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Circulating immune complexes and severe sepsis: duration of infection as the main determinant

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SUMMARY

The relation between the duration of bacterial infection and circulating immune complexes (CIC) level was evaluated using the C1q binding assay in a group of patients with well defined clinical sepsis. Fifty-four patients with endocarditis and 35 with post-open heart surgery mediastinitis were prospectively studied over a period of 2 years. CIC were detected in 42% of patients studied. Interindividual variations were observed but it was found that the level of CIC increased statistically with time ($P < 0{\cdot}001$). CIC were statistically linked with cryoglobulinemia ($P < 0{\cdot}001$), rheumatoid factor ($P < 0{\cdot}001$) and a decreased CH_{50} ($P < 0{\cdot}05$). CIC were more frequent in patients with endocarditis (53%) than in patients with mediastinitis (24%). However, when the duration of the infection was taken into account the difference was no longer significant. No relation could be evidenced between the incidence of CIC and clinical symptoms including prognosis and renal signs. In our experience, determination of CIC does not have a critical clinical value.

INTRODUCTION

In patients with sepsis, the occurrence of clinical symptoms possibly related to immune complex pathology such as retinitis, cutaneous vasculitis or glomerulonephritis is well known (Phair & Clarke, 1979). Using several methods, circulating immune complexes (CIC) have been detected during the course of bacterial infections (Bayer *et al.*, 1976; Mohammed *et al.*, 1977; Cabane *et al.*, 1979; Bayer *et al.*, 1979b), even in the absence of shock, disseminated intravascular coagulation or endocarditis (Bayer *et al.*, 1979b).

The aim of this work was to measure immune complexes in a group of infected patients with a well defined clinical sepsis, in whom an early sampling was possible. We therefore choose to examine prospectively patients suffering from post-open heart surgery mediastinitis, and bacterial endocarditis. Simultaneously, clinical symptoms, and biological markers of immune complex disease such as cryoglobulinemia, rheumatoid factor and complement activation were also studied. Analysis of the data was done using a correspondence analysis as multivariate method (Hill, 1974).

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PATIENTS AND METHODS

Patient selection. The 89 adult patients with well established sepsis suffering from endocarditis or mediastinitis were prospectively studied over a period of 2 years. Patients suffering from other diseases known to be possibly associated with CIC, were excluded from this study as well as patients with shock prior to the first examination (Füst, Petras & Ujkelyi, 1976). We also excluded patients under either corticosteroid or cytotoxic therapy.

A total of 54 patients had infective endocarditis. Infective endocarditis was diagnosed when patients had septicemia, (three blood cultures positive to the same pathogenic organism or five blood cultures to 'non pathogenic' bacteria), associated with one or more other criteria = valvular lesions at surgery or on pathological examination, or embolic phenomena (septic purpura, retinitis, neurological manifestations). Four patients with history of subacute endocarditis (fever longer than 60 days associated with positive blood culture and known valvulopathy) were also included in the absence of other portal of entry for persistent septicemia. Nine patients with negative blood culture were also included in this study when fever was associated with detectable macroscopic valvular lesions and for a significative modification of the cardiac status estimated by a new regurgitant murmur or change in echocardiographic study. Thirty-five patients had an acute suppurative mediastinitis following open heart surgery. This diagnosis was established when purulent fluid was found in the mediastinum at second look surgery associated with positive culture from the surgical specimens. Twenty-three out of 35 patients had positive blood cultures. Distribution of the micro-organisms was different in endocarditis (27 streptococci) and mediastinitis (27 staphylococci).

Study protocol. The following clinical and biological data were recorded: duration of sepsis as time elapsed between the first symptoms (fever as a rule or eventually other clinical symptoms which may evoke sepsis) and the date of sampling; presence or absence of: valvular prothesis, septic purpura, retinitis, neurological manifestations (including focal signs, major strokes or non-metabolic encephalopathy), septicemia, proteinuria above 200 mg per day, renal failure (creatinine clearance below 50 ml/min), severe renal failure (requiring haemodialysis). The short term survival rate which refers to the number of patients leaving the intensive care unit was also recorded.

Blood samples were specifically collected for CIC detection on dry glass tubes, allowed to clot for at least 1 hr at 37°C, centrifuged 20 min at room temperature, and kept frozen in small aliquots in a deep freeze at −70°C. CIC were detected by the Clq binding assay (Zubler *et al.*, 1976). Assays were done in duplicate for each serum, the results expressed in percent of Clq precipitated and compared to a pool of normal human serum (normal value $\pm$ s.d. $= 6 \pm 1\%$). Results were considered positive when binding was higher than 10%.

Total haemolytic complement (CH_{50}) was estimated according to Kabat & Mayer (1961) and expressed as percentage of pooled normal sera (normal value $\pm$ s.d. $= 100 \pm 20\%$). Rheumatoid factor (RF) was detected on microplate by the latex agglutination test and the Waaler–Rose test, modified with rabbit haemagglutinating antibodies to human red blood cells (Institut Pasteur, France). The results were considered positive when latex titre was higher than 80 and Waaler–Rose titre was higher than 16. Cryoglobulins (CG) were detected and analysed according to methods previously described (Adam, Morel-Maroger & Richet, 1973). C3d was measured in the plasma of 30 patients (21 with endocarditis and nine with mediastinitis) according to Perrin, Lambert & Miescher (1975). Levels higher than 30 μg/100 ml were abnormal.

Statistical analysis. The relationship between time and CIC level was analysed for correlation coefficient. For further analysis the patients were taken into account if all the above defined data were recorded on the same day, just after entering the unit (80 patients). The statistical analysis also used correspondence analysis. This method (Benzecri, 1969; Benzecri, 1973; Nakache, 1976) is a particular case of multifactorial analysis. This analysis is available if data are strictly binary or arranged into a contingency table. So continuous variables such as duration of the disease were coded by the input program into meaningful classes. Active modalities of any variable were only taken into account by the program when the size of the corresponding class was at least equal to nine. When a class included less than nine items the other classes were rearranged according to their relative frequencies.

The multicorrespondence analysis included the 14 informative clinical and biological variables mentioned above. The duration of the disease was divided in three classes: Du_0 less than 10 days, Du_1 between 10 and 40 days, Du_2 greater than 40 days. Causative organisms were divided in two classes: staphylococcus and streptococcus sp.

Moreover relationships between all data were analysed by the chi-square (χ^2)-test. Yate's correction for continuity was applied when the expectations were less than five. The level of significance is given when P is below 0·05 by reading in Pearson's tables.

RESULTS

Incidence of immunological abnormalities

Forty-two per cent of patients had initial levels of CIC greater than 10%. Among them 14 patients had levels above 20%. A cryoglobulinemia was detected for 26% of patients. For three patients the level of cryoglobulinemia was higher than 100 mg/l. Detectable levels of RF was observed in 22% of patients. The total haemolytic complement was lower than 100% in 50% of patients and lower than 60% in 22% of patients. C3d levels were elevated in 27/30 patients tested including 13 above 50 μg/100 ml.

Relationship between duration of sepsis and level of CIC

The level of CIC on the first serum sample studied short after the admission in the unit is related to the duration of bacterial infection (89 patients, $r = 0{\cdot}392$, $P < 0{\cdot}001$). This relation is also found in the group of patients with endocarditis ($r = 0{\cdot}368$) as well as in the subgroup of patients in whom the infection lasted less than 90 days ($r = 0{\cdot}365$). The incidence of positive CIC was 17% when duration of sepsis was below 10 days, 32% when the duration was between 10 and 40 days, 75% when duration of sepsis was above 40 days ($P < 0{\cdot}001$ between each group). These results are in agreement with the multicorrespondence factorial analysis. As shown on Fig. 1, two areas can be clearly

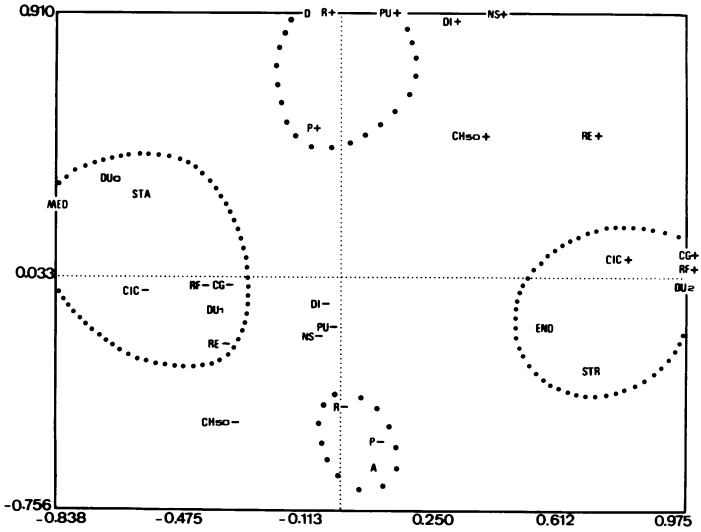

Fig. 1. Multicorrespondence factor analysis of data obtained on first examination (80 patients). The figure is a two dimensional projection of the clinical and biological data checked in the input program. The horizontal line is the main discriminant axis. The frame is drawn at two standard deviations from centre of gravity of the cloud. Dotted lines encircle areas containing linked items. END = endocarditis; MED = Mediastinitis; DU_0 = duration less than 10 days, DU_1 = duration between 10 and 40 days; DU_2 = duration greater than 40 days; + = presence or positivity of the item; − = absence of negativity of the item; CG = cryoglobulinemia; CH_{50} = total haemolytic complement; CIC = circulating immune complexes; DI = dialysis; NS = neurological signs; P = proteinuria; PU = purpura; R = renal failure; RE = retinitis; RF = rheumatoid factor; STA = staphylococcus sp.; STR = streptococcus sp.; A = alive; D = dead.

delineated on the left and right extremities of the main horizontal axis: on the left, short durations of sepsis (Du_0-Du_1) are not associated with circulating immune complexes (CIC^-) whereas on the right, long duration of sepsis (Du_2) is associated with immune complexes in the circulation (CIC^+).

CIC were measured serially in 34 patients (Fig. 2 & 3) who were all treated with antibiotics, associated in some instances with surgical drainage. It is noticeable that 14 patients (five with endocarditis) had no detectable CIC during the course of the disease whereas eight patients had very high levels of CIC (>35%). No systematic trend could be detected in the evolution of CIC levels. Using for each patient the latest available sample, the duration of infection appears to be of great importance = 35 of 49 sera were positive for CIC after 40 days of infection (including some patients seen before 20 days). By contrast when the duration of infection was less than 20 days only seven of 41 patients had detectable CIC.

Relation between CIC and immunological data (Table 1)

Circulating immune complexes were associated with cryoglobulins and Rheumatoid factor. Multicorrespondence factorial analysis (Fig. 1) shows that along the horizontal axis representative points of CIC^+, CG^+ and RF^+ are included in the same area, far from the centre of gravity of the cloud and opposite to the negative points (CIC^-, CG^-, RF^-). These data are related two by two ($P<0{\cdot}001$) using the chi-square test but no quantitative correlation could be evidenced. CH_{50} projection in the multicorrespondence analysis is on the first bisectrix, equally distant from the main axis; nevertheless CH_{50} abnormalities are related to the other immunological data using the chi-square test ($P<0{\cdot}05$ at least). In 19 of 30 patients raised C3d levels were associated with decreased CH_{50} values. No relation could be demonstrated between C3d and other immunological data.

Relation between CIC and clinical data

On the first serum sample tested CIC were significantly more frequently found in patients with endocarditis (25 of 47) than in patients with mediastinitis (eight of 33; $P<0{\cdot}01$). However after 40 days of sepsis, although 28 of 36 patients with endocarditis had CIC compared to seven of 13 with mediastinitis, the difference was no longer statistically significant. This suggests that the duration of infection rather than its initial localization is of major importance for the presence of CIC. Similarly streptococcal infections are associated with CIC ($P<0{\cdot}05$) but this organism is almost exclusively

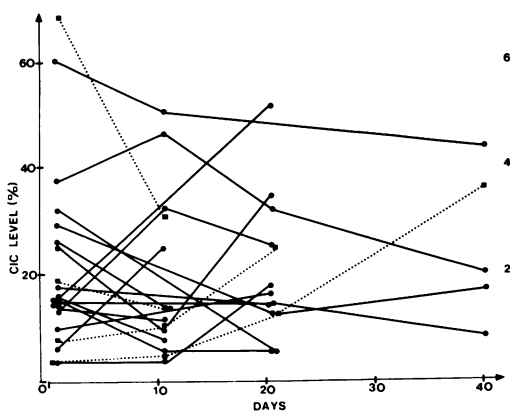

Fig. 2.

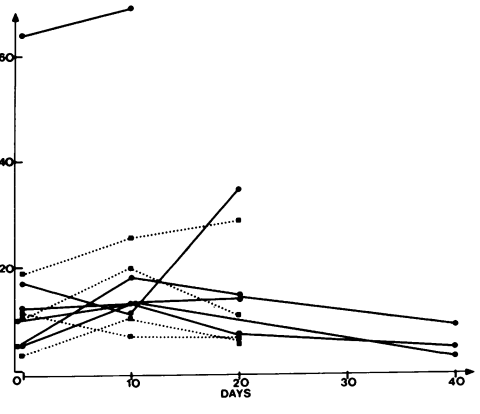

Fig. 3.

Fig. 2. Endocarditis: serial CIC levels in 25 patients (five patients with serial CIC measurements lower than 10% were excluded of the graph). Results are expressed in % of C1q precipitated. (●———● = alive; □- - - - - - - - - -□ = death).

Fig. 3. Mediastinitis: serial CIC levels in 19 patients (nine patients, four deaths, with serial CIC measurements lower than 10% were excluded of the graph). Results are expressed in % of C1q precipitated. (●———● = alive; □- - - - - - - - - -□ = death).

Table 1. Relationship between circulating immune complexes (CIC) detected by the C1q binding assay and immunological data (measured on the initial blood sample)

	CIC positive	CIC negative	Statistical significance (P)
Cryoglobulins			
+	17	4	
−	16	43	$<0{\cdot}001$
Rheumatoid factor			
+	16	2	
−	17	45	$<0{\cdot}001$
Total haemolytic complement			
$<100\%$	23	17	
$\geqslant 100\%$	10	30	$<0{\cdot}01$

responsible for endocarditis ($P<0{\cdot}001$) and linked to long duration of disease ($P<0{\cdot}02$) (Fig. 1). Staphylococcal endocarditis is associated with CIC in four of 14 patients on the first examination and six of 10 after 40 days.

The positivity of CIC is not related to the other clinical data studied which include renal failure (26 patients), proteinuria (41 patients), need for extrarenal dialysis (10 patients), cutaneous signs (12 patients), septic retinitis (26 patients) and prognosis (34 deaths). Multicorrespondence factorial analysis confirms these results since most of the clinical representative points are located along the vertical axis (Fig. 1).

DISCUSSION

The main, important finding in the present study is a relationship between the duration of bacterial infection and CIC level. This observation may be clinically important since it has been suggested that determination of CIC could have diagnostic and/or prognostic value in endocarditis (Bayer *et al.*, 1979a; Bayer *et al.*, 1979b). In this study, CIC are indeed more frequently found in serum samples from patients with endocarditis than mediastinitis but the difference is statistically significant only for patients with less than 40 days of sepsis. It could be argued that patients suffering from mediastinitis went on to develop endocarditis and hence CIC. However, this is unlikely for patients who underwent coronary bypass surgery. Furthermore in the three patients in whom infection of valvular prothesis was demonstrated, CIC levels were normal. We would therefore suggest that there is no specific immunological profile for endocarditis and that duration of infection is the main determinant for the appearance of CIC. This conclusion does not disagree with the observation of Bayer *et al*, (1979b) who demonstrated a significant difference between endocarditic and non-endocarditic septicemia, but noticed a statistically longer duration of the disease in the former group.

The particular statistical method which we used allowed us to study the relationship between CIC and various clinical and biological parameters. On multicorrespondence analysis, the representative points for CIC, Rheumatoid factor and cryoglobulinemia are closely associated. However detection of CIC is the most sensitive test. But, there was no relation between clinical symptoms and occurrence of CIC. In particular, there was no correlation between renal failure, proteinuria and CIC although renal lesions in bacterial infections are usually attributed to the presence of IC deposited in the kidney (Gutman *et al.*, 1972).

Complement synthesis is increased in inflammatory process (Phair & Clarke, 1979). Therefore CH_{50} values lower than 100% may be considered as low values. In our results low CH_{50} values were associated with proteinuria ($P<0{\cdot}02$), retinitis ($P<0{\cdot}001$) and close to significance with renal failure and cutaneous signs. Complement activation assessed by the presence of C3 breakdown

products was present in most patients in whom it was searched for. It is interesting to note that raised C3d levels were often found in the absence of hypocomplementemia suggesting increased complement synthesis.

Previously published data on CIC in endocarditis stressed the fact that adequate treatment was associated with a decrease in CIC levels. Indeed, it has been experimentally suggested that antibiotic therapy suppresses bacterial antigenic release and hence IC formation, although the antibody response may persist or increase with time. In this study however, although a decrease in CIC did occur in some patients, this was not the general trend. Several explanations can be put forward to explain this observation. Firstly in our patients persistent vegetations could be demonstrated by echocardiographic study and antigenic material may therefore persist long after living organisms have been killed by antibiotics. A similar phenomenon may occur in osteitis. Secondly, most of the patients which we studied were critically ill. It has been previously shown that such patients had markedly abnormal cellular immune responses (MacLean *et al.*, 1975) and it is conceivable that they could also have abnormalities in the control of B cell responsiveness or RES function.

A particular point of interest is that some patients developed CIC whereas others did not, even after a prolonged systemic infection. Such interindividual variation, reflected in the 0·4 correlation coefficient between CIC level and duration of disease may be explained by differences in the rate of removal of CIC or in the rate of formation of CIC. Both RES activity and immune responses have been shown to be under genetic control in mice. In man the importance of genetic make up in the immune response to bacterial polysaccharides has been emphasized recently (Pandey *et al.*, 1979; Siber *et al.*, 1980). Further studies along these lines are in progress.

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