Journal of Clinical Immunology, Vol. 7, No. 5, 1987

Helper Activity by Human Large Granular Lymphocytes in *in Vitro* Immunoglobulin Synthesis

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Accepted: March 24, 1987

In the present study we have examined the effect of human large granular lymphocytes (LGL) from healthy donors on Ig synthesis by autologous B lymphocytes. The results showed that this cell population has a consistent helper activity in pokeweed mitogen-activated cultures even when added at very low numbers. LGL can mediate their effect by secreting soluble helper factors capable of modulating B-cell responses as evidenced by the enhancement of IgG and IgM production by supernatants obtained from LGL cultures. Preincubation with interferon gamma further potentiated the helper activity by LGL.

KEY WORDS: Large granular lymphocytes; B lymphocytes; immunoglobulin synthesis; helper lymphocyte function.

INTRODUCTION

Human large granular lymphocytes (LGL)[3] represent a phenotypically heterogeneous cell population containing most of the natural killer (NK) activity in peripheral blood (1). Recent studies suggest that LGL are involved in the regulation of the immune response either directly or through the secretion of diverse soluble mediators (2). Also, they have accessory-cell function for mitogen- and antigen-induced T-cell activation (3). More recently, evidence has been presented indicating a regulatory role of LGL in immunoglobulin (Ig) production by B cells (4–8). These studies suggest a suppressor activity by this subpopulation in mitogen- and antigen-driven B-cell responses. On the other hand, LGL are known to secrete diverse soluble factors such as interleukin 2 (IL2) (2), interleukin 1 (IL1) (9), interferon (IFN) (2, 10), and B-cell growth factor (BCGF) (11, 12) with known facilitatory rather than inhibitory effects on Ig synthesis. These cells may potentially be involved in regulating humoral responses *in vivo*, as suggested by their vicinity to B lymphocytes in lymphoid tissues (13). Therefore we sought to examine the effect of LGL on spontaneous and mitogen-induced Ig production by blood B lymphocytes. Our results indicate that LGL from healthy subjects can have a helper effect on mitogen-induced Ig synthesis by autologous B cells in a T cell-independent manner.

MATERIALS AND METHODS

Separation of Peripheral Blood Mononuclear Cells (PBMC)

PBMC were obtained from healthy donors aged 25 to 48 years. Cells were separated from heparinized venous blood samples by Ficoll–Hypaque gradients and depleted from adherent cells by incubation in glass petri dishes with 20% fetal calf serum (FCS) at 37°C for 45 min. E-rosetting cells were obtained after treatment of glass-nonadherent PBMC with 2-aminoethylisothiouronium bromide hydrobromide (AET)-treated sheep erythrocytes followed by centrifugation on Ficoll–Hypaque for 30 min at 550*g*. E-rosetting cells were recovered

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[3]Abbreviations used: LGL, large granular lymphocytes; NK, natural killer; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; Ig, immunoglobulin; IFN, interferon; FCS, fetal calf serum; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; AET, aminoethylisothiouronium bromide hydrobromide; PBS, phosphate-buffered saline; RIA, radioimmunoassay; IL, interleukin.

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after red blood-cell lysis with Tris–ammonium chloride buffer. This population contained 95 ± 2% OKT3-positive cells as tested by indirect immunofluorescence with OKT3 monoclonal antibody (Ortho Diagnostics Systems, Raritan, NJ) and 1.4 ± 0.3% monocyte contamination as assessed by peroxidase stain. Non-T-cell fractions (hereinafter called B cells) were tested for proportions of surface Ig (sIg)-bearing cells by direct immunofluorescence with B-cell sIg marker fluorescein isothiocyanate (FITC)-conjugated goat anti-human immunoglobulin (IgG, IgM, IgA) $F(ab')_2$ fraction (Ortho Diagnostics Systems, Raritan, NJ) (54 ± 2.7%), by E rosetting with AET-treated SRBC to check for contaminating T cells (less than 4%), and by peroxidase stain to determine the degree of monocyte contamination (9.8 ± 1.1%). Also, B-cell fractions contained 11.1 ± 0.95 and 7.8 ± 0.65% B73.1+ and 3G8+ cells, respectively, as tested by indirect immunofluorescence. As expected, these preparations showed some degree of NK activity as evaluated by the chromium-51 release assay (PBMC, 45 ± 3%; non-T cells, 15 ± 4%; figures in percentage of release as calculated in Ref. 1).

In some experiments B cells were further purified by a second cycle of E rosetting followed by treatment with OKT3 monoclonal antibody plus complement as detailed below. By this method less than 1% of T cells were present in the B-cell subpopulation as tested by indirect immunofluorescence with OKT3 monoclonal antibody. Cells were washed five times in RPMI 1640 and resuspended to 1×10^6 cells/ml in complete medium (RPMI 1640 supplemented with 2 mmol/ml L-glutamine, 200 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS).

Percoll Fractionation of PBMC and LGL Purification

Plastic- and nylon wool-nonadherent cells were fractionated by centrifugation on a discontinuous density gradient of Percoll (Pharmacia Chemicals, Uppsala, Sweden) as previously described (1, 14). Percoll and complete medium osmolality were corrected to 285 mOsm/kg H_2O with 10× concentrated phosphate-buffered saline (PBS) and distilled water, respectively. Seven different concentrations of Percoll in complete medium were prepared, with each varying from the next by 2.5% concentration steps. Percoll concentrations ranged from 40 to 57%. Gradients were carefully layered in 15-ml Falcon 2095 conical test tubes (Falcon Plastics, Oxnard, CA). Cells (50 to 70×10^6) were placed on top of Percoll gradients and tubes were centrifuged at 550*g* for 30 min at room temperature. Cells were recovered with a Pasteur pipette and washed in RPMI 1640. LGL were present in fractions 2 to 4 (peak in fraction 3) and contained most of the NK-cell activity when tested by the chromium release assay with K562 target cells as described (1). Morphological analysis of cells present in each fraction was performed by Cytospin centrifugation of 2×10^6 cells onto microscope slides, followed by fixation with methanol and Giemsa staining. LGL were identified by their high cytoplasmic/nuclear ratio and weakly basophilic cytoplasm with azurophilic granules. To purify LGL further, low-density fractions (2 and 3) were pooled and depleted of high-affinity E-rosetting lymphocytes by incubation with sheep erythrocytes at 29°C for 1 hr and centrifugation on Ficoll–Hypaque gradients. By this method highly enriched preparations containing 90 ± 1% LGL were obtained. A slight monocyte contamination (3.2 ± 2.7%) as tested by peroxidase stain and 2.2 ± 0.7% surface Ig-positive cells were observed. Phenotypic characterization of LGL preparations was done by indirect immunofluorescence with monoclonal antibodies as detailed below. The predominant subpopulations were those reactive with B73.1 and 3G8 monoclonal antibodies, representing 64 ± 3 and 81 ± 3%, respectively ($N = 15$). A low proportion of LGL also showed reactivity with Leu 4 (9 ± 1%) and T101 (12 ± 3%) monoclonal antibodies. Only background levels of IgG (89 ± 14 ng/ml) and IgM (31 ± 1.7 ng/ml) were observed in supernatants from 7-day cultures of relatively high numbers of LGL (1×10^6/ml) stimulated with PWM.

Monoclonal Antibodies

OKT3, Leu 4, and T101, three pan-T monoclonal antibodies, were obtained, respectively, from Becton & Dickinson (Becton & Dickinson Monoclonal Antibody Center, Burlingame, CA), from Ortho Laboratory (Ortho Diagnostic Systems, Inc., Raritan, NJ), and as a kind gift from Dr. John Ortaldo (National Cancer Institute, Frederick, MD). 3G8 and B73.1, two monoclonal antibodies recognizing the Fc portion of NK cells within LGL (15, 16), were donated, respectively, by Dr. Ortaldo and Dr. Giorgio Trinchieri (The Wistar Institute, Philadelphia, PA).

Indirect Immunofluorescence with Monoclonal Antibodies

PBMC (2×10^6) or LGL (1×10^6) were pelleted, incubated with 10 μl monoclonal antibody for 30 min at 4°C, and washed twice in cold PBS plus 1% sodium azide (PBS/azide). An appropriate dilution of fluoresceinated goat anti-mouse IgG (Tago, Inc., Burlingame, CA) was added. After incubation for 40 min at 4°C cells were washed in cold PBS/azide and slides were read using a Zeiss fluorescence microscope with epiillumination. At least 200 cells were scored. Controls for background fluorescence were set up similarly but using nonreactive mouse monoclonal IgG of the corresponding class (Coulter Immunology, Division of Coulter Corp., Hialeah, FL).

Complement Lysis of Lymphocyte Subpopulations with Monoclonal Antibodies

Cells (1×10^6) were incubated with 10 μl of monoclonal antibody for 30 min at 4°C. After two washes in cold PBS/azide in a refrigerated centrifuge, low-toxic rabbit complement (Cedarlane Lab, Hornby, Ontario, Canada) was added and cells were incubated for 45 min at 37°C. Cells were washed thrice in PBS/azide and resuspended to 1×10^6/ml in complete medium. Less than 5% contaminating cells were observed in the depleted population as assessed by indirect immunofluorescence with the corresponding monoclonal antibody used for complement-mediated lysis.

Preparation of LGL Supernatants

LGL (1×10^6) were incubated in complete medium with or without pokeweed mitogen (PWM) (Gibco Lab, Grand Island Biological Corp., Grand Island, NY) at a final dilution of 1/200 for 2 hr at 37°C. Cells were extensively washed in RPMI/FCS, 5%, resuspended at 1×10^6/ml in fresh complete medium, and incubated for 48 hr at 37°C. Cell-free supernatants obtained after centrifugation of LGL cultures were passed through 0.45-μm Millipore filters and stored at −20°C before use.

Preincubation of LGL with IFN Gamma

In some experiments LGL were incubated for 18 hr at 37°C in complete medium alone or in the presence of increasing concentrations of highly purified human IFN gamma (Interferon Sciences, Inc., New Brunswick, NJ). After several washes LGL were resuspended in fresh medium before coculture with autologous PBMC.

Cell Cultures

Cells in complete medium were cultured in duplicate in 12 × 75-mm plastic capped tubes (Falcon 2054, Becton Dickinson, Sunnyvale, CA) at 37°C and 5% CO_2 in a humidified atmosphere. B cells (2.5×10^5) were cultured alone or in the presence of increasing numbers of autologous T cells or LGL. In some experiments B cells maximally depleted of T cells were cocultured with unfractionated LGL or LGL depleted from OKT3+ or Leu 4+ subpopulations. Cultures were set up with or without PWM (final dilution, 1/200). Net synthesis was calculated by subtracting spontaneously released from mitogen-driven Ig production as examined in unstimulated and PWM-activated cultures. To test the effect of LGL supernatants on Ig synthesis, 0.5×10^6 B cells were added with serial dilutions of supernatants obtained from unstimulated or PWM-activated LGL cultures as outlined above.

Enzyme-Linked Immunosorbent Assay (ELISA) for Measurement of Ig

The assay for measurement of IgG has been previously described in detail (17). Briefly, flexible microtiter plates were incubated with human IgG (20 μg/ml). After several washes and incubation for 1 hr in PBS/BSA, RIA grade 1%, samples and peroxidase-labeled goat anti-human IgG (Tago, Inc., Burlingame, CA) were added, followed by incubation for 2 hr. For IgM quantitation plates were incubated overnight with goat anti-human IgM (Tago, Inc., Burlingame, CA). After several washes and incubation in PBS/BSA, RIA-grade plates were added with samples, followed by incubation for 2 hr and the addition of peroxidase-labeled goat anti-human IgM. Standard curves were prepared with human IgG purified from human Cohn fraction II (Sigma Chemical Co., St. Louis, MO) by DEAE chromatography and normal human control reference serum (Tago, Inc., Burlingame, CA). The substrate was prepared with citric acid, 3.2 g%, pH 4 (Eastman Kodak Co., Rochester, NY), hydrogen peroxide (1/100) (E. Merck, Darmstad), 2,2-azino-di-[3-ethyl-benzthiazolin-sulfonate] (6) (Boehringer & Sohne, Manheim, West Germany), and wells were filled with 100 μl. The reaction was stopped

Table I. Human LGL Enhance PWM-Induced Ig Synthesis by Autologous Blood B Cells[a]

Expt No.	IgG (ng/ml), <B plus LGL>				IgM (ng/ml), <B plus LGL>			
	Nil	5×10^3	5×10^4	1×10^5	Nil	5×10^3	5×10^4	1×10^5
1	3103	4245	9316	773	2	8	89	145
2	831	1073	1776	2456	170	117	555	1031
3	109	515	345	—	52	481	606	418
4	430	604	1216	1769	299	784	725	886
5	35	154	505	398	633	1083	1745	163
6	35	503	2119	1717	99	572	314	262
Mean	757	1182	2546	1422	209	507	672	484
±SE	485	624	1383	370	94	164	233	156
		($P < 0.02$)[b]	($P < 0.02$)	NS		($P < 0.05$)	($P < 0.02$)	($P < 0.05$)

[a]B cells (2.5×10^5) were cultured alone or in the presence of autologous LGL. PWM was added at the beginning of the culture. Supernatants were tested on day 7 for Ig concentrations by ELISA. Figures represent net (spontaneous release minus mitogen-activated) IgG production. Spontaneous Ig release was measured in parallel unstimulated B-cell cultures.
[b]Wilcoxon signed-rank test.

after 30 min with citric acid buffer, pH 2.8. Plates were read in an automated Titertek Multiskan MC (Flow Laboratories, McLean, VA).

Statistical Analysis

The Wilcoxon signed-rank test was used for statistical analysis.

RESULTS

LGL Show Helper Activity on Mitogen-Induced Ig Synthesis by Autologous B Cells

B cells (2.5×10^5) were cultured alone or in the presence of increasing numbers of LGL. PWM was added at the beginning of the culture and supernatants were tested on day 7 for IgG and IgM concentrations by ELISA. LGL increased the net mitogen-induced IgG and IgM synthesis even when added in very low numbers. The degree of enhancement varied from 3- to more than 10-fold over baseline production (Table I). Maximal enhancement was observed when adding 5×10^4 to 1×10^5 LGL. Under the conditions of our assay these cell ratios lead to an optimal Ig production in B- plus T-cell cocultures (not shown). We also examined the effect of LGL in unstimulated cultures. B plus LGL cocultures were set up as described above but in the absence of PWM. LGL enhanced spontaneous IgG synthesis by B cells in only two of seven experiments (not shown).

The preceding set of experiments was set up to test helper activity by LGL on B cells, in the absence of T cells. In order to rule out potential suppressor activity by LGL we set up cultures under optimal help conditions, that is, in the presence of T cells. Thus, LGL were added to autologous B- plus T-cell cocultures and PWM-driven Ig synthesis was examined on day 7. As shown in Fig. 1, LGL did not abrogate PWM-driven Ig synthesis in five consecutive experiments.

LGL Enhance Ig Synthesis by a T Cell-Independent Mechanism

We next tested whether the enhancing effect by LGL was dependent on residual T cells present in

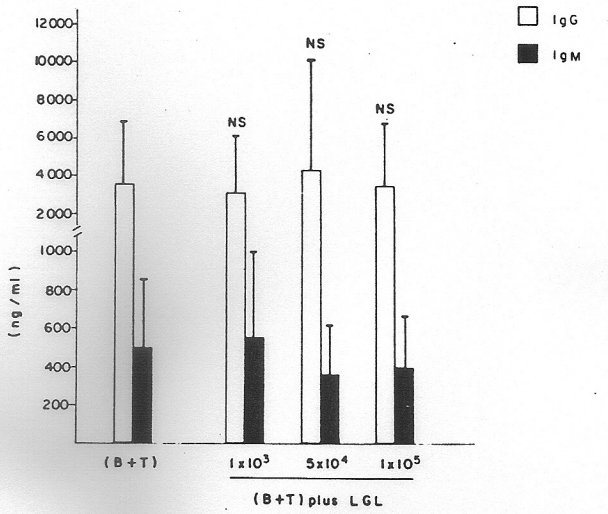

Fig. 1. B (2.5×10^5) plus T (2.5×10^5) cells were cocultured alone or in the presence of increasing numbers of autologous LGL. Cultures were activated with PWM, and supernatants tested for Ig concentrations on day 7 by ELISA (mean ± SE of five experiments).

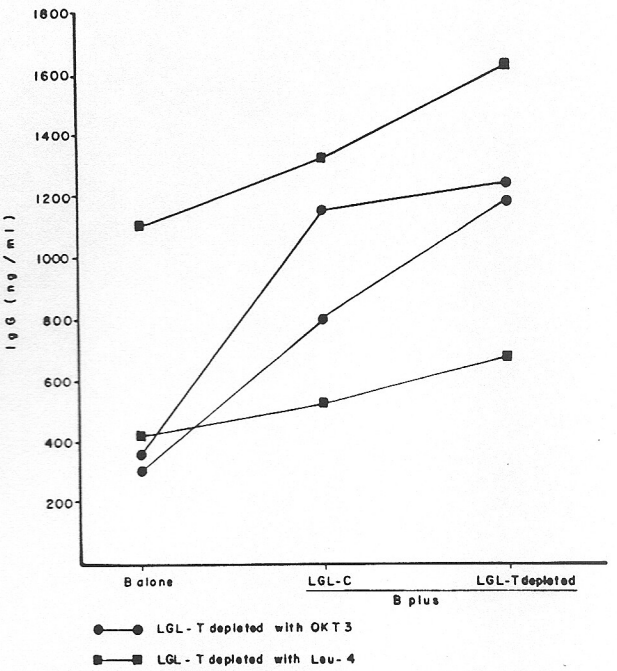

Fig. 2. B cells were depleted of T cells by a double E-rosetting cycle followed by complement-mediated lysis with OKT3 monoclonal antibody (0.77 ± 0.24% remaining OKT3+ cells). B cells (2.5×10^5) were cultured alone or in the presence of autologous LGL maximally depleted of T cells (LGL-T depleted) by complement-mediated lysis with either OKT3 ($N = 2$) or Leu 4 ($N = 2$) monoclonal antibodies. These LGL contained only 0.58 ± 0.21% contaminating OKT3+ cells. PWM-driven IgG production was tested on day 7 by ELISA and compared to that of B cells plus LGL pretreated with complement alone (LGL-C) as the control. A total of four separate experiments is shown.

the B-lymphocyte preparations. B cells were maximally depleted of T lymphocytes by a double cycle of E rosetting followed by complement-mediated lysis with OKT3 monoclonal antibody. These preparations contained 0.77 ± 0.13% T lymphocytes as tested by indirect immunofluorescence with OKT3 monoclonal antibody. These highly enriched B-cell preparations were unable to secrete Ig above baseline levels upon PWM activation (see below). As depicted in Fig. 2, LGL still enhanced Ig synthesis by B cells maximally depleted of T cells. In the same group of experiments we examined the possibility that subpopulations of LGL bearing T-cell markers or potentially contaminating T lymphocytes were responsible for the enhancement of Ig synthesis. Cocultures were performed with B cells plus LGL depleted of OKT3+ or Leu 4+ cells by complement-mediated lysis and compared to B cells plus LGL pretreated with complement alone. Figure 2 shows that LGL maximally depleted of OKT3+ or Leu 4+ cells retained and sometimes even displayed a stronger helper effect on autologous B cells.

Supernatants from LGL Cultures Can Directly Enhance Ig Synthesis by Autologous B Lymphocytes

To delineate further the mechanisms whereby LGL may enhance Ig synthesis, we examined whether these cells may regulate B-cell responses through secreted soluble helper factors. B cells (0.5×10^6) in complete medium were cultured with increasing concentrations of supernatants obtained from LGL incubated for 48 hr with medium (LGL-M) or PWM (LGL-PWM). Figure 3 shows that supernatants from either unstimulated or PWM-activated LGL cultures enhanced spontaneous IgG synthesis by B lymphocytes ($P < 0.05$). Supernatants from PWM-activated but not unstimulated LGL cultures also increased IgM synthesis ($P < 0.02$). To rule out potential contamination by PWM carried over in LGL supernatants, we examined Ig synthesis in unstimulated and PWM-activated B-cell cultures. B cells maximally depleted of T cells were unresponsive to mitogen activation: B alone = 630 ± 141 ng/ml vs B + PWM = 649 ± 143 ng/ml IgG; B alone = 35 ± 4 ng/ml vs B + PWM = 41 ± 7 ng/ml IgM ($N = 5$).

Preincubation with IFN Gamma Potentiates the Helper Activity by LGL

IFN gamma is known to boost NK-cell activity by PBMC or LGL populations (18, 19). It was of interest to test whether this lymphokine could also modulate LGL helper activity. PBMC (0.5×10) were cocultured in the presence of LGL preincubated for 18 hr with medium in the absence of (LGL-IFN, 0) or with increasing concentrations of IFN gamma (LGL-IFN, 50 to 1000 IU). As shown in Table II, IFN gamma markedly increased the helper activity by LGL on PWM-induced Ig production by autologous PBMC. The boosting effect of IFN gamma on LGL helper activity was dose related and consistent at different numbers of cells added (Fig. 4).

DISCUSSION

In this study we have examined the role of LGL from healthy young donors in the control of Ig

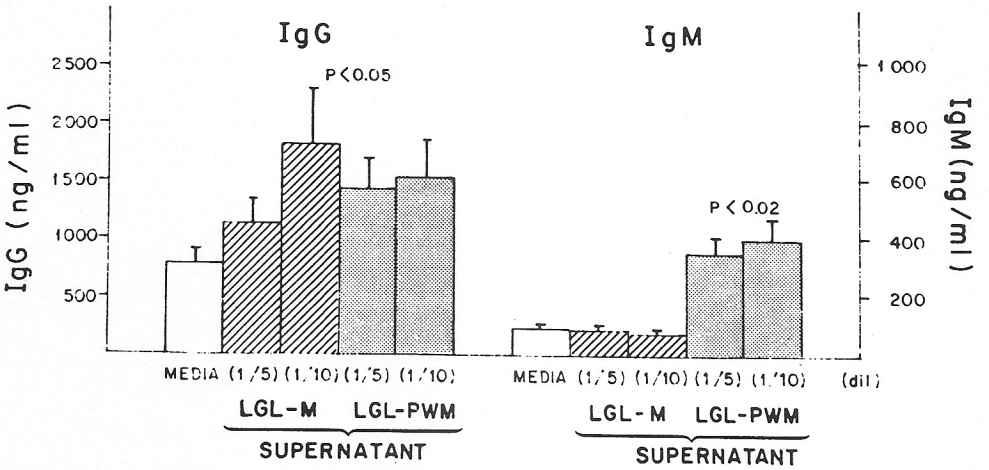

Fig. 3. Duplicate cultures of B cells (0.5×10^6) were added with medium or two different concentrations of supernatants obtained from unstimulated (LGL-M) or PWM-activated (LGL-PWM) LGL cultures as detailed under Materials and Methods. Cultures were harvested on day 7 and tested for IgG and IgM concentrations by ELISA. Results express means ± SE of six experiments.

secretion by peripheral blood B cells. Our results indicate that LGL have a helper effect on IgG and IgM synthesis by a T cell-independent mechanism that involves the secretion of soluble factors. Supernatants obtained from unstimulated or PWM-activated LGL cultures also induced Ig secretion by B cells. Preincubation of LGL with highly purified human IFN gamma markedly increased their helper effect on B-cell responses.

LGL represent a heterogeneous cell population containing most of the NK activity in peripheral blood (1). Recent evidence supports a broader immunoregulatory role for this cell population (2, 3, 18, 19). For example, it has recently been suggested that NK cells can regulate Ig synthesis (4–8). The presence of NK cells in germinal centers of secondary follicles (13) further argues for a physiological role of this subpopulation in the control of antibody responses.

In our study, LGL showed a consistent enhancing effect on PWM-stimulated Ig synthesis by autologous B cells or PBMC. In fact, in some experiments as few as 1×10^3 LGL could significantly increase PWM-driven Ig synthesis (Fig. 4). Increases in the net Ig production over baseline production were 3- to more than 10-fold. It is possible that actual B/LGL ratios varied among individual experiments depending on the numbers of NK cells already present in B-cell fractions. Moreover, the fact that the number of autologous

Table II. Preincubation with Interferon Gamma Increases the Helper Activity by Human LGL[a]

Expt No.	PBMC alone	PBMC plus LGL (5×10^4)			
		LGL-IFN 0	LGL-IFN 50	LGL-IFN 100	LGL-IFN 1000
1	1,165	3,551	6,235	7,896	10,830
2	5,133	7,910	18,642	9,346	18,379
3	5,975	8,283	12,343	10,977	ND
4	1,840	3,224	2,418	3,718	5,316
5	2,437	1,151	3,942	3,538	ND
6	1,583	1,356	2,804	2,197	3,303
7	7,069	13,565	18,364	23,625	19,586
Mean ± SE	3,600 ± 906	5,577 ± 1,715	9,249 ± 2,700	8,756 ± 2,772	11,482 ± 3,305
		($P < 0.05$)[b]	($P < 0.01$)	($P < 0.01$)	($P < 0.05$)

[a] PBMC (0.5×10^6) were cultured alone or with 5×10^4 autologous LGL that had been preincubated at 37°C for 18 hr with medium (LGL-IFN 0) or increasing concentrations of highly purified human IFN gamma (LGL-IFN 50 to -IFN 1000). Cultures were stimulated with PWM (final dilution, 1/200) and supernatants tested on day 7 for IgG concentrations (ng/ml) by ELISA.
[b] Wilcoxon signed-rank test.

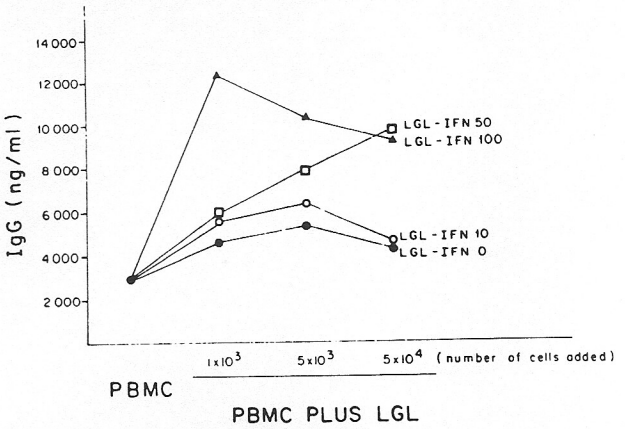

Fig. 4. PBMC (0.5×10^6) were cultured alone or in the presence of autologous LGL preincubated for 18 hr with medium (LGL-IFN 0) or increasing concentrations of human IFN gamma (LGL-IFN 10 IU to LGL-IFN 100 IU). All cultures were stimulated with PWM, and supernatants tested for IgG concentrations on day 7 by ELISA. The means of five experiments are depicted.

LGL needed to provide optimal helper activity varied in individual experiments may be explained by the presence of a low but variable number of NK cells present in target B-cell preparations. At any rate, within the range of total LGL numbers used in this study, comprising both LGL added and LGL already present in B-cell fractions, this subpopulation always showed a facilitatory effect for Ig production in mitogen-activated cultures.

It has been previously reported that LGL or NK cells can suppress Ig production (4–8) but technical differences may account for the apparently contradictory results. First, in two of these studies relatively high numbers were necessary to show significant suppression (5, 8). In the report by Brieva *et al.* (8) suppression was observed only at B/LGL ratios equal or greater than 1/1, a proportion higher than that existing in peripheral blood or lymphoid tissue. Second, the purification of putative suppressor cells was not comparable. Thus, either LGL were not further depleted of high-affinity E-rosetting cells (6) or only fluorescence-activated sorted HNK-1+ cells, and not whole LGL, were examined (5). Also, in the study by Tilden *et al.* (5), HNK-1+ cells showed suppressor activity only after preactivation with Ig-coated erythrocytes. Third, in one study *in vivo* antigen-induced lymphoblastoids but not whole B cells were used as targets for suppression (8). In such a system, PWM, a well-known inducer of Ig synthesis when added to fresh PBMC or B- plus T-cell cocultures (17, 20), activates predominantly suppressor cells (21). Consonant with our results, one of the first studies assessing the role of NK cells in Ig synthesis in a murine model showed that low numbers of such cells significantly enhanced the numbers of plaque-forming cells by autologous splenocytes (7). Further, a recent study by Vyakarnam *et al.* (22) shows that human clones with NK-cell function can promote B-cell differentiation.

It could be argued that the helper effect by LGL may be mediated by contaminating T cells present in the B-cell subpopulation. However, LGL enhanced Ig synthesis by B cells maximally T cell depleted and unable to respond by themselves to PWM stimulation (Fig. 2). It is not clear as yet whether T-cell markers are actually expressed on LGL or in T cells contaminating LGL preparations. However, recent studies using two-color flow cytometry have revealed that pan-T- and NK-cell markers can simultaneously be expressed in cells with predominant NK activity (23, 24). Therefore, it could be suggested that T-cell markers expressed on NK cells or even in contaminating helper T lymphocytes may be responsible for PWM enhancement of Ig synthesis in B-cell plus LGL cocultures. Two sets of evidence argue against this possibility. First, to obtain a comparable level of PWM-driven Ig synthesis, similar numbers of T cells or LGL were required (not shown). Fewer than 1×10^3 T cells or LGL did not provide enough help in PWM-activated cultures. Thus, a few contaminating T lymphocytes still remaining in LGL preparations would clearly be insufficient to support optimal levels of PWM-driven Ig synthesis. Second, the removal of OKT3+ or Leu 4+ cells did not abolish, and sometimes even enhanced, the helper activity by LGL preparations on Ig synthesis (Fig. 2).

Our experiments suggest that PWM is required to activate and induce the secretion of soluble helper factors by LGL. This model is suggested by the enhancement of IgM and IgG synthesis by LGL in mitogen-driven cultures. However, supernatants from both unstimulated and PWM-driven LGL cultures significantly augmented IgG production by B cells (Fig. 3). These supernatants were obtained from cultures containing relatively large numbers of LGL (1×10^6 cells), whereas a maximum of only 1×10^5 LGL was added to cell cocultures. Thus, it is possible that in the range of cell numbers added, mitogen activation was needed to generate enough helper factors from LGL.

LGL are known to secrete a number of lympho-

kines such as IL1, IL2, and IFN (2) capable of promoting B-cell proliferation and differentiation into Ig-secreting cells (25–27). More recently, Pistoia *et al.* (11) and Procopio *et al.* (12) demonstrated the production of B-cell growth factor by human LGL. Also, NK-cell clones from normal subjects have recently been shown to spontaneously secrete BCDF for differentiation of IgG (22). Our results support these findings since supernatants from unstimulated LGL cultures were able to enhance IgG synthesis by nonactivated B cells, suggesting spontaneous secretion of class-specific BCDF for IgG. On the contrary, secretion of BCDF for IgM synthesis required preactivation of LGL by PWM. The subpopulations responsive to LGL supernatants are probably B cells preactivated *in vivo* or a subset that can differentiate into Ig-secreting cells without the need of antigen or mitogen stimulation (28).

NK-cell function, contained predominantly within LGL (1), can be enhanced by IFN (18, 19). As shown in Table II and Fig. 4, the enhancement of LGL helper activity after preincubation with IFN gamma suggests that these cells can also be recruited by this cytokine to promote B-cell differentiation. The mechanism responsible for this effect is currently under study in our laboratory.

Given the heterogeneity of this subpopulation we cannot completely exclude that NK cells may secrete both helper and suppressor factors under conditions different from our *in vitro* system. The final effect of LGL on B-cell function may be dependent on the type of stimulus needed for NK-cell activation, the predominant LGL subset triggered, the balance of helper versus suppressor factors released, and the timing for them to influence a particular phase of the B-cell cycle.

In summary, we have shown that low numbers of LGL from healthy subjects enhance Ig synthesis by a T cell-independent mechanism involving the secretion of soluble helper factors. The helper activity by LGL on Ig synthesis can be potentiated by preincubation with IFN gamma. This study further supports the notion of a broader involvement of NK cells in regulation of the immune response.

ACKNOWLEDGMENTS

We thank Mrs. Egle de Hersen for helping in the preparation of the manuscript and Mrs. Omaira de Troconis for skillful secretarial assistance. We are also very grateful to Dr. John Ortaldo (National Cancer Institute, Frederick, MD) and Dr. Giorgia Trinchieri (The Wistar Institute, Philadelphia, PA) for kindly donating the 3G8 and B73.1 monoclonal antibodies. This work was supported by Grants S1-1409 y de Fortalecimiento de Centros del Consejo Nacional de Investigaciones Cientificas y Tecnologicas (CONICIT).

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