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Detection of anti-contractile antibodies after cardiac surgery using ELISA assay

I. DE SCHEERDER,* J. VANDEKERCKHOVE,‡ G. DE SCHRIJVER,† M. HOSTE,* D. CLEMENT,* R. WIEME† & R. PANNIER* **Department of Cardiology and* †*Laboratory of Internal Medicine, University Hospital and* ‡*Laboratory of Histology and Genetics, Gent, Belgium*

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SUMMARY

Sera from 196 patients were collected before and after cardiac surgery to measure antibodies against heart tissue and against actin and myosin. In the post-operative period antibodies were found in 87 patients (44%) producing a cross-striated fluorescence pattern in heart tissue. Antibodies against the major contractile proteins were found in 91 patients (46%), anti-actin antibodies in 49 patients (25%) and anti-myosin antibodies in 65 patients (33%). We found a significant correlation ($P < 0.0001$) between the antibodies producing a cross-striated fluorescence pattern and antibodies recognizing contractile proteins. These results suggest that contractile proteins evoke an immune response after cardiac surgery and that this response may compromise cardiac function.

Keywords cardiac surgery anti-heart antibodies anti-myosin antibodies anti-actin antibodies

INTRODUCTION

Previously anti-heart antibodies (AHA) were measured by the tanned red cell technique on non-human myocardium (Robinson & Bridgen, (1963), and still earlier by a passive agglutination technique with human heart extract (Davies & Gery, 1960; Gery, Davies & Ehrenfeld, 1960). Van der Geld (1964), Engle *et al.* (1974) and Maisch, Berg & Kocksiek (1979) used an indirect immunofluorescence technique and different patterns have been described (Maisch *et al.*, 1979; Maisch, Maisch & Kocksiek, 1982).

Using all these tests, AHA were found in the sera of patients who developed the post-pericardiotomy syndrome (PPS) after cardiac surgery. Furthermore the presence of AHA correlated well with the clinical manifestations of the syndrome suggesting that a laboratory test was available to supplement clinical findings in making the diagnosis of PPS (McCabe *et al.*, 1973; Engle *et al.* 1974, 1975, 1980, 1981; Maisch *et al.*, 1979; De Scheerder *et al.*, 1984). The indirect immunofluorescence technique is at best only a semi-quantitative approach for evaluating AHA and is not able to discriminate clearly between the diverse antigens evoking autoimmune responses after cardiac surgery. Although a cross-striated pattern of fluorescence suggests involvement of antibodies against contractile proteins, more specific techniques are needed. In this report we further characterize the antibodies producing a cross-striated pattern in heart tissue (CS-AHA). We show, using an ELISA technique on sera of 196 long-term survivors of cardiac surgery, that these antibodies are mainly directed against the major contractile proteins, actin and myosin.

Correspondence: Dr I. De Scheerder, Department of Cardiology, University Hospital, De Pintelaan 185, 9000 Gent, Belgium.

MATERIALS AND METHODS

This study was undertaken over a period of 1 year and included 196 adult patients (46 female and 150 male). Thirty-five patients underwent aortic valve replacement, 22 mitral valve replacement, eight combined valve replacement and 128 aortic–coronary bypass procedures. One patient was operated for an atrial myxoma, another for combined septum defect and one underwent a commissurotomy.

Serum samples were collected prior to surgery and 1, 5, 10, 20, 30 and 60 days after operation. Sera from 40 patients showing no cardiovascular disturbance, and matched for sex and age, were used as controls. All sera were stored at −20°C for up to 1 year.

AHA. The indirect technique of Coons & Kaplan (1950) was used: monkey heart muscle frozen in liquid nitrogen was cut in sections of 4 μm thickness in a cryostat at −20°C, mounted on slides and incubated for 20 min with 1/10 diluted test serum. Four different patterns of immunofluorescence staining were distinguished: cross-striated, diffuse intracellular, intercalated disc and heart muscle membrane fluorescence.

In this study we concentrate on the cross-striated fluorescence. The intensity of fluorescence staining was graded from 0 to +4; only fluorescence of ⩾ +2 was considered positive in this study.

Anti-actin (AAA) and anti-myosin antibodies (AMA). We have used the indirect enzyme linked immunosorbent assay (ELISA) for the quantitation of anti-actin autoantibodies (AAA) and anti-myosin autoantibodies (AMA) (Voller, Bartlett & Bidwell, 1978). The conjugate used for the assay was alkaline phosphatase conjugated goat anti-human IgG (Sigma). Rabbit muscle heavy meromyosin (HMM, Sigma) or bovine cardiac actin, prepared according to Spudich & Watt (1971), were coated overnight at 4°C onto glutaraldehyde coated immunobeads (Plastic ball Company) in phosphate-buffered saline (PBS) 0·15 M, pH 7·2. Sera were added in dilution ranging from 1/10 to 1/10,000 in PBS/0·5% gelatine (DIFCO) and incubated at 37°C for 2 h. After washing with PBS/Tween 0·05%/gelatine 0·25% the amount of enzyme bound was assayed using *p*-nitrophenyl phosphate disodium (Sigma) as substrate. The reaction was stopped by the addition of 1 N NaOH. The absorbance of test beads was measured on a Vitatron spectophotometer at a wavelength of 410 nm. A dilution above the means of the control patients was considered positive.

Specificity test. Five millilitres of a 1/100 dilution of serum containing AMA or AAA was absorbed with 0·5 mg of bovine cardiac actin and rabbit skeletal muscle myosin for 2 h at room temperature. The absorbed serum, recovered by centrifugation at 10,000*g* for 30 min, was tested for reactivity for actin and myosin by ELISA and results were compared to control unabsorbed serum. Results showed neutralization of anti-actin and anti-myosin activity by heart muscle actin and HMM, respectively.

Statistics. The significance of differences between incidence was evaluated by the chi-square test.

RESULTS (Fig. 1)

AHA

Of the 196 patients studied in the pre-operative period (1–3 days before operation) 23 (8%) showed antibodies producing CS-AHA. In the post-operative period, CS-AHA were found in 87 patients (44%); they appeared from 10 days to 2 months post-operatively, the maximum number being found 20 days after operation (41%). This number slowly decreased, but even at 2 months post-operatively 52 patients (27%) showed positive CS-AHA. The control group showed two patients (5%) with CS-AHA.

AAA

When the pre-operative sera were examined for antibodies against actin, seven out of 196 (4%) had AAA. After operation, AAA were found in 49 patients (25%). These antibodies appeared after day 5 and were found in 45 patients (23%) 20 and 30 days after surgery. Two months post-operatively

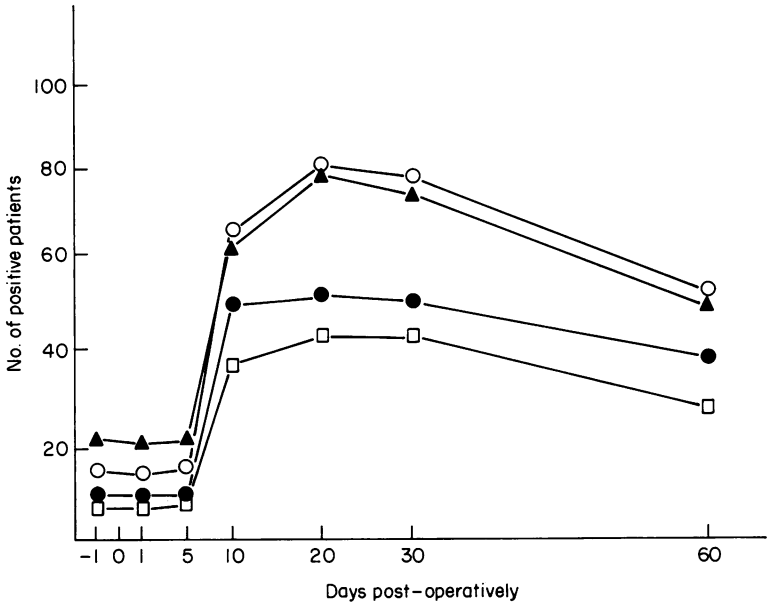

Fig. 1. Occurrence and evolution of AAA (□), AMA (●), AHA (△) and AAA and/or AMA (○) in the post-operative period.

AAA were still found on 29 patients (15%). In the control group AAA were observed in two patients (5%).

AMA

A screening of the sera for AMA showed 10 positive pre-operative sera (5%) increasing to 65 (33%) after operation. Like CS-AHA and AAA, the maximum level was reached at 20 days post-operatively (30%). The level of myosin antibodies dropped slightly in the later period, so that two months post-operatively 40 patients (20%) still had positive AMA. In the control group only one patient (2·5%) showed AMA.

Association of AHA and anti-contractile protein antibodies

Of the 196 patients studied, CS-AHA were found in 87 patients (44%) 10 or more days post-operatively; AAA and/or AMA were found in 91 patients (46%). In the group of patients with positive CS-AHA, eighty patients (92%) had also AAA and or AMA antibodies ($P < 0{\cdot}001$). In the group of patients with positive AAA and/or AMA, 80 patients (88%) also had CS-AHA ($P < 0{\cdot}001$).

DISCUSSION

The differences in immunofluorescent staining reactions suggest that AHA is a heterogeneous group of autoantibodies reactive with different heart muscle antigens. Little is known about the antigens triggering immune response and the prevalence of specific autoantibodies after cardiac surgery. Our results clearly indicate that contractile proteins are important antigens evoking immune response after cardiac surgery and that autoantibodies generated after cardiac surgery, giving a cross-striated fluorescence pattern, are mainly directed against the major cardiac proteins, actin and myosin.

Whether these autoantibodies play a role in the pathogenesis of post-operative complications like the post-pericardiotomy syndrome remains unclear. A cytotoxic role against myocardial cells

seems unlikely because of the localization of these antigens in the cytoplasm making them relatively inaccessible. Formation of precipitation immune complexes must be considered as a possible mechanism. In a previous study (De Scheerder *et al.*, 1984) we demonstrated a good correlation between PPS, AHA and circulatory immune complexes (CIC) suggesting a possible role of these CIC in the pathogenesis of PPS by precipitation in the pericardium and pleura.

Anti-contractile protein antibodies do not only occur after cardiac surgery and are not specific for PPS. Bretherton *et al.* (1983) showed that a significant number of patients with autoimmune chronic active hepatitis had autoantibodies to G-actin. Wada *et al.* (1983) found that 90% of patients suffering from polymyositis had a mean titre of AMA which was significantly higher than a control group. We have recently found anticontractile protein autoantibodies in five out of nine patients who were followed after traumatic muscle injury (unpublished observations).

In summary, cardiac surgery is accompanied by the release of autoantigens which sometimes trigger an immune response and give rise to AHA. Contractile proteins are important antigens in evoking this response. Detection of anti-contractile protein antibodies using an ELISA assay makes it possible to quantitate these autoimmune phenomena.

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