short report
Haematologica 1995; 80:146-149

MONOCLONAL EXPANSION OF LARGE GRANULAR LYMPHOCYTES WITH A CD4$^+$ CD8dim$^{+/-}$ PHENOTYPE ASSOCIATED WITH HAIRY CELL LEUKEMIA

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ABSTRACT

Peripheral blood lymphoid cell expansions with an unusual CD3$^+$, CD4$^+$, CD8dim$^{+/-}$, CD11b$^+$, CD57$^+$ immunophenotype have recently been reported. They frequently have the morphology of large granular lymphocytes (LGL) and can be either monoclonal or polyclonal. Their significance is still unclear and no association with hematological neoplasms has been described. We report the case of a patient with a monoclonal expansion of LGL associated with a B-cell-derived hairy cell leukemia. The two lymphoid clones were not physically associated since T-LGL were found in the peripheral blood and hairy cells were detected in the bone marrow and kidney.

Key words: large granular lymphocytes, CD4$^+$ CD8$^+$ lymphocytes, NK cells, T-cell receptor, hairy cell leukemia

Expansions of large granular lymphocytes (LGL) are immunologically heterogeneous;[1,2] rare cases with a CD4$^+$CD8$^+$ phenotype have been described. Recently, two series of patients with a distinct abnormality characterized by persistent expansion of CD4$^+$ lymphocytes partially coexpressing CD8 at low density (dim) as well as LGL-associated (LGLa: CD11b, CD56, CD57) markers have been reported.[3,4] TCR genotypic studies revealed both polyclonal and monoclonal expansions; no association with other lymphoid malignancies was described.

We describe here a patient who presented with a monoclonal expansion of LGL with a CD3$^+$, CD4$^+$, CD8dim$^{+/-}$, LGLa$^+$ phenotype, associated with a classical B-cell-derived hairy cell leukemia (HCL).

Case report

T.A., a 66-year-old male, presented with macrocytic anemia and neutropenia. On admission, hepatomegaly was found. Laboratory tests showed: hemoglobin 9.2 g/dL, MCV 114 fL, reticulocytes 0.2%, WBC count 2.28×10^9/L with neutrophils 18%, lymphocytes 79%, monocytes 0%, eosinophils 2%, basophils 0%, ESR 132 mm/h, creatinine 1.8 mg/dL with a clearance of 36 mL/min and microscopic hematuria.

Cytological evaluation showed that 82% of peripheral blood lymphocytes had LGL morphology, whereas 60% of marrow cells were medium-sized lymphoid cells with *hairy* cytoplasmic projections, no azurophilic granules and tartrate-resistant acid phosphatase. The patient underwent bone marrow and kidney biopsy.

Morphological, immunophenotypic and genotypic results demonstrated the presence of two separate lymphoid clones.

One clone, present in the peripheral blood but not in the bone marrow and kidney (Table 1), consisted of CD4$^+$ T LGL, the majority of which coexpressed LGLa markers (CD11b, CD57, CD8dim). Southern Blot analysis on

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Acknowledgments: the authors wish to thank Dr. A. Rambaldi for performing genotypic studies and Mrs. L. Salvi for performing immunohistochemical analysis.
Received July 19, 1994; accepted January 2, 1995.

Table 1. Immunological studies. Data from the phenotypic analysis are expressed as % of positive cells. The cytotoxic activity of unfractionated PBMC either unstimulated or after activation with rIL-2 (1000 U/mL for 72h)[@] was studied against the NK-sensitive K562 and NK-resistant Daudi cell lines at different effector/target (E/T) ratios. Data are expressed as percentage of specific lysis.

Immunophenotypical analysis

Monoclonal antibody	*Total PBL (CD4+ cells)*	*Bone marrow*	*Kidney*
CD1a	< 1	n.a.	n.a.
CD3	88	< 5 (f)[#]	n.a.
TCR α/β	83	n.a.	n.a.
TCR γ/δ	< 1	n.a.	n.a.
CD2	95	n.a.	n.a.
CD5	91	n.a.	n.a.
CD7	42	n.a.	n.a.
CD4	76	n.a.	n.a.
CD8	42 (23dim)*	n.a.	n.a.
CD11b	58 (62)	n.a.	n.a.
CD16	3 (0)	n.a.	n.a.
CD57	56 (60)	< 1 (p)	< 1 (p)
CD19	3	50-80 (f)	n.a.
CD20	3	50-80 (f,p)	50 (p)
HLA-DR	25	n.a.	n.a.
CD25	2 (1)	50-80 (f)	n.a.
CD11c	2	50-80 (f)	n.a.
HML-1	< 1	50-80 (f)	n.a.
DBA.44	n.a.	50-80 (p)	50 (p)
κ light chain	n.a.	< 1(f)	n.a.
λ light chain	n.a.	50-80 (f)	n.a.
CD43	n.a.	< 5 (p)	50 (p)
CD45RO	27 (30)	< 5 (p)	50 (p)
CD45RA	71 (68)	n.a.	n.a

Cytotoxic activity analysis

Target cells	*E/T*	*Patient*	*Healthy controls (3)*
K562	100 : 1	35%	34-73%
	30 : 1	16%	17-55%
Daudi	100 : 1	16%	8-18%
	30 : 1	3%	1-8%
Daudi[@]	100 : 1	57%	55-85%
	30 : 1	23%	27-71%

*data between parentheses refer to $CD4^+$ cells, as evaluated by two-color immunofluorescence; dim: diminished expression. [#]f: frozen section; p: paraffin section; n.a. not analyzed.

peripheral blood lymphocytes after DNA digestion with the restriction enzymes EcoRi and HindIII showed a biallelic rearranged configuration of the TCRβ chain gene, and demonstrated the monoclonal nature of this T-cell population.

The second neoplastic cell population consisted of a classical HCL, demonstrated by morphological and immunohistochemical analysis of bone marrow that showed diffuse reticulin fibrosis and infiltration of hairy cells of B-lymphocyte origin expressing DBA.44 (Figure 1a) and other HCL-associated markers (CD25, CD11c, HML-1), with λ light chain restriction. No HCL cells were detected in peripheral blood.

Renal biopsy showed nephroangiosclerosis and interstitial nephritis; about 50% of the lymphoid cells in the kidney biopsy exhibited morphological and phenotypical features of hairy leukemic cells (Figure 1b).

There were no associated immune abnormalities except for polyclonal hypergammaglobulinemia and weak positivity of antinuclear antibodies (1/40). Proliferative response to mitogens (not shown) was normal; likewise, spontaneous cytotoxicity against NK-sensitive cell lines and IL-2-activated cytotoxicity against NK-resistant cell lines were observed (Table 1).

The patient was treated with subcutaneous α2a interferon 3 MU daily, but this was discontinued after eight months because of severe depression. At this time 30% of PBL were LGL, the percentage of $CD4^+$ lymphocytes with $CD8^+$dim coexpression had decreased to 10%, and marrow infiltration by hairy cells was still greater than 50%. Hematological parameters were as follows: hemoglobin 11.5 g/dL, WBC 2.82×10^9/L with 54% neutrophils, platelets 67×10^9/L. Both the WBC and platelet counts normalized one month after discontinuation of IFN.

Discussion

Proliferating LGL exhibit a rather heterogeneous phenotype. To date, this phenotypic heterogeneity has not been clearly correlated with relevant clinical and biological findings.[1,2] Two series of patients with persistent expansions of $CD4^+CD8^+$ lymphocytes immunophenotypically identical to our case have recently been reported.[3,4] The rarity of this phenotype is documented by the Yorkshire Leukemia Group study, in which it was observed in less than 2%

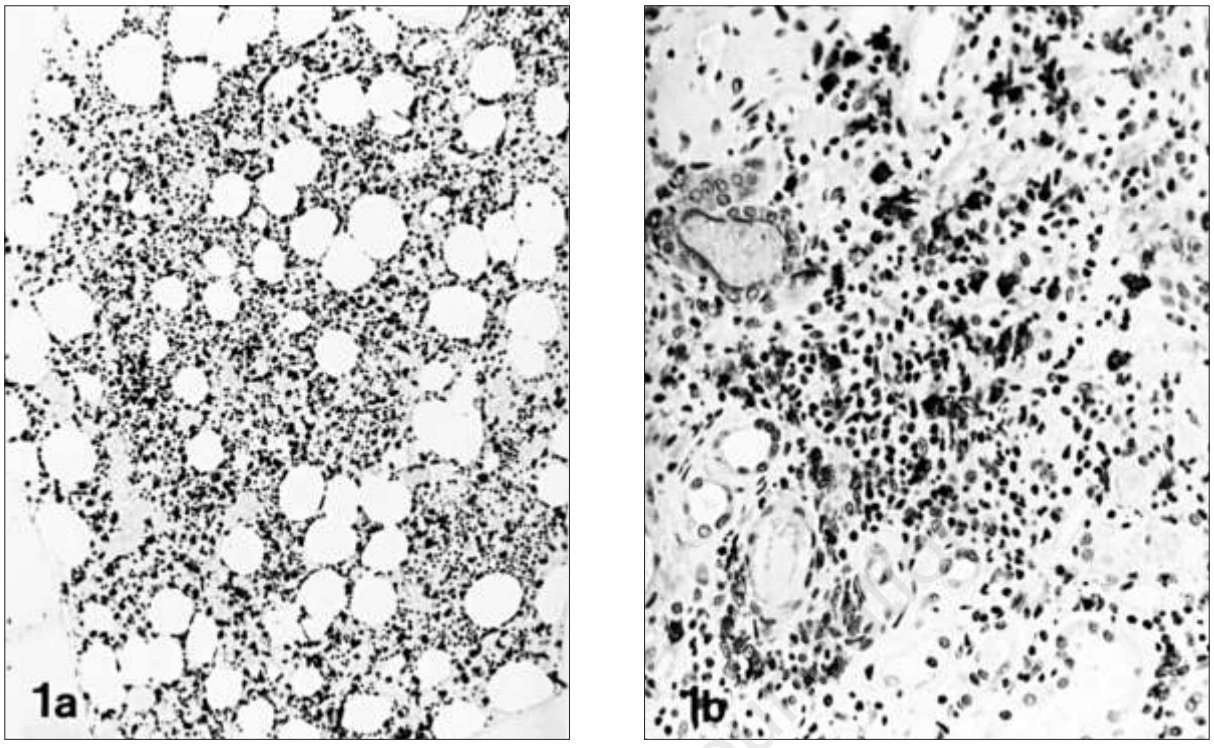

Figure 1. Bone marrow (1a) and kidney (1b) biopsies stained with DBA.44 recognizing hairy leukemia cells. Positive cells in the bone marrow show a typical interstitial distribution. Cell size and nuclear morphology of positive cells are better recognized in the kidney biopsy. (Streptavidin-Biotin complex immunoperoxidase technique for DBA.44; original magnification: x110, 1a; x250, 1b).

of patients with lymphocytosis, an increased proportion of LGL and/or neutropenia.[3] About 50% of the expansions of $CD4^+CD8^+$ cells previously described had LGL morphology. Monoclonal, oligoclonal and polyclonal cases were observed; none was associated with B-cell disorders. The existence of close relationships between autoimmunity, autoimmune diseases and lymphoproliferative disorders is well established.[5] It has recently been suggested that $CD3^+CD4^+$ LGL proliferations are less frequently associated with neutropenia and autoimmune diseases than those with a $CD3^+CD4^-$ phenotype.[6]

The origin and function of the $CD4^+$ $CD8dim^{+/-}$ $LGLa^+$ population is undetermined. In the present study normal response to mitogens, as well as NK and LAK activity were observed. Given that the functional assays were performed using unfractionated PBMC populations, residual normal cells could have been responsible for the normal results observed; however, the number of *true* NK cells ($CD16^+$) was extremely low, and therefore it seems likely that $CD4^+$ LGL did contribute to the NK and LAK activity observed.

Expansions of $CD3^+$ LGL can be found in association with other hematological malignancies,[7] including HCL.[8] Since LGL expansions and HCL are relatively rare entities, their association (which may even be underestimated considering that in the cases described the total lymphocyte count was within normal limits) might be not casual and suggests the possibility of some interrelationship between the two cell populations. Clonal T cell populations have been described in patients with B-cell malignancies,[9] and it has been hypothesized that they are reactive in nature and may be involved in the control of B-cell proliferation. Moreover, it has been reported that LGL from B-CLL patients are able to inhibit normal B-cell proliferation.[10] In the present case the somewhat indolent course of the HCL (absence of leukemization and splenomegaly; no evolution during 6 months of observation before the start of interferon treatment) may

indirectly support this hypothesis; it is noteworthy, however, that the two neoplastic cell populations were not physically associated in the bone marrow and kidney.

In conclusion, we thought it worthwile to report this case as the first description of the association of an expansion of LGL with a $CD4^+$ $CD8dim^{+/-}$ $LGLa^+$ phenotype and B-cell HCL. This might be useful for further delineating the correlation (or lack thereof) between the phenotype of proliferating LGL and clinical or biological findings.

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