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Clinical significance of immunopathological findings in patients with post-pericardiotomy syndrome

II. THE SIGNIFICANCE OF SERUM INHIBITION AND ROSETTE INHIBITORY FACTORS

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SUMMARY

Serum inhibition factors (SIF) that suppress phytohaemagglutinin-induced blast transformation of normal lymphocytes, and lymphocyte E-rosette inhibitory factors (RIF) that inhibit the T cell-specific property of E-rosette formation were determined in sixty-five patients before and after cardiac surgery.

SIF was found in the first post-operative week in almost all patients; patients with complete post-pericardiotomy syndrome (PPS) still had these factors in the fourth postoperative week. The appearance of SIF correlated well with the intensity of the PPS. Persistence of SIF in eleven out of eighteen patients with clinically incomplete PPS reaffirms the probability that they had an 'immunologically' positive PPS. RIF was to be found in one third of the patients with complete or incomplete PPS and may be of prognostic value. The two factors were not identical.

INTRODUCTION

The role of serum factors and lymphokines in the pathogenesis of the PPS has not been examined. Only a few studies have been performed to demonstrate lymphocytotoxicity towards heart antigens. There is little convincing evidence to date for cell-mediated immune reactions in autoimmune perimyocarditis and PPS (Laufer & Davies, 1969; Laufer, Friedman & Ron, 1975a, b; Friedman *et al.*, 1970; Hainaut *et al.*, 1974).

Recently, non-antigenic serum inhibition factors (SIF) have been demonstrated in various diseases (Tomasi, 1977), especially in autoimmune liver disease (Newberry *et al.*, 1973; Newble *et al.*, 1975; Macsween & Thomas, 1976; Brattig & Berg, 1976a, b). Chisari & Edgington (1974; 1975) were able to isolate a low density lipoprotein as a lymphocyte E-rosette inhibitory factor (RIF) from the sera of patients with acute viral hepatitis released from the liver into the serum during the inflammatory process. RIF has also been demonstrated in patients with chronic glomerulonephritis (Gluckman, Bonfils & Sanchez, 1976) and seems to be of prognostic relevance, since the presence of RIF correlated with the progression of the disease. We were interested, therefore, to study SIF and RIF during the course of the PPS to see whether they have diagnostic and prognostic significance.

MATERIALS AND METHODS

Details of both patients and their clinical classification are described in part I (Maisch *et al.*, 1979).

Lymphocyte transformation test. The lymphocyte transformation test used was a modification of the method described by Penhale *et al.* (1974). Lymphocytes from a panel of two healthy donors were separated from heparinized blood by centri-

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fugation on Ficoll–Amido trizoe acid (sp. gr. 1077 g/ml) and washed three times in Hanks' balanced salt solution. $0{\cdot}25 \times 10^6$ lymphocytes were cultivated in the presence of 8 μg of phytohaemagglutinin (PHA) per ml (Difco Laboratories) for 90 hr in 0·25 ml of TC 199 (Difco Laboratories) containing 20% test serum, 100 μg of streptomycin/ml and 100 units of penicillin per ml. After the addition of 1·0 μCi/ml ^{3}H-thymidine (sp. act. 2 Ci/mmole) (Radiochemicals Amersham, Buchler), incubation was continued for 6 hr. The cultures were transferred into glass fibre filters (Whatman GF/C), washed with 5% trichloroacetic acid and ethanol. The dried filters were counted in a liquid scintillation counter (Tricarb 3375) for incorporated thymidine.

The serum inhibitory activity was expressed as an index comparing PHA-induced lymphocyte transformation of normal lymphocytes in the presence of patient serum and transformation in the presence of normal serum:

$$\text{Inhibition index} = \frac{\text{mean cpm of stimulated control lymphocytes} + \text{test serum}}{\text{mean cpm of stimulated control lymphocytes} + \text{control serum}}$$

Lymphocyte cultures from two controls were set up in triplicate and mean cpm values of these triplicates were calculated. In the presence of normal serum the mean index was 0·95. Inhibitory activity was demonstrated when the index was lower than 0·65.

To exclude the interference of haemolysis with lymphocyte stimulation after valve replacement (Björk Shiley), ten samples from normal donors were tested before and after mechanical haemolysis. The mean SIF index of the sera before haemolysis was $0{\cdot}88 \pm 0{\cdot}05$ and after $0{\cdot}83 \pm 0{\cdot}12$ ($2P > 0{\cdot}2$). Lactate dehydrogenase (LDH) increased from pre-haemolytic levels of 121 ± 32 u/l to 273 ± 45 u/l after haemolysis.

Lymphocyte E-rosette inhibitory factor (*RIF*). Lymphocytes were isolated from two healthy donors as described above. The RIF assay was performed using a modification of the method by Chisari & Edgington (1975). Lymphocytes were suspended in concentrations of $1{\cdot}5 \times 10^6$/ml in TC 199 (Difco Laboratories) supplemented with penicillin (100 u/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM). Cells were dispensed in 400 μl aliquots into sterile tubes with U-bottom wells, containing 100 μl of either test serum or control AB-serum. The tubes were covered with sterile lids and incubated for 18 hr at 37°C in a humid atmosphere containing 5% CO_2. For the assay of percentage E-rosette-positive lymphocytes, cells were resuspended, washed three times with 400 μl TC 199 by centrifugation at 200 *g* for 5 min. After the last washing all but 100 μl TC 199 was removed and 100 μl of a 1·25% suspension of fresh sheep erythrocytes which had been washed three times was added. The tubes were closed, incubated for 10 min at 37°C, centrifuged for 5 min at 200 *g* and incubated for 12–18 hr at 4°C. 5 μl of 0·02 trypan blue was added. The pellet was gently resuspended, aspirated and transferred to a microslide. Viability was always greater than 95%. 400 viable lymphocytes were counted. Lymphocytes with three or more erythrocytes were counted as rosettes. The arithmetic mean was calculated from duplicate assays and accepted if the s.d. was less than $\pm 5\%$.

Significant inhibition was considered to be present if the number of E-rosettes in the test serum was lower than in the control AB serum by at least 2 standard deviations (normal: $63{\cdot}2 \pm 4{\cdot}0\%$ rosettes, $n = 20$; no PPS: $62{\cdot}4 \pm 3{\cdot}8\%$ rosettes, $n = 10$).

RESULTS

The appearance of serum inhibition factors (SIF) and rosette inhibitory factors (RIF) was analysed in patients with complete PPS (group 1, $n = 19$), incomplete PPS (group 2, $n = 18$) and no PPS (group 3, $n = 28$).

Demonstration of SIF

All patients who underwent cardiac surgery showed a significant inhibition of PHA-induced lymphocyte transformation ($2P < 0{\cdot}005$) with a SIF index between 0·3 and 0·4 in the first post-operative week. Pre-operative SIF indices were normal (0·68–0·8). In the first and second post-operative week no difference could be found for average SIF indices between all three groups (Fig. 1a). In the fourth week, the sera of patients with complete PPS still showed strong inhibitory activity with a SIF index of $0{\cdot}48 \pm 0{\cdot}19$, whereas patients with incomplete or without PPS already showed normal indices ($2P < 0{\cdot}05$, Fig. 1a).

Eighty-nine per cent of the patients in group 1 demonstrated an increase of serum inhibition (= decrease of SIF index of more than 0·1) prior to or at the time of pericarditis, fever and leucocytosis. Six out of nineteen patients with complete PPS and six out of eighteen with incomplete PPS had serum inhibition factors (SIF < 0·6) before the operation, whereas only four out of twenty-eight patients without PPS had these factors pre-operatively. When patients with incomplete PPS were redistributed according to the immunological criteria of Table 3 in the companion paper (Maisch, Berg & Kochsiek, 1979) and eleven patients with immunologically positive PPS were included, the SIF indices differed even

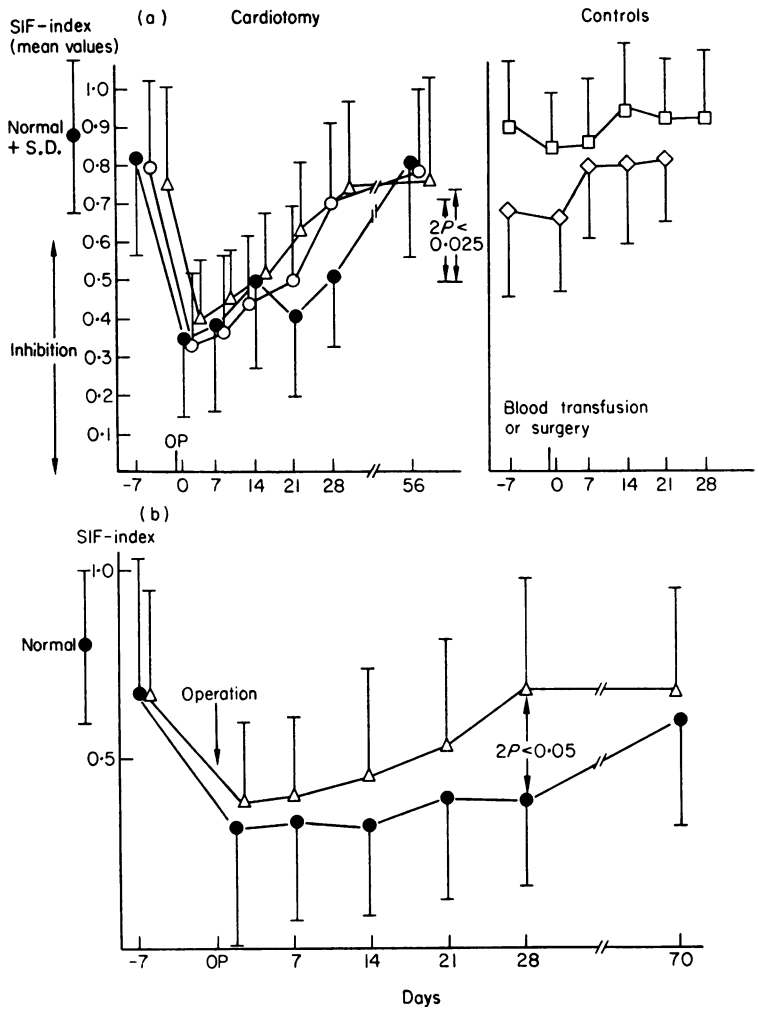

FIG. 1. (a) Serum inhibition factors (SIF) in patients after cardiac surgery and in controls, (●) Complete PPS ($n = 19$); (○) incomplete PPS ($n = 18$); (△) no PPS ($n = 28$); (□) non-cardiac surgery ($n = 19$); (◇) blood transfusions ($n = 9$). (b) Serum inhibition factors (SIF) in patients immunologically positive (●) and negative (△) for a PPS.

more clearly. The mean index of immunologically positive PPS patients was $0{\cdot}39 \pm 0{\cdot}22$, and of negative patients $0{\cdot}67 \pm 0{\cdot}29$ ($2P < 0{\cdot}05$) in the fourth post-operative week (Fig. 1b). No significant difference was found for SIF pre-operatively and in the first post-operative and the tenth post-operative weeks in the different groups.

In the control groups of nineteen patients who underwent non-cardiac surgery (group 4) and nine patients before and after blood transfusion (group 5) the mean inhibition index remained constant between 0·72 and 0·96 before and after the intervention. Only one patient in group 4 showed inhibitory activity after non-cardiac surgery.

Correlation of SIF with the course of the disease

There was no correlation between the demonstration of SIF in the pre-operative phase and post-operative clinical course (SIF-positive pre-operatively: 45 ± 43 days in hospital, SIF-negative pre-operatively: 42 ± 43 days in hospital). However, patients whose sera still showed inhibitory activity 4 weeks after cardiac surgery were hospitalized for longer ($P < 0{\cdot}05$) than those without SIF (SIF-positive: 51 ± 33 days, SIF-negative: 38 ± 40 days).

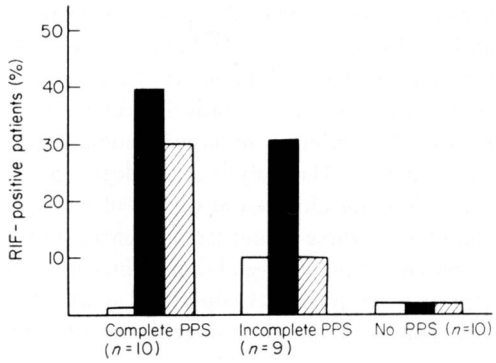

FIG. 2. Rosette inhibitory factors (RIF) in patients after cardiac surgery. (□) Pre-operative; (■) 1–4 post-operative weeks; (▨) after the fourth post-operative week.

Correlation of SIF to serological data

No correlation between SIF indices (y) and the following parameters (x) could be found: (1) C3 complement ($r = 0{\cdot}19$, $n = 164$, $y = 0{\cdot}002x + 0{\cdot}35$); (2) C4 complement ($r = 0{\cdot}31$, $n = 164$, $y = 0{\cdot}03 \pm 0{\cdot}19$); (3) α_1-globulin serum levels ($r = 0{\cdot}38$, $n = 164$); (4) α_2-globulin serum levels ($r = 0{\cdot}34$, $n = 164$); (5) α_2-macroglobulin ($r = 0{\cdot}41$, $n = 164$); (6) C-reactive protein ($r = 0{\cdot}37$, $n = 24$); and (7) serum cholesterol ($r = 0{\cdot}18$, $n = 82$).

Demonstration of lymphocyte E-rosette inhibitory factors (RIF)

Only sera from patients with complete and incomplete PPS inhibited lymphocyte E-rosette formation. However, ten patients selected at random with complete PPS did not all show rosette inhibition: only four developed RIF post-operatively. Three out of ten randomly selected patients of group 2 (incomplete PPS) demonstrated RIF, but no patients in group 3 did so.

The percentage of RIF-positive patients in all three groups with respect to time can be seen in Fig. 2. When patients with incomplete PPS were redistributed into immunologically positive and negative PPS cases, two out of the three RIF-positive patients could be transferred to the PPS-positive group.

RIF and hospitalization time

Patients who developed RIF pre- or post-operatively showed a prolonged clinical course. Patients with pre-operative RIF were hospitalized for 110 ± 105 days, with postoperative RIF 108 ± 92 days as compared to only 48 ± 14 days for the RIF-negative patients ($2P < 0{\cdot}05$). One patient with PPS developed an increase in rosette inhibition a few weeks before he died.

Clinical relevance of SIF and RIF in patients with PPS

The appearance of SIF and RIF in the early post-operative phase ($r = 0{\cdot}27$, $n = 29$) and in the fourth post-operative week ($r = 0{\cdot}23$, $n = 29$) did not correlate. This dissociation indicates that the factors were not identical.

DISCUSSION

SIF and RIF were analysed in sixty-five patients after open heart surgery. These factors interfere directly or indirectly with lymphocyte transformation after stimulation with PHA (Nelson & Gatti, 1976; Cooperband *et al.*, 1976 a, b; Tomasi, 1977) or the forming of E-rosettes. We were able to demonstrate that SIF appeared fairly uniformly after thoracotomy and persisted in almost all patients with a PPS after the third week. α_1-, α_2-globulins and α_2-macroglobulins could not be implicated, nor was there a correlation with the occurrence of C-reactive protein, which is known to have an immunosuppressive

effect on lymphocytes (Elster, Wood & Seely, 1954; Mortensson, Osmand & Gewurz, 1975). The use of anaesthetics during the operation (Espanol, Todd & Soothill, 1974; Riddle & Berenbaum, 1967; Paronetto & Popper, 1970) may have influenced lymphocyte transformation during the first few post-operative days, but not in subsequent weeks. Post-operatively decreased but later increased complement levels did not correlate satisfactorily. The influence of immune complexes cannot be excluded, however, since they may inhibit blast transformation. Haemolysis of the degree found after valve replacement did not inhibit lymphocyte transformation, nor did the transfusion of 500 ml of blood.

The nature and biological function of these serum factors remain unknown. They probably belong to the group of immunosuppressive factors in the α_2-globulins, which may be produced during an immune response either by lymphocytes or macrophages (Cooperband *et al.*, 1976; Nelson & Gatti, 1976). It has been suggested that they may play a role in immunoregulation after infection (Tomasi, 1977; Fitzgerald & Hosking, 1976) or act at the stage of amplification of the immune response. They may be early products of an immune response, especially during viral infection, and may be responsible for the anergic phase during the incubation period (Kantor, 1975).

A factor which inhibits the formation of E-rosettes has been demonstrated in patients with active chronic hepatitis (Chisari, *et al.*, 1976; Chisari & Edgington, 1974; 1975) and chronic glomerulonephritis (Gluckman *et al.*, 1976). It remains to be proven whether the RIF found in patients with PPS is similar to the low density protein isolated by these authors. The persistence of RIF activity seems to be clinically unfavourable (Chisari *et al.*, 1977) and in our patients we were able to correlate the persistence of the factor with a protracted clinical course. The loss of SIF and RIF activity corresponded with clinical improvement in five out of six patients treated with 1 mg of prednisolone per kg of body weight per day.

Since 89% of the patients with complete PPS exhibited a strong inhibitory activity (SIF) before or during pericarditis which persisted to the fourth post-operative week, we re-examined the eighteen patients with a clinically incomplete PPS. Ten out of eighteen patients showed a SIF index $<0{\cdot}6$ in the fourth post-operative week and nine of these had an 'immunologically' positive PPS.

Since RIF was found in patients of groups 1 and 2 only, it is probably a useful marker of an active immune reaction. A tentative grading system including SIF and RIF reaffirmed the distribution of immunologically positive and negative PPS patients achieved in part I (Maisch *et al.*, 1979). Eleven out of eighteen patients with incomplete PPS remained immunologically positive. Fifteen out of nineteen patients with complete PPS were also positive in this grading system and only four out of twenty-eight patients without symptoms were falsely positive.

Immunological criteria applied to patients after cardiac surgery may enable us to classify patients into two definite groups. (1) Patients with PPS demonstrate heart-associated antibodies against sarcolemma and non-organ-specific antibodies against endothelium and connective tissue. Their sera contain immunosuppressive serum factors (SIF), even as late as 4 weeks after surgery, and in a complicated and prolonged clinical course rosette inhibitory factors (RIF) may persist. (2) In patients with no PPS antibody titres are low or absent, SIF can be found only in the early post-operative period (1–2 weeks) and RIF cannot be detected.

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