CONCISE REPORT

Rearrangement and Expression of T-Cell Receptor Genes in Large Granular Lymphocyte Leukemia

By Thomas P. Loughran, Jr, Gordon Starkebaum, and Joseph A. Aprile

Ten patients with large granular lymphocyte (LGL) leukemia were studied for rearrangement and expression of T cell receptor (TCR) genes. Eight patients with CD3+ LGL proliferation had unique TCR β-gene rearrangements, supporting a clonal process. Each of five patients studied with CD3+ disease had evidence for expression of full-length TCR α-, β-, and γ-gene transcripts. In contrast, patients with CD3− LGL proliferation had no evidence for rearrangement or expression of TCR genes. These studies suggest that leukemic LGL arise from two different cell origins. Leukemic LGL may be a useful model for studying natural killer (NK) cell (CD3− LGL) and non-MHC-restricted cytotoxic T lymphocyte (CD3+ LGL) activation and differentiation.

INITIAL PHENOTYPIC studies of large granular lymphocytes (LGL) concluded that the surface antigen phenotype of these cells was heterogeneous.[1] However, it is now clear that LGL can be divided into two discrete subsets: CD3− LGL and CD3+ LGL.[2] CD3− LGL, comprising approximately 10% of peripheral blood mononuclear cells (PBMC), do not rearrange T cell receptor (TCR) genes, supporting a non-T lineage for these cells.[3,4] CD3+ LGL, constituting approximately 2% of PBMC, are of T lineage, since they rearrange TCR genes.[5] Both LGL subsets mediate non-MHC-restricted cytotoxicity. Because of their phenotypic and molecular features, it has been proposed that these two subsets be recognized as discrete cell types: NK cells (CD3− LGL) and non-MHC-restricted cytotoxic T lymphocytes (CD3+ LGL).[2]

LGL leukemia results from a chronic proliferation of LGL that is associated with chronic neutropenia and rheumatoid arthritis.[6] Leukemic LGL may be either CD3− or CD3+, suggesting that neoplastic cells may represent proliferation of either of the two normal cell types. In this study we examined rearrangement and expression of TCR genes in cells from patients with LGL leukemia. Our results show that leukemic LGL have molecular features similar to their CD3− and CD3+ normal counterparts.

MATERIALS AND METHODS

Patients. All patients met clinical criteria for diagnosis of LGL leukemia.[7] LGL counts varied from 3,300 to 20,000/μL (normal: 223 ± 99 cells/μL, n = 10). Previous publications have detailed the clinical course of patients 1, 2, 5, and 8,[6,8] lymphocyte surface markers for patients 1 through 5 and 7,[9] and TCR β-gene rearrangement studies for patients 1, 2, and 8.[8] Patients 1 through 8 had CD3+ LGL disorder (reactivity of PBMC with anti-CD3 monoclonal antibody [MoAb] ranging from 78% to 99%), whereas patients 9 and 10 had CD3− disease (27% and 16% of PBMC reacting with anti-CD3 MoAb, respectively).

Blot hybridization analyses. Genomic DNA was extracted from PBMC as described[8] and was digested with restriction enzymes *Bam*HI, *Eco*RI, or *Hind*III. Digested DNA was separated on 1.1% agarose gels and transferred onto nitrocellulose filter by method of Southern.[10] RNA was extracted from PBMC in the presence of guanidine thiocyanate, size fractionated by agarose/formaldehyde gel electrophoresis, and transferred onto nitrocellulose filter.[11] Filters were then hybridized to DNA probes ^{32}P-labeled by nick translation and visualized by autoradiography.[8]

The cDNA clones for TCR α, β, and γ genes were kindly provided by Dr Tak Mak (Ontario Cancer Institute, Toronto, Canada). Clone pY14 contains a full length copy of TCR α gene cDNA,[12] clone Jurkat β_2 contains the constant and joining regions of TCR β gene,[13] and the clone for TCRγ gene contains a full length copy of TCRγ gene cDNA.[14] The β-actin cDNA probe[15] was kindly provided by Dr Steven Collins (Fred Hutchinson Cancer Research Center, Seattle, WA).

RESULTS

Results of Southern blot hybridization analyses using TCR β gene probe are shown in Fig 1. Analysis of DNA after digestion with *Bam*HI or *Hind*III showed unique rearrangements of TCR β gene in each of eight patients with CD3+ disease. In contrast, germ-line pattern was observed in patients 9 and 10 with CD3− LGL leukemia using both these enzymes (Fig 1) as well as *Eco*RI (not shown). The rearrangement pattern for patient 1 after *Bam*HI analysis differs somewhat from that previously published[8] in that an additional faint band at 6 kilobase (kb) was noted. This finding has been confirmed in two subsequent experiments. TCRα-gene rearrangement was noted in one patient (CD3+ LGL leukemia); another patient (also with CD3+ disease) had rearrangement of TCRγ gene (not shown). The inability to detect TCRα- and γ-gene rearrangement in the other CD3+ patients may result from such rearrangements using different joining regions occurring over large distances of DNA.[16,17] Detection of these rearrangements might require use of multiple joining region probes and/or pulsed-field–gradient-gel electrophoresis.[16,17] Alternatively, one can examine for TCR messenger RNA (mRNA; see below), since expression of full-length TCR gene transcripts is evidence of prior rearrangement of TCR genes.

From the Fred Hutchinson Cancer Research Center, the Division of Rheumatology, Department of Medicine, University of Washington School of Medicine; and the Veterans Administration Medical Center, Seattle.

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Address reprint request to Thomas P. Loughran, Jr, MD, Fred Hutchinson Cancer Research Center, 1124 Columbia St, Seattle, WA 98104.

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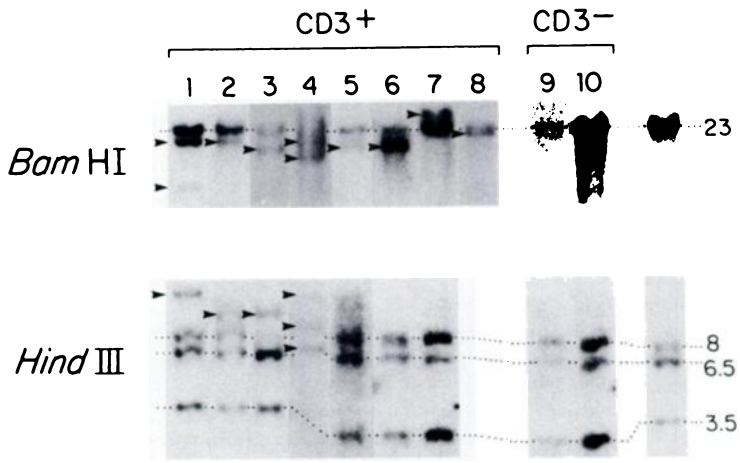

Fig 1. Results of Southern blot hydridization analyses using TCRβ-gene probe showing rearrangement patterns after digestion with *Bam*HI or *Hind*III. Lanes 1 through 8 represent CD3+ LGL leukemia patients, lanes 9 and 10 CD3− LGL leukemia patients, and lane 11 the germ-line pattern obtained when analyzing DNA extracted from neutrophils of a normal volunteer. Arrowheads indicate position of rearranged bands. The gels are not aligned because the studies were carried out at different times. Sizes of rearranged fragments are as follows. For *Bam*HI: patient 1, 16 kb and 6 kb; patient 2, 16 kb; patient 3, 11 kb; patient 4, 16 kb and 9 kb; patient 5, 12 kb; patient 6, 8 kb; patient 7, 25 kb; and for patient 8, 20 kb. For *Hind*III analysis: patient 1, 22 kb; patient 2, 14 kb; patient 3, 14 kb; and for patient 4, 23 kb, 9 kb, and 6.8 kb.

Results of Northern blot hybridization analyses using TCRα-, β-, and γ-gene probes are shown in Fig 2. Expression of full-length transcripts for TCRα-, β-, and γ-genes was seen in three patients with CD3+ LGL leukemia (similar results were observed in the other two patients examined with CD3+ disease, not shown). Two of five CD3+ LGL leukemia patients also expressed a 1.0-kb TCR β-gene transcript. In contrast, in the one CD3− patient examined, there was no expression of TCR α-, β-, or γ-gene transcripts. This was not due to a lack of RNA loaded in the gel, as shown by hybridization to β-actin probe.

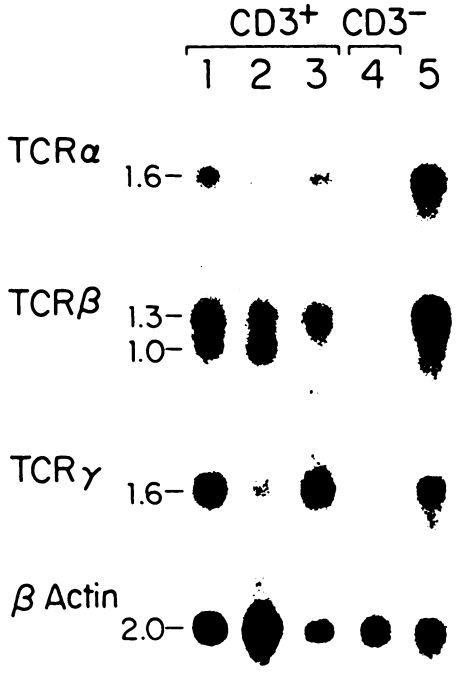

Fig 2. Results of Northern blot hybridization analyses using TCR gene probes and β-actin gene probe. Lanes 1 through 3 represent CD3+ patients (lane 1, patient 6; lane 2, patient 2; and lane 3, patient 5). Lane 4 represents patient 10 with CD3− LGL leukemia, and lane 5 represents RNA extracted from tumor cell line HPB-ALL, used as control.

DISCUSSION

These results show that CD3− and CD3+ leukemic LGL have molecular characteristics that suggest different cell origins. Previous TCRβ-gene rearrangement studies have shown germ-line configuration of TCRβ gene in all five patients studied with CD3− LGL leukemia,[18-20] whereas 19 of 23 patients with CD3+ disease had TCRβ-gene rearrangement.[18-22] Our studies confirm and extend those findings by demonstrating germ-line configuration of TCR α, β, and γ genes in two patients and absence of expression of these gene transcripts in one patient examined with CD3− LGL leukemia. These results are similar to those seen in normal CD3− LGL (NK cells), supporting a non-T lineage for these cells.[3-5]

In contrast, we found expression of TCR α-, β, and γ-gene transcripts in cells from patients with CD3+ LGL leukemia. Similar findings of expression of TCR α-, β-, and γ-gene transcripts have been noted in cloned murine Thy-1+ LGL cell lines.[23] We had previously shown that these CD3+ leukemic LGL may exhibit non-MHC-restricted cytotoxicity.[9] Some recent evidence suggests that TCRγ-protein may serve as a functional receptor involved in non-MHC-restricted lysis.[24] The precise role of expression of TCRγ-gene transcripts in CD3+ leukemic LGL awaits further study.

Rearrangement of TCRβ gene could be used as evidence for clonality or, alternatively, might reflect a stage of normal LGL differentiation. For example, one similar TCRβ-gene rearrangement has been observed in murine LGL cell lines,[23] suggesting that the similar rearrangement might be associated with a differentiation stage. In contrast, our finding of unique TCRβ-gene rearrangements in each of eight patients with CD3+ LGL leukemia supports the clonal nature of this disorder.[6] Our studies support a non-T cell origin for CD3− leukemic LGL and a T cell origin for the CD3+ leukemic LGL. Therefore leukemic LGL may be useful for studying activation of two different classes of cytotoxic lymphocytes: NK cells and non-MHC-restricted cytotoxic T lymphocytes.

NOTE ADDED IN PROOF

Similar molecular findings for CD3− and CD3+ leukemic LGL have been described by Pelicci et al.[25]

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