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FURTHER ASPECTS OF T CELL FUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Generation of effector cells, the expression of cell mediated lympholysis (CML) and the reactivity of mononuclear cells in solid cultures, were explored in patients with Systemic Lupus Erythematosus (SLE). While proliferative responses to alloantigens were comparable to the controls, a significant decrease in CML capacity was found in SLE T lymphocytes; further, T cells stimulated with a T cell mitogen in solid cultures showed a disminished proliferative response to soluble factors. Functional cell interaction defects rather than intrinsic T cell abnormalities may be operating in SLE.

INTRODUCTION

Cell mediated immune reactions (CMI) in SLE have been studied in an attempt to establish their role in the etiopathogenesis of the disease (1,2,3,4). Several reports have focused on possible defects at the level of immunoregulation, namely abnormalities of T suppressor cells (5,6,7,8). In our laboratory, Pérez-Rojas *et al*. (9) found normal delayed skin reactions to common recall antigens, adequate proliferative responses to mitogens and alloantigens, marked influence of autologous SLE sera on *in vitro* CML reaction and preliminary evidences of T killing activity in patients with SLE. In this paper, we report further observations on generation and expression of CML as well as preliminary results on SLE responses to a T cell mitogen in solid cultures.

MATERIAL AND METHODS

Twelve patients fullfilling the American Rheumatism Association diagnostic criteria for SLE (10)were selected; 11 were female. The age range of the group was 21-45 years; 9 were untreated. As controls, 15 healthy blood bank donors were included. CMI reactions *in vitro* were performed simultaneously in patients and controls.

PBL Isolation and Allogenic Response

Peripheral blood lymphocytes (PBL) were isolated from heparinized blood on Ficoll-hypaque(R) density gradients (11). Mixed lymphocyte response to alloantigens was performed between patient or control lymphocytes as responder cells and normal PBL from at least 3 healthy donors, used as stimulators, previously treated with mitomycin C (50 ug/ml, 37°C for 30 min). Triplicate cultures for each combination were set up in Falcon microtiter plates. $50x10^3$ responding and $100x10^3$ stimulating cells, in a total volume of 0.2 ml of RPMI 1640 (Penicillin-Streptomycin, 4% hepes, supplemented with 20% pooled heat-inactivated normal human serum, (NHS) were incubated for 6 days at 37°C in a 5% CO_2 air mixture. Eighteen hours prior to harvesting, each well was labelled with 1 µCi of ^3H thymidine. Cultures were harvested in a Mash II harvester and thymidine incorporation was counted in a B counter (Packard, 3330). Results were expressed as relative proliferation index (RPI) as described by Dean *et al*. (12)

In Vitro Generation of Cytotoxic Effector Cells

PBL from patients and controls, isolated as above, were set up in 12x75 mm tubes. Triplicate cultures containing $1.5x10^6$ responder and $3x10^6$ stimulators cells in 2 ml of RPMI 1660 (supplemented as above) were incubated for 6 days at 37°C in a 5% CO_2 air mixture.

Eighteen hours prior to harvesting, an aliquot of each tube was labelled with 1 µCi of ^3H thymidine to investigate cell proliferation. All tubes with the same experimental combination and evidence of proliferation were pooled.

Target Cells

Phytohemaglutinin (PHA) blasts were used as target cells. Briefly, $1x10^6$ cells from the control cells used as stimulators in

the generation of effector cells, were set up at the same time in RPMI 1640 (suplemented as above); 72 hours prior to harvesting, 10 ug/ml of PHA (Wellcome) were added to induce blast transformation.

Cell Mediated Lympholysis (CML)

CML was performed using Brunner's technique (13). Briefly, U-bottom microplates and an effector to target cell ratio of 100:1 was employed. Target cells were labelled with sodium chromate (^{51}Cr, New England Nuclear); $5x10^3$ target cells were added to the wells containing effector cells to obtain the experimental release of ^{51}Cr. Maximal ^{51}Cr release was achieved by freezing and thawing $5x10^3$ target cells in distilled water. Spontaneous release was determined using a similar number of target cells, diluted in RPMI medium with either 10%, 1% or 0.1% of the patient serum. The plates were incubated at 37°C for 18 hours; prior to harvesting, the plates were centrifuged for 10 min at 200 g. One hundred ul of the supernatant were taken from each well and counted (Packard Scintillation Counter). The percentage of lysis of the target cells was calculated by the following equation:

$$\% \text{ of lysis} = \frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Maximal release} - \text{Spontaneous release}} \times 100$$

In our laboratory, values of over 5% lysis are considered as an index of positive killing, since 50 normals (non-sensitized controls) showed values less than 2%.

Lymphocyte Stimulation in Solid Cultures

Lymphocyte stimulation in solid cultures was performed following Kondracki's method (14). Briefly, two percent (w/v) agarose (Marine Colloids, Springfield, N.J.) in distilled water was autoclaved at 121°C for 20 min and cooled in a water bath at 40°C. The agarose was then mixed with an equal volume of twice concentrated RPMI 1640, supplemented with 200 units/ml penicillin and 100 µg/ml streptomycin (Gibco, Grand Island, N.Y.), and warmed in a water bath at 40°C. The resulting 1% agarose solution RPMI 1640, was kept in a water bath at 40°C. Fifty microliters of the 1% agarose, were added to 10x75 mm glass tubes (Bellco, Vineland, N.Y.) and the tubes were placed in the 40°C water bath. Human

leukocytes were adjusted to the desired lymphocyte concentration in RPMI 1640. Fifty microliters of this leukocyte suspension, were transferred to glass tubes containing 50 ul of 1% agarose in RPMI 1640. The tubes were agitated, removed from the 40°C water bath, and left undisturbed at room temperature. The tubes contained at this step, a suspension of non-aggregated lymphocytes in 100 µl in 0.5% agarose (solid medium). The leukocyte suspension was then overlaid with 100 µl of RPMI 1640 containing the desired dose of mitogen and 20% autologous serum. In the present study, twelve-o-tetradecanoylphorbol-13 acetate (TPA), a T cell mitogen was utilized, with an optimal dose of 10^{-6M}. Six hours prior to culture termination, 0.2 µCi of ^{3}H-thymidine (New England Nuclear, Boston, Mass), in 10 µl PBS were added to each glass tube. Cultures were terminated by placing them in a -20°C freezer where, if not processed immediately, they were stored for up to 1 month.

Cultures were then processed by a modification of the method described by Peters (15). Tubes with cultures were allowed to thaw at room temperature. Thereafter, 1 ml of a 3 M potassium iodine in 5% trichloroacetic acid (TCA) solution was added to each tube, and the tubes were heated at 60°C for 2 min. Immediately after heating, the contents of the tubes were stirred on a Vortex mixer, and then centrifuged at 600xg for 5 min at room temperature. After centrifugation, the supernatants were removed, and the pellets washed twice with 5% TCA. After the last washing, TCA was removed and the pellets dissolved with 0.5 ml NCS of solubilizer(Amersham Searle, Arlington Heights, III). The solubilizer material was transferred to 7 ml scintillation vials (Fisher, Fair Lawn, N.J.) to which 6 ml of a scintillation fluid mixture were added. The scintillation fluid mixture was composed of 6g of 2.5 diphenyloxazole (Amersham-Searle) and 0.6 g of 1.4 bis-2.4 (4 methyl-5-phenyloxazolyl) benzene (Packard) in 1 liter of toluene (Fisher, Fair Lawn, N.J.). The scintillation vials were placed in a liquid scintillation counter to measure the amount of ^{3}H-thymidine present.

Statistical Analysis

T-statistical evaluation for paired observations and two sample test and Student's distribution program were used.

RESULTS

MLC and CML in both SLE and Controls

MLC and CML responses are shown in table I; while the MLC responses in both groups were comparable, a significant diffe -

TABLE I

ALLOGENEIC RESPONSE (MLC) AND CELL MEDIATED LYMPHOLYSIS (CML) IN SLE PATIENTS AND CONTROLS

MLC (RPI)		CML (% OF LYSIS)	
Patients n=12	Controls n=15	Patients n=12	Controls n=15
1.73	1.63	67	85
1.11	1.56	61	85
0.93	1.56	57	80
0.80	1.34	43	78
0.77	1.07	37	74
0.73	1.05	25	72
0.70	0.97	25	72
0.70	0.92	22	70
0.66	0.90	22	47
0.65	0.79	15	43
0.58	0.69	14	31
0.57	0.65	13	25
	0.64		17
	0.62		13
	0.54		7
$\bar{X}\pm SD$ 0.83±0.21	1.00±0.37	33.4±19	53.3±28
	t=1.24; p>0.1		t=2.07; p<0.025

TABLE II

EFFECT OF SLE SERA ON CML OF CONTROLS AND PATIENTS LYMPHOCYTES

	Controls (n=9) (mean±S.D.)*	Patients (n=12) (mean±S.D.)*
NHS	46 ± 33	33 ± 19
SLE sera 1/10	43 ± 24	33 ± 15
SLE sera 1/100	48 ± 30	38 ± 21
SLE sera 1/1000	41 ± 30	39 ± 19

* : % of lysis

TABLE III

LYMPHOCYTE RESPONSE TO TPA IN SOLID CULTURE

SLE (n=7) c.p.m.	Controls (n=6) c.p.m.
13.289	55.340
1.753	37.892
14.663	17.000
2.401	26.289
1.271	28.657
1.960	8.768
3.400	
$\bar{X}\pm SD$ 5.5.34±5.817	28.991±16.332

rence in CML capacity was found among the SLE patients ($p<0.025$).

Effect os SLE sera on the generation of CML by both SLE and controls lymphocytes

The possible influence of serum factors, present in SLE sera, on CML capacity of both SLE and control lymphocytes was investigated and compared with results obtained in NHS. SLE sera was diluted 1/10, 1/100 and 1/1000; no differences were found in the percentage of lysis in either group (table II).

Lymphocyte proliferation in solid cultures

Only 2 out 7 SLE patients showed proliferative response to TPA in solid cultures, comparable to the controls (table III). Results are expressed in cpm; the mean for SLE patients was 5.534 while in the controls was 28.991 being the difference significant ($p<0.025$).

DISCUSSION

Many attempts have been made to characterize the immuno - pathology of SLE; conflicting results concerning *in vivo* and *in vitro* CMI responses have been reported (1,2,3). Further more, recent investigations suggested abnormalities of cell responses in autologous MLC and loss of T_5 positive cells (subset of su - pressor cells defined by a monoclonal antibody) among patients with SLE (16,17).

Our laboratory has approached the study of CMI responses in SLE from the functional viewpoint. PBL reactions are tested using precultured cells. Several CMI *in vitro* tests are carried out simultaneously, in order to examine the various phases of immune response at the cellular level. Pérez *et al.* (9) reported intact *in vivo* and *in vitro* CMI responses in our SLE population. Pre - liminary evidence of generation of CML effector cells was also described stressing the inhibitory influence of autologous serum factors on SLE lymphocyte reactivity.

In the present investigation, we corroborated the capacity of T cells in SLE patients to proliferate against alloantigens and offer additional evidence in the generation of T killer cells and expression of T-mediated lympholysis, even thouhg the latter function was reduced when compared with the control group. These

findings tend to suggest the absence of intrinsic abnormalities of SLE T cell subsets. Further, the inhibitory influence observed with SLE autologous serum factors at the level of proliferative response either to mitogens or alloantigens (9) did not alter the generation of effector cells or their killing capacity. One possible explanation of our findings might be disturbances of cell interactions through soluble factors (18). Since the use of solid cultures allows the study of cell behavior, by responding to regulatory signals, we proceeded to explore SLE T lymphocytes responding to such signals.

TPA has been described as a T cell mitogen in solid agarose cultures by Kondracki and Milgrom (19). Lymphocyte proliferation in such cultures is dependent on at least 2 signals: one delivered by the mitogen and another provided by accessory cells through soluble factors (14); the low responses obtained by TPA stimulation of SLE patients lymphocytes could be due to functional deficiencies in either lymphocytes, accesory cells or both. The possible generation of responding suppressor cells under solid culture conditions can not be ruled out, even though their loss in SLE has been suggested (17). Further experiments presently under way, are required to elucidate which of the previous hypotheses will adequately explain our results. Certainly, investigations of cell communications between lymphocytes and accessory cells may offer new approaches to the study of the function of SLE lymphocytes.

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