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Failure to detect brain reactivity of lymphocytotoxins in cerebral lupus

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SUMMARY

Lymphocytotoxic activity (LCA) was examined in the sera of 29 patients with systemic lupus erythematosus (SLE), including eight with cerebral involvement. LCA was elevated in 80% of samples and was significantly higher in the group with cerebral disease ($P < 0{\cdot}001$). No correlations were observed between LCA and immune complexes or complement components. Sera from 10 patients (six with cerebral SLE) were absorbed with homogenates of normal human frontal cortex and liver using protein standards to control for dilutional effects. No serum sample showed selective depletion of LCA following incubation with brain homogenate. It is concluded that no single parameter, including brain absorption of LCA, is effective in monitoring disease activity in cerebral lupus.

INTRODUCTION

The majority of sera from patients with systemic lupus erythematosus (SLE) exhibit lymphocytotoxic activity (LCA) (Mittal *et al.*, 1970; Terasaki, Mottironi & Barnett, 1970). This effect occurs optimally at 15°C in the presence of rabbit serum as a source of complement and is usually considered to be caused by a cold-reactive IgM antibody to the lymphocyte surface membrane (Winchester *et al.*, 1974; Winfield *et al.*, 1975). Recently, an IgM-containing immune complex has also been implicated (Quin *et al.*, 1980). Although lymphocytotoxicity has been reported in SLE patients with various organ involvement, the association between neurological manifestations and disproportionately high titres of LCA has led to the suggestion that cross-reaction with brain antigens plays a role in the development of CNS complications (Bluestein & Zvaifler, 1976; Bresnihan *et al.*, 1977). This proposal has been supported by the observation that the absorption of SLE sera with homogenized cerebral cortex causes a selective reduction in LCA titres.

Further work by Winfield, Brunner & Koffler (1978) has cast doubt on the specificity of LCA absorption by cerebral homogenates. This group found LCA to be comparable in SLE patients with and without cerebral involvement. We therefore examined the specificity of the reaction using brain and liver homogenates and the measurement of two non-immune serum proteins (caeruloplasmin and albumin) to control for inevitable dilutional effects. LCA was also correlated with other laboratory parameters of disease activity (immune complexes and complement components) in patients with or without cerebral disease.

PATIENTS AND METHODS

Patients. All patients fulfilled the American Rheumatism Association's criteria for diagnosis

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(Cohen & Canuso, 1972). Sera from 29 patients were studied for LCA, immune complexes and complement components. Twenty-one patients had no evidence of cerebral disease (mean age 38 years, range 12–65 years) and eight were diagnosed as having CNS involvement (mean age 22 years, range 10–41 years). The manifestations of cerebral lupus included neuropsychiatric disturbances, major seizures and transverse myelitis. None of these features were considered to be attributable to intercurrent infection, systemic hypertension, corticosteroid therapy, uraemia or haematological disease. The sera of 10 of these patients were selected for absorption studies—six from the cerebral group and four from the non-cerebral group. All had LCA greater than 50%, there being no significant difference between the two groups.

Lymphocytotoxins. The assay was based on the microdroplet technique of Terasaki & McClelland (1964) and was performed with the modifications described by Charlesworth *et al.* (1978). The effect of serial dilutions of SLE sera on LCA was studied in five patients (two from the cerebral group and three from the non-cerebral group). In all cases serum was diluted just before testing with RPMI 1640 with 10% complement fixation diluent (CFD, Oxoid).

Absorption studies. Disease-free human frontal cortex and liver were collected within 24 hr of death. Meninges and surface vasculature were stripped from the frontal cortex which was then diced and homogenized. Similarly, the capsule, major blood vessels and bile ducts were removed from the liver before homogenization. Preparations were then washed twice in equal volumes of Hanks' balanced salt solution (HBSS) and stored in 0·5-g aliquots at −20°C until used. Immediately before use they were washed in an equal volume of CFD and centrifuged at 1,600 ***g*** for 15 min at 15°C. Absorption was performed by adding 0·5 ml of test serum to each homogenate and incubating for 30 min at 15°C with intermittent mixing.

Following centrifugation (2,000 ***g***) for 15 min at 15°C, the supernatant was removed and a small aliquot saved for testing. The remaining serum was subjected to two further absorptions. Control samples were treated in the same fashion except that equal volumes of CFD were used instead of homogenate of liver or brain. This dilutional control provided a reference for measuring the relative loss of LCA, caeruloplasmin and albumin. In one sample a further aliquot of serum was diluted 1:10 with RPMI/CFD before being subjected to the same absorptions with brain and liver and CFD control (Fig. 1).

Serum protein concentrations. C1q, C4, C3, caeruloplasmin and albumin were measured by radial immunodiffusion using monospecific antisera. Complement component levels were expressed as a percentage of pooled normal human serum (NHS) and caeruloplasmin and albumin as a percentage of that found in the unabsorbed control.

Circulating immune complexes. The assay used was the C1q binding assay (C1qBA) described by Zubler *et al.* (1976).

RESULTS

None of the 10 sera showed selective loss of LCA with brain absorption (see Table 1). Only one sample in each group exhibited significant depletion of LCA by absorption with brain homogenate and in both cases a comparable loss occurred with liver absorption. The five remaining samples from the cerebral group showed a rise in LCA following absorption with brain homogenate and a similar rise occurred in two of the non-cerebral group. Brain and liver absorption caused dilution of all 10 sera. Caeruloplasmin concentration decreased by a mean of 50% (range: brain = 71–42%; liver = 63–49%) and albumin concentration decreased by a mean of 43% (range: brain = 71–40%; liver = 71–44%). In all CFD-diluted control samples a greater loss of protein concentration occurred than with brain and liver absorption. In the three samples where LCA decreased after brain absorption, there was no correlation between the degree of dilution and the loss of LCA.

Serial dilutions of five SLE sera (two from the cerebral group and three from the non-cerebral group) showed that LCA behaved in a complex manner. Fig. 2 shows that, at a 1:10 dilution, LCA rose in four sera and remained unchanged in the fifth. A progressive reduction in LCA then followed to a 1:1,000 dilution and at 1:10,000 rose or remained static. There was no distinctive pattern between the cerebral and non-cerebral groups.

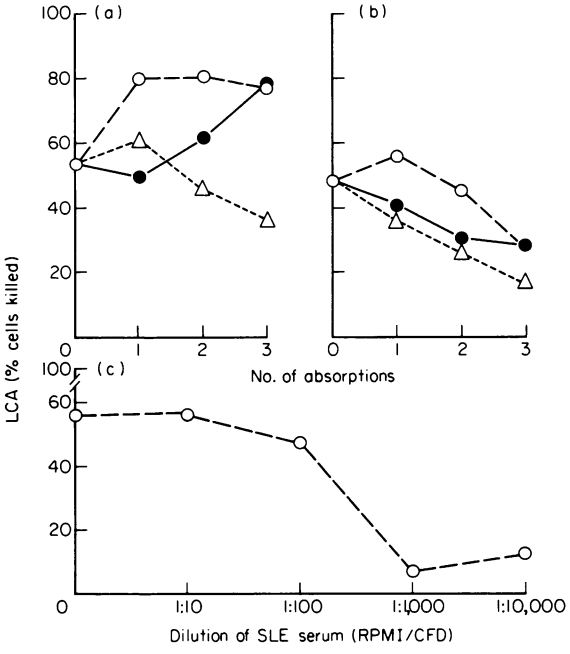

Fig. 1. Changes in LCA with tissue absorption and serum dilution. (a) Absorption of undiluted serum, (b) absorption of serum first diluted 1:10 (in RPMI/CFD), (c) serial dilutions of same serum. (o- - - -o) CFD, (●——●) frontal cortex, (▵· · · ·▵) liver.

Table 1. Per cent lymphocytotoxic activity in SLE patients with and without cerebral involvement

	Per cent LCA		
Test group	Preabsorption	Frontal cortex (3 absorption)	Liver (3 absorption)
Cerebral SLE	67	24	27
	42	66	72
	92	96	30
	55	68	38
	60	66	58
	65	77	23
Non-cerebral SLE	58	49	67
	62	7	8
	56	87	5
	53	77	37

LCA was increased in 80% of SLE sera (see Fig. 3) and was significantly higher in the cerebral group [mean 65 *vs* 41% in the non-cerebral group, $P < 0{\cdot}001$ (t-test)]. There was no correlation between the levels of immune complexes, complement components and LCA in the two groups.

DISCUSSION

Patients with cerebral SLE exhibited a higher mean serum LCA than those without cerebral involvement. Similar findings have been reported by others (Butler *et al.*, 1972; Bluestein & Zvaifler,

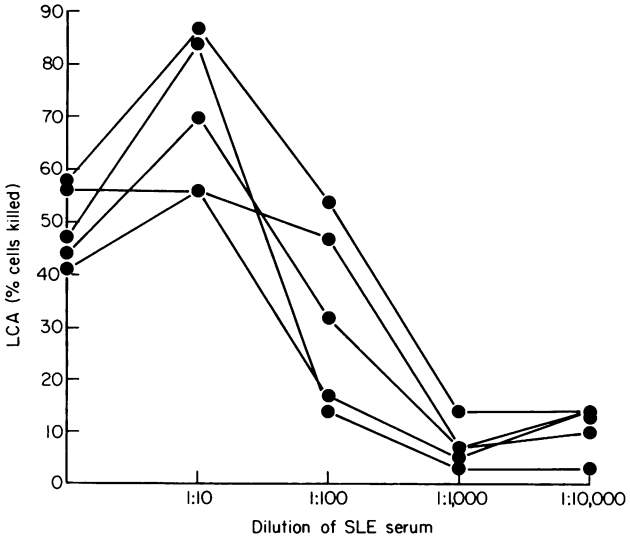

Fig. 2. Effect of serial dilutions on the LCA in five SLE sera.

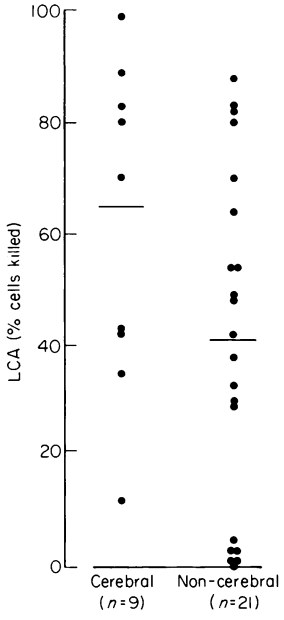

Fig. 3. LCA in SLE sera from patients with and without cerebral involvement. $P < 0{\cdot}001$ using the t-test.

1976; Bresnihan *et al.*, 1977). However, high levels of lymphocytotoxicity could be demonstrated in both groups, suggesting that this reaction was not specific for cerebral lupus. Indeed, we have been unable to confirm selective LCA depletion by brain absorption as reported by Bluestein & Zvaifler (1976) and Bresnihan *et al.* (1977). These striking differences in results may be explained by problems in methodology. We experienced great difficulty in controlling for dilutional factors as well as non-specific absorption by tissue. This was highlighted by the complex behaviour of LCA in individual sera following simple serial dilution (Fig. 2) and in one case where absorption was performed on diluted serum (Fig. 1). In this case it was shown that the enhancement of LCA by brain absorption was due to complex dilutional behaviour rather than an intrinsic effect of the brain

homogenate (the effect was reversed at the higher dilution of 1:10). We conclude that the measurement of LCA absorption by tissue homogenates is not an accurate index of an immunological reaction between lymphocytotoxins and cerebral or liver antigen, but more one of a non-specific dilutional effect or entrapment of serum proteins by the variable density of the homogenates. It is interesting that Bresnihan *et al.* (1977) showed that DNA-binding activity in sera with brain-absorbable LCA was unchanged following the absorption procedure and in one case the DNA binding was slightly enhanced. It is possible that this was also a manifestation of a complex dilutional effect of the DNA-binding capacity of sera as we have shown with LCA.

Although we demonstrated significant differences between levels of LCA in the cerebral and non-cerebral groups, other parameters of disease activity (immune complexes and complement) did not differ significantly. This supports the data reported by Winfield *et al.* (1978) that established markers of generalized disease activity in SLE were poor markers of cerebral lupus. That cerebral damage is secondary to immune complex vasculitis in SLE is supported by our findings of the non-specificity of LCA. Even in clinically quiescent cerebral lupus, Pinching, Travers & Hughes (1978), using *in vivo* scanning techniques, were able to show abnormalities in oxygen consumption and blood flow, indicating active cerebral vasculitis. More recently, Bresnihan *et al.* (1979) and Bluestein (1979) have reported reactivity of anti-lymphocyte antibodies with multiple cerebral antigens. These reports suggest that exposure of cerebral tissue during vascular damage may be the primary antigenic stimulus but such issues remain to be clarified.

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