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## PRODUCTION OF B CELL GROWTH FACTOR BY A LEU-7<sup>+</sup>, OKM1<sup>+</sup> NON-T CELL WITH THE FEATURES OF LARGE GRANULAR LYMPHOCYTES (LGL)<sup>1</sup>

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The present study reports the characterization of a non-T cell from human peripheral blood which is capable of releasing BCGF. This BCGF-producing non-T cell had a T3<sup>-</sup>, T8<sup>-</sup>, Leu-7<sup>+</sup>, OKM1<sup>+</sup>, HLA-DR<sup>-</sup>, Leu-11<sup>-</sup> surface phenotype and was likely to belong to the so-called large granular lymphocyte (LGL) subset because: 1) after fractionation of non-T cells according to the expression of Leu-7 or HLA-DR markers, it was found in the Leu-7<sup>+</sup>, HLA-DR<sup>-</sup> fractions that were particularly enriched in LGL; 2) it co-purified with LGL on Percoll density gradients; and 3) it expressed Leu-7 and OKM1 markers that are shared by a large fraction of LGL. Although co-purified with cells with potent NK capacities, the BCGF-producing cell was not cytotoxic, because treatment of Leu-7<sup>+</sup> cells with Leu-11 monoclonal antibody and complement abolished the NK activity but left the BCGF activity unaltered. The factor released by this LGL subset was not IL 1 or IL 2 mistakenly interpreted as BCGF, because: a) cell supernatants particularly rich in BCGF activity contained very little or no IL 1 or IL 2; b) BCGF-induced B cell proliferation was not inhibitable by anti-Tac antibodies (this in spite of the expression of IL 2 receptor by a proportion of activated B cells); and c) BCGF activity was absorbed by B but not T blasts.

Proliferation and differentiation of B lymphocytes into plasma cells is a complex multistep process that is, in part, under the control of T cells (as reviewed in References 1-3). Such control is exerted by direct cell-cell interaction (4, 5) or through the release of a number of soluble factors (6-9). Of these factors, B cell growth factor (BCGF),<sup>3</sup> which supports cell proliferation, and B cell differentiation factor, which induces transformation of lymphocytes into plasma cells, are probably the most

widely studied. Both factors are active on cells activated by contact with antigen (or other ligands *in vitro*), but have no effect on resting B cells. Although it is now clear that these factors are released by T cells, it is not known whether or not they can be produced also by other cells. In human peripheral blood, most of the natural killing (NK) activity is mediated by the so-called large granular lymphocytes (LGL) (10-12). Besides cytotoxic properties, LGL have been shown to participate in the regulation of the proliferation of other cell types (13-16), a finding that is consistent with other data from animal models demonstrating similar functions for NK cells (17-21). This regulatory control can be exerted by an apparently direct cytotoxic effect on proliferating cells or through the release of soluble factors. Of particular note in this connection is the finding that defined LGL subsets can release lymphokines such as burst-promoting activity (BPA), IL 1, IL 2, or colony-stimulating factor (15, 22, 23).

In the present study, we investigated the capacity of human peripheral blood non-T cells of releasing BCGF. We found that a non-T cell with LGL characteristics which expresses the OKM1 and Leu-7 markers, but displays very little or no NK activity, can release BCGF.

### MATERIALS AND METHODS

**Cell fractionation procedures.** Leukocyte-rich buffy coats were obtained from the plateletpheresis of 300 to 500 ml of blood and mononuclear cells (MNC) separated on Ficoll-Hypaque (F-H) density gradients. Monocytes were removed by adherence on plastic petri dishes (24). T cells were separated from adherent cell-depleted MNC by rosetting with neuraminidase-treated sheep erythrocytes (E), followed by fractionation on two subsequent F-H density gradients (24). Sheep E were removed from rosetting cells (T cells) by hypotonic lysis (24). Nonrosetting cells (non-T cells) were re-rosetted and were depleted of the remaining T cells through another F-H gradient (24).

Cells that expressed surface HLA-DR antigens or Leu-7 determinants were detected by rosetting with ox E coated with the PTF 29/12 anti-HLA-DR monoclonal antibody or the Leu-7 reagent, respectively. PTF 29/12 was a generous gift of Dr. G. Damiani (25). The clone producing Leu-7 (HNK-1) antibody, originally raised by Abo and Balch (26, 27), was obtained from the American Type Culture Collection (Rockville, MD). These murine monoclonal antibodies were purified from ascitic fluids on a Sephadex G-200 column, were dialyzed against saline, and were coupled to ox E, according to the technique of Ling et al. (28). Cells were rosetted at 4°C and rosettes were separated on F-H density gradients.

In all of the above experiments, the rosette-enriched fractions contained a minimum of 94% rosetting cells and the rosette-depleted fractions never had more than 4% rosettes. Non-T cells never contained more than 3% T cells, as assessed by staining with OKT3 monoclonal (see below). T cells never contained more than 2% cells with surface immunoglobulin (sig) or HLA-DR antigens.

LGL were isolated from adherent cell-depleted MNC on a discontinuous Percoll density gradient, as reported previously (12). In brief, seven different Percoll concentrations (in 2-ml aliquots), ranging

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<sup>3</sup> Abbreviations used in this paper: BCGF, B cell growth factor; BPA, burst-promoting activity; F-H, Ficoll-Hypaque; LU, lytic units; LGL, large granular lymphocytes; Ly-CM, lymphocyte-conditioned medium; MNC, mononuclear cells; SAC, *Staphylococcus aureus* I Cowan Strain.

from 40 to 55% and each differing from the next by 2.5%, were layered in 15-ml conical test tubes, starting from the solution with the highest density. Cells ( $5 \times 10^7$ ) were layered on the top of the gradient and were centrifuged at 550  $\times$  G for 30 min. Fractions 2 and 3, which contained the highest proportion of LGL as assessed by morphology, cytochemistry, and surface marker analysis (see below), were pooled. To remove any remaining T cells, LGL were subsequently depleted of E rosettes as described above.

**Cell phenotype analysis.** Cells with E receptors were detected by a rosette technique as detailed previously (24). Cells with sIg were detected by staining with a rabbit F(ab')<sub>2</sub> anti-human Ig (polyvalent) serum conjugated with fluorescein isothiocyanate (FITC) (29). Mouse monoclonal antibodies to human MNC were obtained from different sources. Anti-T cell reagents (OKT3, OKT4, and OKT8) were from Ortho Pharmaceutical Corp. (Raritan, NJ). Anti-HLA-DR antibodies (HLA-DR or PTF 29/12) were from Becton Dickinson, Sunnyvale, CA, and from Dr. G. Damiani, respectively. An anti-macrophage/monocyte monoclonal reagent which also stains NK cells (OKM1) was purchased from Ortho Pharmaceutical Corp. An anti-B cell monoclonal antibody (B1) was obtained from Coulter Electronics (Hiialeah, FL). Two LGL-specific monoclonal antibodies were used, namely Leu-7 (HNK1), described above, and Leu-11b, which was purchased from Becton Dickinson. All of the above reagents were used in indirect immunofluorescence with an FITC-conjugated rabbit F(ab')<sub>2</sub> anti-mouse Ig (polyvalent). Preparations were observed under a Leitz Orthoplan fluorescence microscope. The percent of positive cells was calculated on counts of a minimum of 200 cells per preparation.

LGL were identified by staining with Giemsa or for the cytochemical localization of acid  $\alpha$ -naphthyl esterase or acid phosphatase as reported previously (11). In the preparations stained for these enzyme activities, LGL display a paranuclear staining pattern, T cells show a dot-like staining, and monocytes show a diffuse staining; B cells are unstained.

**Cytotoxicity assays.** Cell cytotoxicity with monoclonal antibodies and complement was carried out as detailed previously (15). In brief, cells were suspended at a concentration of  $1 \times 10^7$ /ml in RPMI 1640 medium (GIBCO-Biocult, Glasgow, Scotland) which contained OKT3 or OKT8 (both at a final dilution of 1/100), OKM1 (at a final dilution of 1/20), or Leu-11 (at a final dilution of 1/50) monoclonal antibodies or no antibody (control). After a 1-hr incubation on ice, nontoxic rabbit complement (Pel-Freez, Rogers, AR) was added at a final dilution of 1/2 in RPMI 1640. The cells were incubated at 37°C for 1 hr, were washed twice, were resuspended in fresh complement, and were incubated again at room temperature for 30 min. Dead cells were removed by centrifugation of the cell suspension on F-H gradients. When this procedure was not feasible, owing to the small cell numbers obtained, dead cells were detected by trypan blue staining and were excluded from the counts carried out for the determination of cell concentration. To assess the efficiency of complement-mediated cytotoxicity, the proportion of dead cells in the suspensions treated with a given monoclonal antibody was determined by staining with trypan blue or aethidium bromide, and was compared to that of the cells stained by the same antibody in immunofluorescence.

In the preparations used, complement-mediated lysis killed at least 80% of the cells that stained in immunofluorescence.

NK activity was measured in a conventional 4-hr assay against <sup>51</sup>Cr-labeled K562 cells (30). In brief,  $4 \times 10^6$  K562 cells were incubated with 100  $\mu$ Ci <sup>51</sup>Cr (Na<sub>2</sub> <sup>51</sup>CrO<sub>4</sub>; Amersham, Arlington Heights, IL) in 1 ml of Hanks' balanced salt solution (HBSS) for 60 min at 37°C, were washed four times, and were resuspended in HBSS-FCS. Different effector to target (E:T) ratios were selected, ranging from 40:1 up to 0.6:1. E:T mixtures were incubated for 4 hr, were centrifuged, and the supernatants were recovered and were counted. Maximal release was determined by lysis of labeled K562 cells with 0.1 N HCl. The percentage cytotoxicity was calculated as follows:

$$\% \text{ Cx} = \frac{\text{Exp} - \text{Sp}}{\text{Max} - \text{Sp}} \times 100$$

where Exp is radioactivity release induced by effector cells, Sp is radioactivity spontaneously released by <sup>51</sup>Cr-labeled K562 cells, and Max is radioactivity released by HCl-lysed K562 cells.

Alternatively, results were expressed as lytic units (LU)/10<sup>7</sup> cells; 1 LU is defined as the number of the effector cells required to release 10% specific radioactivity. This calculation gives a good comparison of the cytotoxicity activity of effector populations tested in the same experiments against the same targets, providing that the slopes of the dose-response curves are similar.

**Preparation of culture supernatants.** Different cell fractions were cultured at the concentration of  $1 \times 10^6$  cells/ml in RPMI 1640 containing 10% FCS in 24-well plates for 48 hr. Phytohemagglutinin

(PHA-P; Difco, Detroit, MI) was included in culture media at the final concentration of 1  $\mu$ l/ml. All supernatants were collected subsequently and were stored at -20°C.

**IL 2-enriched supernatants.** Lymphocyte-conditioned medium (Ly-CM) enriched with IL 2 was obtained by stimulating normal peripheral blood MNC with 1  $\mu$ l/ml PHA-P. After 40 hr, supernatants were recovered, were aliquoted, and were stored at -20°C until use.

**Lymphokine assays: BCGF assay.** Surgically removed tonsils were minced with a fine needle and MNC were isolated on F-H density gradients (29). T cells were removed by E rosetting as described above. Non-T cells were re-rosetted with E and were depleted of the remaining T cells on another F-H gradient. Non-T cells were fractionated on a Percoll density gradient as follows (31). Cells ( $5 \times 10^7$ ) were resuspended in 2 ml of 100% Percoll solution in a 15-ml conical test tube. Four additional Percoll concentrations (diluted in RPMI 1640 supplemented with 10% FCS), 60, 50, 40, and 30%, were layered over the 100% fraction starting from the bottom of the tube. Cells were spun at 3000 rpm in a tabletop centrifuge. Four cell fractions were obtained: the first contained low density cells, predominantly constituted by large B cells and monocytes, whereas the fourth band was highly enriched for small resting B cells. The second and the third cell fractions comprised cells of intermediate characteristics. High density cells from fraction 4 were collected, were washed twice, and were stimulated at the concentration of  $1 \times 10^6$  cells/ml in culture medium for 3 days with killed *Staphylococcus aureus* of the I Cowan strain (SAC) (kindly donated by Dr. S. Romagnani). SAC-activated B cells were washed subsequently and were cultured for an additional 3 days in 96-well, flat-bottomed plates ( $5 \times 10^4$  cells/well) in the presence or absence of the different supernatants to be tested. At the end of this time, cells were pulsed with 1  $\mu$ Ci of [<sup>3</sup>H]thymidine for 8 hr, were harvested, and were counted. In some experiments, BCGF assays were performed in the presence of anti-Tac monoclonal antibody (32) (kindly provided by Dr. T. Waldmann) that identifies IL 2 receptor. This reagent was included in the culture medium at time 0, using a final dilution of 1/1000.

**IL 2 assay.** IL 2 activity was measured by determining the capacity of the following cells to be stimulated: 1) a PHA-induced normal T cell line that had been expanded and kept in culture for 2 mo by adding exogenous IL 2; this line was unresponsive to PHA in the absence of any added irradiated accessory cell; and 2) the murine cell line CTLL-2 (33).

Human T blasts or CTLL cells were washed twice, were resuspended in RPMI 1640 containing 10% FCS, and were cultured for 24 hr in 96-well, flat-bottomed plates at the concentration of  $2 \times 10^4$ /well for human T cells or  $1 \times 10^4$ /well for CTLL, in the presence or absence of various dilutions of the supernatants to be tested. At the end of this time, cells were pulsed and were processed as described above. Results obtained in the two different experimental systems were completely overlapping.

**IL 1 assay.** IL 1 activity was assayed by using the conventional thymocyte assay (34). In brief, thymocytes, recovered from surgically removed thymuses of C3H/HeJ mice, were cultured for 72 hr in 96-well, flat-bottomed plates at the concentration of  $1.5 \times 10^6$ /well, in the presence of PHA-P (1  $\mu$ l/ml) alone or of PHA plus serial dilutions of the supernatants to be tested. Subsequently, the cells were processed as described above. One unit of IL 1 was defined as the amount of supernatants capable of doubling the [<sup>3</sup>H]thymidine incorporation by  $1.5 \times 10^6$  PHA-stimulated thymocytes.

**Absorption experiments.** Absorption experiments were performed as follows. Selected supernatants were subdivided into two aliquots. The first was used as a control (unabsorbed supernatant), whereas 2 ml of the second one were incubated for 4 hr at 4°C with  $4 \times 10^7$  B blasts, generated by a 3-day stimulation with SAC, or equal numbers of T blasts, obtained by PHA stimulation of peripheral blood MNC for 48 hr. At the end of the incubation period, absorbed supernatants were recovered by centrifugation and were tested in parallel with the control supernatants.

## RESULTS

**BCGF production by non-T cells.** Unfractionated MNC, T cells, and non-T cells were pulsed with PHA for 48 hr. T cells (that contained 1 to 2% contaminant monocytes) proliferated vigorously, as judged by light microscopy, whereas occasional blasts were seen in the non-T cells. When stimulated with PHA, all cell fractions produced BCGF (Fig. 1), whereas no BCGF was released in the absence of PHA. Furthermore, PHA alone at the concentration used failed to sustain the proliferation of SAC-stimulated cells (data not shown). In three experiments,

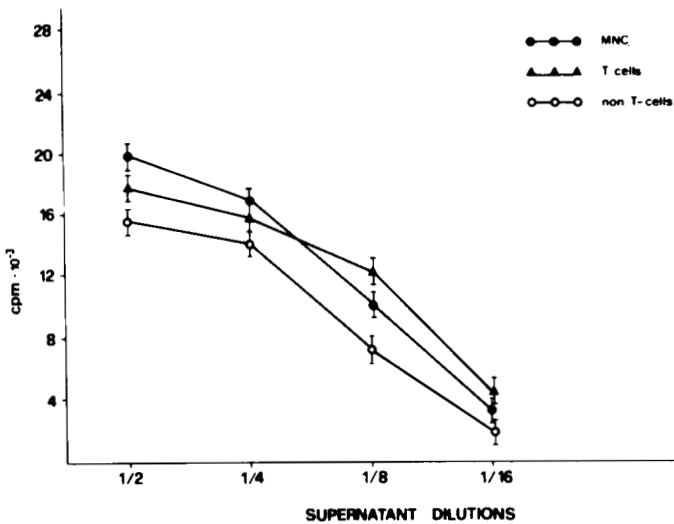


Figure 1. BCGF production by unfractionated MNC and non-T cells. The results represent the mean  $\pm$  SE of three experiments.

TABLE I  
Surface phenotype of SAC-stimulated tonsil non-T cells

Surface Marker	% Positive Cells <sup>a</sup>
HLA-DR	97.5 $\pm$ 1.1
sIg	91.2 $\pm$ 2.7
B1	79.5 $\pm$ 5.7
Tac	13.0 $\pm$ 3.1
OKT3	3.3 $\pm$ 1.1
OKM1	4.4 $\pm$ 0.3
Leu-7	1.4 $\pm$ 0.9

<sup>a</sup> Mean  $\pm$  SE from three different experiments. The SE is calculated as the square root of the pooled variance divided by the overall number of samples. The homogeneity of variance between experiments was demonstrated and no significant differences between the means were documented.

non-T cells were treated with OKT3 antibody and complement to remove any remaining T cells. After this treatment, the BCGF titration curve was unchanged (data not shown).

Tonsil B cells were fractionated on Percoll gradients to remove large (probably pre-activated in vivo) B cells that proliferated spontaneously in vitro (35). Surface marker analysis demonstrated that the vast majority of SAC-stimulated cells were B cells, with a negligible contamination of T cells (Table I). This control was necessary to ensure that the major target of the factors contained in the supernatants tested were indeed B cells.

**Characterization of the non-T cells that produce BCGF.** Non-T cells were separated into HLA-DR<sup>-</sup> and HLA-DR<sup>+</sup> cells, and the two fractions were tested for BCGF production. BCGF activity was detected in the HLA-DR<sup>-</sup> cell supernatants only, in amounts that were somewhat higher than those found in the unfractionated non-T cells (Fig. 2). Dilutions of 1/4 or 1/8 of the HLA-DR<sup>-</sup> cell supernatants were more efficient in sustaining B cell proliferation than the 1/2 dilution, a finding that may suggest the presence of small quantities of a lymphokine with inhibitory properties. Table II shows the phenotype of HLA-DR<sup>-</sup> cells together with that of the other cell fractions used in the present experiments. The large majority of HLA-DR<sup>-</sup> cells were OKM1<sup>+</sup>, Leu-7<sup>+</sup>, and Leu-11<sup>+</sup>. Cells identifiable as B cells or monocytes were less than 2%. Likewise, as reported previously (15), there were no cells with T cell markers, except for a subset of cells expressing the OKT8 marker.

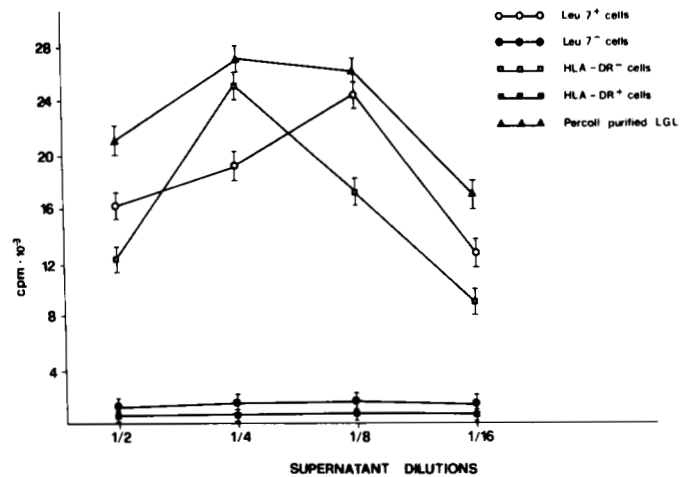


Figure 2. BCGF production by different non-T cell subsets. The results represent the mean  $\pm$  SE of five experiments.

The finding of a high proportion of Leu-7<sup>+</sup> cells in the HLA-DR<sup>-</sup> fraction prompted experiments in which Leu-7<sup>+</sup> cells were isolated from non-T cells and were tested for BCGF production. Most of these cells had morphologic features and surface markers of LGL (Table II), and released BCGF in a quantity comparable to that of HLA-DR<sup>-</sup> cells (Fig. 2). In contrast, Leu-7<sup>-</sup> cells released very little or no BCGF (Fig. 2). Comparative analysis of the properties of HLA-DR<sup>-</sup> non-T cells and Leu-7<sup>+</sup> cells showed that they comprised LGL with similar phenotype, with the exception that Leu-7<sup>+</sup> cells also contained a number of HLA-DR<sup>+</sup> cells. Furthermore, HLA-DR<sup>-</sup> cells had a much higher NK activity than Leu-7<sup>+</sup> cells (Table II), a finding which is also corroborated by the observation that non-T cells depleted of Leu-7<sup>+</sup> cells (Leu-7<sup>-</sup> cells) retained a high cytotoxic activity (Table II).

In another series of experiments, LGL were prepared on Percoll density gradients. These fractions were enriched in cells with Leu-7 and Leu-11 markers, and displayed a potent NK activity (Table II). When tested for BCGF, they were found to produce BCGF in amounts comparable to those of HLA-DR<sup>-</sup> and Leu-7<sup>+</sup> cells (Fig. 2).

The surface phenotype of the BCGF-producing cell was studied further in the following experiments. Leu-7<sup>+</sup> cells were treated with Leu-11, OKT8, and OKM1 monoclonal antibodies and complement or with complement alone (control). The cells were tested for NK activity and BCGF production. As apparent from Figure 3, treatment with OKM1 monoclonal antibody largely decreased BCGF production and NK function. Treatment with Leu-11 monoclonal almost abolished the cytotoxic activity, but did not influence BCGF production. Treatment with OKT8 had no effect on both functions. The same data were obtained by treating Percoll-purified LGL with the same monoclonals and complement (data not shown). Together, the above data show that BCGF was released by an OKM1<sup>+</sup>, Leu-7<sup>+</sup>, Leu-11<sup>-</sup> LGL with very little or no NK activity. Furthermore, the data confirm that most NK activity is in the Leu-11<sup>+</sup> cells, and that the cytotoxicity found in the Leu-7<sup>+</sup> cell fractions is mainly attributable to the cells also expressing the Leu-11 determinant (36, 37).

**HLA-DR<sup>-</sup> non-T cells do not produce IL 1 or IL 2.** All cell fractions that released BCGF were tested for their capacity of producing IL 1, because IL 1 may be involved in the control of B cell proliferation (3, 38). The simulta-

TABLE II  
Characteristics of the different non-T cell subpopulations used

Surface Marker	HLA-DR <sup>-</sup> Cells <sup>a</sup>	Leu-7 <sup>+</sup> Cells <sup>a</sup>	Percoll-purified LGL <sup>a,b</sup>	HLA-DR <sup>+</sup> Cells <sup>a</sup>	Leu-7 <sup>-</sup> Cells <sup>a</sup>
OKM1	70 ± 7	83 ± 4	70 ± 5	46 ± 6	53 ± 4
Leu-7	73 ± 5	92 ± 4 <sup>c</sup>	65 ± 8	14 ± 3	<2
Leu-11	78 ± 7	51 ± 6	73 ± 5	<2	39 ± 4
OKT3	<2	<2	<2	<2	<2
OKT4	<2	<2	<2	<2	<2
OKT8	14 ± 2	7 ± 2	11 ± 3	10 ± 4	5 ± 3
E	<2	<2	<2	<2	<2
slg	<2	<2	<2	49 ± 4	47 ± 5
HLA-DR	<2	28 ± 3	<2	93 ± 6 <sup>c</sup>	70 ± 8
Cytochemical markers <sup>a,d</sup>					
Acid hydrolases:					
1) paranuclear (LGL)	91 ± 8	93 ± 5	85 ± 10	10 ± 3	ND <sup>e</sup>
2) dot-like (T cells)	<2	<2	<2	<2	ND
3) diffuse (monocytes)	<2	<2	5 ± 2	29 ± 3	ND
4) negative (B cells)	<2	<2	4 ± 1	60 ± 8	ND
NK activity <sup>f</sup>	908 ± 150	310 ± 50	954 ± 103	80 ± 10	1240 ± 160

<sup>a</sup> Mean percentage of positive cells ± SE from three different experiments.

<sup>b</sup> Fractions 2 and 3 from Percoll gradients were pooled and were depleted of cells forming E rosettes.

<sup>c</sup> Detected by rosetting during the separation procedures.

<sup>d</sup> See *Materials and Methods* for details on criteria of cell identification according to their staining pattern.

<sup>e</sup> ND, Not done.

<sup>f</sup> The results are expressed as LU calculated at 10% specific lysis per 10<sup>7</sup> effector cells (mean + SE of three different experiments).

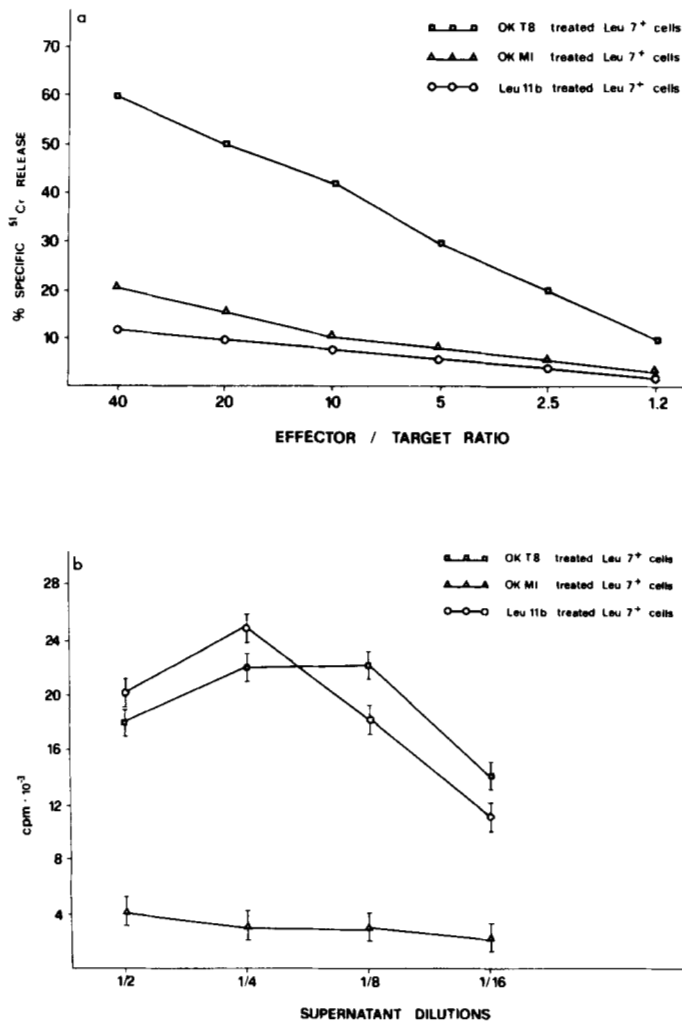


Figure 3. Leu-7<sup>+</sup> non-T cells were treated with OKT8, OKM1, and Leu-11b monoclonal antibodies, and were tested for NK activity (a). The same cell suspensions were also stimulated with PHA for 48 hr, and the supernatants were tested for BCGF activity (b). a. The results of one representative experiment of the four performed. The results reported in b represent the mean ± SE of the same four experiments. The results obtained after treatment with complement alone were superimposable to those obtained with OKT8 plus complement (and hence not reported).

neous presence of the two lymphokines could raise the problem of how much of the B cell proliferation observed was due to IL 1. HLA-DR<sup>-</sup> cells consistently failed to produce IL 1, yet their supernatants had a vigorous BCGF activity (Table III and Fig. 2). In contrast, HLA-DR<sup>+</sup> and Leu-7<sup>-</sup> cells, which produced consistently more than 90 U/ml of IL 1, never released BCGF (Fig. 2). Leu-7<sup>+</sup> cells released variable, but sometimes abundant, amounts of IL 1 (Table III). Recently, it has been reported that a subset of HLA-DR<sup>+</sup> LGL release IL 1 (23). The Leu-7<sup>+</sup> cells tested here contained variable proportions of HLA-DR<sup>+</sup> cells (Table II); therefore, the data on IL 1 production are not unexpected.

Non-T cell fractions were also tested for IL 2 production. Again, HLA-DR<sup>-</sup> cells consistently failed to release IL 2, whereas small amounts of IL 2 were produced by Leu-7<sup>+</sup> cells (Table III). However, both fractions had the same capacity of releasing BCGF. A proportion of SAC-activated B cells expressed IL 2 receptors, detectable by staining with anti-Tac monoclonal antibody (Table I and Reference 39). Because activated B cells can proliferate in response to IL 2 (39), this finding warranted further studies on the role possibly played by IL 2 in our system. SAC-activated B cells were cultured with HLA-DR<sup>-</sup> or Leu-7<sup>+</sup> cell supernatants in the presence or absence of anti-Tac monoclonal. In these cultures, the proliferative response to Leu-7<sup>+</sup> cell supernatants decreased only marginally, and that to HLA-DR<sup>-</sup> cell supernatants did not change at all (Fig. 4). These findings further confirm that B cell proliferation was not due to IL 2. Treatment with anti-TAC monoclonal antibody inhibited the proliferation of PHA-induced T blasts to IL 2-rich Ly-CM (data not shown).

The supernatants of HLA-DR<sup>-</sup> or Leu-7<sup>+</sup> cells were also absorbed with SAC-activated B cells or PHA-activated T cells before being tested for BCGF activity. BCGF was absorbed by B but not T blasts (Fig. 4).

#### DISCUSSION

The present study demonstrates BCGF production by a non-T cell from human peripheral blood. BCGF was de-

TABLE III  
IL 1 and IL 2 production by different non-T cell fractions

Supernatants	IL 1 Activity U/ml			IL 2 Activity [ <sup>3</sup> H]thymidine Incorporation cpm					
	Expt. 1	Expt. 2	Expt. 3	Expt. 1		Expt. 2		Expt. 3	
Leu-7 <sup>+</sup> cells <sup>a</sup> and supernatants <sup>b</sup>	18	33	28	4,156 ± 616	3,768 ± 308	3,261 ± 284			
HLA-DR <sup>-</sup> cells <sup>a</sup> and supernatants <sup>b</sup>	<2	<2	<2	2,084 ± 167	1,597 ± 197	1,638 ± 196			
Ly-CM <sup>c</sup>	ND <sup>d</sup>	ND	ND	38,824 ± 1,427	42,382 ± 2,127	41,225 ± 1,981			
Macrophage supernatant <sup>e</sup>	67	94	75	ND	ND	ND			
Medium	—	—	—	1,824 ± 98	1,683 ± 286	1,427 ± 124			

<sup>a</sup> Fractionated cells ( $1 \times 10^6$ ) were cultured for 48 hr with 1  $\mu$ g/ml PHA-P.

<sup>b</sup> Supernatants were tested at different dilutions, and the reported values are relative to those yielding the highest IL 2 activity.

<sup>c</sup> IL 2-enriched supernatants were obtained by PHA-P stimulation of MNC, as described in *Materials and Methods*, and were used at 1/32 dilution.

<sup>d</sup> ND, Not determined.

<sup>e</sup> Peripheral blood adherent cells, detached from plastic, were cultured at  $1 \times 10^6$  cells/ml with 1  $\mu$ g/ml PHA-P for 48 hr.

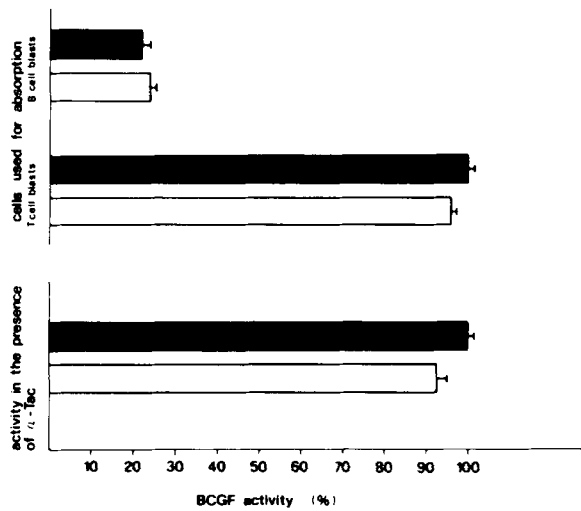


Figure 4. Upper section: percentage reduction of BCGF activity present in HLA-DR<sup>-</sup> (■) and Leu-7<sup>+</sup> (□) cell supernatants after absorption on SAC-generated B cell blasts and PHA-activated T cell blasts. The results represent the mean  $\pm$  SE of three experiments. Lower section: percentage reduction of BCGF activity present in HLA-DR<sup>-</sup> (■) and Leu-7<sup>+</sup> (□) cell supernatants when tested on SAC-activated B cell blasts in the presence of anti-Tac monoclonal antibody. The results represent the mean  $\pm$  SE of three experiments.

tected in supernatants of suspensions depleted of T cells by E rosetting and monoclonal antibody treatment. This finding, together with the observation that the titration curve of BCGF production was similar for T and non-T cells, excludes the possibility that T cells contaminating the non-T suspensions could be responsible for the results obtained.

The BCGF-producing cell was likely to be an LGL because it was found in LGL-enriched cell fractions, copurified with LGL on Percoll density gradients and, like a large number of LGL, expressed the OKM1 and Leu-7 markers. This BCGF-producing cell was, however, incapable of NK activity. This conclusion is based on the results of the experiments in which treatment of Leu-7<sup>+</sup> cells with Leu-11 monoclonal and complement failed to inhibit BCGF production and yet inhibited cell cytotoxicity. Furthermore, the observation that Leu-7<sup>-</sup> cells (which contained approximately 40% Leu-11<sup>+</sup> cells) had a high NK activity but did not produce BCGF reinforces the concept that NK activity and BCGF production may be functions of different cells. LGL are heterogeneous and include cytotoxic cells together with other cells with dif-

ferent functions. A number of specialized LGL subsets can be identified through the expression of different surface markers. For example, LGL that produce IL 1 are HLA-DR<sup>+</sup>, M1<sup>+</sup>, B.73.1<sup>+</sup> (23), those that produce IL 2 are HLA-DR<sup>+</sup>, T11<sup>+</sup> (22), and those that release BPA are HLA-DR<sup>-</sup>, M1<sup>+</sup>, Leu-11<sup>+</sup>, Leu-7<sup>+</sup> (15). The BCGF-producing cell with a Leu-7<sup>+</sup>, M1<sup>+</sup>, HLA-DR<sup>-</sup>, E<sup>-</sup>, Leu-11<sup>-</sup>, T3<sup>-</sup> phenotype seems to belong to an LGL subset different from all of the above. The finding of a marked heterogeneity of phenotypes and functions makes it increasingly difficult to solve the problem of whether or not LGL are cells from a single lineage which specialize in different functions during their maturation. Alternatively, that of LGL could be an operational definition to indicate granulated cells, possibly from different lineages, which share a number of properties.

A particular issue to be discussed here is represented by the role possibly played by IL 1 or IL 2 in our experiments. Previously, it was proposed that IL 1 can be involved in sustaining the growth of preactivated B cells (3, 38). A number of observations exclude that the BCGF activity measured in our experimental system could be attributable to IL 1. First, the supernatants of HLA-DR<sup>-</sup> cells, which were rich in BCGF activity, contained very little or no IL 1. Second, IL 1-rich supernatants, such as those of HLA-DR<sup>+</sup> cells, failed to promote B cell growth. Third, as mentioned above, the surface phenotype of the BCGF-producing cell is different from that of IL 1-producing LGL and that of monocytes. Finally, it is perhaps worth noting that, in most experimental systems, IL 1 cooperates with, rather than substituting for, BCGF (3, 38). The possibility that IL 2 was responsible for the growth of SAC-activated B cells (that express IL 2 receptors) was ruled out by the following observations. First, Leu-7<sup>+</sup> only (and not HLA-DR<sup>-</sup> cells) released small amounts of IL 2, yet the supernatants of the two cell fractions had comparable BCGF activity. Incidentally, the finding that Leu-7<sup>+</sup> but not HLA-DR<sup>-</sup> cells released IL 2 was not unexpected in consideration of the phenotype of IL 2-producing LGL (22). Second, anti-Tac monoclonal antibody failed to inhibit B cell proliferation induced by HLA-DR<sup>-</sup> cell supernatants and inhibited only marginally that induced by the supernatants of Leu-7<sup>+</sup> cells. Third, the BCGF activity of both HLA-DR<sup>-</sup> and Leu-7<sup>+</sup> cell supernatants was absorbed by B but not T blasts.

At present, it cannot be decided whether BCGF released by LGL is identical to that produced by T cells or else

represents a different interleukin with similar functions. Unfortunately, the number of cells obtained in each experiment was too small to allow the preparation of supernatants in amounts sufficient for a chemical analysis of the factor. Studies with LGL lines or clones expanded in continuous cultures may be of help to solve this problem, although of the various lines we have so far obtained, very few have an OKM1<sup>+</sup>, Leu-7<sup>+</sup> surface phenotype, and this is not always a stable one.

Recently, a number of regulatory functions on the proliferation of other cell types have been attributed to various subsets of human LGL. These cells can promote (15) or inhibit (14, 19) hemopoiesis, depending on the different experimental conditions used; they can also regulate T lymphocyte proliferation (16) and inhibit Ig production in vitro (40, 41). The demonstration of the capacity of releasing BCGF adds further weight to the regulatory role of LGL.

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