitrogen 50 mg/100 ml (17.9 mmol/l); serum creatinine 2.5 mg/100 ml (221 $\mu\text{mol/l}$); creatinine clearance 25 ml/min; and 24-hour urinary protein 2.1 g. Percutaneous renal biopsy showed arteriosclerosis with

scattered (predominantly subcapsular) ischaemic obsolescent glomeruli on light microscopy. Immunofluorescent study showed positive glomerular staining for immune reactants (IgG, IgA, IgM, C3 (B_1C), and C4) and characteristic of immune complex-induced mesangiopathy. Roentgenogram of the chest showed cardiomegaly with normal pulmonary vasculature, and electrocardiogram a left bundle branch block.

At operation the aortic valve was widely insufficient with thickened leaflets; vegetations were visible on the central portions of all three leaflets. Similar vegetations were present immediately below the aortic valve annulus; the mitral valve appeared normal. The aortic valve was removed and replaced with a porcine xenograph. Postoperatively, cardiac status improved, but the patient required readmission because of recurrent seizures and paranoid behaviour.

COMMENT

SLE was diagnosed according to the preliminary ARA criteria (Cohen *et al.*, 1971). In addition, both patients had significant native DNA-binding antibodies and, on one or more occasions, hypocomplementaemia. Neither patient gave a history of

rheumatic fever, and blood cultures were repeatedly negative throughout their courses. Interestingly, neither patient had ever received systemic steroids or immunosuppressive agents.

Materials and methods

SEROLOGICAL STUDIES

Antinuclear antibodies were detected by indirect immunofluorescence using leucocytes as a source of nuclei and fluorescein-isothiocyanate conjugated antihuman IgG, IgM, and IgA (Behring Diagnostics, Somerville, N.J.). Antibodies to double-stranded (native) DNA were measured in a binding assay (Ginsberg and Keiser, 1973), using a tritium (^3H) labelled KB cell DNA (Electronucleonics, Bethesda, Maryland); normal sera binds $<6.1\%$ of the DNA used in the assay. Serum immunoglobulins and C3 and C4 were quantitated by radial immunodiffusion (Behring Diagnostics).

HISTOLOGICAL STUDIES

Specimens of aortic valve cusps were obtained at the time of operation and immediately processed for light and immunofluorescent microscopy.

LIGHT MICROSCOPY

Half of each biopsy was fixed in Helly's solution and processed in the usual manner. Sections were cut at $2\mu\text{m}$ and stained with haematoxylin and eosin.

Fig. 2 Case 1. Prominent horn-shaped verruca extends in the direction of blood flow and involves the ventricular aspect of an aortic valve cusp. Haematoxylin and eosin. $\times 20$.

Fig. 3 Case 1. Three distinct zones are seen within the verrucous lesion. Zone of exudation (ZE) consists of amorphous proteinaceous material and fibrin. Zone of organization (ZO) contains proliferating blood vessels. Zone of neovascularization (ZN) shows maturing fibrous connective tissue. Small thin-walled junctional vessels (arrow) occupy the ZN. H. and E. $\times 95$.

Fig. 4 Case 1. Two regions in the ZE are depicted. Linear strands of fibrin, scattered neutrophils, and karyorrhectic nuclear debris (left) and distinct haematoxylin-stained bodies (right) are apparent. H. and E. $\times 260$ (left); $\times 405$ (right).

Fig. 5 Case 1. ZO shows organization of a fibrinous exudate by proliferating capillaries and fibroblasts. H. and E. $\times 185$.

IMMUNOFLUORESCENT MICROSCOPY

Half of each biopsy specimen was immediately snap frozen in a slurry of dry ice and acetone, briefly stored at -60°C , and then sectioned in a -20°C cryostat at $6\text{ }\mu\text{m}$. The first and last specimens were stained with haematoxylin and eosin. Additional sections were then stained with fluorescein-isothiocyanate conjugated antihuman IgG, IgM, IgA, IgD, C3, C4, fibrinogen, and albumin. Monospecificity of these preparations was proved by appropriate blocking studies. The stained sections were examined in a Zeiss Ultraphot II microscope equipped for immunofluorescent microscopy.

Results**LIGHT MICROSCOPY**

Haematoxylin and eosin-stained sections from the aortic valves of both patients showed prominent horn-shaped, verrucous lesions which extended in the direction of blood flow and involved the ventricular aspect of the aortic valve cusps (Fig. 2). These lesions were characterized by three apparently distinct zones (Fig. 3). The zone of exudation (ZE), an outer peripheral layer immediately adjacent to the endothelium covering the flow surface of the valve, was comprised of amorphous proteinaceous material and fibrin. At higher magnifications linear strands of fibrin containing scattered polymorphonuclear leucocytes, karyorrhectic nuclear debris, and distinct haematoxylin-stained bodies were seen (Fig. 4). The zone of organization (ZO), the middle layer (Fig. 3), showed organization of a fibrinous exudate by proliferating capillaries and fibroblasts (Fig. 5). The zone of neovascularization (ZN), the innermost layer (Fig. 3), showed maturing fibrous connective tissue

containing fibroblasts. Several distinct thin-walled vessels were seen in this zone at its junction with the ZO (Fig. 3). These small junctional vessels were frequently surrounded by numerous polymorphonuclear leucocytes (Fig. 6) and occasionally showed areas of endothelial disruption with partially degranulated neutrophils within their walls and lumen (Fig. 7).

IMMUNOHISTOLOGY

The results of immunofluorescent studies of the valvular lesions were remarkably similar in both patients. Sections stained with fluorescein-conjugated antihuman fibrinogen showed prominent staining within the ZE and ZO and there was patchy staining about small vessels within the ZN (Fig. 8). Discrete granular deposition of IgG (Fig. 9A), IgM (Fig. 9B),

Fig. 7 Case 1. Endothelial disruption with partially degranulated neutrophils within vessel wall and lumen are present within the ZN. H. and E. $\times 415$.

Fig. 6 Case 2. Small junctional vessel within the ZN is surrounded by neutrophils. H. and E. $\times 530$.

Fig. 8 Case 1. Prominent deposition of fibrinogen within the ZE and ZO zones and patchy staining about small vessels within the ZN (arrows) are shown by direct immunofluorescence using antihuman fibrinogen fluorescein-conjugated serum. H. and E. $\times 75$.

and C3 were found in the walls of small junctional vessels within the ZN of Case 1. Similar vascular deposits of IgG, IgM, IgA, and C3 (Fig. 9C) were noted in Case 2.

The immunoglobulin deposits found in the valvular junctional vessels of both patients are summarized in the Table. The immunoglobulin classes of serum antinuclear antibodies and immunoglobulin deposits in glomeruli and skin were similar to the valvular vessel wall deposits within the same patient. We used as a nonlupus control valve a case of healed rheumatic aortic valvulitis. Multiple sections failed to

Table Summary of immunofluorescent studies in 2 patients with Libman-Sacks endocarditis

	Case 1			Case 2		
	IgG	IgM	IgA	IgG	IgM	IgA
Valvular vessel wall deposits	+	+	-	+	+	+
Serum ANA	+	+	-	+	+	+
Glomerular deposits	No biopsy			+	+	+
Dermoeplidermal junction deposits	+	+	-	No biopsy		

+ = present; - = absent.

show IgG, IgM, IgA, or C3, within the walls of the small blood vessels in areas of discrete neovascularization.

Discussion

The verrucous endocardial lesions seen in our patients with SLE conform to the classical gross and histological descriptions of Libman-Sacks endocarditis (Libman and Sacks, 1924; Gross 1948; Klemperer *et al.* 1941). The dominant endocardial pathology differed morphologically and topographically from that usually found in the rheumatic and bacterial forms of endocarditis (Robbins, 1967). In addition, multiple histological sections of valve specimens obtained from both patients failed to show any evidence of Aschoff's bodies or micro-organisms.

A striking finding in our study was the apparently selective deposition of immune reactants identified by direct immunofluorescence within small blood vessels in the active portions of the verrucae. Deposition from

Fig. 9 Case 1. Conglomerate deposition of IgG within the wall of a junctional vessel in the ZN. $\times 410$. Case 1. Deposits of IgM within the wall of a junctional vessel within the ZN. $\times 410$. (C) Case 2. Discrete deposits of C3 within the wall of a junctional vessel in the ZN. $\times 410$.

IgG, IgM, and C3 was noted in both patients. Case 2 also had deposition of IgA within these vessel walls. The immunoglobulin classes of anti-nuclear antibodies and immunoglobulin deposits in glomeruli, skin, and valvular vessel walls were similar within the same patient.

SLE is a model system for immune complex disease in man. Sophisticated techniques have shown immunoglobulins and/or complement in the kidney (Koffler and Kunkel, 1968), skin (Pohle and Tuffanelli, 1972), and choroid plexus (Atkins *et al.*, 1972) of patients with SLE. Articular (Pekin and Zvaifler, 1970), pleural (Hunder *et al.*, 1972), pericardial (Hunder *et al.*, 1974), and cerebrospinal (Petz *et al.*, 1971; Hadler *et al.*, 1973) fluids from SLE patients show evidence of consumption of complement components, and a variety of techniques (Agnello *et al.*, 1971; Harbeck *et al.*, 1973; Nydegger *et al.*, 1974) have indicated that their sera contain circulating immune complexes. Similarly, the finding of apparently selective deposition of immunoglobulins and complement within vessel walls of the Libman-Sacks vegetations in our 2 patients with SLE suggests that immune complexes may well mediate the development of this classical pathological finding.

Our findings suggest a role for immune complexes in the pathogenesis of Libman-Sacks lesions. The initial insult is disruption of the valvular endothelium by turbulent flow (Germuth, 1953; Kniker and Cochrane, 1967). The damaged endothelium then allows the insudation of plasma proteins containing phlogogenic immune complexes which are capable of complement fixation. Subsequent activation of the complement sequence provides the mediators of inflammation, resulting in enhanced permeability of the surface endothelium with oedema, leucocyte infiltration, and fibrin deposition within a zone of exudation (ZE). During the next phase there is invasion by proliferating capillaries and fibroblasts which organize the fibrin deposits. The new capillaries have permeable walls (Gamble *et al.*, 1970) which provide an access for the deposition of more circulating immune complexes. As a result there is potentiation of the inflammatory process with increased exudation of fibrin, enhanced stimulus to organization, and the establishment of a vicious cycle leading to the progressive growth and proliferation of the verrucous lesion. As the verruca increases in size, maturation of fibroblasts and deposition of collagen result in fibrosis with progressive thickening of the valve.

The above pathogenic scheme is an explanation for the occurrence of valvular lesions in patients with SLE. Recent observations (Bulkley and Roberts, 1975) indicate the Libman-Sacks endocardial lesions

are smaller in patients treated with corticosteroids, suggesting that the cyclic pathogenetic sequence can be interrupted by treatment. It is notable that in our 2 untreated patients large verrucous lesions resulted in symptomatic valvular disease.

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