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Responses of Fractionated Cells from Patients with Systemic Lupus Erythematosus and Normals to Plant Mitogen: Evidence for a Suppressor Population of Monocytes[1] (40127)

JOSEPH A. MARKENSON, JOHN W. MORGAN, MICHAEL D. LOCKSHIN, CATHARINE JOACHIM,[2] AND JOHN B. WINFIELD[3]

The Hospital for Special Surgery, Department of Medicine of Cornell University Medical College, New York, New York 10021, and [3] Department of Medicine of the University of Virginia School of Medicine, Charlottesville, Virginia 22904

Impairment of cell-mediated immunity (CMI) has been postulated as an important mechanism in the pathogenesis of systemic lupus erythematosus (SLE) (1–6). Prior studies of CMI response in patients with SLE have often shown abnormal *in vitro* lymphocyte responses to mitogen even when untreated patients have been studied (7–12). In this test, impaired lymphocyte proliferation may be due to abnormal function of T cells (either suppressor or helper), or B cells; a second possibility is that the lymphocyte population is normal but other cells, such as monocytes or macrophages, are abnormal in either quantity or function. Although cell populations have been identified by membrane criteria, the function of subpopulations has not been studied directly in SLE. The present report concerns studies examining mitogen responses of unfractionated cells and of cell populations from which B cells and/or adherent cells have been removed. The results demonstrated that an adherent cell population mediated the poor lymphocyte response to phytohemagglutinin (PHA) in some subjects.

Materials and methods. Patients. Thirty-two patients with definite SLE as defined by criteria of the American Rheumatism Association (13) and 19 normal volunteers were studied. The patients with SLE were not selected on the basis of activity of disease or concurrent therapy and represent a spectrum of patients with this disease.

Cell separation and purification. Fifty milliliters of heparinized blood were obtained by venipuncture (after informed consent) and layered on a Ficoll–Hypaque gradient in order to isolate the mononuclear cells (referred to in this report as FH) (14), which were then washed three times in Hank's Balanced Salt Solution. In order to identify more clearly the suppressor cell, two additional populations of cells were studied: B *and* monocyte depleted (hereafter referred to as Fab Col), and monocyte depleted (referred to as NR Ig Col). To obtain these populations, twenty million cells per ml were suspended in RPMI 1640 media with 5% fetal calf serum, penicillin, and streptomycin and layered on a G-200 Sephadex column to which either purified rabbit anti-human $F(ab)_2$ (Fab Col) or normal rabbit immunoglobulin (NR Ig) was conjugated (15). The column was washed until the effluent was cell-free; the cells collected from the Fab Col were "T" cells as judged by the absence of staining for cell surface immunoglobulins (<1%) and by formation of E-rosettes (>85%) (15). Cells collected from NR Ig Col were depleted of monocytes as shown by the absence of cells (<3%) staining positive by peroxidase (16), but contained B and T cells proportional to those added.

Mitogen cultures. FH, Fab Col, or NR Ig Col cells from the same patient were cultured in microtiter plates according to previously published methods (1), except that RPMI 1640 and AB human serum were used. Response was measured at 3 days by tritiated thymidine pulse and recorded as the sum of responses of eight concentrations of PHA between 0.5 and 300 μg/ml. Calculations were also done for individual mitogen concentrations and are presented where relevant. The Fab Col response was divided by the FH response to obtain the Fab Col/FH ratio. In

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some experiments, incubation for 1 hr at 37° was carried out prior to testing to elute antilymphocyte antibodies. In other experiments cells were cultured in autologous serum as a control for serum inhibitory factors. Antilymphocyte antibody determinations were done by previously published methods (17).

Results. The response of Fab Col cells obtained from normal subjects was less than that of FH cells in 17 of 19 instances (90%) (Table I). The converse occurred for lymphocytes obtained from patients with SLE. The response of SLE FH cells was depressed relative to that of normal lymphocytes, but increased to normal values when isolated Fab Col cells were tested, giving a Fab Col/FH ratio greater than unity in 14 out of 32 SLE patients (44%) (Table I). The Fab Col/FH ratio inversely varied with the absolute response of the FH cell population for each patient population (p for correlation coefficient <0.001 for each population) (Fig. 1). There was no difference for Fab Col/FH ratios when the results were calculated at optimum response or as the sum of responses at each concentration of mitogen used.

Two groups of SLE patients were distinguished: those whose FH response was lower than their Fab Col response (Fab Col/FH ratio >1.0) and those whose values for both FH and Fab Col were both below normal but FH was >Fab Col (Fab Col/FH ratio <1.0) (Fig. 2). In the first group the Fab Col/FH ratio of >1.0 was due primarily to an increase in the Fab Col response. Improvement of the response of the Fab Col was seen at all concentrations of mitogen tested but was most marked at optimal concentrations (Fig. 2).

All patients' records were reviewed for clinical status, therapy and serological activity of

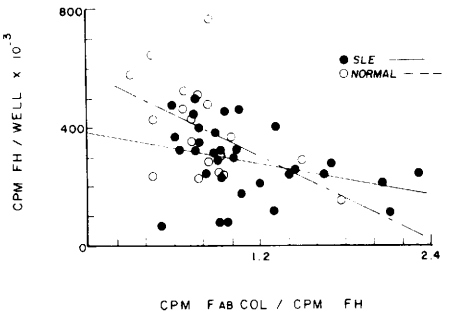

FIG. 1. The Fab Col/FH ratio varied inversely with the response of the FH cell population to PHA. P value for correlation coefficient <0.001 for both normal and SLE.

TABLE Ia. FREQUENCY DISTRIBUTION OF FAB COL/FH CELL RATIO[a] FOR SLE[b] PATIENTS AND NORMAL SUBJECTS.

		Ratio Fab Col/FH					
		Number of subjects					
Subject	No. tested	<0.50	0.51–.75	0.76–1.00	1.01–1.25	1.26–1.50	>1.50
SLE	32	0	6	12	5	4	5
Normal	19	4	5	8	0	1	1

[a] PHA response in CPM of Ficoll-Hypaque cells after passage over anti $F(ab)_2$ column divided by PHA response in cpm of unfractionated Ficoll-Hypaque cells.
[b] Systemic lupus erythematosus.

TABLE Ib. MEAN RESPONSES TO PHA OF FAB COL[a] AND FH CELLS[a] FOR NORMAL AND SLE[a] PATIENT POPULATIONS.

	All subjects			Subjects with ratio <1.00			Subjects with ratio >1.00		
	No. tested	FH	Fab col	No. tested	FH	Fab col	No. tested	FH	Fab col
SLE	(32)	287334 ±20036[b]	292113 ±40772	(18)	315009 ±31331	249752 ±24887	(14)	257630 ±26496	366124 ±35105
Normal	(19)	399874 ±8876	299680 ±26982	(17)	420287 ±38365	288789 ±30152	(2)	227863 ±70785	362241 ±83801

[a] Abbreviations same as Table Ia.
[b] Response to PHA, cpm ± SE of mean.

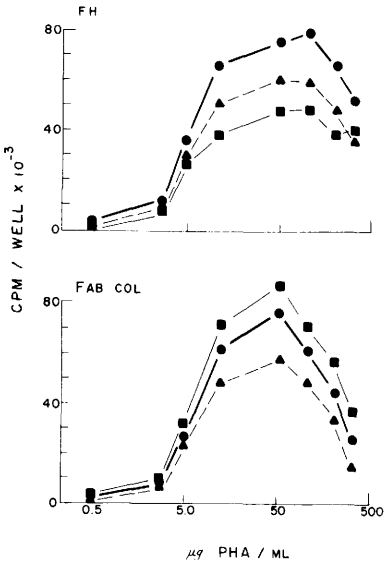

FIG. 2. Mean mitogen dose-response curves for FH cells and Fab Col cells in 19 normals ●, 14 SLE patients with Fab Col/FH ratio >1.0 ■ and 18 SLE patients with ratio <1.0 ▲. FH cell responses for both groups of SLE patients differ significantly from normal at most mitogen concentrations but the Fab Col response of patients with ratio >1.0 is equivalent to, or greater than, normal. The shape of the dose-response curve is not altered in either patient group. Top: FH cells; bottom: Fab Col cells.

disease as measured by anti-DNA antibody and complement. There was no correlation between Fab Col/FH ratio >1.0 and any parameter of disease activity or therapy. In addition, three patients studied serially during therapy tended to show identical responses repetitively with Fab Col/FH ratios of >1.0 regardless of disease status. Finally, there was no correlation between cell responses and the presence of antilymphocyte antibodies, nor did elution of these antibodies consistently improve responses.

In some SLE patients, but not in normal controls, the lower FH cell responses could be corrected equally well by passage of FH cells over NR Ig columns which depletes monocytes. In this set of experiments with three normals and three SLE patients, the normal FH cell response diminished after passage over NR Ig G-200 columns. By contrast, SLE FH cell responses showed marked improvement after passage over anti-$F(ab)_2$ and NR Ig columns suggesting that removal of adherent cells alone is effective in improving the poor FH cell response (Table II).

The possibility that low response of FH SLE cells is a function of dilution of Fab Col cells by monocytes was considered. There was no statistical relationship between response of FH cell populations and percent monocytes for either normal or SLE patients, despite the fact that some SLE patients had greater than 50% peroxidase staining cells. The response of Fab Col cells for both normal and SLE subjects was a linear function of the number of cells present in the well (incubation volume constant). This was also true for FH cells for normal subjects and for SLE subjects with a normal (Fab Col/FH ratio <1.0) response. However, for SLE subjects with an abnormal response (ratio >1.0) the response of FH cells varied *inversely* with the number of cells present, implying that a soluble inhibitor is easily dilutable or that close cell-to-cell approximation is necessary for inhibition to occur. Since incubation volumes were kept constant, the data do not distinguish these possibilities (Fig. 3), but since the converse was seen with Fab Col cells, the pivotal role of a cell removed by the Fab Col is implied.

In other experiments, dilution of lymphocytes from normals within the range expected from calculations of percent monocytes did not consistently lower the unfractionated cell response to levels seen in SLE patients.

TABLE II. PERCENT CHANGE IN PHA RESPONSE IN THREE NORMALS AND THREE SLE PATIENTS AFTER PASSAGE OF FICOLL-HYPAQUE (FH) MONONUCLEAR CELLS OVER ANTI-$F(ab)_2$ (FAB COL) AND OVER NORMAL RABBIT Ig (NR Ig) SEPHADEX G-200 COLUMNS.

		Percent change of response to PHA of FH cells after passage over	
	Response of FH cells to PHA (cpm)	Anti-$F(ab)_2$ columns	Normal rabbit Ig columns
Normals			
1	279994	−51	−16
2	235271	−7	−5
3	354961	−27	+5
SLE			
1	175670	+11	+38
2	258364	+44	+48
3	209367	+19	+52

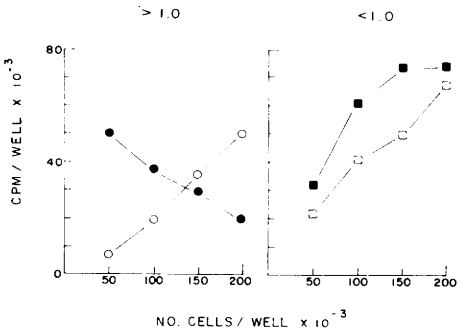

FIG. 3. Response of Fab Col and FH cells as a function of cell concentration. Each point is the average of two experiments of cells cultured in the presence of 50 μg/ml PHA. Circles, SLE patient with Fab Col/FH ratio = >1.0 at 200,000 cells/well. Squares, patient with ratio = <1.0. Closed figures, FH cells; Open, Fab Col cells.

Discussion. CMI is depressed in patients with active SLE and this is generally not related to treatment (1–12). The question of whether abnormal CMI is related to intrinsic lymphocyte abnormalities, depletion of functionally important subsets, antibody-mediated blockade of surface receptors, or a combination of these factors cannot be resolved by available data (12, 18–20).

Recently classes of peripheral blood lymphocytes have been identified which enhance immunoresponsiveness (helper T cells) (21) and populations which decrease immunoresponsiveness (suppressor cells) (22). Barthold and coworkers have described the age-related loss of both suppressor and helper T cells in NZB mice with lupuslike disease (23). They have postulated that this is related to the onset and increased severity of autoimmune disease in these mice.

A third cell population, the adherent cell or monocyte–macrophage which plays an important role in the immune response has not been well studied in SLE (24–28). The present data suggest that the decreased responsiveness of peripheral blood lymphocytes from patients with SLE to PHA may be mediated by an adherent cell population, since removal of these cells corrected the abnormally low responsiveness of unfractionated SLE lymphocytes in approximately one-half of the patients tested. Other, as yet unidentified, cell populations removed by the Fab columns may also exert suppressive effects. The fact that dilution of FH cells in patients with Fab Col/FH ratio >1.0 improves responses while dilution diminishes responses in patients with Fab Col/FH ratio <1.0 and in normals implies that cell-to-cell interaction may also be important in mediating suppression. By contrast, defective T cells, excess function of suppressor T cells (not removed by the column), or defective helper T cells seem unlikely as an explanation of low responsiveness of SLE FH cells, since purified T cells had responses equal to those of normal Fab Col cells.

Experimental evidence to support a suppressor role for the monocyte exists in other systems. A similar inhibition of PHA responses in patients with Hodgkin's disease by mononuclear cells has been observed (41). Excessive numbers of macrophages may inhibit the induction of primary antibody formation (30, 31), the responses of T cells to allogeneic cells (32), PHA (33), and the response of B cells to lipopolysaccharide (34). Removal of macrophages can restore *in vitro* lymphocyte responsiveness in the graft-versus-host reaction (35), in reaction to BCG (36), and in some animal tumor models (37). In addition, two recent studies (42, 43) report a decreased PHA response to monocyte depleted cell populations from normal donors with one (43) observing an increased response to PHA in the FH fraction.

Certain other factors possibly related to the hyporesponsiveness of SLE lymphocytes to PHA did not appear to be involved. Corticosteroid administration (38, 39) in agreement with our prior experience (1), did not correlate with decreased responsiveness of unfractionated SLE cells to PHA, nor did the presence of lymphocytotoxic antibodies influence PHA responsiveness, an observation noted previously (20, 40, 43).

In conclusion, our data suggest that a nonlymphocyte mononuclear cell population, probably monocytes, is responsible for the depressed response to PHA seen in some patients with SLE. This phenomenon is probably not limited to patients with SLE, but may represent a more generalized mechanism of suppression of CMI in disease states where alterations in the immune system are evident.

Summary. In systemic lupus erythematosus (SLE) three populations of blood mononuclear cells from patients with systemic lupus erythematosus (SLE) and from normal controls were examined for their responses to

graded concentrations of phytohemagglutinin (PHA). In normal controls, H_3 thymidine uptake of unfractionated cells (FH) was greater than that of T cells (Fab Col). In contrast, SLE Fab Col cells responded better than FH cells in 44% of patients tested. SLE mononuclear cells devoid of adherent monocytes (NRIg) also responded better than FH cells.

The results indicate that hyporesponsiveness to PHA seen in some SLE patients is moderated by an adherent cell which may be functioning as a suppressor cell.

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