Lymphocytotoxic antibodies in systemic lupus erythematosus: their clinical significance

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Cold-reactive lymphocytotoxic antibodies are present in the serum of most patients with systemic lupus erythematosus (SLE) (Mittal *et al.*, 1970; Terasaki *et al.*, 1970). They appear to be equally cytotoxic for autologous and heterologous lymphocytes (Stastny and Ziff, 1971). Studies of the nature of these antibodies showed that their cytotoxic effect is temperature-dependent and maximal at 15°C, that their immunoglobulin class is IgM, and that they are equally reactive with T and B lymphocytes (Winfield *et al.*, 1975a). Studies of the clinical significance of cold-reactive lymphocytotoxic antibodies in SLE have shown a consistent association with neuropsychiatric complications (Butler *et al.*, 1972; Bluestein and Zvaifler, 1976; Bresnihan *et al.*, 1977b). In other respects, however, clinical studies have been inconclusive, which may partly be related to the retrospective nature of most series. Previous studies have suggested an association between cold-reactive lymphocytotoxic antibodies and lymphopenia (Winfield *et al.*, 1975b; Utsinger, 1976). A detailed prospective clinical analysis of SLE began at Hammersmith Hospital in 1973. In all, 50 patients have been investigated, and their clinical features have been described elsewhere (Grigor *et al.*, 1978). This report relates the clinical features in these patients to the presence of lymphocytotoxic antibodies.

Patients and methods

Fifty successive patients with SLE, seen at Hammersmith Hospital between 1973 and 1977, were intensively investigated during at least one hospital admission and subsequently followed as outpatients. A strict study protocol had been designed which included a large number of serological and non-invasive investigative procedures (Grigor *et al.*, 1978).

LYMPHOCYTOTOXIC ANTIBODY ASSAY

Serum was obtained at each hospital visit and stored in aliquots at -20°C until the time of testing. The

two-stage microdroplet dye exclusion cytotoxicity assay was used (Mittal *et al.*, 1968). Target lymphocytes from at least 20 of a panel of 27 normal subjects were used to test each serum sample. All incubations were at 15°C. Fresh rabbit serum was used as a source of complement. The degree of cytotoxicity was assessed independently by two observers using phase-contrast microscopy and without knowledge of which serum was being tested. Cytotoxicity was expressed as an index which represented the mean percentage of lymphocytes killed by each serum. The cytotoxicity index ranged between 1 and 8: 1 = <20% lymphocytes killed (regarded as normal); 2 = 20-40% killed; 4 = 40-60% killed; 6 = 60-80% killed; and 8 = >80% killed. Each serum with a score of 2 or greater resulted in at least 20% lymphocyte killing in 10 or more donor lymphocyte suspensions.

SEROLOGICAL METHODS

Antibodies to DNA were measured by the Farr ammonium sulphate precipitation technique (Pincus *et al.*, 1969). ¹⁴C-DNA was obtained from the Radiochemical Centre, Amersham. C3 concentrations were measured by radial immunodiffusion in agar (Kohler *et al.*, 1967).

Statistical significance was determined by Student's *t* test for unpaired samples.

Results

CLINICAL FEATURES

In all, 336 serum samples, together with clinical, haematological, and serological information, were available from 49 of the patients. Of these, 235 sera (70%) showed abnormal lymphocytotoxicity and sera from 40 patients (80%) had lymphocytotoxic antibodies at some time during the course of their illness. The Table lists the major clinical manifestations of SLE. The mean ($\pm$ SD) of the maximum cytotoxicity indices observed for each patient who at some time showed a particular feature is compared to the mean of those who did not. Thus the mean

Table Lymphocytotoxic antibodies and individual clinical features

	No. of patients	Cytotoxicity*		P value
		Involved	Uninvolved	
CNS	21	5.4 ± 2.1	3.1 ± 1.4	0.001
Cutaneous	39	4.4 ± 2.1	2.3 ± 1.0	0.004
Arthritis	43	4.1 ± 2.2	3.7 ± 1.1	NS
Serositis	28	4.1 ± 2.1	4.0 ± 2.1	NS
Renal	18	3.9 ± 1.9	4.1 ± 2.3	NS

*Results are expressed as the mean (± SD) of the maximum cytotoxicity indices observed for each patient with and without individual clinical features.

maximum cytotoxicity index of the 21 patients who at some time developed evidence of CNS involvement was 5.4 ± 2.1, compared with 3.1 ± 1.4 in those patients who remained free of neuropsychiatric disease. This difference was statistically significant (P < 0.001). Similarly, there was a significant difference (P < 0.004) between the mean cytotoxicity index of those who developed cutaneous manifestations (4.4 ± 2.1) and those who did not (2.3 ± 1.0). There was no statistical difference between the indices of those who had, or had not, other major manifestations.

There was considerable fluctuation in the degree of cytotoxicity in individual patients throughout the study. However, there was no association between the changes in lymphocytotoxicity and the appearance of disease exacerbations. Indeed, in some patients the maximum cytotoxicity coincided with periods of apparent remission. Furthermore, the degree of lymphocytotoxicity was not noticeably altered by corticosteroid therapy. This is shown in Fig. 1, which compares the mean cytotoxicity (± SD) of sera obtained from groups of patients taking various doses of prednisone or none. The lymphocytotoxicity was similar in each group and there was a considerable degree of overlap.

HAEMATOLOGICAL FEATURES

It was possible to compare lymphocyte counts with the lymphocytotoxicity of 114 sera. This figure excludes sera obtained from patients receiving cytotoxic agents such as cyclophosphamide or azathioprine. The results are shown in Fig. 2. No association between lymphopenia and the degree of lymphocytotoxicity was seen. Thus the lymphocytotoxicity of sera obtained from patients with severe lymphopenia (< 750/mm³) was not different from patients with either moderate lymphopenia (750-1500/mm³) or normal lymphocyte counts (> 1500/mm³). Furthermore, there was no association between lymphocytotoxicity and anaemia, leucopenia, or thrombocytopenia.

Fig. 1 Lymphocytotoxic antibodies and corticosteroid therapy. Mean (± SD) lymphocytotoxicity of sera obtained from patients receiving various doses of prednisone.

SEROLOGICAL FEATURES

Comparison between lymphocytotoxicity and DNA-binding values was possible in 315 sera, and with C3 concentrations in 215 sera. Sera with normal DNA-binding values (< 30%) tended to be less cytotoxic than sera with raised DNA-binding values (Fig. 3), but the difference was not statistically significant. Furthermore, the mean lymphocytotoxicity of sera with DNA-binding values of 30-50%, 50-75%, and 75-100% were almost identical. Similarly, the lymphocytotoxicity of sera with low C3 concentrations (< 60% normal) and normal C3 concentrations showed an equivalent degree of overlap.

Discussion

Cold-reactive lymphocytotoxic antibodies are almost universally present in patients with SLE. Nevertheless, their role in pathogenesis remains unclear. The present study was undertaken to overcome the inherent problems of retrospective analysis so that further insight into the clinical relevance of lymphocytotoxic antibodies might be obtained. The most striking observation was that serum from patients with neuropsychiatric manifestations was more lymphocytotoxic than serum from patients without

Fig. 2 Lymphocytotoxic antibodies and lymphocyte counts. Mean ($\pm$ SD) lymphocytotoxicity of sera shows no correlation with total lymphocyte counts determined at time of obtaining each serum sample.

Fig. 3 Lymphocytotoxic antibodies, anti-DNA antibodies, and C3 levels.

these manifestations. This finding is in agreement with others (Butler *et al.*, 1972; Bluestein and Zvaifler, 1976). These results, in association with the observation that lymphocytotoxic antibodies from

patients who have neuropsychiatric symptoms cross-react with human brain (Bresnihan *et al.*, 1977b), strongly suggest a possible pathogenic role in cerebral lupus. Our study suggests that of the other clinical manifestations only cutaneous lesions correlate with lymphocytotoxicity. Serological evidence of disease activity, as represented by DNA-binding values and C3 concentrations, does not appear to correlate with the lymphocytotoxicity of individual sera.

An association between lymphopenia and the presence of lymphocytotoxic antibodies has been previously reported (Winfield *et al.*, 1975a; Utsinger, 1976). This observation was not supported by our study. It should be noted that our lymphocytotoxicity assays were performed at 15°C using undiluted serum samples. Assays performed under similar conditions by Winfield *et al.* (1975a) also failed to show an association with lymphopenia. When they used undiluted serum samples they found an association with lymphopenia only when assays were performed at 25°C, suggesting the possibility that a distinct subpopulation of antilymphocyte antibodies may be cytotoxic at higher temperatures. Previously, these authors had demonstrated that the predominant cold-reactive antilymphocyte antibody in SLE was maximally cytotoxic at 15°C and that the activity rapidly declined at higher temperatures (Winfield *et al.*, 1975b). Utsinger (1976) reported an inverse linear correlation between serum cytotoxicity measured at 15°C and peripheral blood lymphocyte counts. However, this correlation was made with a relatively small number (50) of matched samples and the degree of lymphopenia was modest (all greater than 750/mm³). Others have observed associations between lymphocytotoxic antibodies and various other haematological disorders, such as haemolytic anaemia or thrombocytopenia, or both (Bluestein and Zvaifler, 1976), and leucopenia (Butler *et al.*, 1972), but not specifically with lymphopenia. Again, these observations were not confirmed by us. It would indeed be surprising if an antibody whose cytotoxic capacity was so temperature-dependent could lead to substantial cell lysis at physiological temperatures. Clearly, other antibodies with different physical requirements may yet be incriminated in the lymphopenia of SLE.

The *in-vivo* action of cold-reactive lymphocytotoxic antibodies on lymphocyte function remains unclear. A spontaneously occurring thymocytotoxic antibody appears in New Zealand mice (Shirai and Mellors, 1972), and it has been suggested that this antibody may result in altered lymphocyte function, particularly impaired suppressor lymphocyte function (Gelfand and Steinberg, 1973). Wernet and Kunkel (1973) and Williams *et al.* (1973) have demonstrated an assortment of human antilympho-

cyte antibodies that may modulate lymphocyte function, though these are apparently distinct from the characteristic IgM cold-reactive antibody.

The possibility that lymphocytotoxic antibodies cross-react with other tissue antigens and thereby mediate tissue injury has also been suggested. In this way reactivity with human brain antigens may result in cerebral manifestations (Bluestein and Zvaifler, 1976; Bresnihan *et al.*, 1977b). In addition, cross-reactivity with trophoblast-reactive lymphocytotoxic antibodies in SLE patients having spontaneous abortions suggested a possible role for these antibodies in the high spontaneous abortion rate (Bresnihan *et al.*, 1977a). The demonstration of cross-reactivity with fetal erythrocytes but not adult erythrocytes suggested that cold-reactive lymphocytotoxic antibodies may share certain characteristics with cold agglutinins, such as specificity for i antigen (Goldberg *et al.*, 1978). Possibly, therefore, lymphocytotoxicity is a feature common to many antibodies whose primary specificity is for antigens present on several different tissues. As suggested by our study, lymphocytotoxicity mediated by cold-reactive antilymphocyte antibodies may never occur *in vivo*. The pathogenic effects of lymphocytotoxic antibodies may arise through reactions with other tissues.

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