Clin. exp. Immunol. (1975) **20,** 187–192.

REDUCED ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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(*Received* 9 *September* 1974)

SUMMARY

The peripheral blood mononuclear cells from twenty-three patients with SLE were studied. They showed a reduction in antibody-dependent cell-mediated cytotoxicity. This reduction was significantly related to disease activity. No correlations were found with other clinical features. Some of the possible explanations for this finding are discussed.

INTRODUCTION

Since the initial description of antibody-dependent cell-mediated cytotoxicity in 1965 (Moller, 1965), there have been few reports relating this to human disease states. These reports have shown specific cytotoxicity in Hashimoto's thyroiditis (Calder *et al.*, 1973) and in transitional cell carcinoma of the bladder (O'Toole *et al.*, 1974); a possible role in renal transplant rejection (Hersey, Cullen & MacLennan, 1973); a selective deficiency of effector cells in a case of macroglobulinaemia (Campbell *et al.*, 1972), and a decrease of the effector cell during immunosuppressive therapy in acute lymphocytic leukaemia (Campbell *et al.*, 1973), multiple myeloma (Mellstedt, Jondal & Holm, 1973) and in inflammatory bowel disease (Campbell *et al.*, 1974). Recently, antibody-dependent cell-mediated cytotoxicity was studied and found to be reduced in NZB mice (Greenberg & Playfair, 1974). In the present study, we describe similar findings in human SLE. Correlations will be made with clinical observations.

MATERIALS AND METHODS

Patient and control populations

Twenty-three patients (twenty-two females and one male) with SLE were selected for study. Clinical and laboratory features were fully evaluated by standard techniques. The mean age of patients was 29 years. Seventeen patients had active and six had inactive

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disease. Eleven patients were taking corticosteroids; six were receiving no medication; and six were receiving other therapeutic agents. There were no patients receiving other types of immunosuppressive therapy, nor had any received such treatment within the past 2 years. Each patient had a minimum of four of the fourteen preliminary criteria proposed by the American Rheumatism Association for the classification of SLE (Cohen *et al.*, 1971).

Fourteen normal female volunteers served as the control group. The mean age of volunteers was 30 years.

Test system (effector cells, target cells, antibody and cytotoxicity assay)

The method used was that of Holm (Holm & Perlmann, 1967) and Perlmann (Perlmann & Perlmann, 1970) with the following modifications. The medium used in all testing was supplemented Eagle's minimal essential medium. The target cells were Chang cells (human polyploid liver cells) grown in continuous culture in 8-ounce Falcon Tissue Culture flasks and harvested after incubation with 0·25% trypsin (Gibco) for 30 min. A single lot of heat-inactivated rabbit antiserum to Chang cells was used at a 1:10,000 dilution throughout the entire study. 5×10^5 Peripheral blood mononuclear cells and 2×10^4 target cells were used in each tube, providing a mononuclear:target cell ratio of 25:1. Results were assayed at 18 hr.

Calculation of cytotoxicity

At the end of the incubation period the tubes were centrifuged at 300 *g* for 10 min and half of the supernatant was transferred to another tube. The tubes were counted in a well scintillation counter for 1 min. The percentage ^{51}Cr release was calculated using the equation: percentage ^{51}Cr release = $\{[2 \times (\text{counts in supernatant} - \text{background})]/[(\text{counts in supernatant} - \text{background}) + (\text{counts in cells button} - \text{background})]\} \times 100$. Taking into account the maximum ^{51}Cr release, obtainable by lysing the cells with saponin, of 87% (mean of thirty determinations) and the baseline ^{51}Cr release of Chang cells alone, the percentage cytotoxicity was calculated using the equation: percentage cytotoxicity = $[(\text{percentage } ^{51}Cr \text{ release})/(87 - \text{baseline})] \times 100$. The percentage antibody-dependent cell-mediated cytotoxicity was determined by subtracting the cytotoxicity obtained with sensitized Chang cells from that obtained with unsensitized Chang cells.

Statistical analysis

Differences were analysed using the two-tailed Wilcoxon Rank Sum *t*-test.

RESULTS

Table 1 compares the total cytotoxicity (that observed in the presence of antibody) and direct cytotoxicity (that observed in the absence of antibody) in patients with SLE and controls. It can be seen that direct cytotoxicity makes a relatively large contribution to total cytotoxicity. The cause of the direct cytotoxicity is not definitively known, but one of the causes may be antibody production by a proportion of lymphocyte donors which cross-reacts with an antigen on the Chang cell. MacLennan, Loewi & Howard (1969) reported that twelve of seventy-eight patients and controls had an IgG in their sera which could sensitize Chang cells. This IgG could be produced in significant amounts by the peripheral blood lymphocytes during the course of the 18 hr of incubation (Harding & MacLennan, 1972).

For this reason it was decided to subtract the direct cytotoxicity from the total cytotoxicity in an attempt to eliminate this component. In many cases this reduced the apparent extent of antibody-dependent cell-mediated cytotoxicity. The statistical significance of the difference between the populations was calculated on both the total cytotoxicity and on the calculated antibody-dependent component of cytotoxicity. The manoeuvre of subtracting the direct cytotoxicity did not alter the statistical significance of the difference between the different populations.

TABLE 1. Comparison of the total, direct antibody-dependent cytotoxicity in patients with SLE and controls

	Total cytotoxicity			Direct cytotoxicity			Antibody-dependent cytotoxicity		
Patients	Mean	s.e.m.	Significance	Mean	s.e.m.	Significance	Mean	s.e.m.	Significance
Group 1 (SLE)	21·40	2·63	<0·01	10·96	1·46	<0·01	10·44	3·11	<0·01
Group 2 (Controls)	40·93	6·44		19·00	5·49		21·93	7·08	

Using the antibody-dependent component, the twenty-three patients with SLE (Group I) had a mean cytotoxicity of 10·44% (s.e.m. = 3·11) compared to fourteen normal controls (Group II) with a mean cytotoxicity of 21·93% (s.e.m. = 7·08). The difference was statistically significant ($P < 0·01$) (Fig. 1). When the group of patients with SLE was divided according to activity, significant differences can be seen. Patients with active disease (Group IA) had a mean cytotoxicity of 9·63% (s.e.m. = 3·90), while those with inactive disease (Group IB) had a mean value of 14·33% (s.e.m. = 6·34). These differed at the 98% level of confidence. Patients with active disease (Group IA) also differed from controls (Group II)

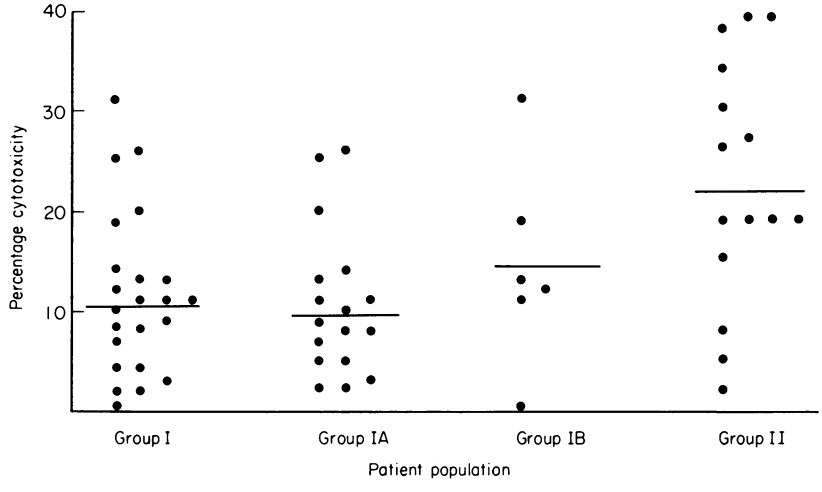

FIG. 1. Antibody-dependent cell-mediated cytotoxicity in patients with SLE. Group I = total SLE. Group IA = active SLE. Group IB = inactive SLE. Group II = controls. Group I *vs* Group II, $P < 0·01$. Group IA *vs* Group II, $P < 0·01$. Group IA *vs* Group IB, $P < 0·02$. Group IB *vs* Group II, $P = 0·10$.

($P<0{\cdot}01$) (Fig. 1). However, inactive patients (Group IB) and controls (Group II) showed no difference ($P = 0{\cdot}10$) (Fig. 1).

Total peripheral lymphocyte counts were obtained in seventeen of the patients with SLE. Twelve of these patients had disease which was active and five inactive. The difference in total peripheral lymphocyte counts between active and inactive patients was below the level of significance.

Within the group of patients with SLE, the degree of cytotoxicity did not differ when related to age at onset of the disease, age of the patient, disease duration, presence, absence, or titre of anti-DNA antibodies, or the level of serum complement. Differences in drug therapy were evaluated. Cytotoxicity did not relate to corticosteroid therapy (prednisone above or below 5 mg/day), antimalarials, aspirin, or combinations of these drugs. Specific types of organ system involvement, using both clinical and histological parameters, did not show any relationship to results. There was no correlation between the degree of activity, assessed by the number of organ systems involved, and the reduction in cytotoxicity.

DISCUSSION

The results of our study demonstrate a reduction in antibody-dependent cell-mediated cytotoxicity in SLE, occurring primarily in active disease. Identification of the effector cell remains speculative, somewhat limiting our ability to evaluate the full significance of our findings. Nevertheless, there are several alternative explanations of the nature of the impaired cytotoxic function. Leading possibilities include either a reduction in the number of circulating effector cells of normal activity, or the presence of normal numbers of functionally ineffective cytotoxic cells.

A reduction in the number of circulating effector cells might result from a progressive permanent depletion of these cells, or a transient depletion related to activity of the disease. Our data, which shows a relationship of cytotoxic function to disease activity, suggests the latter as the more likely alternative, since a permanent defect would be unlikely to be selectively present during periods of increased disease activity. Reduction in the number of circulating effector cells could be produced in several ways. Marked reductions in this type of cytotoxic activity have been demonstrated during therapy with cytotoxic drugs in acute lymphocytic leukaemia (Campbell *et al.*, 1973), multiple myeloma (Mellstedt *et al.*, 1973), and inflammatory bowel disease (Campbell *et al.*, 1974). None of our patients were receiving cytotoxic drugs, but since the above mentioned reports have shown a somewhat selective suppressive effect on this cytotoxic function, the question should not be disregarded in future clinical studies. Destruction of the effector cell by lymphocytotoxic antibodies could take place. Such antibodies do occur in SLE, and their presence is thought to correlate with disease activity. Although these antibodies appear to act principally on T lymphocytes, there is also activity against other lymphoid cell subpopulations, and this could affect the cytotoxic cell involved in our study (Lies, Messner & Williams, 1973). The number of circulating effector cells could also be reduced as a result of diversion to extravascular sites. The presence of substantial numbers of lymphoid cells in the tissue of patients with SLE (Cruickshank, 1974), when lymphopenia is frequent, is consistent with this possibility.

Cytotoxic cells could be functionally ineffective, although present in normal numbers. This could be due to the presence of humoral inhibitory factors, an intrinsic defect in the effector cells, or the presence of cell-bound immune complexes. In *in vitro* systems, soluble

immune complexes of appropriate composition have been shown to block cytotoxic activity by competing with target cell-bound immunoglobulin for the Fc receptors of effector cells (MacLennan, 1972). Sera of patients with rheumatoid arthritis and inflammatory bowel disease have been reported to have inhibitory activity, and there is some evidence that this may be due to the presence of immune complexes (Barnett & MacLennan, 1972; Hallberg, 1972; Jewell & MacLennan, 1973). Circulating immune complexes (DNA–anti-DNA) occur in SLE (Harbeck *et al.*, 1973). In our study, cytotoxic activity was clearly reduced in patients with active disease, at a time when immune complexes are most likely to be present. However, in a limited number of tests, serum obtained from our patients with reduced cytotoxic activity was not inhibitory for normal mononuclear cells in our test system. We cannot, therefore, attribute the reduced cytotoxic activity observed in our patients to a simple inhibitory action of circulating immune complexes, nor to the presence of other types of inhibitory activity in the serum.

Our observations establish that a cellular cytotoxic function, clearly not an activity of thymus-processed lymphocytes, is reduced in SLE. There has been considerable emphasis placed on impaired thymus-derived lymphocyte functions in current concepts of pathogenic mechanisms of the disease (Quismorio & Friou, 1974). Our results may have an important bearing on these concepts, especially if, as has been proposed, the effector cell in man is demonstrated to be a B-lymphocyte precursor (Forman & Moller, 1973). It may be that the lymphocyte defect in SLE is much broader in nature than has been suspected. Further studies will be necessary to confirm or deny this hypothesis.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Mr Gary R. Mirick.

This work was supported by grants from The Arthritis Foundation and the National Institutes of Health.

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