Depressed primary in vitro antibody response in untreated systemic lupus erythematosus. T helper cell defect and lack of defective suppressor cell function.

J F Delfraissy, … , P Massias, J Dormont

Research Article

The in vitro antibody response of peripheral blood lymphocytes (PBL) from 19 patients with untreated systemic lupus erythematosus (SLE) was compared with that of 20 control patients and 44 normal subjects. Trinitrophenyl polyacrylamide beads (TNP-PAA) were used to induce IgM anti-TNP plaque-forming cells. SLE patients displayed a markedly depressed, and in most instances virtually absent, response. This was not due to an unusual kinetics of the response; nor could it be induced by preincubation of SLE patients' PBL. In co-cultures of SLE patients and normal PBL, the former, with few exceptions, did not exert a suppressive effect. In four patients the anti-TNP response of either unfractionated or T-depleted SLE PBL could be restored by T cells from a normal individual. Conversely in three of these patients, SLE T cells could not support the response of normal B cells, suggesting a T helper cell defect in SLE PBL. Concanavalin A (Con A)-induced suppressor cells of the antibody response could be assayed by two approaches: (a) in responder SLE patients, by the direct addition of Con A to TNP-PAA-stimulated cultures; (b) in seven patients by transfer of Con A-activated cells to the responding culture of a normal allogeneic donor. In both cases SLE PBL were able to exert a suppressive effect to the same extent as normal PBL.

Find the latest version:

https://jci.me/109827/pdf

Depressed Primary In Vitro Antibody Response in Untreated Systemic Lupus Erythematosus

T HELPER CELL DEFECT AND LACK OF DEFECTIVE SUPPRESSOR CELL FUNCTION

J. F. DELFRAISSY, P. SEGOND, P. GALANAUD, C. WALLON, P. MASSIAS, and J. DORMONT, *Service de Médecine Interne, Service de Rhumatologie Hôpital Antoine Béclère, Institut National de la Santé et de la Recherche Medicale U 131 and Université Paris-Sud, Clamart, France*

A B S T R A C T   The in vitro antibody response of peripheral blood lymphocytes (PBL) from 19 patients with untreated systemic lupus erythematosus (SLE) was compared with that of 20 control patients and 44 normal subjects. Trinitrophenyl polyacrylamide beads (TNP-PAA) were used to induce IgM anti-TNP plaque-forming cells. SLE patients displayed a markedly depressed, and in most instances virtually absent, response. This was not due to an unusual kinetics of the response; nor could it be induced by preincubation of SLE patients' PBL. In co-cultures of SLE patients and normal PBL, the former, with few exceptions, did not exert a suppressive effect. In four patients the anti-TNP response of either unfractionated or T-depleted SLE PBL could be restored by T cells from a normal individual. Conversely in three of these patients, SLE T cells could not support the response of normal B cells, suggesting a T helper cell defect in SLE PBL. Concanavalin A (Con A)-induced suppressor cells of the antibody response could be assayed by two approaches: (a) in responder SLE patients, by the direct addition of Con A to TNP-PAA-stimulated cultures; (b) in seven patients by transfer of Con A-activated cells to the responding culture of a normal allogeneic donor. In both cases SLE PBL were able to exert a suppressive effect to the same extent as normal PBL.

INTRODUCTION

Systemic lupus erythematosus (SLE)[1] is characterized by several immunologic abnormalities, usually at-

tributed to a general lack of regulation of the immune response. The precise function of the immune system in SLE patients, however, is only partially defined. Recent studies on the ability of active SLE patients to mount an antibody response have provided conflicting results regarding defects demonstrated in vivo (1–3) or in vitro (4, 5). The latter studies have been performed with the nonspecific stimulating agent pokeweed mitogen (PWM). We have studied the ability of SLE patients' peripheral blood lymphocytes (PBL) to mount a primary in vitro antibody response to trinitrophenyl-polyacrylamide beads (TNP-PAA). This model of specific in vitro antibody response (5) has proven to be a functional assay in normal (6–8) or pathological conditions (9, 10). Moreover, we have been able to demonstrate a virtually abolished response to TNP-PAA in normal aged individuals (>70 yr) (8), who respond normally to PWM (11). This discordance stresses the importance of specific tests for a comprehensive evaluation of human B cell response.

The in vitro response to TNP-PAA requires monocytes (7) and T cells (9). Thus, our model can be used to identify the cellular basis of a defective response. This approach allowed us to show in a previous study that allogeneic T-B mixtures can function as well as autologous mixtures, and to characterize the T cell origin of the age-related impairment of the in vitro response to TNP-PAA (8). In addition, the response to TNP-PAA can be used as the target of Concanavalin

A (Con A)-induced suppressor cells (9, 12) to study at least one aspect of the T-suppressor cell function.

METHODS

Subjects

Normal control population. 44 healthy subjects from the medical and laboratory staff with an age range of 19 to 53 yr (14 females, 30 males) were studied.

Patients

39 patients who had never received either immunosuppressive drugs or corticosteroid therapy were selected for study. Administration of aspirin or nonsteroidal antiinflammatory agents was stopped at least 72 h before blood collection. These patients belonged to a control group and a SLE group.

Control patients. 20 patients (9 females, 11 males, age range 19 to 52 yr) with osteoarthrosis, cardiac, or neurologic diseases were studied.

SLE group. 19 patients fulfilling the American Rheumatism Association criteria for SLE (13) were studied. All of these patients had circulating anti-DNA antibodies as evaluated by a Farr test (14) and low serum levels of C_3 and C_4 complement components. There were 13 females and 6 males ranging in age from 19 to 52 yr. 15 of these patients were studied at the time of initial diagnosis of SLE and had active disease, the last 4 had moderate disease activity.

Lymphocyte cultures

Blood was heparinized with Liquemine (Roche Laboratories, Neuilly-Sur-Seine, France) and lympho-monocytic cells (PBL) were isolated on Ficoll-metrizoate. The technique for in vitro induction of a primary antibody response toward TNP-PAA has been previously reported (6). Briefly, PBL were cultured at a 5×10^6-cells/ml concentration in culture medium containing 10% fetal bovine serum. They were incubated either without antigen (control cultures), or with TNP-PAA beads (stimulated cultures). After 7–8 d of culture, PBL were recovered, separated from the TNP-PAA beads, and the number of anti-TNP antibody-forming cells (AFC) evaluated. Each experimental group was composed of triplicate cultures assayed separately. In some experiments, 2-mercaptoethanol (2-ME, Schwarz/Mann, Div. Becton, Dickinson & Co., Orangeburg, N. Y.) was added to the culture medium at a 50-μM final concentration (7). In co-culture experiments PBL from a patient and from a normal donor were cultured either separately at a 5×10^6-cells/ml concentration or together (2.5×10^6 cells of each individual/ml).

Assay for the anti-TNP response

The in vitro antibody response was assessed by enumeration of the anti-TNP AFC as already described (6). Both TNP-sheep erythrocytes (TNP-SRBC) and SRBC were used as targets in a Jerne-type plaque assay. Indirect plaque-forming cells were assayed by using an anti-human Fc γ serum diluted 1:100 together with guinea pig complement. Results are expressed as the number of anti-TNP AFC per million collected cells (AFC/10^6). Approximately $3-4 \times 10^6$ cells were recovered in each culture. In some instances, cell viability was assessed by trypan blue exclusion.

Cell separation experiments

Cells forming E rosettes with neuraminidase-treated SRBC (E-RFC) (15) were centrifuged on Ficoll-metrizoate. This results in T-depleted (interface) and T-enriched (bottom) cell fractions. The latter cells were separated from SRBC by agitation at 37°C for 30 min without serum, followed by hemolysis with NH_4Cl. Both preparations were then washed and cultured either alone (5×10^6/culture), or in recombination experiments (2.5×10^6 of each/culture).

Con A-induced suppression of the antibody response

Con A (Sigma Chemical Co., St. Louis, Mo.) was added at a 0.5-μg/ml final concentration with the antigen, to TNP-PAA-stimulated cultures. This induced a suppression of the anti-TNP response that is maximum at the peak (12). In transfer experiments, PBL were cultured for 48 h, without antigen, either in the presence of Con A (2 μg/ml), or without Con A (control cultures). At the end of this incubation period, the same amount of Con A was added to control cultures and cells were collected, washed twice in a 0.1-M O-methyl-alpha-D-mannopyranoside solution and once without it. Control and Con A-stimulated cells were adjusted to the same cell concentration and 1.5×10^6 of these cells were added to cultures of 5×10^6 allogeneic responding cells. The latter cultures, established 2 d earlier, were stimulated with TNP-PAA. We previously demonstrated (12) that, under these conditions, Con A-stimulated PBL (or T cells) suppress the anti-TNP response of allogeneic (or autologous) responding cells. The addition of incubated cells to a responding culture on day 2 permits a maximum demonstration of the suppressive effect of Con A-stimulated PBL, at a time when spontaneously generated T-suppressor cells do not affect the response (9, 12).

RESULTS

Defective antibody response in SLE patients. The in vitro antibody response of PBL from 19 SLE patients was compared with that of 20 matched control patients and with that of 44 normal controls (Fig. 1). The mean response of SLE PBL cultures was 19 ± 6 AFC/10^6 (mean$\pm$SEM), virtually abolished in comparison with the response of control patients (169 ± 32 AFC/10^6; $P < 10^{-4}$) and with that of normal individuals (210 ± 25 AFC/10^6; $P < 10^{-5}$). The total cell recovery was similar in all three groups as was the percentage of viable cells ($75\pm3\%$; $73\pm4\%$, $75\pm4\%$, respectively). The background anti-TNP response in unstimulated cultures was low and comparable in the three groups (1 ± 1; 5 ± 1; 3 ± 1 AFC/10^6, respectively). A study of the kinetics of the response performed in two SLE patients gave results similar to those of controls: the response peaked on day 7 to day 8. A search for anti-TNP and anti-SRBC AFC on freshly isolated SLE PBL was negative in three patients. Similarly, the search for immunoglobulin (Ig)G anti-TNP forming cells at the end of the stimulated cultures was negative in 2 SLE patients, 6 control patients, and 10 normal controls. The addition of 2-ME to the culture medium did not
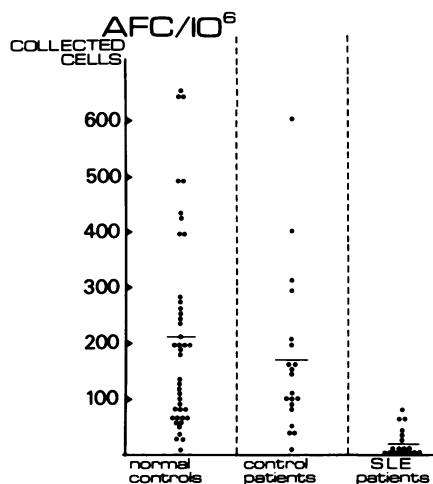
FIGURE 1 Defective antibody response in SLE patients. The in vitro antibody response to TNP-PAA was measured in 44 normal controls, 20 control patients, and 19 SLE patients. Individual responses (expressed as AFC per 10^6 cells) are plotted and mean response in each group is represented by the horizontal bar.

modify the in vitro antibody response of stimulated PBL from two SLE patients (37±30 without 2-ME vs. 46±37 AFC/10^6 in the presence of 2-ME). In two experiments, PBL were incubated for 2 or 20 h in fetal bovine serum containing culture medium at 37°C. Cells were then washed extensively and cultured in the presence of TNP-PAA. Preincubation under these conditions did not restore an anti-TNP response to SLE PBL, nor did it modify the response of control PBL (results not shown).

Antibody response of co-cultures from SLE and normal PBL. Co-cultures involving 2.5 × 10^6 PBL from normal subjects and 2.5 × 10^6 PBL from either SLE patients or control patients were performed. The anti-TNP response from TNP-PAA stimulated cultures was evaluated in individual cultures and in co-cultures from 17 SLE patients and 16 control patients. In each experiment, the response observed was compared to the expected response (i.e., arithmetic mean of the responses of cells from each donor cultured at 5 × 10^6/ml) and a stimulation index (observed/expected) was calculated. As a mean, the observed response in co-cultures of control patients and normal donor was 407±114 AFC vs. 183±31 AFC for the expected response. In co-cultures from SLE patients and normal donors the observed response was 230±58 AFC/10^6 vs. 156±35 AFC/10^6 for the expected response. This indicated that, as a mean, a positive allogeneic effect takes place in such co-cultures and enhances the anti-TNP response above the expected level. The mean stimulation index in co-cultures from SLE plus normal PBL did not differ from that of control patients plus

normal PBL (1.76±0.49 vs. 2.13±0.56, t = 0.5424). However, it is of interest to note that PBL from four SLE patients profoundly inhibited the response of normal PBL: the mean stimulation indices of these co-cultures respectively, were 0.03, 0.04, 0.05, and 0.07 (Fig. 2). It should be pointed out that the PBL from one of these four patients were able to respond to TNP-PAA (62 AFC/10^6).

SLE B cells are functional. In four patients, T-depleted and T-enriched PBL populations were prepared as described in materials and methods from PBL of two normal donors and one SLE patient. As shown in Table I, 5 × 10^6 T-depleted cells from normal donors and from the SLE patient were unable to mount an anti-TNP response upon stimulation with TNP-PAA for 7 d (two cultures assayed in duplicate for each experiment). Similarly, normal T-enriched PBL (96% E-R-FC) were unable to respond to TNP-PAA (see legend to Table I). However, when 2.5 × 10^6 T-depleted normal cells were cultured with 2.5 × 10^6 T-enriched normal cells for 7 d in the presence of TNP-PAA, an anti-TNP response was obtained in either autologous or allogeneic mixtures. In the same way, the addition of 2.5 × 10^6 T-enriched PBL from the same normal donor restored an anti-TNP response from 2.5 × 10^6 of either unfractionated or T-depleted SLE PBL.
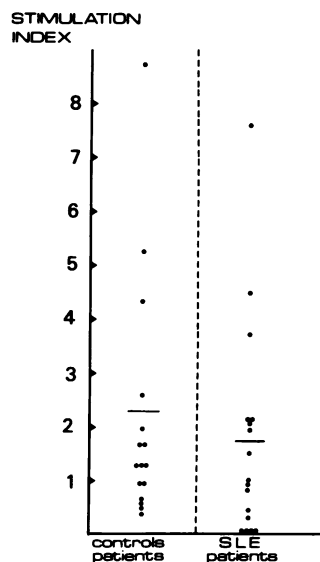

FIGURE 2 Antibody response of co-cultures from SLE and normal PBL. Co-cultures involved 2.5 × 10^6 normal PBL and 2.5 × 10^6 PBL from either control patients or SLE patients, and their anti-TNP response was measured (observed response). For each experiment the stimulation index was determined by dividing this observed response by the expected response (see Methods). Individual stimulation indices are plotted and the mean of each group is represented by a horizontal bar. The absolute means of observed and expected responses are provided in the text.

In Vitro Antibody Response in SLE **143**

TABLE I
Functional SLE B Cells

Responding cells Expt.	1		2		3		4	
	−	+*	−	+	−	+	−	+
Normal autologous B cells	0	76	0	146	0	100	0	112
Normal allogeneic B cells	3	120	0	192	3	80	0	83
SLE B cells	0	97	0	60	1	85	6	68
Unfractionated SLE cells	0	202	8	130	0	103	10	106

In four patients, the antibody response of diverse cell populations was measured either alone (5 × 10⁶ responding cells) or in the presence of T-enriched cells from a normal donor (2.5 × 10⁶ of each). The mean response of 5 × 10⁶ T-enriched cells (97±1% E-RFC) was 2 PFC/10⁶ in these four experiments. Results (AFC per 10⁶ cells) are the mean±sem of three cultures in each experimental group.
* Normal T cells in culture.

Moreover, in experiments 3 and 4, a similar restoration was obtained with T cells from another normal donor (results not shown).

SLE T cells do not restore an anti-TNP response from normal B cells. In three of these recombination experiments, we studied the function of SLE T cells. Normal B cells were stimulated with TNP-PAA in the presence of either normal (autologous or allogeneic) T cells or SLE T cells (Table II). As already shown in Table I, the B cell response was restored to the same extent by autologous or allogeneic normal T cells. In contrast, SLE T cells did not restore this response. Additionally, in experiments 3 and 4, when SLE T cells were added to cultures of unfractionated normal PBL they did not affect the anti-TNP response (111±21 plaque-forming cells/10⁶ as compared with 108±32 PFC/10⁶), which argues against the presence of suppressor cells of the allogeneic B cell response, in SLE T cells.

TABLE II
Anti-TNP Response of Normal B Cells Not Supported by SLE T Cells

	Response of normal B cells		
T cells added Expt.	2	3	4
0	0	3	0
Normal autologous	146	100	112
Normal allogeneic	192	80	83
SLE	14	0	0

Normal B cells were cultured either alone (5 × 10⁶/ml) or in the presence of T cells (50% each) from the same donor, a normal allogeneic donor, or one SLE patient. The mean response of normal T cells was 2 PFC/10⁶ and that of the SLE T cells were 0, 2, and 14 PFC/10⁶ in patients 2, 3, and 4, respectively. Response are represented as PFC per 10⁶ cells in three patients (mean of three cultures) the mean percentage of E-RFC in SLE T cells was 92±1%.

Con A-induced suppression of the in vitro antibody response in SLE patients. Con A was added at the initiation of TNP-PAA stimulated cultures, in order to induce nonspecific T cell suppression of the anti-TNP response. The effect of Con A could be evaluated only in the five SLE patients' PBL capable of mounting a detectable in vitro anti-TNP response (Fig. 3). A significant inhibition was observed in the presence of Con A (7±2 AFC/10⁶, as compared with 54±10 AFC/10⁶ in control cultures; $P < 0.002$). The mean suppression
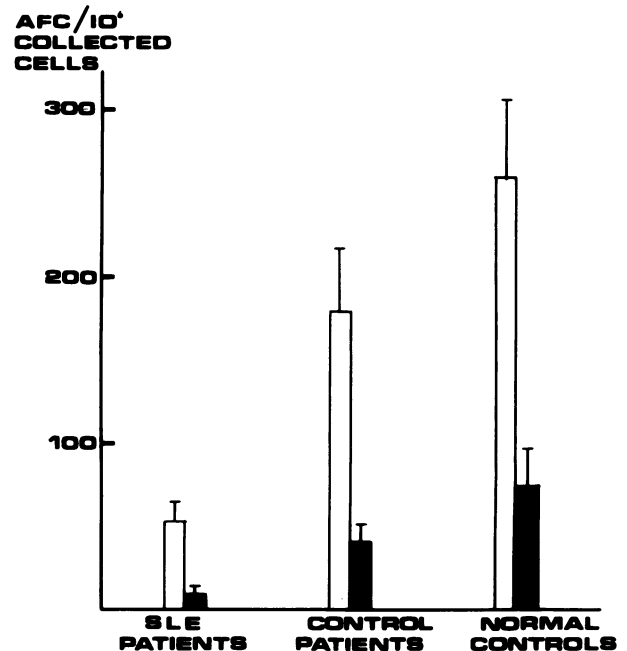

FIGURE 3   Con A-induced suppression of the in vitro antibody response. The anti-TNP response was measured in the absence of Con A (□) and in the presence of 0.5 μg/ml Con A (■) in 5 SLE patients, 17 control patients, and 22 normal individuals. Results are the mean anti-TNP responses±SEM expressed as AFC per 10⁶ cells in each experimental group.

index (88±3%) was similar to that observed in parallel cultures from 17 control patients (75±4%) and from 22 normal individuals (76±5%).

Con A-activated SLE PBL suppress the anti-TNP response of normal allogeneic PBL. Cell transfer experiments, using normal allogeneic PBL responding cells, were performed as detailed in Methods. PBL from six normal subjects and from seven SLE patients were incubated for 48 h either without Con A (control cells) or in the presence of Con A (Con A cells). The anti-TNP response of normal allogeneic PBL was enhanced by the addition of SLE control cells as well as normal control cells (Fig. 4). In contrast, the addition of Con A cells suppressed the anti-TNP response in both cases. The mean suppression index developed by SLE PBL was 85±5% as compared with 86±7% for normal PBL. When the results were expressed as AFC per culture, the same conclusion could be drawn: 80±5% and 85±4%, respectively. It should be pointed out that among these seven SLE patients, three were able to mount an anti-TNP response and their responses were suppressed by the direct addition of Con A in the culture medium (mean suppression 83%).

DISCUSSION

We have shown that PBL from untreated SLE patients have an impaired ability to mount a primary in vitro antibody response. Similarly the PWM-induced non-
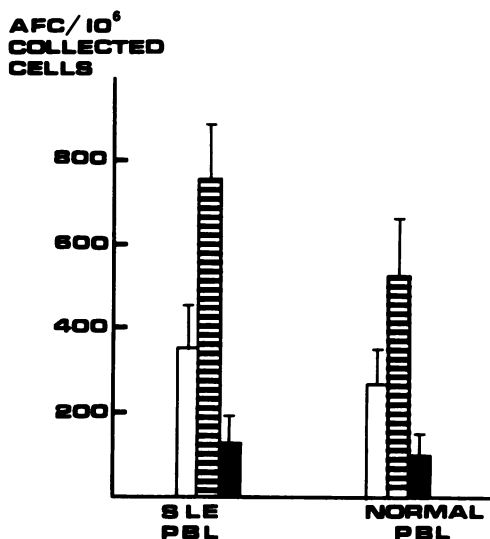

FIGURE 4 Cell transfer experiments. PBL from either normal controls or SLE patients were incubated for 48 h without Con A (control cells) or in the presence of 2 μg/ml Con A (Con A cells). These cells were transferred to TNP-PAA-stimulated cultures of normal allogeneic PBL. The results expressed as AFC per 10^6 cells are the mean±SEM of responses in cultures containing: no incubated PBL (□); control cells (▨), and Con A cells (■), in experiments with seven SLE patients and six normal donors.

specific in vitro B cell response of NZB × NZW mice (16) and of SLE patients (4, 5, 17, 18) is depressed. The only other study reporting results of a specific in vitro antibody response in human SLE indicated increased anti-TNP PFC (19). In the experimental model used by Morimoto et al. (19), no response was obtained in normal PBL cultures and the AFC response in SLE PBL cultures occurred unusually early (19, 20), when no AFC were detectable in our PBL cultures of normal individuals or SLE patients. Thus, one may wonder what are the respective contributions of an in vivo preactivation and of an in vitro response in Morimoto's results.

Our in vitro findings are in agreement with those of in vivo studies showing that SLE patients with active disease have an impaired antibody response (1–3, 21). In rheumatoid arthritis where a polyclonal hypergammaglobulinemia is observed, we have demonstrated an impaired ability of PBL to respond to TNP-PAA (10). In this previous study, control patients, including subjects with ankylosing spondylitis, displayed a normal in vitro response. This shows that our results in SLE and in rheumatoid arthritis patients cannot simply be explained by the presence of an inflammatory state. Several mechanisms may account for the profound impairment of the in vitro antibody response in SLE. A role for lymphocytotoxic antibodies (22, 23) cannot be excluded, although preincubation of SLE PBL in fetal bovine serum-containing culture medium does not improve their functional capacity. Experiments testing the effect of active SLE sera on the response of normal PBL are in progress.

Spontaneous suppressor cells have been described in murine SLE (24) and in a few patients with SLE (25). In the present work the PBL from few SLE patients suppressed the response of normal PBL, whereas in most cases the response of such co-cultures was unchanged or enhanced. Our experimental results do not allow us to distinguish between two possible explanations for these variable results: (*a*) suppressor cells may be inconstant in SLE PBL; (*b*) all SLE PBL may contain suppressor cells but the expression of this suppressive activity toward the response of normal PBL may be restricted by histocompatibility and thus could be detected only in few patient-control pairs. Several reports have demonstrated an in vitro preactivation of SLE B cells (5, 18, 26, 27). This point was not thoroughly examined in our study, as we did not use the reverse plaque assay. We looked for anti-TNP AFC in freshly isolated PBL of SLE patients, with negative results. This does not exclude a general in vivo B cell preactivation in SLE, but indicates that this phenomenon is unlikely to be the only explanation of the decreased response toward this specific antigen.

Because none of the mechanisms mentioned above could fully account for our results, it was important to

investigate the cellular defect responsible for the impaired in vitro antibody response in SLE. The response of TNP-PAA requires monocytes that can apparently be replaced by 2-ME (7). The addition of 2-ME does not modify the response of SLE PBL which tends to exclude a major functional defect of monocytes. The B cell response to TNP-PAA requires T cells (9), and the respective T and B cell functional abilities in SLE PBL had to be tested. The B cell competence in SLE had previously been evaluated by testing whether the PWM-induced response of SLE PBL could be enhanced upon addition of either mitomycin-treated normal T cells (5) or irradiated normal PBL (4). In the first study, the reconstitution of a normal response could be obtained in two patients whereas, in the latter, variable results were observed. We investigated the functional capacity of SLE B cells more directly by adding normal T cells to B cells from four patients. In the presence of normal allogeneic T cells, SLE B cells respond to TNP-PAA to the same extent as normal B cells. This suggests that peripheral B cells from active SLE patients are potentially normal. In addition, SLE T cells, in the three patients in whom they were studied, were unable to support the anti-TNP response of normal B cells. It should be pointed out that in these experiments, as well as in another study from our laboratory (8), allogeneic T-B mixtures functionally behave as autologous mixtures. Two lines of evidence suggest that SLE T cells do not exert a suppressive effect on the antibody response of normal allogeneic cells: SLE T cells do not decrease the response of normal PBL; normal T cells can restore the response of SLE unfractionated PBL and of SLE B cells to the same extent. These results confirm and extend previous reports (4, 5) of a functional T helper cell defect in SLE. This defect could be one aspect of an overall T cell dysfunction (28). However, it should be mentioned that our results with SLE T cells are at variance with those of Chiorazzi et al. (29) using another method of in vitro antibody production.

Our experiments using Con A to induce nonspecific suppressor cells of the in vitro antibody response, show that normal suppressor cell activity can be generated from SLE PBL. This contrasts with the fact that PBL from aged individuals are unable to generate Con A-induced suppressors in the same conditions (8), in agreement with a recent report (30). Thus, the suppressor function appears to be affected differently in SLE and aging, two conditions in which a general T cell defect is assumed to be present. Our observation in SLE is in apparent contradiction with the general idea that suppressor cell function is decreased in this disease. Such a decrease has been observed in several studies of T cell proliferative responses (23, 31–34).

In the other reported studies of human B cell response, Con A-induced suppression was found to be decreased. Fauci et al. (4) observed that SLE PBL were unable, as a mean, to suppress the PWM-induced response of normal subjects, although results varied from one patient to another. Morimoto (20) and Morimoto et al. (35) reported that Con A-activated SLE PBL were unable to suppress the normal B cell response. The same results were obtained when studying the Con A-induced suppression of PWM-driven immunoglobulin production (36, 37). The reasons for this difference may be found in the patient population or in the in vitro model: (a) Our patients were untreated, whereas in most of the above mentioned studies, some of them received corticosteroids. Although the effect of corticosteroids on the cells involved in the regulation of B cell response is complex and far from being elucidated (38), they may interact with suppressor cell function (39); (b) More probably the Con A-induced suppression in these models of B cell response correspond to at least partially different phenomena, according to the nature of the stimulus used. Our model of specific response may be more susceptible to suppressive signals than nonspecific activation by PWM as suggested by the following facts: lower concentrations of Con A are required to suppress (12, 40); and the response is still susceptible to suppression on day 2 (12). As Con A activates more than one population of suppressor cells (41, 42) the response to TNP-PAA could be selectively influenced by negative signals from a subpopulation of T cells which remains unaffected in SLE. Alternatively, this response could be suppressed by a decreased (4, 43, 44) but functionally active suppressor population (44).

In murine in vitro models of antibody response, similar discrepancies have been reported (45, 46). Again the major difference between these studies rests on the stimulating agent used to trigger the B cell response: a defect in T-suppressor function has been evidenced with PWM (45). These apparent contradictions underscore the complexity of the abnormal regulation of the immune response in NZB × NZW mice as well as in SLE patients. They may, in part, be explained by the recent demonstration that murine lupus is associated with a decrease in the Lyt-$1^+2^+3^+$ population, rather than a disappearance of the suppressor cells themselves (47). Awaiting a more precise definition of the T cell subsets regulating the antibody response in man, clinical studies of suppressor function should be based on the assessment of several parameters, as recently suggested by Goodwin and Williams (48). In cultures of normal PBL the response to TNP-PAA can be differently affected by Con A-induced suppressors, by in vitro spontaneously generated suppressors (12) and by sodium periodate-induced sup-

pressors (49). Further studies of these regulatory phenomena should be useful in the interpretation of the immunological defect in SLE.

In this study we found no correlation between the disease activity and the magnitude of the in vitro antibody response. An important advantage to our study will be the sequential examination of these immune parameters in patients under treatment, taking into account the reproducibility of the in vitro response to TNP-PAA (6). Our preliminary results in rheumatoid arthritis patients show that the moderate impairment of the in vitro antibody response disappears with effective treatment (10). In SLE patients such a study could provide a better evaluation of the immune status, a prerequisite for a more rational use of therapeutic agents.

ACKNOWLEDGMENTS

REFERENCES

1. Baum, J., and M. Ziff. 1969. Decreased 19 S antibody response to bacterial antigens in systemic lupus erythematosus. J. Clin. Invest. 48: 758–767.
2. Williams, G. W., A. D. Steinberg, J. L. Reinertsen, L. W. Klassen, J. L. Decker, and R. Dolin. 1978. Influenza immunization in systemic lupus erythematosus A double blind trial. Ann. Intern. Med. 88: 729–734.
3. Jackson, C. G., H. D. Ochs, and R. J. Wedgwood. 1979. Immune response of a patient with deficiency of the fourth component of complement and systemic lupus erythematosus. N. Engl. J. Med. 300: 1124–1129.
4. Fauci, A. S., A. D. Steinberg, B. F. Haynes, and G. Whalen. 1978. Immunoregulatory aberrations in systemic lupus erythematosus. J. Immunol. 121: 1473–1479.
5. Ginsburg, W. W., F. D. Finkelman, and P. E. Lipsky. 1978. Circulating and pokeweed mitogen-induced immunoglobulin secreting cells in systemic lupus erythematosus. Clin. Exp. Immunol. 35: 76–88.
6. Delfraissy, J. F., P. Galanaud, J. Dormont, and C. Wallon. 1977. Primary in vitro antibody response from human peripheral blood lymphocytes. J. Immunol. 118: 630–635.
7. Delfraissy, J. F., P. Galanaud, J. Dormont, and C. Wallon. 1978. Primary in vitro antibody response of human peripheral blood lymphocytes: role of phagocytic mononuclear cells. J. Immunol. 120: 1283–1288.
8. Delfraissy, J. F., P. Galanaud, J. Dormont, and C. Wallon. 1980. Age-related impairment of the in vitro antibody response in the human. Clin. Exp. Immunol. 39: 208–214.
9. Galanaud, P. 1979. In vitro antibody response to TNP-PAA beads. Immunol. Rev. 45: 141–161.
10. Segond, P., J. F. Delfraissy, P. Galanaud, C. Wallon, P. Massias, and J. Dormont. 1979. Depressed primary in vitro antibody response in rheumatoid arthritis. Clin. Exp. Immunol. 37: 196–204.

11. Kishimoto, S., S. Tomino, K. Inomata, S. Kotegawa, T. Saito, M. Kiroki, H. Mtsuya, and S. Hisamitsu. 1978. Age-related changes in the subsets and functions of human T lymphocytes. J. Immunol. 121: 1773–1780.
12. Galanaud, P., M. C. Crevon, J. F. Delfraissy. P. Segond, C. Wallon, and J. Dormont. 1979. Regulation of the in vitro antibody response in human peripheral blood lymphocytes: different effects of mitogen-induced and spontaneously-generated T-suppressor cells. Clin. Exp. Immunol. 38: 106–115.
13. Cohen, A. S., W. E. Reynolds, E. C. Franklin, J. P. Kulka, M. W. Ropes, L. E. Shulman, and S. L. Wallace. 1971. Preliminary criteria for the classification of systemic lupus erythematosus. Bull. Rheum. Dis. 21: 643–648.
14. Pincus, T., P. H. Schur, J. A. Rose, and N. Talal. 1969. Serum DNA binding in systemic lupus erythematosus. N. Engl. J. Med. 281: 701–705.
15. Bach, J. F. 1973. Evaluation of T cells and thymic serum factors in man using the rosette technique. Transplant. Rev. 16: 196–224.
16. Cohen, P. L., and M. Ziff. 1977. Abnormal polyclonal B cell activation in NZB × NZW F1 mice. J. Immunol. 119: 1534–1537.
17. Bobrove, A. M., and P. Miller. 1977. Depressed in vitro B lymphocytes differentiation in systemic lupus erythematosus. Arthritis Rheum. 20: 1326–1333.
18. Nies, K. M., and J. S. Lovie. 1978. Impaired immunoglobulin synthesis by peripheral blood lymphocytes in systemic lupus erythematosus. Arthritis Rheum. 21: 51–57.
19. Morimoto, C., T. Abe, M. Hara, and M. Homma. 1977. In vitro TNP-specific antibody formation by peripheral lymphocytes from patients with systemic lupus erythematosus. Scand. J. Immunol. 6: 575–579.
20. Morimoto, C. 1979. Loss of suppressor T-lymphocyte function in patients with systemic lupus erythematosus. Clin. Exp. Immunol. 32: 125–133.
21. Niva, Y., and T. Kanoh. 1979. Immune deficiency states and immune imbalance in systemic lupus erythematosus. Clin. Exp. Immunol. 32: 125–133.
22. Horwitz, D. A., M. A. Garrett, and A. H. Craig. 1977. Serum effects on mitogenic reactivity in subjects with systemic lupus erythematosus, rheumatoid arthritis and scleroderma. Technical considerations and lack of correlation with anti-lymphocyte antibodies. Clin. Exp. Immunol. 27: 100–110.
23. Sakane, T., A. D. Steinberg, J. P. Reeves, and I. Green. 1979. Studies of immune functions of patients with systemic lupus erythematosus. Complement-dependent immunoglobulin M anti-thymus-derived cell antibodies preferentially inactivate suppressor cells. J. Clin. Invest. 63: 954–965.
24. Roder, J. C., D. A. Bell, and S. K. Singhal. 1977. Regulation of the immune response in autoimmune NZB/NZW F1 mice. I. The spontaneous generation of splenic suppressor cells. Cell. Immunol. 29: 272–284.
25. Tardieu, M., and J. M. Dupuy. 1978. Suppressor activity of lymphocytes from patients with systemic lupus erythematosus. Clin. Immunol. Immunopathol. 11: 125–130.
26. Jasin, H. E., and M. Ziff. 1975. Immunoglobulin synthesis by peripheral blood cells in systemic lupus erythematosus. Arthritis Rheum. 18: 219–228.
27. Budman, D. R., E. B. Merchant, A. D. Steinberg, B. Doft, M. E. Gershwin, E. Lizzio, and J. P. Reeves. 1977. Increased spontaneous activity of antibody forming cells in the peripheral blood of patients with active

systemic lupus erythematosus. *Arthritis Rheum.* **20:** 829–833.

28. Gottlieb, A. B., R. G. Lahita, N. Chiorazzi, and H. G. Kunkel. 1979. Immune function in systemic lupus erythematosus. Impairment of in vitro T cell proliferation and in vivo antibody response to exogenous antigen. *J. Clin. Invest.* **63:** 885–892.

29. Chiorazzi, N., S. M. Fu, and H. G. Kunkel. 1979. Induction of human antibody responses in vitro with emphasis on allogeneic helper factors. *Immunol. Rev.* **45:** 219–241.

30. Antel, J. P., and B. G. W. Arnason. 1979. Suppressor cell function in man: evidence for altered sensitivity of responder cells with age. *Clin. Immunol. Immunopathol.* **13:** 119–124.

31. Bresnihan, B., and H. E. Jasin. 1977. Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. Clin. Invest.* **59:** 106–116.

32. Horowitz, S., W. Borcherding, A. V. Moorthy, R. Chesney, H. Schulte-Wisserman, R. Hong, and A. Goldstein. 1977. Induction of suppressor T-cells in systemic lupus erythematosus by thymosin and cultured thymic epithelium. *Science (Wash. D. C.).* **197:** 999–1001.

33. Newman, B., S. Blank, R. Lomnitzer, P. Disler, and A. R. Rabson. 1979. Lack of suppressor cell activity in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **13:** 187–193.

34. Sakane, T., A. D. Steinberg and I. Green. 1978. Failure of autologous mixed lymphocyte reactions between T and non T cells in patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. U. S. A.* **75:** 3464–3468.

35. Morimoto, C., T. Abe, and M. Homma. 1979. Altered function of suppressor T lymphocytes in patients with active systemic lupus erythematosus. In vitro immune response to autoantigen. *Clin. Immunol. Immunopathol.* **13:** 161–170.

36. Miller, K. B., and R. S. Schwartz. 1979. Familial abnormalities of suppressor cell function in systemic lupus erythematosus. *N. Engl. J. Med.* **301:** 803–809.

37. Krakauer, R. S., J. D. Clough, S. Frank, and J. T. Sundeen. 1979. Suppressor cell function defect in idiopathic systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **14:** 327–333.

38. Haynes, B. F., P. Katz, and A. S. Fauci. 1979. Mechanisms of corticosteroid. Action on lymphocyte subpopulation. V. Effects of in vivo hydrocortisone on the circulatory kinetics and function of naturally occurring and mitogen-induced suppressor cells in man. *Cell. Immunol.* **44:** 169–178.

39. Saxon, A., R. H. Stevens, S. J. Ramer, P. J. Clements, and D. T. Y. Yu. 1978. Glucocorticoids administered in vivo inhibit human suppressor T lymphocyte function and diminish B lymphocyte responsiveness in in vitro immunoglobulin synthesis. *J. Clin. Invest.* **61:** 922–930.

40. Haynes, B. F., and A. S. Fauci. 1978. Activation of human B lymphocytes. V. Kinetics and mechanisms of suppression of plaque-forming cell responses by Concanavalin A-generated suppressor cells. *J. Immunol.* **120:** 700–708.

41. Haynes, B. F., and A. S. Fauci. 1978. Activation of human B lymphocytes. X. Heterogeneity of Concanavalin A-generated suppressor cells of the pokeweed-mitogen-induced plaque forming cell response of human peripheral blood lymphocytes. *J. Immunol.* **121:** 559–565.

42. Hayward, A. R., L. Layward, P. Lydyard, L. Moretta, M. Dagg, and A. R. Lawton. 1978. Fc-receptor heterogenicity of human receptor T cells. *J. Immunol.* **121:** 1–5.

43. Alarcon-Segovia, D., and A. Ruiz-Arguelles. 1978. Decreased circulating thymus-derived cells with receptors for the Fc portion of immunoglobulin G in systemic lupus erythematosus. *J. Clin. Invest.* **62:** 1390–1394.

44. Moretta, L., M. C. Mingari, and A. Moretta. 1979. Human T cell subpopulations in normal and pathologic conditions. *Immunol. Rev.* **45:** 163–193.

45. Krakauer, R. J., T. A. Waldman, and W. Strober. 1976. Loss of suppressor T cells in adult NZB/NZW mice. *J. Exp. Med.* **144:** 662–673.

46. Primi, D., L. Hammarström, and C. I. E. Smith. 1978. Genetic control of lymphocyte suppression. I. Lack of suppression in aged NZB mice is due to a B cell defect. *J. Immunol.* **121:** 2241–2243.

47. Cantor, H., L. McVay-Boudreau, J. Hugenberger, K. Naidorf, F. W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits amont T-cell sets. II. Physiologic role of feedback inhibition in vivo: absence in NZW mice. *J. Exp. Med.* **147:** 1116–1125.

48. Goodwin, J. S., and R. C. Williams. 1979. Suppressor cells. A recent conceptual epidemic. *J. Clin. Lab. Immunol.* **2:** 89–91.

49. Galanaud, P., M. C. Crevon, M. Guenounou, J. Agneray, and J. Dormont. 1980. Sodium periodate-induced suppressor T cells of the human in vitro antibody response. *Cell. Immunol.* **51:** 85–91.