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Production of multiple cytokines by clones of human large granular lymphocytes

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Summary. *LGL in addition to mediating natural killer (NK) activity, can secrete a variety of lymphokines, depending on the stimulus used: interleukin-1 (IL-1), interleukin-2 (IL-2), interferon α and γ (IFN), and B-cell growth factor (BCGF). To define more directly whether cells with NK activity can also secrete one or more cytokines, we obtained clones by limiting dilution assays from highly purified preparations of human LGL and cultured them in IL-2-containing medium for several weeks. All the clones tested spontaneously produced detectable levels of IFN-γ and 35 of 40 clones (87%) produced higher levels when stimulated with PHA. A smaller proportion (16%) of clones (9 of 54) secreted IL-1 after stimulation with LPS, while 34% of the clones (17 of 49) produced IL-2 in response to PHA stimulation. Cytokine production was associated with both cytotoxic and noncytotoxic clones and did not correlate with their surface phenotype, as has been observed for fresh LGL. The ability to produce IL-1 or IL-2 was not usually found within the same clones following PHA and LPS stimulation, respectively; however two clones produced both IL-1 and IL-2 when stimulated in different experiments, but not at the same time. In addition, two of nine cloned LGL simultaneously produced IFNγ and IL-1. These results indicate that LGL-derived clones have the ability to produce multiple cytokines, suggesting that the LGL population may play an important immunoregulatory role and may also be capable of self-regulation of cytolytic activity.*

Introduction

Natural killer (NK) activity [14] is exerted predominantly, if not exclusively, by cells with the morphological features of large granular lymphocytes (LGL) [33, 34]. A large proportion of human LGL have been shown to mediate NK activity [34], but in addition to their cytotoxic activity, LGL have recently been shown to have the ability to secrete a variety of cytokines, including interferon α and γ (IFN), interleukin 1 (IL-1), interleukin 2 (IL-2), B-cell growth factor (BCGF), colony-stimulating factor(s) (CSF), NK-cytotoxic factor (NKCF) [15], and other lymphotoxins (J. Djeu et al., unpublished observations).

In 1978. Trinchieri et al. reported that human Fc receptor-posistive lymphocytes enriched for NK activity produced IFN when stimulated by some tumor cell lines or by virus-infected allogeneic fibroblasts [37, 38]. Djeu et al. [8, 9] confirmed a close association between cells with NK activity and IFN-producing capabilities, finding that human purified LGL produced α- and/or γ-IFN in response to mitogens, bacterial antigens, mycoplasma-infected tumor cell lines, and both influenza and herpes simplex viruses.

The possibility that human LGL might produce IL-2 after mitogen stimulation was first addressed by Domzig et al. [10]. These studies were extended by Kasahara et al. [16], who reported that human LGL produced IL-2 as well as IFN_{γ}, and also produced CSF. Further, Scala et al. [30] have reported that stimulation of LGL with lipopolysaccharide (LPS) induces the production of a cytokine with the biological and biochemical properties of IL-1.

We have recently extended these studies by examining cytokine production associated with distinct subsets of LGL [30, 31]. These studies, however, were performed with fresh LGL populations and were not able to determine whether an LGL with NK activity may also have the capability of secreting one or more cytokines. Therefore, in the present study, we have investigated the ability of clones of human LGL to produce cytokine(s) as well as mediate NK activity.

LGL can grow in vitro for at least several generations when the culture medium is supplemented with IL-2 [35, 36]. Cultured LGL maintain the characteristic morphology and tumoricidal activity of fresh LGL; however, some changes in the surface phenotype occur with culturing [21]. We have recently reported [3] a study in which clones derived from purified preparations of human LGL were studied for cytotoxic activity against a panel of NK-susceptible tumor targets and for their surface antigenic profiles. Those results, and results of other investigators [13, 25], showed that LGL-derived clones displayed heterogeneity in terms of target specificity pattern and surface markers, similar to that associated with the entire NK-cell population [17, 22].

In the present study, 64 LGL-derived clones were studied for (a) their ability to produce IFN, IL-1, IL-2, and BCGF; (b) cytotoxicity against a panel of NK-susceptible tumor cells; and (c) surface phenotype. LGL-derived clones were found to have the ability to produce various cytokines, and this secretory function was associated with cytotoxic as well as

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Abbreviations: NK, natural killer; LGL large granular lymphocytes; IL-2, interleukin 2; IFN, interferon; IL-1, interleukin 1; BCGF, B-cell growth factor; ADCC, antibody-dependent cellular cytotoxicity; PHA, phytohemagglutinin A; LPS, lipopolysaccharide; FBS, fetal bovine serum; MoAb, monoclonal antibody; Staph A, *Staphylococcus aureus* protein A; FcR, receptor for the Fc portion of Ig

noncytotoxic clones. Almost all clones were shown to be able to secrete more than one type of factor, and IFN_γ production was associated with most of the clones. The production of IL-1 and IL-2 was not usually associated with the same clone. However, clones were able to secrete both of these cytokines, with one clone able to secrete both IL-1 and IL-2 at the same time and the other two clones secreting these cytokines at different times of culture. These findings indicate that LGL may not only be important cytotoxic effector cells but also immunoregulatory cells, with the potential to regulate their own activity and that of other lymphoid cells.

Materials and methods

Isolation of large granular lymphocytes (LGL). Normal peripheral blood lymphocytes were separated on discontinuous gradients of Percoll (Pharmacia, Sweden) [33]. LGL were collected from low-density fractions, and contaminant T lymphocytes were removed by rosetting with sheep red blood cells (SRBC) at 29° C for 1 h [33]. Cell preparations consisted of an average of 80%–90% LGL, as determined by morphological analysis on Giemsa-stained cytopreps [33], and were 75% $OKMI^+$ [23], 80%–85% $B73.1^+$ [26], and < 5% $OKT3^+$ [23], as determined by flow cytometry.

Culture medium. Culture medium was RPMI 1640 (Biofluids, Inc., Rockville, Md, USA), supplemented with 10% human AB serum (M. A. Bioproducts, Walkersville, Md, USA), 0.1 m*M* nonessential amino acids (GBICO, Grand Island Biological Company, Grand Island, NY, USA), 2 m*M* sodium pyruvate (GIBCO), 4 m*M* glutamine (NIH media unit), 50 μg/ml gentamicin (Sigma, St. Louis, Mo, USA), 0.2 m*M* 2-mercaptoethanol-buffered solution (Sigma), and with IL-2 [50% v/v (final concentration, 100 U/ml) of the supernatant from a gibbon lymphosarcoma cell line: MLA 144 [kindly provided by Dr H. Rabin, NCI-Federick Cancer Research Facility, Frederick, Md, USA)]. This cell line has been shown to be constitutively able to produce IL-2 [27].

LGL cultures. Uncloned cultures of LGL were initiated as described elsewhere [3]. Cells (5×10^5/well) were plated in 24-well plates (Costar, Cambridge, Mass, USA) together with irradiated (3,000 R) autologous mononuclear cells (5×10^5/well) in a total volume of 1 ml culture medium + 0.1 μg/ml phytohemagglutinin (PHA) HA16 (Wellcome, Beckman, England).

Cloning procedures. LGL were cloned by limiting dilution at 0.5 cell/well in 96-well round-bottomed microtiter plates (Cooke, Dynatech Lab, Alexandria, Va, USA) [3]. Autologous irradiated (3,000 R) mononuclear cells, 2×10^4/well, and 0.1 μg/ml PHA were dispensed in to each well to give a final volume of 0.2 ml of culture medium containing IL-2. Fresh autologous feeder cells and IL-2-containing medium were added weekly. After 14–20 days of growth the plates were checked with an inverted microscope, and wells were considered positive when visible colonies were seen.

Preparation of cytokine-rich supernatants. Cells from each clone were washed twice in RPMI 1640 + 5% fetal bovine serum (FBS) (Gibco) and then plated in 0.1 ml of the same medium at the concentration of 2×10^6/ml in a round-bottomed 96-well plate. The stimuli [PHA (2.5 μg/ml) or LPS (25 μg/ml)] were diluted in RPMI medium + 5% FBS were added in 0.1 ml volume, then after 48 h of incubation at 37° C the supernatants were harvested and stored at −70° C.

The supernatants from unstimulated clones provided an indication of baseline cytokine production; fresh or cultured uncloned LGL were used as positive control populations. The medium control cultures contained PHA or LPS without cells.

Cytokine assays. The levels if IFN in the culture supernatants were determined by the inhibiton of cytopathic effects by vesicular stomatitis virus in human foreskin fibroblasts (for IFN_α) or human WISH amniotic cells (for IFN_γ) [8]. The titers are expressed in international units, standardized with NIH reference human IFN_γ (G-023-901-527). The type of IFN was identified by neutralization with specific antibodies [8].

IL-2 activity of supernatants was determined by their ability to support the growth of murine IL-2-dependent CT6 cell line [10, 16]. In every assay the results with the experimental supernatants were compared with those obtained with a standard IL-2 supernatant prepared in our laboratory [32].

IL-1 activity of supernatants was determined in a C3H/HeJ(H-2^k) mouse thymocyte co-stimulator assay, as already described [30]. In every assay the results with the experimental supernatants were compared with those obtained with a standard IL-1 supernatant prepared in our laboratory [29].

BCGF activity was determined as the ability to enhance the growth of purified human B lymphocytes primed with 15 μg/ml affinity purified $(Fab)'_2$ goat-anti-human μ-chain of IgM (Cappel, West Chester, PA, USA), as previously, described [18].

Cytotoxicity. Cytotoxicity assays were performed against a panel of targets, including four human-NK-susceptible targets: K562 (derived from a patient with chronic myelogenous leukemia in blast crisis), MOLT-4 (a T-cell line derived from a patient with acute lymphocytic leukemia), Daudi (a B-cell line derived from a patient with Burkitt's lymphoma), and Alab (derived from a patient with breast carcinoma). The target for antibody-dependent cellular cytotoxicity (ADCC) was RL♂1 (a murine T lymphoma) coated with 1 : 100 dilution of a rabbit antimouse brain serum (Flow Labs, Rockville, Md, USA). Effector cells were washed and 'rested' in medium without IL-2 for 18 h prior to the cytotoxicity assay to minimize any nonspecific lysis that might occur [35].

Target cells were labeled with 100 μl chromium-51 (New England Nuclear). The effector-to-target ratio varied from 3 : 1 to 10 : 1, depending on the available number of effector cells. The duration of the assay was 6–8 h. Cytotoxicity was determined by the amount of chromium-51 released from target cells, according to the formula: % specific lysis = (cpm in experimental wells − cpm in wells with target cells alone/cpm incorporated in target cells) × 100. A 6% increase in isotope release above baseline was always statistically significant at $P < 0.05$ (Student's *t*-test).

Membrane markers. LGL-derived clones were studied for their surface phenotype with the following monoclonal antibodies: OKT3 (Pan T antigen), OKT8 (cytotoxic-suppressor T lymphocytes), OKM1 (monocyte and LGL), and B73.1 (FcR on LGL and neutrophils). The reactivity with monoclonal antibodies was performed by the *Staphylococcus aureus* protein A assay [28]. Giemsa-stained cytocentrifuged cell preparations

[34] were scored by microscopic examination. A clone was considered positive when ≤ 30% of the cells were coated with ≤ 10 staphylococci per cell.

Results and discussion

The general characteristics of LGL-derived clones obtained by the procedures used in this study have been described in detail elsewhere [3]. The clones studied here were derived from the LGL of five different donors. The median plating efficiency, defined as percentage of wells with proliferating cells compared with number of wells seeded, was 17% (range 15%–25%). Clones were maintained in culture for periods varying from 40 to 70 days. Although the plating efficiency was only 17%, the LGL employed were devoid (< 1%) of T cells. In addition, cytokine production from both cytotoxic and noncytotoxic LGL clones was similar.

LGL-derived clones were concomitantly tested for: (a) production of cytokines, (b) cytotoxicity against tumor targets, and (c) surface markers. The results from some representative clones (in culture 40–70 days) are presented in Table 1. Some clones had NK activity as well as cytokine-producing ability, whereas others secreted cytokines but had no detectable NK or ADCC. Various combinations of cytokines were produced by the same clone, but no clones simultaneously produced all the factors examined. For example, clones LM-E4 and LM-D4 produced IL-1 and IFN_γ, but not IL-2; clones LM-E5 and BB-58 produced IL-2 and IFN_γ, but not IL-1; clone BB-58 also produced BCGF; and clone BB-7 produced IFN_γ and BCGF, but no detectable IL-1 or IL-2 activity. Only one clone, RF-E, as shown in Table 2, concomitantly produced IL-1 when stimulated with LPS, and IL-2 (a low but significant level of 4 U/ml), when stimulated with PHA. It should be noted that no IL-2 activity was ever

Table 1. Multiple functions of LGL-derived clones

Clone[a]	Cytokine[b] (units/ml)				Phenotype[c] (% positive cells)				Cytotoxicity[d] against				
	IFN_γ	IL-1	IL-2	BCGF	OKT3	OKT8	OKM1	B73.1	K562	ADCC	MOLT-4	Daudi	Alab
LM-E5	625	0	165	NT[e]	90	5	20	20	20	5	NT	1	3
LM-E4	50	31	0	NT	100	10	80	5	3	2	NT	5	10
LM-D4	25	85	0	NT	100	20	80	50	1	0	NT	2	8
RF-3	125	50	0	NT	30	20	70	80	74	62	7	38	44
MM-31	NT	NT	120	NT	100	NT	80	0	5	1	35	3	18
BB-5	125	0	84	0	NT	NT	NT	NT	1	3	2	NT	3
BB-58	125	0	60	90	NT	NT	NT	NT	1	0	40	NT	3
BB-7	25	0	0	166	NT	NT	NT	NT	0	1	− 2	NT	− 3
BB-158	200	0	0	65	NT	NT	NT	NT	1	0	3	NT	− 1
MM-6	NT	0	0	NT	90	NT	80	30	20	0	10	2	33
BB-156	25	0	0	58	NT	NT	NT	NT	2	− 1	0	4	1
LM-D1	125	0	25	NT	90	50	50	10	0	0	NT	7	5
LM-C4	80	0	31	NT	100	10	40	15	7	5	NT	5	9
Uncloned LGL culture	200	140	70	80	50	35	50	40	65	60	58	28	49

[a] Clones were obtained by limiting dilution at 0.5 cells/well, from highly purified preparation of LGL, and expanded in culture medium + IL-2
[b] Cytokine production was induced after culture for 48 h with 2.5 µg/ml PHA (for IFN, IL-2, BCGF) or with 25 µg/ml LPS (for IL-1)
[c] Reactivity with monoclonal antibodies was revealed by the Staph A assay. A clone was considered positive when > 30% of the cells were coated with 10 or more bacteria
[d] Cytotoxicity was measured by release of chromium-51 from target cells in a 6- to 8-h assay. Data are expressed as percent specific lysis at an effector-to-target ratio (E : T) of 10 : 1. Values > 6% above spontaneous release were always significant ($P < 0.05$ Student's *t*-test)
[e] NT, not tested

Table 2. Production of IL-1 and IL-2 by the same clones[a]

Clone[a]	Cytokine[b] (units/ml)				Phenotype[c] (% positive cells)				Cytotoxicity[d] against				
	IFN_γ	IL-1	IL-2	BCGF	OKT3	OKT8	OKM1	B73.1	K562	ADCC	MOLT-4	Daudi	Alab
RF-E (21)[b]	125	60	4	NT[c]	10	70	70	90	71	56	11	NT	44
LM-D1 (21)	125	0	25	NT	90	50	50	10	0	0	NT	7	5
LM-D1 (28)	NT	29	0	0	100	50	70	20	1	7	NT	0	10
LM-C4 (21)	80	0	31	NT	100	10	40	15	7	5	NT	5	9
LM-C4 (28)	NT	36	0	0	98	60	60	10	8	3	NT	3	13
LM-C4 (45)	NT	0	13	NT	90	5	5	5	1	0	NT	0	0
Uncloned LGL Culture (28)	200	140	70	80	50	35	50	40	65	60	58	28	49

[a] Clones were obtained by limiting dilution at 0.5 cell/well from highly purified preparation of LGL. Cytokine production, reactivity with MoAb and cytotoxicity assay were performed as described in *Materials and methods*
[b] In parentheses, days in cultures
[c] NT, not tested

detectable in any of the supernatants of clones that were stimulated with LPS, and therefore detection of IL-1 in these samples was not complicated by the presence of IL-2.

Two clones (LM-D1 and LM-C4), upon repeated testing, were found to shift in their pattern of cytokine production (Table 2). When stimulated at day 21 after the cloning they produced IFN_γ and IL-2 (but not IL-1); when stimulated again 1 week later (day 28) both of them produced IL-1, but not IL-2. Clone LM-C4 was again stimulated at day 45 and produced IL-2, but not IL-1.

It was surprising to find clones producing two cytokines that are usually products of distinct cell types. In addition, two of nine clones that were tested for both factors produced both IFN_γ and IL-1. Production of IFN_γ, IL-2, and BCGF have been associated with T cells [9, 37, 38], whereas production of IL-1 has not been associated with T cells, but rather with monocytes and other cell types, as reviewed [20]. There are a number of possible explanations for this unexpected finding: (1) It is possible that the in vitro culture conditions might preferentially have selected for immature precursor LGL with capability for multiple functions. However, this is not necessarily the case, because cytotoxic patterns of LGL clones (data not shown) [4] are quite similar to those of fresh LGL, with 60% of clones showing a broad pattern of lysis [4]. (2) It is also possible that a particular factor is produced in a given stage of differentiation and that the alternative between production of one cytokine and then another might reflect shifts in stages along the differentiation pathway of LGL. In contrast with this possibility, however, clone LM-C4 after 50 days reverted to production of the same lymphokine (IL-2) as was seen at day 21. (3) The possibility must also be considered that some of the cultures showing such results were not true clones. Because of their short life span, it has been technically difficult to reclone these cultures to be absolutely certain of their clonal nature. However, all the clones tested were obtained by plating at 0.5 cell/well; under these conditions, the probability that each well contained only 1 cell was > 97% as calculated by the Poisson distribution. Moreover, since we found that the median frequency of proliferative progenitors among LGL was 1/55 (range 1/5 to 1/400) [4], the probability that both of two cells in the same well would proliferate is very low. (4) Finally, although feeder cells and controls produced no detectable cytokines, it remains conceivable that feeder cells may have been stimulated in the presence of some activated LGL. However, since fresh LGL make the some factor without feeder cells, this is unlikely. Despite all these reservations, the observation that seven of nine cloned LGLs simultaneously produced IFN_γ and IL-1 suggests that these LGL populations can produce both these T-cell- and non-T cell-associated mediators.

The analysis of the phenotype of the clones discussed above is also shown in Tables 1 and 2. Most clones reacted with MoAb OKT3, OKT8, OKM1, and B73.1, but without a unique pattern of reactivity, and such heterogeneity confirms our previous findings [3]. The pattern of cytotoxic activity also varied among the clones. Some clones were cytotoxic against K562 and also other susceptible targets such as MOLT-4, Daudi, and Alab and were reactive in ADCC. Other clones killed only one or two targets, such als clone MM-31, which reacted only against MOLT-4 and Alab, and clone BB-58, which reacted only against MOLT-4. Some clones were not cytotoxic against any of the targets tested. The percentage of cytotoxic clones among all the clones tested was 12.5%, which is very close to the results previously obtained (9%) with clones derived from highly purified LGL. In the previous study [3] the possible explanations and implications of such a low frequency of cytotoxic clones were extensively discussed.

Table 3 provides an overall summary of the cytokine-producing capability of all the LGL-derived clones that were tested. All the first 12 clones tested spontaneously produced IFN_γ; when the number of clones studied was increased to 40 there were five clones among the noncytotoxic ones that did not produce any detectable IFN even when stimulated with PHA, while the other clones spontaneously produced IFN_γ in even higher quantities when stimulated with PHA.

The incidence of cytotoxic clones secreting IL-1 was higher than that of clones without detectable cytotoxic reactivity. However, more clones will need to be tested to determine to what extent these two functions are really associated. In contrast, the frequency of production of IL-2 was quite similar for the cytotoxic and noncytotoxic clones. With regard to BCGF, some of the noncytolytic and cytolytic clones were positive, but the number of clones tested will have to be increased before conclusions can drawn regarding the possible connection between these two functions.

The possible association of cytokine production with a particular surface phenotype was also examined. In fresh LGL, IL-1-producing cells have been found to comprise a distinct subpopulation ($B73^+$, $OKM1^+$, Ia^+ cells) [15, 30, 31]. It has been more difficult to identify a particular LGL subset that is responsible for production of IFN_γ and IL-2, although the LGL producing IL-2 appear to be predominantly $OKT11^+$ [16, 30, 31].

A summary of the phenotype of the lymphokine-producing clones is presented in Table 4. In agreement with the phenotype of the subset of LGL that is predominantly responsible for production of IL-1, all the clones producing IL-1 were $OKM1^+$, and three clones were also $B73.1^+$.

Table 3. Cytokine production by clones of human LGL[a]

Cytokine[b]	Cytoxicity[e]	No. positive/ No. tested	%	Range (U/ml)
IFN_γ sp.[c]	–	7/7	100	10–120
	+	5/5	100	25–125
IFN_γ[d]	–	32/37	86	10–625
	+	3/3	100	10–250
IL-1	–	7/48	14	20– 85
	+	2/6	33	50– 60
IL-2	–	14/41	34	3–165
	+	3/8	37	4–120
BCGF	–	5/24	21	51–166
	+	1/1	100	90

[a] Clones were obtained by limiting dilution at 0.5 cells/well and expanded in medium + IL-2
[b] Assayed in culture fluids from cells incubated for 48 h with PHA or LPS but in absence of added IL-2
[c] Spontaneous production of IFN was determined in the culture supernatant of clones incubated for 48 h in the absence of PHA or LPS. Culture medium + IL-2 alone contained < 5 U/ml
[d] IFN_γ levels indicated are above the spontaneous levels in control unstimulated
[e] Cytotoxic activity was determined in a 6- to 8-h chromium-51 release assay. A clone was considered positive when a significant level of reactivity ($P < 0.5$, Student's t-test) was observed with at least one of a panel of three to five target cells

Table 4. Monoclonal antibody reactivity of cytokine-producing LGL-derived clones

Cytokine produced	Reactivity with monoclonal antibody[a]			
	OKT3	OKT8	OKM1	B73.1
IL-1	3/9[b]	4/8	9/9	3/9
IL-2	8/9	3/6	8/9	1/9
IFN_{γ}	10/14	3/12	13/14	6/14

[a] Reactivity with MoAb was assessed by the *Staphylococcus aureus* protein A assay. A clone was considered positive when > 30% of the cells were coated with 10 or more bacteria

[b] Number of positive clones out of total clones tested

However, six B73.1⁻ clones also were found to produce detectable levels of IL-1 activity.

Most of the clones, as already reported [3], were $OKT3^+$, a T-cell marker that is expressed by most LGL after being cultured [21, 36] but is not detectable on LGL freshly isolated from the peripheral blood [20]. The OKT8 antigen is also expressed on about 50% of the LGL-derived clones. A previous report of a study in which fresh LGL was used [30] indicated that uncultured $OKT8^+$ cells might have a suppressive effect on IL-1 production by LGL; however, four of the eight clones that produced IL-1 were $OKT8^+$.

The above results are somewhat difficult to interpret in relation to what we know about lymphokine production by distinct phenotypic subsets of fresh LGL. This may be because the phenotype of cultured cells appears to differ significantly from that of the cell of origin, with some surface markers being lost in culture and others, which are undetectable on the surface of fresh LGL but may be present intracellularly in an 'occult' form (A. C. Morgan et al., unpublished data), becoming evident on the surface of cultured LGL. Moreover, studies performed on cloned cells have shown that surface markers are not a constant feature and that their expression may be modulated during culture [40]. (See also Table 2, clone LM-C4.)

Overall, these data show that a considerable proportion of LGL-derived clones including those with NK activity, have the capability of producing one or more cytokines. Cytotoxic clones were at least as effective producers of cytokines as noncytotoxic clones, indicating the potential for these cells to play an important role as immunoregulatory cells as well as effector cells.

Most of the clones produced IFN_{γ}, either constitutively or in response to PHA. This is in line with the results of Handa et al. [12], who reported that IL-2 induced IFN_{γ} production in all the murine NK clones they have tested. Such a large proportion of clones producing IFN_{γ} may have a physiological relevance, since IFN_{γ} is involved in the boosting of NK activity [37] as well as IL-1 production [6]. Moreover, since IL-2 can also stimulate NK cells [10, 24] and this effect may synergize with that of IFN and IL-1 [7], there is the possibility that cytotoxic clones with secretory function might positively self-regulate their own cytotoxicity. On the other hand, noncytotoxic LGL might be helper cells and regulate the NK activity of cytotoxic LGL through secretion of cytokines.

The effects of the cytokines associated with LGL could also be directed towards other lymphoid cell populations, including T cells, macrophages, and B cells. In fact, both IL-1 and IL-2 are involved in the generation of cytotoxic T cells [11], while IFN_{γ} has been shown to activate monocyte-mediated cytotoxicity [19]. We also observed that LGL-derived clones produced BCGF activity, confirming results obtained with fresh LGL [15] and indicating that LGL may participate in the positive regulation of B cell growth. Other investigators have reported that $HNK\text{-}1^+$ cells [5], a marker expressed on a large proportion of human LGL [1], as well as murine NK cells [2], may also have a negative regulatory effect on B lymphocytes.

These data on cytokine production by clones derived from human LGL indicate that LGL may be involved in many different cytokine-mediated mechanisms of inflammation and immunity. In addition, recloning and retesting must be performed to verify clonality; however, technical problems with long-term culture of LGL prevent these experiments at present. This is especially true of such clones as LM-DL and LM-C4, which produced both IL-1 and IL-2 but were not cytotoxic.

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