Clin. exp. Immunol. (1974) **17**, 237–243.

LYMPHOCYTOTOXIC ANTIBODIES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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(*Received* 9 *November* 1973)

SUMMARY

Lymphocytotoxic activity could be demonstrated in the sera of patients with systemic lupus erythematosus. The number of positive reactions varied with temperature of incubation. Lymphocytotoxicity was present in 88%, 49% and 11% of sera tested at 15°C, 22°C and 37°C respectively. At an incubation temperature of 22°C, the presence of the lymphocytotoxic antibody in the sera could be correlated with significantly higher titres of anti-nuclear factor, anti-single-stranded DNA and the histological appearance of active diffuse glomerulonephritis in renal biopsies.

INTRODUCTION

Lymphocytotoxic antibodies have been demonstrated in the serum of patients with systemic lupus erythematosus (Mittal *et al.*, 1970; Terasaki, Mottironi & Barnett, 1970; Stastny & Ziff, 1971). The exact clinical and biological significance of this antibody is obscure. There is some evidence that the presence of the antibody is related to specific organ damage in SLE (Butler *et al.*, 1972). This communication reports on relationships between the presence of this antibody and (1) titres of anti-nuclear factor, (2) presence of abnormal levels of antibody to single-stranded DNA (anti-SSDNA), and (3) the nature of the renal involvement.

MATERIALS AND METHODS

Fifty-one serum specimens from forty-one patients with SLE as previously defined (Pollak, Pirani & Schwartz, 1964) were studied. Control subjects consisted of fifty patients matched by sex and age who were attending a general medical outpatient clinic for 'non-collagen' illnesses. All serum specimens were stored at −70°C until examined.

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Detection of lymphocytotoxic activity

Allogeneic lymphocytes from healthy subjects were isolated by the Ficol–Hypaque flotation method (Boyum, 1968), and cytotoxic activity determined by the micro-droplet technique (Mittal *et al.*, 1968). Briefly, it consisted of the incubation of 1500–2000 lymphocytes in 0·001 ml of test serum under oil in disposable microtest plates (Falcon Plastics, Los Angeles, California). After incubation for 30 min, 0·005 ml of rabbit complement was added and incubation continued for another $3\frac{1}{2}$ hr. Finally, 0·005 ml of 40% formaldehyde was added and cytotoxicity assessed with an inverted phase microscope. The reactions were graded from 0 to 3 based on percentage cytotoxicity in comparison to negative controls: 0 = <10%, 10–30% = 1+, 30–70% = 2+, and >70% = 3+. Negative and positive controls were included in every experiment.

Titration of anti-nuclear factor

Details of the technique have been described previously (Mandema *et al.*, 1961). Modifications are: (a) Sprague–Dawley rat liver used as a substrate; and (b) peroxidase-conjugated antibody instead of fluorescein-conjugated antibody used as a marker. The preparation of the conjugate and the modified technique used are in accord with reported procedures (Benson & Cohen, 1970; Clyne *et al.*, 1972).

Assay for anti-SSDNA

This was done by a solid-phase enzyme-linked immunoassay. The details of the method will be reported elsewhere (Pesce *et al.*, 1973). Essentially, it consists of the preparation of the antigen (SSDNA) on an insoluble support, layering of test (patient's) serum containing anti-SSDNA onto antigen, and using the anti-SSDNA as an antigen to react with anti-human IgG labelled with peroxidase. The colour developed by the enzymatic reaction of peroxidase with *o*-dianisidine is proportional to the concentration of anti-SSDNA. The actual procedure consisted of the following steps.

(a) Preparation of DNA by the method of Marmur (1961), using commercially obtained calf thymus DNA (Sigma Chemical Company, St Louis, Missouri), starting from the Sevaging step. Single-stranded DNA was prepared by heating 0·5 ml of stock DNA solution (0·20 mg per ml) at 100°C for 10 min followed by immediate dilution into 4·5 ml of ammonium acetate buffer, pH 5.

(b) Goat anti-human globulin peroxidase was prepared by the method of Avrameas (1969).

(c) Procedure for titration consisted of coating each polystyrene tube with SSDNA by incubating with 0·2 ml of diluted SSDNA solution for 3 hr at room temperature. The excess SSDNA was removed, and tubes washed twice with bovine serum albumin (BSA) buffer. Additional possible binding sites were coated with goat serum solution followed by removal and further washings with BSA buffer. Sera of patients at various dilutions were then placed in tubes and allowed to react for 4 hr removed, and tubes washed. Goat anti-human globulin–peroxidase conjugate diluted 1:100 in albumin buffer was added and incubated for 4 hr. Excess conjugate was removed, tubes washed and colour developed by incubation with 0·25 mg of *o*-dianisidine and 0·003% H_2O_2 in 3 ml of 0·01 M phosphate buffer (pH 6) for 20 min. The reaction was stopped with 20 μl of 1% sodium azide and the reaction product was read at 460 nm. A single serum with a high anti-SSDNA titre was used as a reference standard.

TABLE 1. Results of cytotoxic reactions at different temperatures on sera from patients with SLE and matched disease controls

Incubation temperature	Category	Number of sera: Total tested	Lymphocytes exhibiting cytotoxicity: <10%	10–30%	30–70%	>70%
15°C	SLE	51	6	2	7	36
	Control	50	42	0	5	3
22°C	SLE	51	26	4	7	14
	Control	50	50	0	0	0
37°C	SLE	18	16	1	1	0
	Control	50	0	0	0	0

Assessment of histological activity of renal biopsies

This was done by a semi-quantitative method of analysis previously described in detail (Pirani, Pollak & Schwartz, 1964; Dujovne *et al.*, 1972). The biopsies were divided into the following histological groups: normal; minimal glomerular change; focal glomerulonephritis; diffuse glomerulonephritis; and membranous glomerulonephritis (Pollak & Pirani, 1974). The degree of histological activity was estimated as described previously (Dillard *et al.*, 1973).

RESULTS

Frequency of antibody and effect of incubation temperature

The results are shown in Table 1. The frequency of positive reactions on SLE sera was

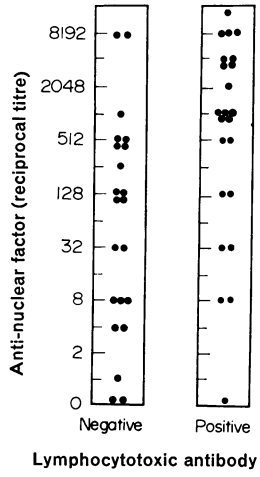

FIG. 1. Relationship of the titre of anti-nuclear factor of SLE patients to presence of lymphocytotoxic antibody at an incubation temperature of 22°C. The difference between the geometric means of titres of the two groups is statistically significant ($t = 2{\cdot}68$, $P < 0{\cdot}02$).

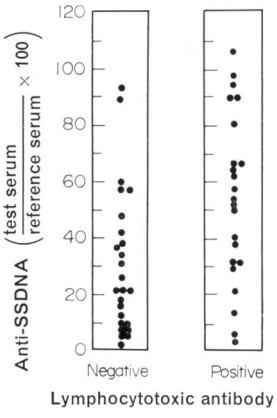

FIG. 2. Relationship of the titre of anti-SSDNA of SLE patients to presence of lymphocytotoxic antibody at an incubation temperature of 22°C. The difference between the means of the two groups is statistically significant ($t = 2{\cdot}79$, $P < 0{\cdot}01$).

88% at 15°C, 49% at 22°C and 11% at 37°C. On control sera, the frequency of cytotoxic reactions was 16% at 15°C; all were negative at 22°C and 37°C.

Relationship of lymphocytotoxic antibody to anti-nuclear factor (ANF), anti-SSDNA, and renal histological findings

At an incubation temperature of 15°C, forty-five of fifty-one sera from patients with SLE gave a positive lymphocytotoxic reaction. Because of the high incidence of positive tests, it was not surprising that no correlation could be established between the presence or absence of cytotoxic antibody and the presence or titre of ANF and anti-SSDNA.

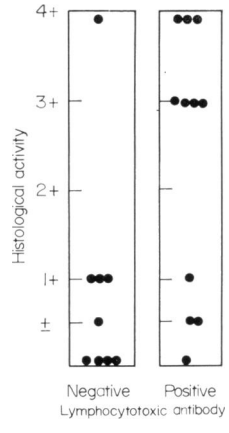

FIG. 3. Relationship of the degree of histological activity of renal biopsies of SLE patients to presence of lymphocytotoxic antibody at an incubation temperature of 22°C. Comparison of both groups shows a good correlation between degree of activity and presence of antibody ($x^2 = 3{\cdot}72$, $P < 0{\cdot}1$).

From Figs 1 and 2, it is apparent that at an incubation temperature of 22°C, lymphocytotoxins were found more frequently in sera with high titres of ANF, as evidenced by a statistically significant difference between the geometric means of titres of the two populations ($t = 2{\cdot}68$, $P<0{\cdot}02$). A significant difference was also observed in the levels of anti-SSDNA between SLE patients with and without lymphocytotoxic antibody ($t = 2{\cdot}79$, $P<0{\cdot}01$).

The relationship between the histological findings and presence of lymphocytotoxic antibody at an incubation temperature of 22°C are shown in Fig. 3 and Table 2. There is a good correlation between the more severe degrees of renal histological activity and the presence of the antibody ($x^2 = 3{\cdot}72$, $P<0{\cdot}1$). The pattern of histological involvement shows a high degree of correlation with the presence of lymphocytotoxic antibody, being significantly associated with active diffuse glomerulonephritis ($x^2 = 4{\cdot}59$, $P<0{\cdot}05$).

TABLE 2. Relationship between presence of lymphocytotoxic antibody at an incubation temperature of 22°C and nature of renal histological involvement in SLE patients

	Lymphocytotoxic antibody		Statistical significance
	Negative	Positive	
Active diffuse glomerulonephritis	2	10	$x^2 = 4{\cdot}59$, $P<0{\cdot}05$
Other histological patterns	6	2	

DISCUSSION

The present study confirms and extends previous observations on lymphocytotoxic antibodies in patients with SLE. The finding of a frequency of 88% of such cytotoxicity at 15°C roughly equals the 86% previously reported (Terasaki *et al.*, 1970), as does a frequency of 51% of positive reactions at 22°C accord with a previous finding of 60% (Stastny & Ziff, 1971). The heightened sensitivity of the reactions in the cold is confirmed both for SLE patients and for other 'non-collagenous' disease subjects. Contrary results on the effect of temperature have been recorded by Stastny & Ziff (1971). The reasons for this discrepancy are not immediately apparent as the techniques used by all workers have been similar.

The association of lymphocytotoxic antibody at 22°C with significantly higher titres of anti-nuclear factor could suggest that lymphocytotoxins are important indicators of disease activity in this disease. This is confirmed by the positive correlation of the antibody with elevated titres of anti-SSDNA; the latter antibody has been found in serums of patients with active SLE, and in disease states showing active tissue destruction (Koffler *et al.*, 1969). Furthermore, histological activity of renal disease could be correlated with the presence of the antibody. These findings corroborate the previous report of an inverse correlation of this antibody with C'3 levels (Butler *et al.*, 1972). These workers could not establish any relationship between renal manifestations and the presence of the antibody. However, specific criteria for renal involvement were not described in the report.

The lack of a definite correlation between the presence of this antibody at 15°C and titres

of ANF or anti-SSDNA is not surprising, in view of our finding that at this lowered temperature a number of subjects with non-collagen diseases also have this antibody, giving rise to 'false positives'. While a positive association was established between the presence of this antibody at 15°C to the presence of anti-nuclear factor in a number of 'autoimmune' disorders, no statistically significant relationship was detected with SLE; nor was association with anti-SSDNA found (Terasaki *et al.*, 1970). From a clinical standpoint, it would appear that the test would be more useful when conducted at 22°C.

Autocytotoxic activity has previously been shown (Terasaki *et al.*, 1970; Stastny & Ziff, 1971) with activity against T cells specifically demonstrated (Lies, Messner & Williams, 1973).

The biological significance of this antibody has been discussed. Among the possibilities described (Terasaki *et al.*, 1970), the most attractive, in the light of our findings, would be to endorse the hypothesis that the action of this antibody on cell membranes may lead to cell injury which in turn results in release of intracellular contents and nuclear antigens (Butler *et al.*, 1972).

ACKNOWLEDGMENT

This study was supported in part by NIH grants AM 10314 and AM 17196.

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