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Abnormal Expansions of Granular Lymphocytes: Reactive Lymphocytosis or Chronic Leukemia? Case Report and Literature Review

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Summary. A case of chronic lymphoproliferative disorder is presented, wherein a morphologically homogeneous population of lymphoid cells displayed properties similar to those described for large granular lymphocytes (LGL). Besides their LGL-like phenotype (VEP 13$^+$, OKM 1$^+$, OKT 10$^+$ Fc-IgG-receptor$^+$, OKT 3$^-$), the proliferating cells were cytotoxic to NK targets as well as to antibody-coated target cells. Clinically, our patient presented low-grade lymphocytosis, splenomegaly, neutropenia, hyperimmunoglobulinemia and recurrent infections. Based upon this and 32 similar cases reported in the literature, we conclude that lymphoproliferative disorders involving GL encompass a variety of clinical entities, ranging from reactive GL lymphocytoses to overt lymphocytic malignancies.

Key words: Large granular lymphocytes – Natural killer cells – Chronic lymphocytic leukemia

Recently, a group of lymphoproliferative disorders characterized by a predominance of granular lymphocytes was described by several investigators (For a survey see Table 4 A, B) [4, 11, 15, 37, 39, 45].

In most patients, the disease follows a chronic course characterized by lymphocytosis with bone-marrow infiltration and neutropenia with recurrent infections. The expanded lymphocytes often have the appearance of LGL [66], and while they may

Abbreviations used in this paper. *GL* granular lymphocytes; *LGL* large granular lymphocytes; *PBL* peripheral blood lymphocytes; *NK* natural killer; *ADCC* antibody-dependent cellular cytotoxicity; *APh* acid phosphatase; *PAS* periodic acid-Schiff; *β-Glu* β-glucuronidase; *ANBE* alpha-naphtyl-butyrat-esterase; *ANAE* acid esterase; *POX* peroxidase; *PTA* parallel tubular arrays; *E-R* surface membrane receptor for sheep erythrocytes

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not have NK activity, they almost always exhibit ADCC activity. Their surface markers are distinct from any of the previously described T-cell malignancies, since these lymphocytes phenotypically resemble mature T-suppressor cells and bear the Fc receptors for IgG. Therefore, this lymphoproliferative disease can be clearly distinguished from neoplastic disease involving T lymphocytes, such as cutaneous T-cell lymphoma, adult T-cell leukemia, T-cell lymphoblastic lymphoma and T-cell ALL. Most authors prefer to classify this GL-lymphocytosis as a "disease" rather than a leukemia/lymphoma, because in most cases no definite evidence exists for monoclonality. Although a clonal expansion of lymphocytes is suggested by the uniform phenotype, chromosomal studies to date have reported normal karyotypes in the majority of cases [7, 15, 25, 42].

This article deals with a patient suffering from a GL lymphocytosis without distinct markers for monoclonality. A review of the relevant literature concerning similar cases reveals a rather homogenous group of lymphoproliferative states as far as clinical and hematological aspects are concerned. The clinical course and cellular characteristics of this disease favor the view that GL-lymphocytosis may occur as a reactive as well as a neoplastic hematologic disorder.

Case Report

In June 1982, a 71-year-old woman was admitted for evaluation of marked lymphocytosis. In 1962 she had undergone a radical mastectomy followed by ovarectomy and local irradiation for treatment of mammary carcinoma (stage I). In 1975 she had been admitted for cholecystectomy. At this time no other abnormalities were observed. Physical examination on admission revealed signs of chronic bronchitis. Neither hepatosplenomegaly nor enlargement of lymph nodes was found. Chest X-rays disclosed a localized pulmonary infiltrate in the paracardial area of the right lower lobe with a small pleural effusion.

The hematocrit (hct) was 40%; hemoglobin (Hb) 13.0 g/dl; white blood cell count (WBC) 7,000/mm^3 with 13% neutrophils, 5% monocytes and 82% lymphocytes. A bone-marrow aspirate was normocellular with patchy infiltration by medium-sized lymphocytes comprising approximately 15% of the nucleated cells. Bone marrow biopsy revealed a diffuse infiltration with medium-sized granular lymphocytes and an arrested myeloid maturation from the myelocytic stage onward. Karyotype analysis by G-banding technique of unstimulated and phytohemagglutinin-stimulated PBL showed a normal 46, XX pattern. Total serum protein was 7.3 g/dl and serum protein electrophoresis showed polyclonal hypergammaglobulinemia. Quantitative immunoglobulin levels were: IgG 1650, IgA 284 and IgM 285 mg/dl. Tests for cryoglobulins (mixed type), rheumatoid factor and antinuclear antibodies (1 : 10) were weakly positive. Autoantibodies to native DNA, smooth muscles, mitochondria and erythrocytes were not detected. EBV and CMV antiviral antibodies were absent. Tests for HBs antigen repeatedly showed negative results.

In June 1982 and November 1984 the Mantoux skin test was found to be weakly positive. In June 1982 therapy was begun with tuberculostatics (3-drug regimen including INH, rifampicin, ethambutol) and prednisone, 32 mg daily, the dosage being tapered in the following months. Under this treatment, the neutrophils increased from 770 to 1,100/mm^3, but blood lymphocytosis remained unchanged. At the same time, the pulmonary infiltration resolved and the patient was discharged in July 1982. In March 1983 she was readmitted because of severe fatigue, bronchitis, and a weight loss of 14 kg over a period of several months. Chest X-ray examination revealed recurrence of pulmonary infiltration. Bronchoscopy and an open lung biopsy was refused by the patient. *Staphylococcus aureus* and *Escherichia coli* were grown from her sputum. Laboratory tests showed Hct 32%, Hb 10.4 g/dl, WBC 10,500/mm^3 with 80% lymphocytes, 80% neutrophils, 6% monocytes and a platelet count of 54,000/mm^3. Physical examination and abdominal sonography revealed splenomegaly, without hepatomegaly or lymphadenopathy.

Treatment with oxacillin and tobramycin did not influence the pneumonic infiltration. Combination therapy with 16 mg daily methyl prednisolone, INH and ethambutol was then reinstituted without success. Alpha-2-recombinant interferon (Bender, Vienna) administered at a dose of 5×10^6 units daily i.m. for 1 month also had no beneficial effect. As no resolution of the pulmonary infiltrate could be attained and splenomegaly increased, the patient was treated with steroids (prednisolone, 24 mg/day) and a weekly dosage of vincristince sulfate (1.5 mg/sqm). Four weeks of chemotherapy resulted in a marked improvement of the patient's general condition and resolution of splenomegaly but blood lymphocytosis and the pulmonary infiltration persisted. After 3 months, chemotherapy was discontinued because of progressive neurotoxicity. Despite discontinuation of cytostatic treatment, the patient's condition stabilized, with a lymphocytosis of 7 to $15 \times 10^3/mm^3$ and a neutropenia consistently below $1 \times 10^3/mm^3$ (last clinical examination in March 1985).

Methods

Preparation of cells. Peripheral blood of the patient was collected prior to, as well as 12 and 36 weeks after the initial 6-month course of tuberculostatics and prednisolone. A bone-marrow sample was taken in June 1982. Mononuclear cells were separated from heparinized blood samples and bone-marrow specimen by means of Ficoll-Isopaque density-gradient centrifugation [50].

Cytochemical staining, light and electron microscopy. Staining with May-Grünwald-Giemsa and cytochemical reactions were carried out on cytocentrifuge smears. The following cytochemical reactions were applied: acid phosphatase (APh), periodic acid-Schiff (PAS), β-glucoronidase (β-Glu), alpha-naphtyl-butyrat-esterase (ANBE), acid esterase (ANAE) and peroxidase (POX) [56]. Peripheral blood cells were further investigated by electron microscopy after fixation with precooled half-strength Karnovsky's fixative and a 3% aqueous osmium tetroxide solution. After Epon embedding and ultrathin sectioning, samples were positively stained with lead hydroxide and uranylacetate [55].

Surface markers. Immunofluorescence tests were performed as described previously [52]. For surface-marker studies [50], a panel of commercially available monoclonal antibodies was applied: OKT 3 and Leu 1 react with mature T cells. Helper/inducer T cells were identified by the monoclonal reagents OKT 4 and Leu 3 a, the suppressor/cytotoxic T-cell subset by OKT 8 and Leu 2 a, respectively. OKT 6 reacts with mature thymocytes. OKT 11 and Lyt 3 bind to the sheep erythrocyte receptor. OKM 1 identifies the C_3bi-receptor expressed on cells from the myelomonocytic lineage and on a subset of LGL [40, 50, 53]. OKIa 1 reacts with Ia-like antigens [50]. OKT 10 reacts with the majority of thymocytes as well as with about 15% of bone-marrow cells; it has also been reported to recognize some LGL [40, 50]. HNK-1 reacts with LGL and with a few fetal bone-marrow and peripheral-blood non-LGL cells [1]. VEP 13 reacts with LGL and granulocytes [52]. VIB-C 5, kindly provided by Dr. W. Knapp (Institute for Immunology, Vienna), identifies mature B cells, polymorphonuclear leukocytes and 70% of bone marrow cells [31]. The anti-Tac monoclonal antibody recognizes the receptor for the human T-cell growth factor [67]. Receptors for the Fc portion of IgM and IgG were assessed by rosette formation with IgM- and IgG-coated ox RBC.

Target cells. The NK-sensitive lines K 562, MOLT-4, U 937 and CCL-227 and the NK-resistant cell lines, HL-60 and REH 6 were used as targets to assess natural cytotoxicity. Therefore 2.5×10^5 target cells were labeled with 100 μCi $^{51}Na\ CrO_4$ for 1 h at 37°C, washed twice and adjusted to a concentration of 5×10^4 cells/ml. For ADCC assays, bencylpenicilloyl (BPO)-coated REH-6 cells were incubated with an affinity-purified anti-BPO rabbit IgG antibody at a final concentration of 3.3 μg/ml [52].

Conjugate assay. To measure the binding ability of different preparations of mononuclear blood cells to target cells, 1×10^4 target cells and mononuclear cells at a ratio of 1 : 5 were mixed in RPMI 1640 (Gibco, New York) supplemented with 10% fetal calf serum in round-bottom plastic tissue-culture tubes (Greiner, FRG). After centrifugation and incubation for 3 min at 37°C,

the pellets were resuspended and the proportion of lymphocytes bound to target cells was determined in a hemocytometer (Bürker chamber). For further details, see [18]. Target-cell binding was assessed by using the following cell lines: MOLT-4, K562, Raji and the lymphoblastoid (EBV) cell line, ChHubl.

Natural killing and ADCC assays. Various concentrations of effector cells were added to triplicates of 150 μl medium containing 1×10^4 radiolabeled target cells to give target: effector ratios of 1 : 10 to 1 : 40. NK activity was tested in a 3-h ^{51}Cr-release assay as described previously [52]. Spontaneous isotope release was determined from control tubes containing only target cells and maximum release was determined by the addition of 1% NP 40. Percent specific lysis was calculated according to the formula:

$$\frac{\%^{51}\text{Cr release in test} - \%^{51}\text{Cr spontaneous release}}{\%^{51}\text{Cr maximum release} - \%^{51}\text{Cr spontaneous release}} \times 100$$

Cytokilling by HNK-1 and VEP13 antibodies. Twenty million PBL were incubated with medium, HNK-1 antibody and purified VEP 13 IgM antibody for 30 min on ice and were further washed once with cold medium. Thereafter, either medium or rabbit C preabsorbed with human buffy coat cells diluted 1/5 was added, and the cells were incubated for 1 h at 37°C. Subsequently, an aliquot was taken and the percentage of killed cells was determined by the addition of 0.5% trypan blue solution in PBS. The samples were layered onto a Ficoll-Hypaque gradient and were centrifuged for 30 min at 400 g to remove dead cells. Interphase cells were washed three times in RPMI/10% calf serum, counted and adjusted to cell concentrations required for NK assay.

Proliferative response to mitogens and alloantigens. For mitogen stimulation, a cell concentration of 1×10^6/ml and 1 μg/ml phytohemagglutinin (PHA) or 10 μg/ml of concanavalin-A (Con-A) were used. Each culture contained 1×10^5 cells. For the alloantigen-stimulated cultures an allogeneic EBV-transformed lymphoblastoid cell line was used as stimulator. In detail, 5×10^4 responder cells were added to 1×10^4 mitomycin-C-treated stimulator cells. In all experiments, proliferative capacity was assessed at various times by a 12-h ^{3}H-thymidine pulse. Mononuclear blood cells of a healthy donor served as a control.

Results

Morphology and cytochemistry. May-Grünwald-Giemsa staining showed that the patient's mononuclear blood cells, whenever tested, comprised 70% – 90% lymphoid cells with a morphology resembling human LGL (Fig. 1). The cells appeared medium-sized with a reniform nucleus surrounded by abundant and pale cytoplasm containing several azurophilic granules of varied diameter. Large-diameter granules were predominant. Activity of APh and β-Glu was extraordinarily strong and also distributed in a granular pattern. APh activity was present in a few azurophilic granules, whereas β-Glu was detected in virtually all granules. Activity of APh was inhibitable with tartrate. The cells were negative for PAS, POX, ANAE and ANBE. In electron microscopy (Fig. 2), the abnormal lymphocytes showed two types of cytoplasmic granules, small granules (0.2 – 0.35 μm in diameter) containing an electron-opaque core within a granular matrix, and large granules (0.33 – 0.35 μm) packed with tubular structures arranged in parallel arrays (PTA). These ultrastructural features have been described in normal human LGL [26]. In cross section, the PTA showed a pentagonal framework. Both types of granules were always bound by unit membranes. GL from peripheral-blood and bone-marrow specimens revealed the same morphological and cytochemical characteristics.

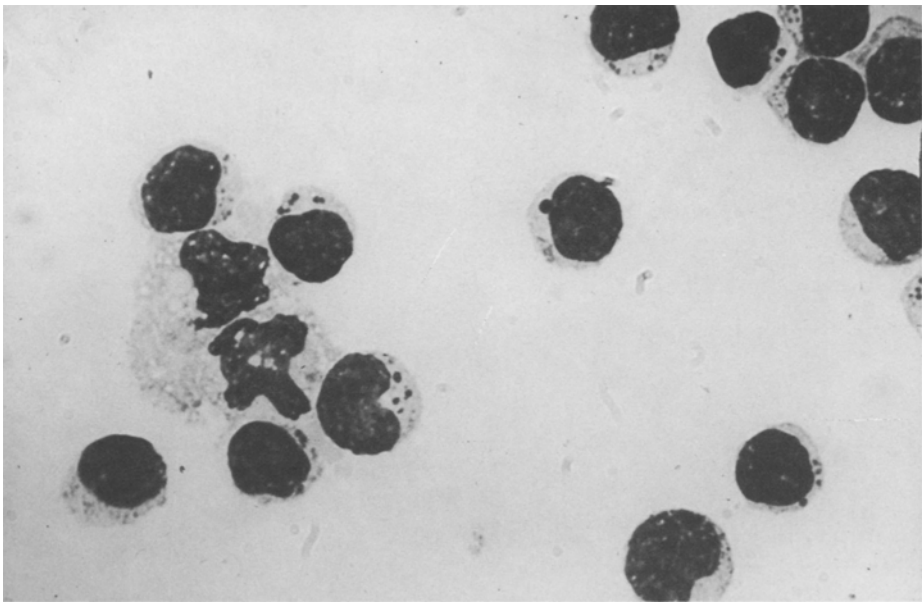

Fig. 1. Peripheral-blood mononuclear cells exhibiting LGL morphology in cytocentrifuge smears after staining according to May-Grünwald-Giemsa; ×1000

Surface-marker analysis. The results of the surface-marker analysis are depicted in Table 1. Reactivity with monoclonal antibodies showed that more than 70% of the PBL reacted with the VEP 13, OKM 1 and OKT 10 reagents. A major subset was stained with HNK-1, Leu 2 a and OKT 8. PBL, however, failed to exhibit pan-T (Leu 1, OKT 3) or T-helper cell (Leu 3 a, OKT 4) markers. The reaction with OKIa 1 was weakly positive in 25% of PBL. Most of the PBL bore receptors for the Fc portion of IgG, and sheep erythrocytes (Lyt 3^+). About 50% of the bone marrow infiltrating GLs were positively stained for Ia-like antigens (OKIa 1^+).

Contrasting to the findings in peripheral blood, a relatively high proportion of bone-marrow mononuclear cells expressed pan-T markers (Leu-1, OKT 3), whereas differentiation markers (OKM 1, VEP 13) were markedly diminished (Table 1).

No striking difference was between blood and bone-marrow lymphocytes found in the expression of the E-receptor and the OKT 8 antigen. Double-marker studies revealed that virtually all of the VEP 13^+ PBL co-expressed the antigens detected by the antibodies OKM 1 and OKM 10. Roughly 50% of the VEP 3^+ were also labeled by the HNK-1 antibody, recognizing a subset of normal NK cells. In contrast, VEP 13^+ cells failed to react with the antisera OKT 4 and Leu 3 a, specific for T-helper/inducer cells and with the monoclonal antibody anti-Tac.

Target cell binding, NK and K activity. Because abnormal lymphocytes from our patient exhibited morphological and antigenic characteristics of LGL, further functional analyses were performed concerning the capacity to mediate NK or ADCC. Target-cell binding studies with NK-sensitive as well as NK-insensitive tumor cell lines revealed that the patient's PBL bound well to the NK-sensitive targets (K 562,

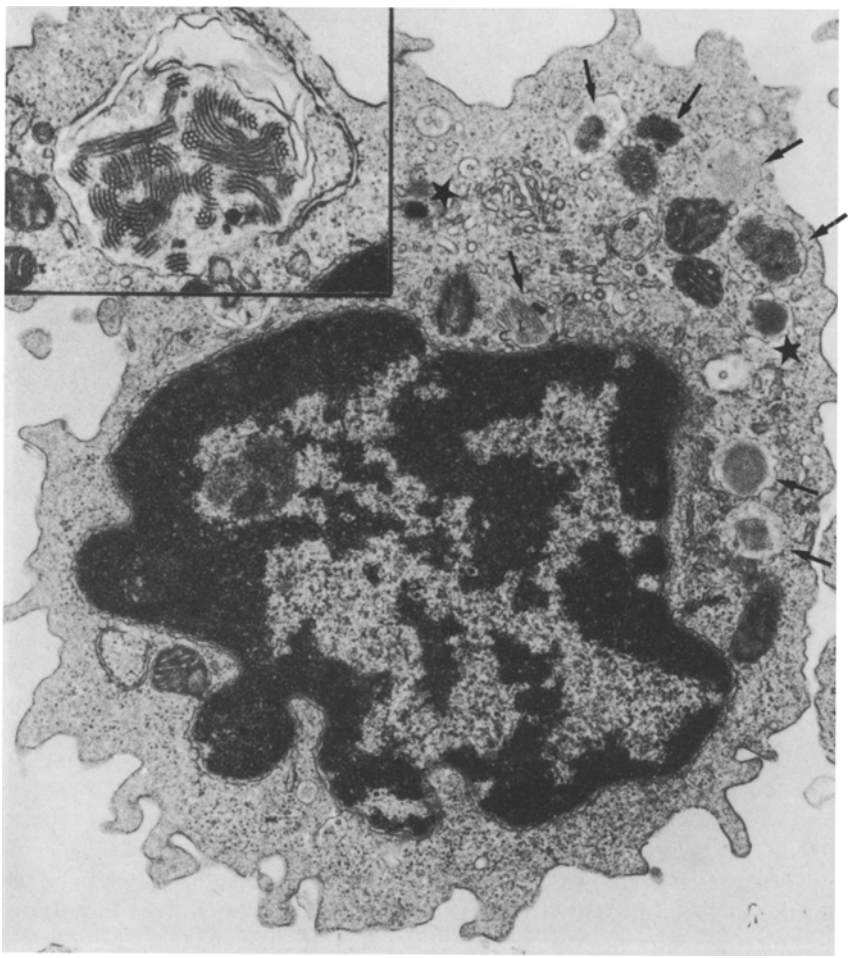

Fig. 2. Ultrastructure of the abnormally expanded cells ×19 500. They are characterized by two types of granules: (a) granules composed of an electron dense core and surrounding granular material (*); (b) granules containing tubular structures (*arrow*) arranged in a parallel array (*PTA*). *Inset* shows a large granule containing parallel tubular arrays (*PTA*); ×26 000

MOLT-4), whereas few or no effector/target cell conjugates were observed with the NK-resistant cell lines (Raji, ChHubl, REH 6) (Table 2). As also depicted in Table 2, the patient's PBL also acted as potent effectors in NK tests against NK-sensitive lines such as K 562, MOLT 4, U 937, CCL 227, and showed ADCC against sensitized BPO-REH 6 cells. To further characterize the cell population active in natural killing towards K 562 cells, PBL were treated either with the HNK-1 or the VEP 13 antibody and rabbit complement. After removal of dead cells, the remainders were tested for cytotoxic capacity against K 562 cells. HNK-1 plus C-treatment caused a 30% decrease of NK-activity, while VEP 13 plus C-treatment resulted in a more than 60% reduction in K 562 lysis.

Table 1. Cell surface markers

	Patient's cells			
Markers	PBMC[a]		BMC[b]	
LGL-morphology	70–90	(10–23)	15	(0–5)
Fc-IgR-R	70–80	(7–14)	ND	
Fc-IgM-R	1	(46–70)	ND	
Lyt-3 (E-R)	83 (weakly)	(75–87)	15 (weakly)	(0–5)
Leu-1	5	(70–85)	10 (weakly)	(0–5)
HNK-1	22–45	(10–18)	5	(0–1)
OKT3	5	(56–88)	9	(0–5)
OKT4 (Leu3a)	5	(36–62)	ND	
OKT8 (Leu2a)	14–26	(17–31)	5	(0–5)
OKT6	1	(0– 5)	1	(0–5)
OKT10	77	(5– 9)	ND	
OKT11 (E-R)	80 (weakly)	(4–15)	2 (weakly)	(0–5)
OKIa1	25 (weakly)	(8–12)	10 (weakly)	(0–1)
OKM1	75–85	(15–30)	3	
VEP13	70 – 80	(13–29)	8	(3–11)
TAC	0	(0– 2)	ND	
VIB-C5 (B cells)	1	(5–11)	ND	

[a] All results are expressed as percentages; normal values (mean ± SD) are given in brackets; results represent the values obtained with peripheral blood mononuclear cell (PBMC) specimens at different times during a 9-month period;

[b] Values from a bone marrow sample obtained in July 1982; BMC = bone marrow mononuclear cells;

ND = not done

Table 2. Profile of target binding capacity, NK and K cytotoxicities of the patient's PBL

Targets	Cell origin	Percent target-binding blood lymphocytes of the patient	Percent lysis of ^{51}Cr-labelled targets at a target: effector cell ratio of 1:10	1:20	1:40
K 562	Erythroleukemia	39 (68 ± 5)[a]	30 (28 ± 7)[a]	36 (28 ± 4)	48 (54 ± 8)
MOLT-4	T	11 (28 ± 4)	10 (16 ± 2)	15 (20 ± 4)	18 (27 ± 4)
Raji	B	6	5	5	5
CHHuBl	Lymphoblastoid cell line (B)	5	5	5	5
U937	Histiocytic	ND	18	20	24
HL60	Myeloid	ND	7	8	8
CCL227	Coloncarcinoma	ND	18	26	33
BPO-REH6	O-cells	ND	5	5	5
BPO-REH6 + anti-BPO	O-cells	ND	43 (23 ± 5)[a]	60 (41 ± 4)	69 (45 ± 3)

[a] In brackets: target cell binding (at 37 °C), NK activity and ADCC of LGL-enriched suspensions after density separation on discontinuous Percoll gradients (65% ± 8% LGL); results represent mean ± SD values of 3 healthy donors (see Materials and Methods in reference [18]

ND = not determined

Table 3. Proliferative response of the patient's PBL[a]

	Medium (day 3)	Con-A (10 μg/ml) (day 3)	PHA (1 μg/ml) (day 3)	MLC[b] (day 6)	MLC[b] (day 8)
Patient	357 ± 213	2102 ± 338	2996 ± 478	37084 ± 3306	80805 ± 3225
Normal donor	434 ± 91	27920 ± 964	23566 ± 3098	81212 ± 4707	41763 ± 1844

[a] Values indicate counts per minute (mean ± SD)
[b] In MLC an allogeneic lymphoblastoid cell line was used for stimulation

Proliferative response to mitogens and alloantigens. As illustrated in Table 3, the patient's PBL were virtually unresponsive to T-cell mitogens, such as Con-A and PHA, whereas stimulation with an allogenic lymphoblastoid cell line resulted in quantitatively normal but markedly delayed ^{3}H-thymidine uptake.

Discussion and review of the literature

This report deals with a rare case of GL-lymphocytosis, in which marked blood lymphocytosis, bone marrow infiltration and splenomegaly without involvement of lymph nodes resembled clinical features observed in cases of T-CLL [19, 27]. In order to classify this disorder, two important questions arise:
1. Do the expanded LGL-like lymphocytes correspond to the normal LGL population as far as morphology, cellular and functional properties are concerned?
2. Does this GL-lymphocytosis represent a reactive or a neoplastic process?

We propose that the proliferating mononuclear cells in the present case represent LGL, i.e. that they exhibit LGL morphology [66] even at the ultrastructural level [26, 66], express cytochemical and membrane markers (VEP 13, OKM 1, Fc-IgG-receptor, HNK-1) of LGL [1, 26] and that these cells are functionally effective in NK and ADCC assays [66]. Similar to normal LGL, the patients lymphocytes share surface markers with myelomonocytic cells (OKM 1, Fc-IgG-receptor) bear markers also present on T cells (E-R, OKT 8) and express a surface antigen (OKT 10) which is normally found on T cells as well as on myelomonocytic cells [40, 47, 53]. Interestingly, the GL of this patient did not express the IL-2 receptor as shown by their nonreactivity with the anti-TAC monoclonal antibody. In accordance with this finding, Gramatzky et al. [20] also demonstrated TAC$^-$ GL in three patients with GL lymphocytosis. In this respect, the expanded cell population resembles resting normal NK cells, which have been shown to express the IL-2 receptor only upon activation [2, 36]. Just as for normal LGL [36], proliferation of the patients GL could not be stimulated by means of T-cell mitogens (PHA, Con-A). A defective mitogenic response in similar lymphoproliferative diseases has been reported [16]. These findings suggest that GL lymphocytosis represents an accumulation of mostly nonproliferating cells rather than an expansion of activated, rapidly proliferating NK cells.

Despite LGL morphology, the bone-marrow lymphocytes exhibit a less-mature phenotype indicated by an increased portion of OKT 3$^+$, OKT 8$^+$, OKIa 1$^+$, OKM 1$^-$ cells [1, 3]. In general, the surface-marker phenotype of the patient's bone

Table 4. Clinical features in 33 patients with GL-lymphoproliferative disorders

Patient	Ref. no.	Age	Sex	Symptoms	RAI[a] stage	Blood cell × 10⁹/l			Hb g/l	Chromosomal abnormalities	Enlarged		skin lesions	survival months D = Death	treatment
						L	G	T			spleen	LN			
1	17	80	M	None	II	10	1,3	no	no	–	+	–	–	12 D	None
2	7	58	M	Infection	0	7	0,3	no	no	–	–	–	+	6 D	None
3	7	67	F	Infection	II	14	0,3	NR	NR	–	+	–	–	240	Splenectomy
4	6	55	M	Infection	II	2	0,05	no	no	–	+	–	–	96	Splenectomy
5	22	48	M	Malaise	II	10	1,3	no	no		+	–	–	24	NR
6	44, 60	41	M	None	0	29	1,5	no	no	–	–	–	–	36	None
7	59	67	M	Infection	II	3	1,3	no	no	–	+	–	–	132	Chlorambucil
8	40	48	M	Icterus	III	6	0,5	no	10	–	+	+[b]	–	NR	Splenectomy
9	26, 52, 53	71	M	Malaise	IV	479	4,8	79	no	NR	+	–	–	8 D	Spleen irradiation
10	21, 23	41	F	Infection	III	12	1,1	no	9	NR	+	–	–	24	Chlorambucil, CVP
11	27	50	F	None	IV	7	0,2	97	9	NR	+	–	–	132	NR
12	61	29	M	Malaise	III	7	1,4	no	7	NR	+	+	+	14 D	Chlorambucil, CP
13	42	83	M	NR	0	18	NR	no	no	NR	–	–	–	31	None
14	42	70	M	NR	I	11	NR	no	no	NR	–	+	–	10	None
15	36	19	M	Infection	II	11	0,2	no	no	NR	+	–	–	60	None
16	36	25	M	Infection	III	34	0,8	no	8	NR	+	–	–	120	Splenectomy, steroids
17	36	58	M	None	II	6	2,1	no	no	NR	+	–	–	8	None
18	36	75	M	Malaise	III	13	1,2	no	10	NR	+/–	–	–	30	Steroids
19	56	42	M	None	0	18	2,8	no	no	–	s[c]	–	–	48	None
20	56	62	M	None	0	8	NR	no	no	–	–	–	–	NR	None
21	10	68	F	RA	III	8	0,5	no	11	NR	+	–	–	NR	P
22	10	43	M	Aphthous stomatitis	III	6	0,4	no	9	NR	–	–	–	13	Spontaneous remission
23	10	28	M	Pyoderma gangrenosum	0	4	0,4	no	no	NR	–	–	–	NR	P
24	10	57	M	RA	III	19	0,4	no	no	NR	+	–	–	48 D	None
25	4	25	M	Malaise	III	8	NR	no	9	hypertriploid	+	+	–	45 D	Steroids, IFN
26	9	56	M	None	III	13	0,6	no	11	NR	–	–	–	NR	NR
27	9	64	M	Malaise	III	11	2,4	no	4	NR	–	–	–	NR	NR
28	35	42	F	Infection	II	10	0	no	NR	–	+	–	–	NR	Splenectomy
29	35	56	M	Infection	IV	10	0	80	NR	47xy, +18	+	–	–	NR	Splenectomy
30	35	88	M	None	IV	4	0,6	60	NR	47xy, +8	+	–	–	NR	NR
31	43	50	F	Malaise	IV	15	NR	90	NR	NR	NR	+	–	44 D	Splenectomy, cytostat.
32	43	52	M	Dyspepsia (gastric mass)	NR	11	NR	NR	NR	NR	+	–	–	84	Splenectomy, steroids Chemotherapy
33	OURS	71	F	Infection	IV	6	1,5	89	no	–	+	–	–	36	Steroids, IFN, VP

Abbreviations: L = leukocytes; G = granulocytes; T = thrombocytes; LN = lymph nodes; NR = not reported; no = normal; C = cyclophosphamide; V = vincristine-sulfate; P = prednisolone; RA = rheumatoid arthritis

[a] Ref. [49]

[b] Parapancreatic lymph nodes

[c] Splenectomized patient (after trauma, 22 years before admission)

Table 5. Hematologic and immunologic features in 33 patients with GL-lymphoproliferative diseases

Patient	Blood lymphocytes				Bone marrow		% Mononuclear blood cells						Function		Serum
	Azurophilic granules	PTA	β-Glu	APh	% Lymphocytes	Maturation arrest	E^+	$T3^+$	$T4^+$	$T8^+$	$(x)^+$	T_G	K	NK	IG
1	+	+	NR	+	45	+	90	93	2	95		NR	+	−	H
2	+	NR	NR	NR	"Moderate"	+	67	NR	NR	NR		51	+	−	NR
3	+	NR	NR	NR	NR	+	87	NR	NR	NR		76	+	−	NR
4	+	NR	NR	+	26 – 31	+	95	79	5	87		41	+	−	H
5	+	NR	NR	+	NR	−	95	95	14	95		93	+	−	NR
6	+	NR	+	+	30	NR	93	98	6	92	2 (OKM1)	57	+	−	NO
7	+ (50%)	——	NR	——	30	NR	40	96	28	72		10	NR	NR	NO
8	+	NR	NR	+	29 – 51	−	11	97	5	96	71 (HNK-1)	68	+	+	NO
9	+	NR	NR	+	"Dense"	NR	48	84	1	95	96 (OKM1)	75	+	+	H
10	+	NR	NR	NR	"Mild"	−	75 – 91	69	24	73	61 OKIa1)	49	−	−	NO
11	+	NR	NR	+	75	−	90 – 98	90	5	5	80 – 90 (HNK-1)	90	−	+	H
12	+	NR	+	+	41	NR	65	2	1	1	86 (OKM1)	3	−	−	
13	+	−	NR	NR	70	NR	97	97	10	85		92	+	+	NR
14	+	−	NR	NR	64	NR	81	79	39	28		17	+	−	NR
15	+	+	+	+	58	−	71	NR	NR	NR		NR	NR	NR	H
16	+	+	+	+	53	−	50	——	NR	——		95	——	NR	H
17	+	+	+	+	22	−	66	——	NR	——		53	——	NR	NR
18	+	+	+	+	61	−	50	——	NR	——		83	——	NR	H
19	+	−	+	+	40	NR	91	91	15	33	40 (HNK-1) 44 (OKM1)	76	+	+	H
20	+	−	+	+	21	−	61	30	18	14	58 (HNK-1) 16 (OKM1)	90	+	+	H
21	+	+	NR	+	26	−	71	79	0	82	1 (OKM1)	30	+	−	H (IgA)
22	+	+	NR	+	37	−	75	99	1	99	1 (OKM 1)	78	+	−	NO
23	+	+	NR	+	33	+	81	80	6	72	10 (OKIa1)	55	+	−	H
24	+	+	NR	+	52	+	84	74	10	68	1 (OKM1)	64	+	NR	H
25	+	+	+	+	"Scarcely"	NR	85	NR			14 (HNK-1)	NR	NR	NR	H
26	NR	NR	NR	NR	30	+	83[a]	99[a]	4[a]	95[a]		90	NR	NR	NR
27	NR	NR	NR	NR	60	−	80[a]	NR	13[a]	77[a]		81	NR	NR	NR

Patient	Blood lymphocytes				Bone marrow		% Mononuclear blood cells						Function		Serum
	Azurophilic granules	PTA	β-Glu	APh	% Lymphocytes	Maturation arrest	E^+	$T3^+$	$T4^+$	$T8^+$	$(x)^+$	T_G	K	NK	IG
28	+	NR	NR	+	"Focal infiltration"	−	85	89	7	78	53 (HNK-1)	6	−	+	H
29	+	NR	NR	+	"Focal infiltration"	+	59	97	19	80	67 (HNK-1)	2	−	+	H
30	+	NR	NR	+	NR	−	72	83	24	42	29 (HNK-1)	13	−	−	H
31	+	NR	+	+	28	NR	82	73	5	20	3 (HNK-1) 76 (OKM1)	94	−	+	NO
32	+	NR	+	+	60	NR	80	84	8	88	80 (HNK-1) 7 (OKM1)	76	+	+	Hypogammaglobulinemia
33	+	+	+	+	15	+	83	5	5	19	70 – 80 (VEP13) 75 – 85 (OKM1)	80	+	+	H

Abbreviations: PTA = parallel tubular arrays; β-Glu = β-glucuronidase; APh = acid phosphatase (resistant to L-tartrate); (×) monoclonal antibody in brackets; K = antibody-dependent cellular cytotoxicity (ADCC); NK = natural killing; IG = immunoglobulins; NR = not reported; H = hyperimmunoglobulinemia;

[a] Percentage cells in E^+ fraction

marrow GL is consistent with that of normal LGL in bone-marrow tissue [3]. Finally, our patient showed no lymph-node enlargement, but involvement of peripheral blood, spleen and bone marrow, which are exactly the organs in which most LGL and NK activity are localized in normal individuals [5]. For a survey of clinical, hematologic and immunologic features from 33 patients with GL-lymphoproliferative disorders, see Tables 4 and 5.

In healthy controls, a close relationship between the stage of maturation and differentiation of LGL and their cytotoxic capacity in NK and ADCC assays was demonstrated [1, 3, 47]. This finding might explain the different results in GL-lymphocytoses when NK and ADCC was tested. Only in a minority of patients did the cells exert NK function, whereas ADCC was detectable in most cases (Table 5). Functionally, three types of GL-lymphocytoses have been previously described: (1) Expansions of OKT 3$^+$, OKM$^-$, E-R$^+$, FcIgGR$^+$ lymphocytes with strong ADCC, but without NK activity [9, 21, 46, 51], (2) GL-lymphocytoses of OKT 3$^+$, OKM 1$^+$, E-R$^+$, Fc-IgG-R$^+$ cells with normal NK and ADCC activity [21, 55], and (3) patients, like ours, with OKT 3$^-$, OKM 1$^+$, E-R$^+$, Fc-Ig-GR$^+$ cells, which exert the strongest NK and ADCC activity [21]. In one study, NK activity could only be demonstrated following incubation of the GL over night at 37°C or after incubation of the cells in the presence of β-interferon [29]. It is, therefore, possible that many of the GL-lymphocytoses, besides exerting ADCC, have NK activity upon activation.

Neutropenia and hypergammaglobulinemia, frequently seen in GL-lymphocytosis, might be secondary due to regulatory effects of the GL population. Although hypergammaglobulinemia could be explained by recurrent infections concomitant with severe granulocytopenia, even patients without clinically apparent infections were found to exhibit polyclonal hypergammaglobulinemia. As LGL can suppress B-cell differentiation and antibody production [10, 65] and also efficiently produce interferons [30], which have been shown to inhibit humoral immune response [60], the expansion of an immature or functionally defective population could give way to polyclonal secretion of immunoglobulins. In this respect, a defect in mitogen-induced interferon production (alpha and gamma interferon) was demonstrated in our patient (R. Berger, Heidelberg, personal communication). Impaired suppression of immunoglobulin production and defective gamma-IFN production in GL-lymphocytosis has also been reported by other investigators [37, 43]. A variety of mechanisms have been discussed which might cause neutropenia in GL-lymphocytosis: autoantibodies against granulocytes (acting directly or via ADCC) [9, 37, 61], or a direct inhibitory effect on myelopoiesis was found in 7 of 18 similar cases, as indicated in Table 5.

To characterize this disorder, our case should be compared to cases of GL-lymphocytosis recently reported. During the last decade, several reports on patients with abnormally expanded GL populations have been published (see Tables 4 and 5; [11, 15, 37, 39, 45]). Such clinical entities with markedly increased numbers of lymphoid cells strongly resembling LGL, have been given variety of designations, for example, "chronic T-cell lymphocytosis", "abnormal expansion of LGL", "chronic lymphoproliferative disorder", "T-cell proliferations", "T-CLL", "T-gamma proliferations", "neutropenia with excess of lymphocytes", and "T-gamma lymphoproliferative disease." Since LGL have previously been described as large lymphocytes with abundant cytoplasm [66], it is important to point our that in some of these disorders, as in our case, not only LGL-like cells, but also consistent populations of small to medium-sized GL were observed.

The majority of reports summarized in Tables 4 and 5 describe clinical and hematological entities very similar to that found in our patient: often asymptomatic disease, splenomegaly without lymphadenopathy, moderate to severe granulocytopenia and recurrent infections as the major complaints. Except for patient 9, only moderate lymphocytosis was found in peripheral blood and bone marrow. Just as in our patient, in the majority of cases, GL-lymphocytosis was persistent (range: 8 months – 20 years) without compelling evidence of malignant origin, such as cytogenetic abnormalities or enzymatic markers for monoclonality [9, 13, 15, 24, 25, 42]. As the expanded GLs in these disease states display some T-cell-associated antigens (E-R, OKT 8), a clear distinction should be made between this disease and expansions of T lymphocytes, strikingly demonstrating a neoplastic nature, such as T-cell-derived lymphoblastic lymphoma, T-cell ALL, the human T-cell leukemia/lymphoma, virus-associated adult T-cell leukemia (ATL) and cutaneous T-cell lymphoma (CTCL). In contrast to these T-cell malignancies, the prognosis of GL-lymphocytoses seems quite favorable. Most patients do not require specific therapy, although alkylating agents, steroids or splenectomy may effectively reduce the lymphocyte count and improve neutropenia. Some authors have included GL-lymphocytosis in the domain of T-CLL [9, 27, 33, 59], since similar cases have been reported to terminate in blast crisis [27, 48] and, very recently, non-random chromosome abnormalities were demonstrated in lymphocytes from individual patients [4, 37]. Recently, the presence of T-cell receptor gene rearrangement could be demonstrated in two out of four patients with chronic suppressor T-cell lymphocytosis and neutropenia, using cDNA probes that were specific for the β-chain of the human T-cell receptor [6, 8]. Although it is probable that most such rearrangements signify clinical malignancy, a reactive basis should not be excluded until a broad spectrum of benign T-cell diseases has been analyzed.

When considered as a T-CLL variant, GL-lymphocytosis was termed "azurophilic" T-cell CLL [32, 59] and distinguished from the "knobby-type" T-cell CLL of Levine et al. [33] and the ATL exemplified by the Japanese cases [68]. Most characteristic, the phenotype of the "knobby" T-cell CLL and ATL cases was OKT 11$^+$, OKT 4$^+$, while "azurophilic" T-cell CLL was described as predominantly OKT 11$^+$, OKT 8$^+$ [32, 59]. The validity of this classification scheme for T-cell CLL is strongly supported by the difference of the three forms in their clinical findings and prognoses. Most of the patients with "azurophilic" T-cell CLL exhibit nonprogressive disease, whereas overall survival for "knobby" T-cell CLL and ATL is 12 months and 4.5 months, respectively [32, 59].

From the patients listed in Table 4 and 5, nos. 2 and 24 died of causes unrelated to their lymphoproliferative disease and only in nos. 9 and 12 was death clearly related to the hematological disease and/or treatment modalities. The latter patients showed particular symptoms in comparison to the other cases with a rather stable course: in patient 9, a high-grade lymphocytosis was seen which decreased rapidly after spleen irradiation (total dose 125 rads), but the patient died of disseminated intravascular coagulation 5 days after the last irradiation [54]. In patient 12, a chlorambucil-induced remission was followed by rapid progression to a "diffuse lymphoma of medium-sized cell type" with increasing resistance to therapy. Before therapy was begun, the lymphocyte surface markers in this patient suggested the expansion of a rather undifferentiated LGL-like cell (T 3$^-$, T 8$^-$, E-R$^+$, FcIgGR$^-$) lacking the capacities for NK and ADCC [63]. From these reports we conclude that at least GL-expansions with high-grade lymphocytosis and/or a phenotypically and/or functionally immature cell

type (or with chromosomal and/or enzymatic markers for monoclonality) should be classified as malignant.

However, certain findings in GL-lymphocytoses also favor the view that a considerable portion of patients suffer from a reactive rather than a neoplastic disease: phenotypical heterogeneity in some of the expanded cell populations, presence of chronic inflammatory processes in a considerable proportion of patients, confirmed by serologic and histologic tests [58], occurrence of spontaneous remission in one patient [12], and concomitant malignancies in some of the cases reported [13, 15, 43, 45, 62, 64].

Previous clinical observations support this view, namely the leukemoid reactions involving "granular lymphocytes" in some patients with infectious diseases [15, 17, 56, 58] or autoimmune states [35] and high numbers of NK cells as well as increased NK activity associated with malignancies [34, 64]. In our case, several findings lead us to assume that our patient exhibits a reactive rather than a neoplastic expansion of LGL: (1) the heterogeneity of the expanded-cell population, which is mirrored by the various immunological phenotypes in blood and bone marrow; (2) the absence of a marker for monoclonality; (3) the fact that the patient had suffered from breast cancer despite any current indications of a relapse; and (4) the persistence of an inflammatory pulmonary infiltration.

Finally, as in the case of our patient, there are no conclusive arguments to assume a malignant expansion of GL in the majority of cases of GL-lymphocytosis yet published. It remains a matter of speculation whether the generally benign course of GL-lymphocytosis represents a polyclonal hyperimmune response to a yet unknown agent or whether it reflects a self-control mechanism whereby the outgrowth of (an) undifferentiated GL clone (s) is prohibited or retarded by more mature GLs preserving their function as NK and/or killer (ADCC) cells in vivo [41]. We propose that until obvious malignant features are documented, the term "GL-lymphocytosis" be applied to this hematologic disorder and treatment be confined to symptomatic measures.

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References

1. Abo T, Cooper MD, Balch CM (1982) Characterization of HNK-1$^+$ (Leu-7) human lymphocytes. I. Two distinct phenotypes of human NK cells with different cytotoxic capability. J Immunol 129: 1752–1757
2. Abo T, Miller CA, Balch CM, Cooper MD (1983) Interleukin 2 receptor expression by activated HNK-1$^+$ granular lymphocytes: a requirement for their proliferation. J Immunol 131: 1822–1826
3. Abo T, Miller CA, Gartland GL, Balch CM (1983) Differentiation stages of human natural killer cells in lymphoid tissues from fetal to adult life. J Exp Med 157: 273–284
4. Amagasaki T, Shibata J, Yao E, Nishino K, Yamada Y, Sadamori N, Tomonaga M, Kinoshita K, Ichimaru M (1985) A lymphoproliferative disorder of T cells with the phenotype of cytotoxic/suppressor T cell. Am J Hematol 19: 85–92
5. Antonelli P, Stewart W, Dupont B (1981) Distribution of natural killer cell activity in peripheral blood, cord blood, thymus, lymph nodes and spleen, and the effect of in vitro treatment with interferon preparation. Clin Immunol Immunopathol 19: 161–169
6. Aisenberg AC, Krontiris TG, Mak TW, Wilkes BM (1985) Rearrangement of the gene for the β-chain of the T-cell receptor in T-cell chronic lymphocytic leukemia and related disorder. N Engl J Med 313: 529–533

7. Aisenberg AC, Wilkes BM, Harris NL, Ault KA, Carey RW (1981) Chronic T cell lymphocytosis with neutropenia: report of a case studied with monoclonal antibody. Blood 58: 818–822
8. Bertness V, Kirsch I, Hollis G, Johnson B, Bunn PA (1985) T-cell receptor gene rearrangements as clinical markers of human T-cell lymphomas. N Engl J Med 313: 534–538
9. Bom-Van Noorloos AA, Pegels HG, Van Oers RHJ, Silberbusch J, Feltkamp-Vroom M, Goodsmit R, Zeijlemaker WP, von dem Borne AEG, Melief CSM (1980) Proliferation of T cells with killer-cell activity in two patients with neutropenia and recurrent infections. N Engl J Med 302: 933–937
10. Brieva JA, Targan S, Stevens R (1984) NK and T cell subsets regulate antibody production by human in vivo antigen-induced lymphoblastoid B-cells. J Immunol 132: 611–615
11. Callard RE, Smith CM, Worman C, Linch D, Cawley JC, Beverly PC (1981) Unusual phenotype and function of an expanded subpopulation of T cells in patients with haemopoietic disorders. Clin Exp Immunol 43: 497–505
12. Chan WC, Check I, Brynes RK, Kateley J, Winton EF (1984) A morphologic and immunologic study of the large granular lymphocyte in neutropenia with T lymphocytosis. Blood 63: 1133–1140
13. Dallapicolla B, Alimena G, Chessa L, Gastaldi R, De Rossi G, Semenzato G, Quinti I, Pandolfi F (1984) Chromosome studies in patients with T-CLL chronic lymphocytic leukemia and expansion of granular lymphocytes. Int J Cancer 34: 171–176
14. Degliantoni G, Perussia B, Mangoni L, Trinchieri G (1985) Inhibition of bone marrow colony formation by human natural killer cell-derived colony-inhibiting activity. J Exp Med 161: 1152–1168
15. Ferrarini M, Romagnani S, Montesoro E, Zicca A, Del Prete GF, Nocera A, Maggi E, Leprini A, Grossi CE (1983) A lymphoproliferative disorder of the large granular lymphocytes with natural killer activity. J Clin Immunol 3: 30–35
16. Foa R, Catovsky D, Incarbone E, Cherchi M, Wechsler A, Luss P, Fierro M, Giubellino MM, Bernengo MG, Semenzato G (1982) Chronic T-cell leukemia. III. T-colonies, PHA-response and correlation with membrane phenotype Leuk Res 6: 809–822
17. Gardner FH, Mettier SR (1949) Lymphocytic leukemoid reaction of the blood associated with miliary tuberculosis. Blood 4: 767–771
18. Gastl G, Schmalzl F, Huhn D, Gattringer C, Huber C (1983) Large granular lymphocytes: morphological and functional properties. I. Results in normals. Blut 46: 297–310
19. Geisler C, Ralfkiaer E, Astrup L, Christensen I, Dickmeiss E, Hansen MM, Larsen JK, Petersen J, Plesner T (1983) Chronic lymphocytic leukemia of T cell origin. Clinical variation possibly due to involvement of different T lymphocyte subpopulations. Scand J Haematol 31: 109–201
20. Gramatzky M, Pandolfi F, Maples J, DeRossi G, Semenzalo G, Quinti I, Bonnard G, Stron DM (1985) Cultured T cells from patients with T cell chronic lymphocytic leukemia demonstrate a normal phenotype. Immunobiology 169: 186–197
21. Griend RJ, Bolhuis RLH (1985) In vitro expansion and analysis of cloned cytotoxic T cells derived from patients with chronic T-lymphoproliferative disorders. Blood 65: 1002–1009
22. Hansson M, Beran M, Anderson B, Kiessling R (1982) Inhibition of in vitro granulopoiesis by autologous allogeneic human NK cells. J Immunol 129: 126–132
23. Hocking WG, Singh R, Schroff R, Golde D (1983) Cell mediated inhibition of erythropoiesis and megaloblastic anemia in T-cell chronic lymphocytic leukemia. Cancer 51: 631–636
24. Hocks JJ, Haynes BF, Detrich Hooks B, Diehl LF, Gerard TL, Fauci AS (1982) Gamma(immune) interferon production by leukocytes from a patient with T_G cell proliferative disease. Blood 59: 198–201
25. Hofmann FM, Smith D, Hocking W (1982) T cell chronic lymphocytic leukemia with suppressor phenotype. Clin Exp Immunol 49: 401–409
26. Huhn D, Huber C, Gastl G (1982) Large granular lymphocytes: morphological studies. Eur J Immunol 12: 985–988
27. Huhn D, Rodt H, Thiel E (1976) T-Zell Leukämien des Erwachsenen. Blut 33: 141–160
28. Huhn D, Thiel E, Rodt H, Schlimok G, Themel H, Riebner P (1983) Subtypes of T-cell chronic lymphatic leukemia. Cancer 51: 1434–1447

29. Itoh K, Tsuchikawa K, Awataguchi T, Shiiba K, Kumagai K (1983) A case of chronic lymphocytic leukemia with properties characteristic of natural killer cells. Blood 61: 940–948
30. Kasahara T, Djeu JY, Dougherty SF, Oppenheim JJ (1983) Capacity of human large granular lymphocytes (LGL) to produce multiple lymphokines: interleukin 2, interferon, and colony stimulating factor. J Immunol 131: 2379–2385
31. Knapp W, Majdic O, Rumpold H (1981) Myeloid differentiation antigens as defined by monoclonal antibodies. In: Knapp W (ed) Leukemia markers. Academic Press, London New York, pp 207–223
32. Lennert K, Stein H, Feller AC, Gerdes J (1981) Morphology cytochemistry, and immunobiology of T cell lymphomas. In: Vitetta ES (ed) B- and T cell tumors. Academic, Orlando, p 9–15
33. Levine AM, Meyer PR, Lukes RJ, Teinstein DI (1981) Clinical and morphological heterogeneity of T-cell chronic lymphocytic leukemia (T-CLL). Blood 58: 144–152
34. Ligler FS, Patel M, Strayer D, Brodsky I, Bonner H, Juhani Linna T (1983) Extremely high levels of natural killer cells in angioimmunoblastic lymphadenopathy. J Clin Immunol 3: 375–381
35. Linch DC, Newland AC, Turnbull AL, Knott LS, MacWhannel A, Beverly PCL (1984) Unusual T-cell proliferations and neutropenia in rheumatic arthritis: comparison with classical Felty's syndrome. Scand J Haematol 33: 342–350
36. London L, Perussia B, Trinchieri G (1985) Induction of proliferation in vitro of resting human natural killer cells: expression of surface activation antigens. J Immunol 134: 718–729
37. Loughran Jr TP, Kadin ME, Starkebaum G, Abkowitz JL, Clark EA, Disteche C, Lum LG, Slichter SJ (1985) Leukemia of large granular lymphocytes: Association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. Ann Intern Med 102: 169–175
38. Mc Kenna RW, Parkin J, Kersey JH, Gajl-Peczalska KJ, Peterson LA, Brunning RD (1977) Chronic lymphoproliferative disorder with unusual clinical morphologic, ultrastructural and membrane surface marker characteristics. Am J Med 62: 588–596
39. Newstand AC, Catovsky D, Linch D, Cawley JC, Beverly P, San Miguel JF, Gordon-Smith EC, Blecher TE, Shariari S, Varadi S (1984) Chronic T cell lymphocytosis: a review of 21 cases. Br J Haematol 58: 433–446
40. Ortaldo JR, Sharrow SO, Timonen T, Herberman RB (1981) Determination of surface antigens on highly purified human NK cells by flow cytometry with monoclonal antibodies. J Immunol 127: 2401–2409
41. Oshimi K, Oshimi Y, Yamada Y, Mizoguchi H (1985) Lysis of lymphoma cells by autologus and allogeneic natural killer cells. Blood 65: 638–643
42. Palutke M, Eisenberg L, Kaplan J, Hussain M, Kithier K, Tabaczka P, Mirchandani I, Tenenbaum D (1983) Natural killer and suppressor T-cell chronic lymphocytic leukemia. Blood 62: 627–634
43. Pandolfi F, Capobianchi M, Matricardi P, Facchini J, Bonomo G, DeRossi G, Semenzato G, Fiorilli M, Dianzani F, Aiuti F (1984) Impaired gamma interferon production by cells from patients with lymphoproliferative disorders of mature T and NK cells. Scand J Immunol 21: 315–320
44. Pandolfi F, De Rossi G, Semenzato G, Quinti I, Ranucci A, De Sanctis G, Lopez M, Gasparotto G, Aiuti F (1983) Immunologic evaluation of T chronic lymphocytic leukemia cells: correlations among phenotype, functional activities, and morphology. Blood 59: 688–695
45. Pandolfi F, Pezutto A, De Rossi G, Pasqualetti D, Semenzato G, Quinti I, Ranucci A, Raimondi R, Basso G, Strong DM, Fontana L, Aiuti F (1984) Characterization of two patients with lymphomas of the large granular lymphocytes. Cancer 53: 445–452
46. Pandolfi F, Strong DM, Slease RB, Smith ML, Ortaldo JR, Herberman RB (1980) Characterization of a suppressor T-cell chronic lymphocytic leukemia with ADCC but no NK activity. Blood 56: 653–660
47. Perussia B, Trinchieri G, Jackson A, Warner NL, Faust J, Rumpold H, Kraft D, Lanier LL (1984) The Fc receptor for IgG on human natural killer cells: phenotypic, functional and comparative studies using monoclonal antibodies. J Immunol 133: 180–185
48. Preud'homme JL, Brouet JC (1980) Chronic lymphocytic leukemia of T cell origin. In: Zittoun R (ed). Les hemopathies lymphoides chroniques. Masson, Paris, pp 23–35

49. Rai KR, Sawitskiy A, Cronkite EP, Chand AD, Levy RN, Pasternak BS (1975) Clinical staging of chronic lymphocytic leukemia. Blood 46: 419–424
50. Reinherz EL, Schlossman SF (1980) The differentiation and function of human T lymphocytes (review). Cell 19: 821–827
51. Rümke HC, Miedema F, Ten Berge RJM, Terpstra F, van der Reijden HJ, Van de Gried RJ, de Bruin HG, von dem Borne AEGKr, Smit JW, Zeijlemaker WP, Melief CJM (1982) Functional properties of T cells in patients with chronic T_G-lymphocytosis and chronic T cell neoplasia. J Immunol 129: 419–426
52. Rumpold H, Kraft D, Obexer G, Böck G, Gebhart W (1982) A monoclonal antibody against a surface antigen shared by human large granular lymphocytes and granulocytes. J Immunol 192: 1458–1464
53. Sanchez-Madrid F, Nagy J, Robbins E, Simon P, Springer T (1983) A human leukocyte differentiation antigen family with distinct α-subunits and a common β subunit: the lymphocyte function associated antigen (LFA-I), the C3bi complement receptor (OKM1/Mac-1) and the p 150.95 molecule. J Exp Med 158: 1785–1798
54. Schlimok G, Thiel E, Rieber EP, Huhn D, Feucht H, Renner D, Riethmüller G (1982) Proliferation of T_G-lymphocytes in two patients: Clinical features and functional properties of the proliferating cells. Klin Wochenschr 60: 133–142
55. Schlimok G, Thiel E, Riebner EP, Huhn D, Feucht H, Lohmeyer J, Riethmüller G (1982) Chronic leukemia with a hybrid surface phenotype (T lymphocytic/myelomonocytic): leukemic cells displaying natural killer cell activity and antibody-dependent cellular cytotoxicity. Blood 59: 1157–1162
56. Schmalzl F, Braunsteiner H (1970) The cytochemistry of monocytes and macrophages. Semin Hematol III/2: 93–102
57. Schuler G, Romani N, Fritsch PO (1982) Differential lectin binding to mammalian keratinocytes and melanocytes in vitro. Eur J Cell Biol 27: 88–95
58. Semenzato G, Pizzolo G, Ranuzzi A, Agostini C, Chilosi M, Quinti I, De Sanctis G, Vercelli B, Pandolfi F (1984) Abnormal expansion of polyclonal large to small size granular lymphocytes: reactive or neoplastic process? Blood 63: 1271–1277
59. Simpkins H, Kiprov DD, Davis JL III, Morand P, Puri S, Grahn EP (1985) T cell chronic lymphocytic leukemia with lymphocytes of unusual immunologic phenotype and function. Blood 65: 127–133
60. Sonnenfeld G, Mandel AD, Merigan TC (1977) The immunosuppressive effect of type II interferon preparations on antibody production. Cell Immunol 34: 193–206
61. Starkebaum G, Martin PJ, Singer JW, Lum LG, Price TH, Kadin ME, Raskind WH, Fialkow PJ (1983) Chronic lymphocytosis with neutropenia: evidence for a novel, abnormal T-cell population associated with antibody-mediated neutrophil destruction. Clin Immunol Immunopathol 27: 110–123
62. Strong DM, Pandolfi F, Slease RB, Budd JE, Woody JN (1981) Antigenic characterization of a T-CLL with heteroantisera and monoclonal antibodies: evidence for the T cell lineage of an Ia-positive, Fc-IgG-positive, suppressor-cell subpopulation. J Immunol 126: 2205–2208
63. Tagawa S, Konishi I, Karatune H, Katagiri S, Taniguchi N, Tamaki T, Inove R, Kanayama Y, Tsubakio T, Machii T, Yonezawa T, Kitani T (1983) A case of T-cell chronic lymphocytic leukemia (T-CLL) expressing a peculiar phenotype (E^+, OKM1$^+$, Leu1$^+$, OKT3$^-$, and IgG EA$^-$). Cancer 52: 1378–1384
64. Taylor HG, Terebelo HR, Gamez A (1983) Lymphocytosis in a patient with malignant fibrous histiocytoma. Cancer 50: 1563–1569
65. Tilden AB, Abo T, Balch C (1983) Suppressor cell function of human granular lymphocytes identified by the HNK-1 (Leu7) monoclonal antibody. J Immunol 130: 1171–1175
66. Timonen T, Ortaldo JR, Herberman RB (1979) Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. J Exp Med 153: 569–582
67. Uchiyama T, Groder S, Waldmann TA (1981) A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. J Immunol 126: 1393–1398
68. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H (1977) Adult T-cell leukemia: Clinical and hematologic features of 16 cases. Blood 50: 481–490

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