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Impaired antigen-specific suppressor cell activity in patients with systemic lupus erythematosus

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SUMMARY

Antigen-specific suppressor cell activity of peripheral blood mononuclear cells was investigated in twenty-nine patients with systemic lupus erythematosus (SLE) and sixteen normal, age- and sex-matched healthy controls. Suppressor cell activity was generated by priming peripheral blood mononuclear cells with high dose antigen (ovalbumin) and adding the washed primed or control (unprimed) cells to autologous optimally stimulated target plaque-forming cell (PFC) cultures. The ability of the primed cells to interfere with an optimal ovalbumin-specific PFC response in the target cultures was used as a measure of antigen-specific suppressor cell activity. The results demonstrated reduced suppressor cell activity in the SLE patients relative to controls—46·8 ± 3·6% *vs* 63 ± 2·4% suppression respectively ($P < 0{\cdot}01$). Consistent with reduced suppressor cell activity was an increase in the plaque-forming cell response to ovalbumin in patients relative to controls (880 ± 73 *vs* 763 ± 102 PFC/10^6 cells respectively [$P = 0{\cdot}10$]). No correlation was demonstrated between suppressor cell activity in SLE patients and disease activity or therapy.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic disease characterized by B lymphocyte hyperreactivity leading to hypergammaglobulinaemia and autoantibody formation (Talal, 1976; Stobo & Loehnen, 1976). Recent evidence has accumulated suggesting that such immunologic aberrations may result from a defect in T-suppressor cell function (Katz & Benacerraf, 1972; Waldman & Broder, 1977). Support for this concept is provided by the NZB mouse model of SLE in which the loss of T-suppressor cell function temporally antedates the detection of autoantibodies (Talal, 1976; Klassen, Krakauer & Steinberg, 1977). A similar T-suppressor cell dysfunction has recently been reported in patients with SLE (Abdou *et al.*, 1976; Bresnihan & Jasin, 1977; Horowitz *et al.*, 1977; Sagawa & Abdou, 1978; Fauci *et al.*, 1978; Kaufman & Bostwick, 1979; Morimoto, Abe & Homma, 1979; Newman *et al.*, 1979; Sakane, Steinberg & Green, 1978). Although a variety of target assay systems have been employed to delineate the effect of T-suppressor cells in SLE, polyclonal activation with concanavalin A has been used almost exclusively to generate these suppressor cells.

Recently, several investigators have described the *in vitro* generation of antigen-specific suppressor cells capable of inhibiting *in vitro* antibody synthesis (Uytdehaag, Heijnen & Ballieux, 1978; Shore, Dosch & Gelfand, 1978). Utilizing high dose antigen priming, suppressor cell activity (SCA) could be generated in human peripheral blood mononuclear cells and its effect measured on target

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plaque-forming cell cultures. The present study was undertaken to assess antigen-specific SCA in patients with SLE.

MATERIALS AND METHODS

Patients. Twenty-nine patients with SLE seen at the Rheumatic Disease Unit of the Wellesley Hospital formed the patient population. There were twenty-six females and three males with a mean age of 38 years (range 20–62). All patients fulfilled ARA criteria for the classification of SLE (Cohen *et al.*, 1971). Fourteen patients were considered to have active disease at the time of the study based on the presence of two or more of the following clinical features: mucous membrane lesions, photosensitivity, malar rash, alopecia, arthritis, serositis, proteinuria, active urinary sediment and neuropsychiatric involvement; and two or more of the following laboratory manifestations: elevated DNA binding (Farr assay using ^{125}I) (Carr, Hoffman & Harbeck, 1975), low complement, i.e. total serum haemolytic complement (Kabat & Mayer, 1964) and/or C3, positive Coombs' test and hypergammaglobulinaemia. Ten patients were receiving corticosteroids in amounts less than 15 mg/day and none were receiving cytotoxins. Controls included seventeen age- and sex-matched healthy volunteers selected among hospital employees.

Cell preparation. Peripheral blood mononuclear cells (PBM) were isolated by Ficoll–Hypaque gradient centrifugation (Perper, Lee & Michelson, 1968). The cells were washed twice with phosphate-buffered saline (PBS), pH 7·4, and resuspended in RPMI 1640 (GIBCO, Grand Island, New York), supplemented with α-glutamine (2 mM), 2-mercaptoethanol (5×10^{-5} M), penicillin (100 u/ml), streptomycin (10 μg/ml), fungizone (25 μg/ml) and 10% heat-inactivated normal human serum.

Antigen. Crystalline ovalbumin (OA) (Sigma Chemical Company, St Louis, Missouri) was prepared as a stock solution (1 mg/ml) in PBS and stored at −20°C. Sheep red blood cells (SRBC) (Woodlyn Farms, Guelph, Ontario) were washed three times in PBS before use.

Complement. Commercially available guinea-pig complement (GIBCO, USA) was reconstituted and adsorbed three times with SRBC.

Plaque-forming cell (PFC) response. PFC were induced and evaluated according to the method of Dosch & Gelfand (1976). Briefly, 10-ml target cultures were set up in 17 × 100-mm plastic tissue culture tubes (Falcon, No. 2057) containing 2×10^6 fresh PBM with 10 μg OA (optimal for PFC generation) in supplemented RPMI 1640 media. After 6 days in moist 5% CO_2 at 37°C the target cultures were washed twice and assayed in triplicate for PFC according to the technique of Dosch & Gelfand (1976). Plaques less than 200 were considered background.

Generation of suppressor cells. Suppressor cells were generated by incubation of 2×10^6 fresh PBM with 100 μg of OA. Control cells were cultured without OA. After 24 hr, the cells were harvested, washed thoroughly with RPMI 1640 and resuspended at a concentration of 1×10^6 cells/ml. Cell viability and recovery of the OA-primed cultures was comparable to control cultures.

Suppressor assay. One million viable OA-primed or control (unprimed) cells were added to a target culture of 2×10^6 fresh autologous PBM. The mixture was cultured with 10 μg of OA for 6 days at 37°C, harvested and assayed for direct anti-OA PFC as described (Dosch & Gelfand, 1976). Suppression was expressed as

$$[1 - \frac{\text{PFC (P)}}{\text{PFC (C)}}] \times 100\% \text{ suppression,}$$

where PFC(P) was the PFC response when primed cells were added and PFC(C) the response when unprimed (control) cells were added to the target culture.

RESULTS

The *in vitro* PFC response of SLE patients and controls was first examined. As may be seen in Fig. 1, the mean PFC response of SLE PBM (880 ± 73 PFC/10^6 cells) was higher than that of normal cells

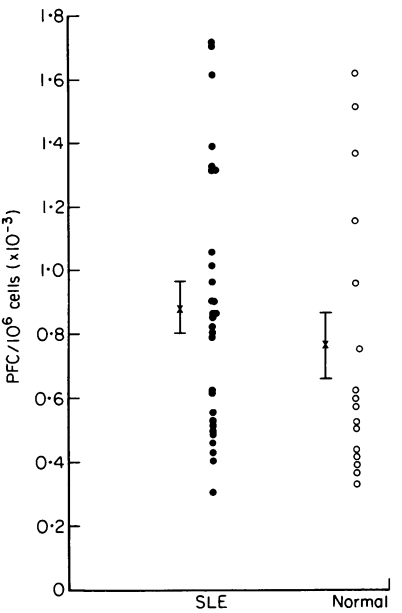

Fig. 1. PFC responses of optimally primed (10 μg OA/culture) target cultures in SLE (•) and normal controls (o). Each point represents the mean PFC response of triplicate cultures. The mean PFC response for the entire group is shown (X) and the error bars indicate ± 1 s.e.

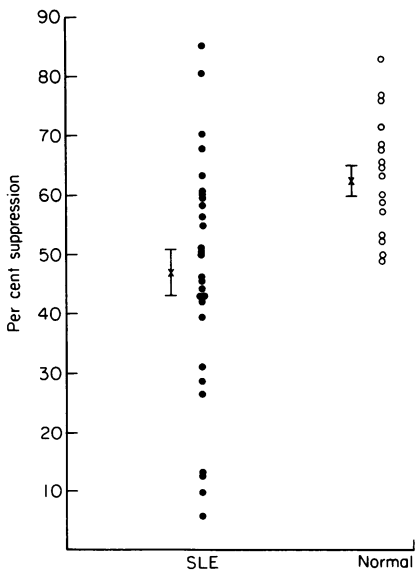

Fig. 2. Suppressor cell activity in SLE patients and normal controls generated by the addition of primed PBM (100 μg/culture) to optimally primed autologous target cultures. Each point represents per cent suppression for the individual patient (•) or control (o) (see Materials and Methods section). The mean per cent suppression for the entire group is shown (X) and the error bars indicate ± 1 s.e.

(763 ± 102 PFC/10^6 cells). The difference, however, did not reach statistical significance ($P = 0{\cdot}1$).

When antigen-primed PBM of normals were added at the initiation of the autologous target PFC culture, suppression of the PFC response was observed with a mean suppression of $63{\cdot}3 \pm 2{\cdot}4\%$ (Fig. 2). In contrast, antigen-activated PBM of SLE patients exhibited less suppression ($46{\cdot}8 \pm 3{\cdot}6\%$) relative to normals ($P < 0{\cdot}01$). Indeed, the majority of patients (i.e. 64·9%) exhibited SCA, 1 standard deviation below the mean of the control group. Moreover, 41·4% (12/29) patients exhibited SCA, 2 standard deviations or more below the mean of the control group.

A number of clinical correlations were attempted with SCA in the patient population. However, no correlation was observed between SCA in SLE patients and disease duration, activity or therapy.

DISCUSSION

The present study demonstrates that peripheral blood mononuclear cells of patients with SLE have defective antigen-specific SCA. The results are consistent with previous reports of impaired suppressor cell function in SLE patients in which suppressor cells were activated in a polyclonal fashion by concanavalin A (Abdou *et al.*, 1976; Bresnihan & Jasin, 1977; Horowitz *et al.*, 1977; Sagawa & Abdou, 1978; Fauci *et al.*, 1978; Kaufman & Bostwick, 1979; Morimoto *et al.*, 1979; Newman *et al.*, 1979; Sakane *et al.*, 1978). The precise relationship, however, between antigen-specific and non-antigen-specific SCA remains obscure. Consistent with impaired SCA was the increased immunoglobulin synthesis observed in some SLE patients. This finding is consistent with the findings of Morimoto *et al.* (1979) but contrasts with those of others (Fauci *et al.*, 1978; Bobrove & Miller, 1977) who noted an impaired PFC response to pokeweed mitogen. The reason for the discrepancy, however, remains unclear.

The relationship between suppressor cell function and disease activity in SLE has been inconsistent in the various reports to date. While some investigators (Abdou *et al.*, 1976; Bresnihan & Jasin, 1977; Sagawa & Abdou, 1978; Morimoto *et al.*, 1979) have noted an inverse relationship, others (Kaufman & Bostwick, 1979; Newman *et al.*, 1979; Horowitz *et al.*, 1977), including the authors of the present study, have not substantiated this relationship. The discrepancy may reflect differences in criteria for disease activity and the diversity of the patient populations studied.

The mechanism(s) of impaired antigen-specific suppressor cell function in SLE remains unclear. Alterations in the antigen–dose kinetics of the priming phase of the assay system did not account for the altered suppressor cell function observed since antigen–dose kinetics of the priming dose of OA were similar in patients and normal controls regardless of the degree of suppressor cell activity (unpublished observations). Although corticosteroid has been shown to impair polyclonal suppressor cell activity (Saxon *et al.*, 1978), no relationship between steroid therapy and antigen-specific SCA was observed in this study. Likewise, other drug therapy could not be implicated in the reduction of the antigen-specific SCA observed.

Recent studies in other systems have demonstrated inhibition of SCA by serum factors from patients with SLE (Alarcon-Segovia *et al.*, 1979; Twomey, Laughter & Steinberg, 1978; Sakane *et al.*, 1979). Preliminary studies of the effect of SLE serum on antigen-specific SCA of normal PBM revealed inconsistent results (unpublished observations).

A reduction in antigen-specific SCA in SLE patients may result from a reduction in the peripheral blood pool of T-suppressor cells and/or impaired function of existing suppressor cells. Recently Moretta *et al.* (1976) have identified a subset of T cells which contains suppressor cells. These cells, designated T_G cells, bear an Fc receptor for IgG and are physically separable from helper cells (T_M) which bear an Fc receptor for IgM. T_G cells have been shown to mediate *in vitro* antigen-specific (Shore, Dosch & Gelfand, 1978; Heijnen *et al.*, 1979) and non-specific (Moretta *et al.*, 1977) SCA. Studies to delineate alterations in this cell population have been carried out in patients with SLE (Fauci *et al.*, 1978; Newman *et al.*, 1979; Hamilton & Winfield, 1979) and have demonstrated a quantitative defect in the T_G suppressor cell population (Fauci *et al.*, 1978; Newman *et al.*, 1979). Whether a qualitative defect in the T-suppressor cell population exists in SLE patients remains to be determined.

The variable impairment of SCA in patients with SLE and lack of correlation with disease

activity observed by ourselves and other investigators (Newman *et al.*, 1979; Fauci *et al.*, 1978; Kaufman & Bostwick, 1979) suggests the possibility of additional immunoregulatory defects in SLE. One consideration is excessive helper T cell activity. To date, information on helper T cell function in patients with SLE is sparse. Fauci *et al.* did observe enhanced *in vitro* antibody production when SLE mononuclear cells were co-cultured with normal cells in the presence of pokeweed mitogen, but the difference between expected and observed responses in these cultures were not significantly different (Fauci *et al.*, 1978). Further studies are warranted to evaluate helper T cell function in patients with SLE.

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