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Stimulating and differentiation factors for human B lymphocytes in systemic lupus erythematosus

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SUMMARY

T cells from 18 untreated SLE patients produced significantly more B cell growth factor (BCGF) than did those from normal subjects. Those from SLE patients with active disease produced significantly more than did those from patients with inactive disease. The response to BCGF of SAC-stimulated B lymphocytes from SLE patients was higher than that of B lymphocytes from normal individuals. Similarly preactivated B cells from five of seven SLE patients also proliferated upon the addition of interleukin 1 (IL-1) whereas those of normal subjects did not. Simultaneous addition of IL-1 and BCGF had a synergistic proliferative effect on B cells from two of seven SLE patients but not on any of the controls. Interleukin 2 (IL-2) had no proliferative effect in either SLE or normal B cells. Supernatant fractions from T cells of seven of 10 patients with active SLE and three of 10 with inactive SLE induced more IgG production by CESS cells than did those of normal subjects indicating a higher production of B cell differentiation factor by SLE T cells than by those of controls. Our findings may explain the reported preactivation and predifferentiation of peripheral blood B cells from SLE patients and give insight into the mechanisms leading to the production of autoantibodies in this disease.

Keywords BCGF BCDF SLE lymphokines B lymphocytes immunoregulation interleukin 1 interleukin 2

INTRODUCTION

Several soluble factors participate in the induction of humoral immune responses (Howard & Paul, 1983). These factors affect cellular proliferation and/or differentiation of B lymphocytes from the resting non Ig-secreting state to that of active Ig secretion (Falkoff, Zhu & Fauci, 1982). Included among them are: interleukin 1 (IL-1), B cell growth factor (BCGF), and B cell differentiation factor(s) (BCDF). IL-1 itself has no direct effect on the proliferative response of unstimulated mature B cells or of in-vivo activated B cells which respond to BCGF *in vitro* but acts in synergy with anti-μ antibody. BCGF is a growth factor, distinct from IL-2, and specific for human B cells (Kehrl *et al.*, 1984). BCDF, formerly called T-cell replacing factor, is involved in the terminal differentiation of B cells into Ig-secreting plasma cells.

Multiple immunological abnormalities have been reported in patients with systemic lupus erythematosus (SLE) (Alarcón-Segovia, Alcocer-Varela & Díaz-Jouanen, 1985). At present, however, the main alterations seem to be hyperactivity of B lymphocytes (Ginsburg, Finkelman & Lipsky, 1979; Blaese, Grayson & Steinberg, 1980; Alarcón-Segovia *et al.*, 1982), defective

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suppressor functions (Abdou *et al.*, 1976; Bresnihan & Jasin, 1977; Ruíz Argüelles *et al.*, 1980) and defective production of and response to interleukins 1 (Alcocer-Varela, Laffón & Alarcón-Segovia, 1983) and 2 (Alcocer-Varela & Alarcón-Segovia, 1982; Linker-Israeli *et al.*, 1985).

The question of whether B cell hyperactivity in SLE and the apparently consequent autoantibody formation result from increased stimulatory and/or differentiative effects from the corresponding T cell factors can be important in elucidating the physiopathogenesis of SLE. We have attempted to answer this question in this study.

METHODS

Subjects. We studied 20 patients who fulfilled criteria for the classification of SLE (Tan *et al.*, 1982). All were females whose age ranged from 17 to 66 years (mean, 40·3). No patient was receiving corticosteroids or immunosuppressive drugs or had received them for at least 3 months. Those patients who were taking nonsteroidal anti-inflammatory agents discontinued them at least a week before the time of the study. Ten patients had active disease with at least two disease manifestations requiring the initiation of treatment thereafter, whereas the other 10 patients had achieved complete remission of their disease and had had all therapy discontinued.

We studied as controls 14 healthy female volunteers whose ages ranged from 20 to 43 years with a mean of 33·4.

Cells. Mononuclear cells (MNC) were obtained from peripheral blood in Ficoll-Hypaque gradients and were washed three times in phosphate-buffered saline, pH 7·4 (PBS). Monocytes were removed from them by layering in plastic dishes held in incubation for 18 h at 37°C. T and B cells were separated by rosetting with erythrocytes as described previously (Alcocer-Varela & Alarcón-Segovia, 1982). B cells were further purified by rosetting and treatment with anti-OKT3 antibody (Ortho Diagnostic Systems Inc., Raritan, NJ) and complement. Over 95% of the B cells thus obtained had surface Ig on fluorescence testing and less than 1% were OKT3 positive upon flow cytometric analysis in an Ortho FC 200 cytofluorograph (Ortho Instruments, Boston, MA) (Melendro *et al.*, 1983). The proliferative response of B cells to PHA or Con-A was less than 1% of that given by the T cell fraction.

Cell culture. All cultures were set up in RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with penicillin 100 i.u./ml, streptomycin (100 μg/ml), glutamine 2 mM, and 5% fetal calf serum (FCS), in microculture plates (Costar, Cambridge, MA) at 37°C in a 5% CO_2, 100% humidity, atmosphere.

Production of B stimulating factors. We stimulated T cells to produce BCGF and BCDF both with phytohemagglutinin-P (PHA) (Difco Laboratories, Detroit, MI) and in autologous mixed lymphocyte reactions (AMLR). PHA stimulation was done essentially as described by both Sredni *et al.* (1981) and Muraguchi & Fauci (1982). Briefly, 1×10^6 unfractionated MNC or T cells were cultured with 1% PHA for 12 h. The cells were washed three times to remove the remaining PHA. We then resuspended them in culture medium and cultured them for 60 h. Supernatants were collected by centrifugation after absorption with human RBC to remove the remaining PHA and with activated T cells to remove any IL-2 activity. Supernatants were filtered and stored at −35°C until used. Testing of these supernatants for IL-2 activity (Alcocer-Varela & Alarcón-Segovia, 1982) showed none.

Stimulation in AMLR was done essentially as described by Falkoff, Zhu & Fauci (1982). For this, we cultured 2×10^6 T cells with the same number of mitomycin-treated autologous non-T cells for 48 h. The supernatants were collected, absorbed with activated T cells to remove IL-2, filtered, and stored as above.

Assay for BCGF activity. For this we cultured 2×10^5 purified B cells with *Staphylococus aureus* Cowan strain I (SAC) at 1:4000 concentration (v/v). After 72 h of culture, we removed 100 μl of medium and replaced it with 25% of T cell factors. B cells growth was measured by determining (^{3}H) thymidine uptake after a pulse of 0·5 μCi, (6·7 Ci/mmol, New England Nuclear, Boston, MA) during the last 12 h of an additional 3 day culture. BCGF activity was standardized with that of purified BCGF produced by a cloned T-cell and devoid of BCDF, IL-1, or IL-2 activities. This factor was a generous gift from Dr B. Sredni of Bar-Ilan University, Israel.

Assay for BCDF activity. Supernatant fractions from T cells stimulated as above were assayed for their BCDF activity by using the CESS cell line (kindly given to us by Dr A. Fauci, NIH, Bethesda, MD), which produces Ig and discharges it into the surrounding medium only upon response to BCDF and in proportion to this stimulus.

We placed ten thousand CESS cells in each culture well for 5 days with the various stimulating factors or their controls. We then collected the culture supernatant fractions and assayed them for their IgG content by an ELISA method.

Assay for B cell response to purified BCGF. We cultured 2×10^5 B cells per well in flat bottomed 96-well tissue culture plates, with SAC at 1:4000 concentration (v/v). After 3 days we removed 100 μl of medium and we added 25% of purified BCGF. The proliferative response after 3 further days of culture was assayed by ^{3}H-TdR incorporation.

Effect of IL-1 on B cell proliferation. To study the effect of exogenous IL-1 on B cell proliferation, we used unstimulated and SAC-stimulated B cells. These cell preparations were exhaustively depleted of T cells and monocytes and were cultured in fresh medium containing 4 units of IL-1 (obtained from the U937 cell line, a generous gift from Dr R. Palacios of Basel Institute for Immunology, Switzerland), BCGF (1:4 dilution), or both. The proliferative response after 3 days of culture was assayed by ^{3}H-TdR incorporation.

Statistical analysis. Experimental results were analysed for their statistical significance by Student's *t*-test.

RESULTS

Production of BCGF activity by lymphocytes from SLE patients or controls. As shown in Table 1, T cells from SLE patients particularly those from patients with active disease produced significantly more BCGF activity than did those from normal subjects. The difference was apparent with either (PHA or AMLR) stimulus but was more striking with the mitogen.

B cell response to BCGF. This was studied in eight SLE patients and six healthy subjects (Table 2). The mean proliferative response of SLE B cells was higher than those of control B cells. It was higher than the mean + 2 s.d. of that found in normal controls in five of eight SLE patients (four active and one with inactive disease).

Synergy of IL-1 and BCGF on B cell proliferation. Figure 1 shows data on the effect of IL-1, BCGF, none or both, on unstimulated B cells from SLE patients and controls. After 3 days of

Table 1. Production of BCGF by T cells from SLE patients or normal controls*

		Stimulus	
Subjects	(*n*)	PHA	AMLR
SLE patients	18	8397 ± 1781*	3848 ± 364
Active	10	11779 ± 1781‡	4956 ± 338†
Inactive	8	4169 ± 957	2739 ± 546
Controls	13	4000 ± 641	2917 ± 273

* Mean CPM ± s.e.m. of ^{3}H thymidine incorporation by normal B cells preactivated with SAC and exposed to 1:2 dilution of supernatant fractions from T cells stimulated with PHA or in AMLR.

† $P < 0.05$ vs normal controls.

‡ $P < 0.0025$ vs normal controls.

Table 2. Response of preactivated B cells from SLE patients or normal subjects to BCGF*

Cells	Disease activity	Without BCGF	Dilution of BCGF 1:4	Dilution of BCGF 1:8
SLE patients				
1	Yes	2055	28910	20694
2	Yes	2750	15301	15317
3	Yes	1527	13389	10321
4	Yes	2813	10412	4331
5	No	3417	21017	2678
6	No	1873	5004	8080
7	No	1326	6807	9736
8	No	—	3789	15641
Mean ± s.e.m.		2251 ± 288	13078 ± 3037†	10874 ± 2841†
Controls (6)		1265 ± 145	4445 ± 2795	3133 ± 684

* Expressed as counts per minute of SAC-activated B cells.
† Difference vs controls $P < 0{\cdot}01$.

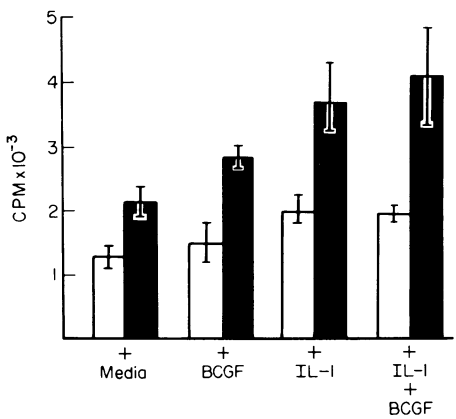

Fig. 1. Effect of BCGF, IL-1 or both on unstimulated B cells from SLE patients (black bars) or normal controls.

culture without these activating signals we found a higher ^{3}H-TdR incorporation by B cells from SLE patients than by those from controls ($P < 0{\cdot}0125$). IL-1 promoted somewhat higher ^{3}H-TdR incorporation by unstimulated B cells than did BCGF in both SLE patients' and controls' cells (difference of both between patients and controls, $P < 0{\cdot}0025$). Addition of BCGF to IL-1 did not increase significantly the mean response had with IL-1 alone in either group.

Experiments done with SAC-preactivated B cells also revealed a mitogenic effect of IL-1, in the absence of BCGF, in five of seven patients with SLE studied. This did not happen in normal B cells (Table 3). B cells from one of the two SLE patients whose B cells had not responded to IL-1 did not respond to BCGF either. Those from the other SLE patient whose B cells were unresponsive to IL-1

Table 3. Effect of BCGF and/or IL-1 on SAC-stimulated B cells from SLE patients or controls*

	Factors added				
Source of cells	None	IL-1	BCGF	IL-1 + BCGF	IL-2
SLE patients					
1	3417	8489	2678	12575	3512
2	1873	3702	8080	6782	1994
3	1325	10812	9736	3350	2015
4	2410	7480	6472	12876	2586
5	1470	5710	15815	10438	1976
6	2146	2273	25860	20673	2643
7	2892	2684	2849	2146	3058
Mean ± SE	2219 ± 285	5878 ± 1211	10212 ± 3109	9977 ± 2347	2540 ± 224
Controls (7)	1251 ± 79	1341 ± 51	3462 ± 223	3412 ± 174	1580 ± 99

* Expressed as counts per minute.

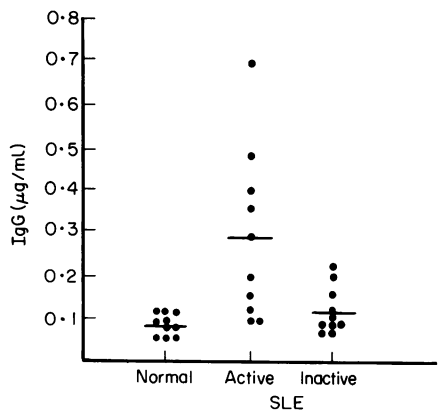

Fig. 2. Production of BCDF activity expressed as the amount of IgG produced by CESS cells added to the supernatant fractions from SLE or normal cells.

had a 12-fold response to BCGF. Conversely, one of the five SLE patients whose cells responded to IL-1 was found to have no B cell response to BCGF. Simultaneous addition of BCGF and IL-1 showed no synergic effect on normal SAC-stimulated B cells. In two of the seven SLE patients' cells, there was demonstrable synergy between the two factors.

Controls experiments done on SAC preactivated B cells added purified IL-2 revealed no mitogenic effect of this lymphokine on either SLE or control B cells.

Production of BCDF activity by lymphocytes from SLE patients or controls. This is shown in Figure 2. The difference between all patients and controls was statistically significant ($P < 0{\cdot}01$). Also significant was the difference found between cells from patients with active or inactive SLE ($P < 0{\cdot}0025$).

DISCUSSION

Immunoregulation is amply disturbed in SLE and, as a result, there seems to be an activation of B cells that leads to the production of autoantibodies, some of which may, in turn, have effects on immunoregulatory T cells and perpetuate their disturbance. The T cell abnormalities that have been found in SLE include decreased suppressor cell functions (Abdou *et al.*, 1976; Breshnihan & Jasin, 1977; Ruíz-Arguëlles *et al.*, 1980), abnormal helper cell functions in at least some patients (Delfraissy *et al.*, 1980), decreased production of IL-2 (Alcocer-Varela & Alarcón-Segovia, 1982), a product mainly of helper T cells, and decreased response to IL-2 (Alcocer-Varela & Alarcón-Segovia, 1982), a function mainly of suppressor cells (Fishbein *et al.*, 1983). Further to this, we find increased production of both BCGF and BCDF in SLE that would explain the state of preactivation and differentiation to Ig-producing cells of peripheral blood B cells found in SLE as judged from plaque forming cell assays (Ginsburg, Finglemann & Lipsky, 1979), the spontaneous production of Ig in culture (Blaese, Grayson & Steinberg, 1980), activation in cytofluorographic studies (Alarcón-Segovia *et al.*, 1982) and, as found here, increased response to BCGF by B cells since only activated B cells are capable of responding to BCGF by proliferating.

The findings made here are attributable to BCGF rather than to IL-2 or IL-1 because both of these factors have been found decreased, rather than increased, in SLE (Alcocer-Varela & Alarcón-Segovia, 1982; Alcocer-Varela *et al.*, 1984). However, we also ruled out a role of these factors in our assays since both have recently been found to also act on B cells (Kishimoto, 1984; Tsudo, Uchiyama & Uchino, 1984).

The dichotomy between the production of IL-2 and BCGF in SLE is of interest for it suggests that in man these two lymphokines may be produced by two different T cell subsets. This would be different from what seems to be the case in the mouse where the Lyt 1+2-cell produces both (Kishimoto, 1985).

Our findings on the effect of IL-1 on B cells are also of interest since they confirm the notion that this monokine may influence the proliferation of human B cells (Kishimoto, 1985). It was peculiar that, whereas T lymphocytes from SLE patients respond poorly to IL-1 (Alcocer-Varela *et al.*, 1984), their B cells responded to this monokine better than those of normal controls both with and without prior stimulus.

Increased production of BCDF in SLE explains the aforementioned spontaneous production of immunoglobulins by peripheral blood B cells from SLE patients.

In a recent study Hirose and his coworkers (1985) presented data on the production and response to BCGF and BCDF in SLE. Their findings with BCDF are akin to ours whereas those with BCGF apparently differ since they found decreased production of and response to BCGF by B cells from patients with SLE, particularly those with inactive disease. The apparent discrepancy can be explained by their inclusion of monocytes in their assays. Indeed, they observed that the removal of monocytes resulted in increased response to BCGF by B cells from patients with active SLE. This is similar to what we found in our assays where we depleted monocytes *a priori*. Another reason for the differences in results between both studies resides in the patients population. Thus, whereas we included only untreated patients in our study, Hirose *et al.* (1985) included prednisone-treated SLE patients particularly among those with inactive disease. Activation and proliferation of SAC-stimulated B cells are profoundly suppressed by corticosteroids *in vitro* (Cupps *et al.*, 1985). This could well explain the low, rather than high, production of BCDF in their study and that this abnormality was found in their patients with inactive disease rather than in those with active disease.

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