Clin. exp. Immunol. (1983) **54,** 248–252.

Relative importance of C4 binding protein in the modulation of the classical pathway C3 convertase in patients with systemic lupus erythematosus

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(Accepted for publication 26 May 1983)

SUMMARY

Serum concentrations of C1q, C4, C4 binding protein (C4bp), C3 and C2 haemolytic activity have been measured in 110 samples from 20 patients with systemic lupus erythematosus (SLE). Significant reductions in comparison to normal levels were found in the mean serum concentrations of C4, C3 and C4bp as well as C2 haemolytic activities. For patients serum concentrations of C4 correlated with C2 haemolytic activities ($r=0{\cdot}91$) and C4bp ($r=0{\cdot}79$); the C2 haemolytic levels correlated with the concentration of C4b ($r=0{\cdot}72$). It is concluded that serum concentrations of the complement components C4 and C2, which are the constituents of the classical pathway C3 convertase, are regulated by C4bp *in vivo*. Further metabolic studies are required to determine the causes of decreased serum concentrations of C4bp in patients with SLE.

Keywords systemic lupus erythematous complement C4 binding protein C3 convertase

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease of unknown aetiology. It is thought that activation of the complement system by immune complexes, may play a significant role in the production of tissue damage in this disease.

Earlier studies (Schur, 1975; Koffler *et al.*, 1969; Gwyn-Williams *et al.*, 1974) have suggested that activation of the complement sequence in SLE primarily occurs via the classical pathway. Reduced levels of factor B and properdin (Gewurz *et al.*, 1969; Hunsicker *et al.*, 1972; McLean & Michael, 1973; Gwyn-Williams *et al.*, 1974; Whaley, Schur & Ruddy, 1979) and the presence of circulating factor B cleavage products (Perrin, Lambert & Miescher, 1975) suggest that amplification of C3 cleavage also occurs in SLE. Furthermore, the increased catabolism of factor B (Charlesworth *et al.*, 1974; Ruddy *et al.*, 1975) and properdin (Zeigler *et al.*, 1975) are consistent with the view that amplification of C3 cleavage in SLE occurs secondarily to C3b formation by the classical pathway.

The classical pathway is initiated by the interaction of antigen–antibody complexes with the first component of complement C1 (Augener *et al.*, 1971; Müller-Eberhard, 1975). Once C1 is activated to C1 it is capable of activating its natural substrates C4 (Lepow *et al.*, 1963) and C2 (Polley & Müller-Eberhard, 1967), which results in the formation of an active enzyme complex C4b2b,a (C42) (Nagasawa & Stroud, 1977), the classical pathway C3 convertase. This convertase has the capacity

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to cleave C3 into C3b and C3a. The C42 enzyme is labile due to inherent decay of C2 (Gigli, Fujita & Nusserzweig, 1979; Daha & van Es, 1980), but it can be regenerated on the residual C4b by the uptake of hemolytically active C2 in the presence of Mg^{++} and its subsequent cleavage by C1. It has been shown that C4b binding protein, C4bp (Gigli *et al.*, 1974; Daha & van Es, 1980) is able to accelerate the decay of the C42 enzyme *in vitro*, by decay–dissociation of C2i from the C42 enzyme.

To investigate the role of C4bp *in vivo*, levels of C1q, C4, C2, C3 and C4bp were determined in serial samples of 20 patients with SLE, and the data analysed in relation to changes in levels of C4bp.

MATERIALS AND METHODS

One hundred and ten sera from 20 patients with SLE were stored at −70°C until used. The number of samples from each patient was between at least two and at most 10. Levels of C1q, C4, C3 and C4bp were determined by radial immunodiffusion using monospecific antisera prepared in this laboratory. C2 activity was measured with a haemolytic method using sheep erythrocytes sensitized with optimal concentrations of rabbit antibody, guinea-pig C1 and human C4 (EAC14) as described (Borsos, Rapp & Mayer, 1961). The results are either presented as concentrations in μg/ml or as percentage of a local standard of normal human serum. The normal values for complement levels were obtained from sera of 25 healthy individuals. The severity of disease activity was determined using the following criteria: manifestation and or severity of clinical signs, increase or decrease of ESR, haemoglobulin values, anti-dsDNA levels, or detoriation of renal function.

When comparing the patients data with those of the normals one can not consider the 110 patients sera as undependent observations as they are obtained from 20 patients only. Formal statistical tests, were therefore applied using from each patient the first observation only. This procedure was applied to compare mean values of normals and patients (*t*-test) and to judge the strength of the correlations within patients. However, the data and statistics presented in tables and graphes are derived from all 110 sera. The correlation between the complement levels and the disease activity, was evaluated for each patient from whom at least eight observations were available. Disease activity was scored as: −(absent), ±(slightly active), +(active) and ++(strongly active). Skin and joint manifestations were treated with antimalarial drugs, whereas haematological, renal and cerebral manifestations were treated with prednisone, frequently combined with azathioprine (Stevens & Hahn, 1982).

RESULTS

Serum concentrations of complement components in SLE

When compared to the mean levels in 25 normal control sera significant reductions of the levels of C3, C4, C2 and C4bp were found in the patients (Fig. 1 and Table 1). The mean concentration of C1q in SLE however was not significantly reduced.

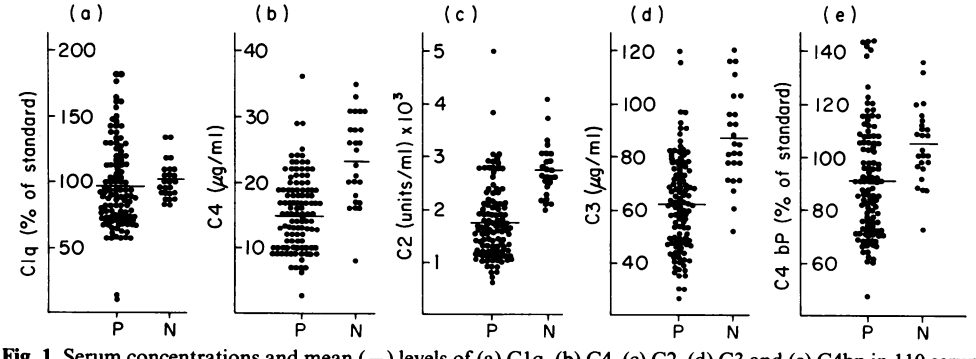

Fig. 1. Serum concentrations and mean (−) levels of (a) C1q, (b) C4, (c) C2, (d) C3 and (e) C4bp in 110 serum samples of 20 patients with SLE (P) and in 25 samples of healthy volunteers (N).

Table 1. Comparison of complement levels in 25 normals with 110 serum samples from 20 patients with SLE.

Component	Groups	Average	s.d.	*P* value
C3 (μg/ml)	Normal	87·3	17·7	0·001
	Patients	62·4	17·9	
C4 (μg/ml)	Normal	23·6	6·8	0·001
	Patients	15·4	5·7	
C1q (% of ST)	Normal	101·3	14·4	0·10
	Patients	96·6	33·2	
C2 (units/ml)	Normal	2,752·4	515·7	0·001
	Patients	1,750·9	648·9	
C4bp (% of ST)	Normal	105·2	14·3	0·01
	Patients	91·8	21·9	

Table 2. Correlation co-efficients calculated for complement levels in sera from normals and in sera of patients with SLE

Correlations between	Normals ($n=20$)	Patients ($n=110$)
C3 and C4	0·29	0·29
C3 and C1q	0·46	0·23
C3 and C4bp	0·01	0·13
C3 and C2	0·11	0·24
C4 and C1q	−0·04	0·08
C4 and C4bp	−0·30	**0·79**
C4 and C2	−0·06	**0·91**
C1q and C4bp	0·45	0·06
C1q and C2	0·03	0·08
C4bp and C2	0·05	**0·72**

In order to achieve more insight in the relationship between the various complement components in normals and in the sera of the SLE patients correlations between the various components were calculated. The results (Table 2) indicate for the patients a significant positive correlation between C4 and C4bp ($r=0·79$), C4 and C2 ($r=0·91$) and between C4bp and C2 ($r=0·72$). Nearly the same levels of correlations are also observed when only the first observation of each patient is taken in account.

All other correlations in the patients sera as well as all correlations in normals turned out to be not significant.

For seven patients enough longitudinal data points were available to investigate within each patient the correlation between complement levels and disease activity. No significant correlation, however, was found.

DISCUSSION

The present study was performed to determine the relationship between levels of C4bp and perturbations of the levels of C1q, C4, C2 and C3 in the sera of patients with SLE. Significantly reduced levels of C4, C2, C4bp and C3 were observed in the patients samples. Although individual

C1q levels were found to be decreased in the patient group the mean level of the whole group was not significantly different from the control group. The latter results which are at variance with previous studies (Lewis, Carpenter & Schur, 1971) in which significant reductions of C1q were found may be dependent on the size of the patient population, the severity of disease or the regimen of drug treatment. Evidence in favour of a role for C4bp in the regulation of the classical pathway in SLE is derived from the correlations between C4, C2 and C4bp concentrations (Table 2), which show that reductions in the serum levels of C4bp coincide with the reduction of C4 and C2. These observations are compatible with the regulatory role of C4bp on the formation and stability of the C42 enzyme, *in vitro*. It was found earlier (Gigli *et al.*, 1979; Daha & van Es, 1980) that C4bp is a potent regulator of C42 activity *in vitro*; C4bp is able to prevent formation of the C42 enzyme and also is capable of accelerating the decay of the preformed C3 convertase, by enhancing release of C2i from C42.

It has been suggested before that complement levels in SLE may fluctuate in association with disease activity (Whaley *et al.*, 1979), but such an evidence was not consistently found in the present investigation. Although a correlation with disease activity was seen in individual patients, not all patients showed such a correlation.

It is possible that diminished levels of certain complement factors may be attributed to diminished synthesis *in vivo*. On the other hand it has been suggested that total serum protein and complement component values may behave individually in many instances. To explain the apparent discrepancies between previous studies metabolic studies are required of the complement factors involved in SLE.

Part of this work was supported by a grant from the Dutch league for Rheumatism. The authors thank Mr W. Haasnoot for excellent technical assistance and Mrs M.E. Mentink for her secretarial help.

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