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Suppressor cell defect in SLE: relationship to native DNA binding

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SUMMARY

Recent evidence suggests the presence of a suppressor T cell defect in systemic lupus erythematosus. We confirm the presence of such a defect and find a strong quantitative correlation between the loss of suppressor T cell function and the activity of SLE as measured by the presence of antibodies to native DNA. In addition, the serum of patients with active SLE contains a soluble factor which when incubated with normal peripheral blood lymphocytes induces a suppressor T cell defect. These data are consistent with the suppressor cell defect being involved in the propagation and possibly the pathogenesis of SLE, and suggests a positive feedback mechanism whereby a suppressor cell defect results in autoantibodies including antibody to suppressor cells.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown aetiology characterized by the production of large quantities of autoantibodies. The immune system of patients with active SLE is characterized by generalized B cell hyperactivity (Budman *et al.*, 1977) and impaired T cell function (Rosenthal & Franklin, 1975). These B and T cell abnormalities could be explained on the basis of loss of suppressor cells which may regulate both T and B cell function. Suppressor cell defects have been reported previously in the murine model of SLE (Krakauer, Waldmann & Strober, 1976).

The concept that a suppressor T cell loss results in autoantibody production by B cells would explain many of the clinical and immunologic findings in SLE. Indeed, suppressor cell defects in human SLE have been reported by ourselves and others (Krakauer *et al.*, 1979; Sakane, Steinberg & Green, 1978; Sagawa & Abdou, 1978).

In order to determine what relationship this suppressor cell defect might have with the extent of disease, we studied this suppressor cell defect as a function of disease activity. The disease activity was determined by measuring the degree of antibody binding to native DNA.

MATERIALS AND METHODS

Patient selection. Sixteen patients with systemic lupus erythematosus were studied. All patients satisfied the ARA criteria for SLE. In addition, all patients had circulating antibody against native DNA, anti-nuclear antibody, reduced total haemolytic complement and active urinary sediments.

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Patients were on no therapy other than salicylates at the time of the study. The patients consisted of fifteen females and one male with a mean age of 32 years. The control group consisted of healthy adults, fifteen females and one male with a mean age of 30 years.

Methods. Peripheral blood mononuclear cells from normals and patients with SLE were separated from peripheral blood by Ficoll–Hypaque density centrifugation.

Overall experimental design. Concanavalin A (Con A), a plant lectin, is a known T cell mitogen which can induce differentiation and function of suppressor T cells *in vitro*. Suppressor T cells were generated by this method in a first culture. These cells were added to normal responder cells in a second assay culture system stimulated by the B cell mitogen, pokeweed. In order to assess the effect of a factor in serum which might affect suppression the incubation with Con A was also accomplished with 50% human serum either from normals or patients with SLE.

Generation of suppressor T cells. Incubation of 8×10^6 lymphocytes was performed in 1 ml of RPMI 1640 with penicillin streptomycin, glutamine, 10% foetal calf serum and 20 μg Con A for 48 hr. These cells were harvested, washed four times and then tested for suppressor activity in a second culture system.

Second culture system. Responder cells used were 8×10^6 lymphocytes in 1 cm^3; pokeweed mitogen was the stimulant in culture. These cells were then co-cultured with an equal number of the above suppressor cells. At the end of 12 days the presence of IgM in the supernatant was measured by a sensitive solid-phase fluorometric assay described previously (Sundeen & Krakauer, 1979). A mean of 15% non-viable cells was noted at the end of the culture period by trypan blue exclusion. This did not constitute a statistical difference between patients and controls.

DNA binding assay. Serum binding of native DNA (nDNA) was measured by a modified Farr assay using calf thymus DNA labelled with ^{125}I by the thallium trichloride method (Harbeck *et al.*, 1973), then passed twice through a 0·45-μm Millipore filter. One microgram of this material was exposed to 0·05 ml of heat-inactivated (56°C for 30 min) test serum in a total volume of 1·0 ml (0·02 M phosphate-buffered saline, pH 7·4) in duplicate and incubated overnight at 4°C. Reaction volumes were then brought to 2·0 ml by adding 1·0 ml saturated $(NH_4)_2SO_4$ solution, pH 7·4, and thoroughly mixing. After 1 hr of incubation in the cold, precipitates were collected by centrifugation, washed twice with cold 50% saturated $(NH_4)_2SO_4$, then redissolved in phosphate-buffered saline and counted in a gamma counter.

Binding percentages were determined on fifty normal sera using this assay. The upper limit of normal was then calculated as mean +2 standard deviations. Since this value varies by a few percentage points among different batches of labelled DNA, we used the following calculation to establish arbitrarily the corrected upper limit of normal as zero binding:

$$\text{Corrected nDNA binding (\%)} = \frac{\frac{\text{c.p.m.}_t}{\text{c.p.m.}_s} \times 100 - \text{ULN}}{100 - \text{ULN}} \times 100$$

where c.p.m._t and c.p.m._s were counts per minute in the precipitate from the test serum and in the total amount of labelled nDNA added respectively, and ULN is the upper limit of normal in percentage binding determined from fifty normal sera.

This assay detects DNA binding activity only in sera with antibody against native DNA, and not in sera which lack this antibody but have high levels of antibody against single-stranded DNA, DNA-histone, ribonucleoprotein, or Sm antigen. As performed in our laboratory this assay has been compared with a solid-phase radioimmunoassay for DNA-binding immunoglobulins (Clough, 1977), and also with the Millipore filter assay, the *Crithidia luciliae* immunofluorescence assay, and a passive haemagglutination assay (Crowe *et al.*, 1978).

RESULTS

When 8×10^6 normal human peripheral blood lymphocytes per cm^3 were cultured in the presence of 20 $\mu g/cm^3$ of Con A for 48 hr, the cells substantially suppressed immunoglobulin synthesis in a 50% co-culture with fresh responding human peripheral blood lymphocytes.

Fig. 1 shows, as reported previously by ourselves and others, that Con A-stimulated lymphocytes from patients with SLE suppress immunoglobulin synthesis by fresh responding peripheral blood lymphocytes to a lesser degree than those of normals ($26 \pm 21\%$ *vs* $86 \pm 12\%$, $P < 0{\cdot}01$). In Fig. 2 the degree of this suppressor cell function is plotted against native DNA binding of the individual at the time that the cells were obtained. A linear correlation is achieved suggesting that the suppressor cell defect is progressive and correlated with the activity of disease as assessed by native DNA binding (significance of regression = 0·001). To assess the possible aetiologic factors in the suppressor cell defect, we investigated the effect of serum from normals and patients with SLE on suppressor cell function by normal peripheral blood lymphocytes.

Serum from patients with SLE induced a decrease in suppressor cell function in normal human

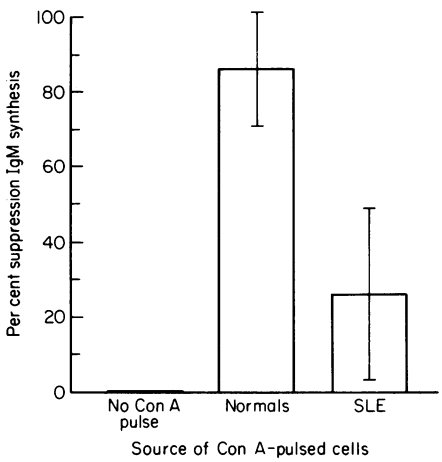

Fig. 1. Putative suppressor cells are harvested from peripheral blood and incubated for 48 hr at $8 \times 10^6/cm^3$ in RPMI 1640 with penicillin, streptomycin, glutamine, 10% foetal calf serum and concanavalin A ($20{\cdot}\mu g^{-1}{\cdot}cm^{-3}$). They are then cultured 1:1 with pokeweed mitogen with fresh responding normal peripheral blood lymphocytes. IgM synthesized *in vitro* is measured by fluorometric assay, and suppression measured against synthesis by lymphocytes which are not suppressed. When the suppressor population is from patients with SLE, significantly less ($P < 0{\cdot}01$) suppression of IgM synthesis is noted compared to normals.

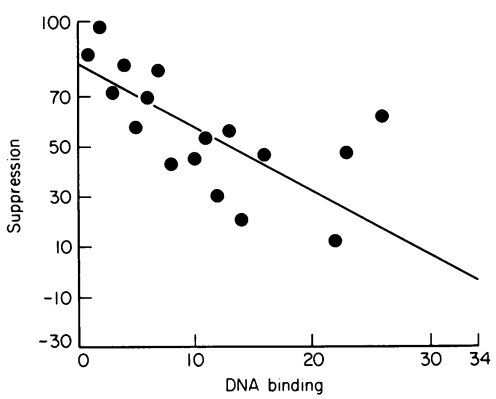

Fig. 2. Suppression by lymphocytes from SLE patients of IgM synthesis of normal lymphocytes is plotted as a function of serum DNA binding by the serum from the SLE patient at the time lymphocytes are harvested. Suppressor function varies inversely with SLE activity as measured by native DNA binding (significance of regression = 0·001).

peripheral blood lymphocytes. The percentage suppression of IgM synthesis was 98·4% when normal peripheral blood lymphocytes were incubated with normal serum. Suppression with SLE serum was substantially reduced to $56 \pm 5\%$, $P < 0{\cdot}01$ (Fig. 3). As seen in Fig. 4, the degree of suppressor cell loss induced by SLE serum in any one patient appears to be related to the degree of DNA binding by that same serum.

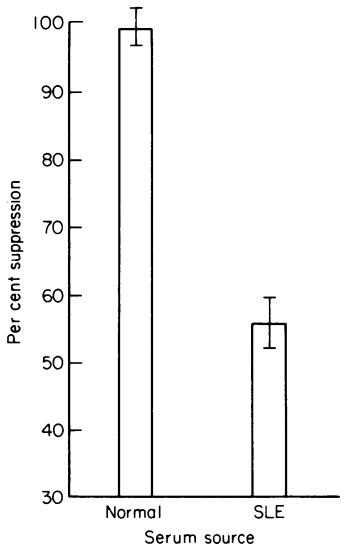

Fig. 3. When putative suppressor cells from normals are incubated in 50% normal human serum (see Fig. 1), substantial suppression of normal lymphocyte IgM synthesis is achieved. When the serum is from SLE patients, significantly ($P < 0{\cdot}01$) less suppression is noted.

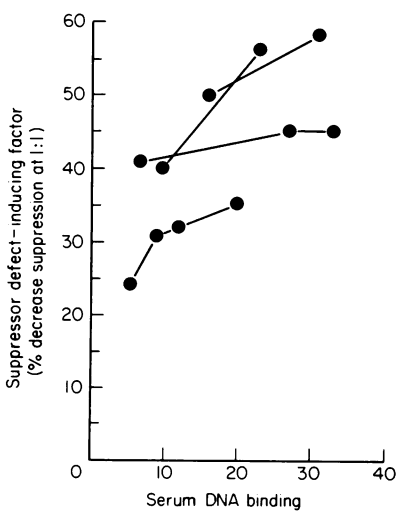

Fig. 4. When the decrease in suppressor function induced by SLE serum is plotted against serum DNA binding of the serum (four patients), increasing SLE activity as measured by DNA binding is associated with an increase in activity of the factor inducing a decrease in suppressor function of normal peripheral blood lymphocytes. This relationship is not, however, statistically significant.

DISCUSSION

Previous evidence of a suppressor cell defect in murine and human SLE has led to the hypothesis that such a defect is directly involved in autoantibody production. Our finding that the degree of suppressor cell defect is correlated with an objective measurement of the activity of disease in SLE lends credence to this concept. There are several plausible explanations for this suppressor cell defect, including selective viral infection or the production of antibodies to suppressor T cells. The fact that natural thymocytotoxic antibodies in NZB mice can induce suppressor cell defects (Klassen, Krakauer & Steinberg, 1977) does not necessarily implicate these antibodies as the proximal cause of the suppressor cell defect in these animals. Nonetheless, our demonstration here that serum from SLE patients can induce a suppressor cell defect in normals *in vitro* suggests that such serum properties are related to the disease itself. Although we have not demonstrated that our suppressor cell defect-inducing factor is, in fact, an antibody, Sagawa & Abdou (1979) have demonstrated the presence of antibodies to suppressor cells in human SLE. We therefore believe this serum factor to be an antibody against suppressor cells. This enables us to construct a hypothesis whereby a suppressor cell defect results in autoantibody production including an antibody to a suppressor cell which causes a further suppressor cell defect and the cycle perpetuates itself. Our preliminary evidence that in an individual patient the trend is for serum to induce increasing suppressor defects with higher anti-DNA titres lends further credence to this hypothesis.

Regardless of whether suppressor cells are involved in the aetiology of SLE, such a cycle could certainly explain disease propagation or 'flare' and this concept is supported by our data. An intriguing question which remains unanswered is what natural homeostatic process may interrupt this 'destructive cycle' since the natural history of an 'SLE flare' is to resolve spontaneously. Perhaps greater understanding of the mechanisms responsible for autoimmunity and interruption of this cycle will enable us to devise better means of adoptive immunotherapy.

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