Published in final edited form as:

Leuk Res. 2010 February ; 34(2): 203. doi:10.1016/j.leukres.2009.06.031.

A novel mouse model for the aggressive variant of NK cell and T cell large granular lymphocyte leukemia

Akihiko Yokohama^{1,2}, Anjali Mishra¹, Takeki Mitsui^{1,2}, Brian Becknell³, Jessica Johns¹, Douglas Curphey¹, Bradley W. Blaser^{4,5}, Jeffrey B. VanDeusen⁵, Hsiaoyn Mao¹, Jianhua Yu¹, and Michael A. Caligiuri^{1,6}

¹Department of Molecular Virology, Immunology, and Medical Genetics, Department of Internal Medicine, Division of Hematology/Oncology, The Ohio State University (OSU) College of Medicine, Gunma University, Maebashi, Japan

²Department of Medicine and Clinical Science, Graduate School of Medicine, Gunma University, Maebashi, Japan

³OSU Department of Pediatrics, Boston MA

⁴The Brigham and Women's Hospital, Boston MA

⁵OSU Integrated Biomedical Sciences Graduate Program, Columbus, OH 43210

⁶The OSU Comprehensive Cancer Center, The James Cancer Hospital and Solove Research Institute, Columbus, OH 43210

Abstract

Murine models of disease are vital to the understanding of pathogenesis and the development of novel therapeutics. We have previously established interleukin (IL)-15 transgenic (tg) mice that demonstrate rapid proliferation of natural killer (NK) and T cells, followed by spontaneous transformation to lethal leukemia. Herein, we have characterized this model, which has many features in common with the aggressive variants of NK and T large granular lymphocyte leukemia (LGLL) in humans. The LGLL blasts are cytolytic and produce IFN- γ *ex vivo*. Cytogenetic analysis revealed trisomy of chromosome 17 and/or 15. This model should provide opportunities to develop effective standard therapies for this fatal disease.

Keywords

Natural killer cell; NK and T large granular lymphocyte leukemia; IL-15; protooncogene

© 2009 Published by Elsevier Ltd.

Address Correspondence to: Michael A. Caligiuri, MD. The Ohio State University, 300 W. 10th Avenue, Suite 521C, Columbus, OH 43210. Phone: (614) 293-7523, FAX: (614)-293-3132, Email: michael.caligiuri@osumc.edu.
Akihiko Yokohama and Anjali Mishra contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors do not have any conflict of interest to declare.

Introduction

Large granular lymphocyte leukemia (LGLL) is a clonal disorder of the NK cell or T cell lineage that presents clinically as indolent or aggressive disease.¹ Aggressive forms of LGLL are often associated with very poor prognosis, with most patients exhibiting rapidly progressive disease that is poorly responsive to multi-agent chemotherapy.² Aggressive variants of human NK cell and T LGLL are characterized by an immunophenotype that includes surface expression of CD56 along with a variable surface expression pattern of NK cell and/or T cell markers.^{3,4}

Since the aggressive variants of LGLL are rare and difficult to manage, rigorous studies are required to identify mechanisms of pathogenesis and opportunities for therapeutic intervention.⁵ We previously showed that IL-15tg mice display early expansions of peripheral blood lymphocytes, specifically NK cells and memory phenotype CD8⁺ T cells. Later, IL-15tg mice develop a striking leukemic expansion with multi-organ infiltration.^{6,7}

In the current study, we describe a murine model of aggressive NK and T LGLL that may eventually provide an opportunity to test and validate new therapeutic advances. We have systematically characterized 39 murine leukemia cases - phenotypically, functionally and cytogenetically - to validate this clinically relevant model of aggressive NK and T LGLL.

Materials and Methods

Mice and *in vivo* studies

IL-15tg mice were generated and maintained as described previously.⁶ Age and sex matched SCID mice were purchased from The Jackson Laboratory (Bar Harbor, ME). To study *in vivo* transplantability of these leukemia cells, 10⁵ leukemia cells from peripheral blood, spleen or bone marrow of moribund mice were intravenously injected into sub lethally (4.5 Gy) irradiated SCID recipient mice. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of The Ohio State University.

Antibody staining and flow cytometry

Peripheral blood, spleen and bone marrow samples were harvested from moribund mice and single cell suspensions were prepared as described previously.⁶ The following fluorochrome-conjugated monoclonal antibodies (mAb) were purchased from BD Pharmingen, San Jose, CA and used for flow cytometry: anti-CD3 (clone 145-2C11), anti-NK1.1 (clone PK136), anti-CD49b, alpha 2 integrin (clone DX5), anti-CD4 (clone RM4-5), anti-CD8a (clone 53-6.7), anti-CD11b (clone M1/70), anti-CD69 (clone H1.2F3), anti-CD122 (clone TM-β1), anti-CD62L (clone MEL-14) and anti- TCR-β (clone H57-597).

Spectral karyotyping (SKY)

SKY analysis was performed on metaphase spreads from spleen samples of leukemia mice using standard procedures as previously described.⁸ SKY images were acquired with COOL-1300 SpectraCube imaging system (Applied Spectral Imaging, Vista, CA) using SKY optical filter (Chroma Technology Corp, Rockingham, VT). For each leukemic mouse sample approximately 20 metaphases were analyzed. Analysis was performed using the SKYView® EXPO v2.1.1 software (Applied Spectral Imaging).

Fluorescent *in situ* hybridization (FISH)

FISH probes specific for chromosomal regions 15E2 and 17B1 were prepared from purified BAC clones RP23-34303 and RP24-271E12 (BACPAC Resources, Children's Hospital Oakland Research Institute, Oakland, CA), respectively. Probes were labeled using standard

nick translation with either SpectrumGreen or SpectrumOrange (Abbott Molecular Inc, Des Plaines, IL). Interphase slides were prepared from spleen samples of leukemic mice. Hybridization and signal detection were performed using standard techniques as described previously.⁹ Interphase nuclei from each leukemic sample were acquired using COOL-1300 SpectraCube camera (Applied Spectral Imaging). Slides were scored and analyzed using FISHView software (version 4.0; Applied Spectral Imaging). FISH signals were scored for ≥ 200 interphase nuclei.

Cytotoxicity and IFN- γ production in aggressive NK cell and T cell LGLL

For cytotoxicity assays, fresh peripheral blood leukocytes (90% NK or T LGLL blasts by flow cytometry) were harvested from symptomatic mice and used as effector cells against ⁵¹Cr-labeled YAC-1 cells as previously described.⁶ For IFN- γ ELISA, whole spleen cells from NK or T LGLL mice were stimulated with 100 ng/ml of recombinant murine IL-18 (R&D Systems, Minneapolis, MN) and 10 ng/ml of recombinant murine IL-12 (R&D Systems). After stimulation for 16 hours, supernatant was evaluated for the release of soluble IFN- γ by ELISA (R&D Systems).

Statistics

All leukemia survival curves were calculated by the Kaplan–Meier method, using the GraphPad Prism software (GraphPad Software Inc., San Diego, USA). All other data were analyzed by unpaired t-test using the same software.

Results

Immunophenotyping identifies both NK and T LGLL

The leukemia identified in the IL-15tg mouse was initially described as a fatal T-NK lymphocytic leukemia which followed a marked leukocytosis in an unknown fraction of the mice.^{6,7} In the current study, we first performed a complete immunophenotypic characterization of the leukemic blasts from the spleens of 39 mice moribund with leukemia (which occurred in approximately 30% of IL-15tg mice). This analysis identified NK1.1 and CD122 as common surface markers in 100% of both NK and T LGLL (Figure 1A and Table 1). CD122 is also referred to as the IL-2 or IL-15 receptor beta chain and is encoded by the gene *Il2rb* in mice. Approximately 50% of these leukemia were CD3⁺ (20 out of 39 leukemia mice tested), and were therefore classified as T LGLL. All the T LGLL cases, with the exception of mouse number 39, exhibited expression of TCR- β . Whereas a CD8⁺ T cell lymphocytosis occurred in all IL-15tg mice, only four of 20 cases (20%) of T LGLL were CD8⁺, and none expressed CD4 (Table 1). All cases of NK LGLL consistently demonstrated the NK1.1⁺CD122⁺CD3[−] phenotype (Figure 1A and Table 1). Expression of the integrin subunit, CD49b, was observed in 15 out of 19 NK LGLL cases and 19 out of 20 T LGLL cases. Comparable phenotypic data was also obtained from bone marrow and peripheral blood consistent with a uniformity of the NK and T LGLL phenotypes in different organs.

CD69+CD62L^{lo} provide a unique phenotype of blast cells in all NK and T LGLL

Leukemic mice were also analyzed for the expression of two antigens, CD69 and CD62L, which are early activation and naïve cell markers, respectively.^{10,11} In striking contrast to the absence of CD69 expression in NK cells and T cells from wild type mice, NK and T LGLL leukemic mice showed significant expression of this activation marker on the blast population (Figure 1B). NK and T LGLL also had absent to low expression of CD62L, unlike the majority of wild type NK cells and T cells that express relatively high expression of CD62L. Therefore, in contrast to wild type NK cells and T cells, LGLL display higher expression of CD69 and lower expression of CD62L.

We next performed Wright-Giemsa staining of the LGLL on peripheral blood from leukemic mice which showed a predominance of blasts (Figure 2A), all exhibiting a large granular lymphoblast morphology (Figure 2B). Thereafter, we investigated the extent of leukemic infiltration by both histology (Figure 2C) and by flow cytometric analysis (data not shown). Both revealed evidence of massive infiltrations of leukemic blasts throughout. Approximately 90% of the bone marrow and spleen lymphocyte gated population were NK1.1+ blasts in both NK and T LGLL.

Identification of chromosomal aberrations in NK and T LGLL

Spectral karyotype (SKY) analysis of two independently derived primary NK and T LGLL revealed trisomy 15 and 17 in each case (Figure 3A and Supplementary data - Table 1 and 2). Interphase fluorescence *in situ* hybridization (FISH) analysis of seven independent cases of NK and T LGLL revealed occurrence of trisomy 17 in every case, and trisomy 15 was noted as an additional aberrant finding in five out of the seven leukemias studied (Figure 3B and Supplementary data - Table 1 and 2).

Leukemic blasts are capable of killing target cells and produce IFN- γ

Functional assays were performed to ascertain if NK and T LGLL blasts retain their LGL properties. We tested peripheral blood mononuclear cells (PBMCs) from leukemic mice (containing approximately 90% blasts) directly in cytotoxicity assays against ^{51}Cr labeled YAC-1 target cells. Blast cells from both NK and T LGLL were highly cytotoxic *ex vivo*, killing with > 45% efficiency at an effector to target (E:T) ratio of only 6.25:1, and doing so without additional *in vitro* cytokines stimulation (Figure 4A). In addition, we also tested the ability of NK and T LGLL blasts to secrete IFN- γ upon stimulation with two monokines. We used the combination of IL-12 and IL-18 since these cytokines have been known to demonstrate synergistic effect in inducing the production of IFN- γ by both murine and human NK cells. We found that both NK and T LGLL blasts secrete high levels of IFN- γ in response to monokine stimulation (Figure 4B). Thus, both NK and T LGLL blasts display the functional characteristics of mature LGL.

NK1.1+ cells transplanted from leukemia mice rapidly develop lethal leukemia

Finally, we plotted the survival curve for IL-15tg mice that spontaneously develop primary NK or T LGLL. Our data indicate that the leukemic mice have a median survival age of approximately 28 weeks (Figure 5A). We also transplanted $\sim 10^5$ NK or T LGLL blasts from spleen, bone marrow or peripheral blood of leukemic mice to sub-lethally irradiated SCID mice to evaluate the capacity of blasts to reconstitute leukemia. Indeed, these animals developed a lethal leukemia with a median latency of approximately 70 days (Figure 5B). Flow cytometric analysis of bone marrow, peripheral blood, and spleen cells of recipient animals demonstrated the immunophenotype of the primary leukemia (data not shown). This data confirms the aggressive nature of the NK and T LGLL *in vivo*.

Discussion

In this report, we have described a novel mouse model for the development of aggressive NK and T LGLL arising in the setting of chronic inflammation driven by constitutive over-expression of IL-15. Previous studies have identified IL-15 as a critical cytokine for the development and homeostasis of NK, NKT cells and memory CD8+ T-cells^{18–20}. In IL-15tg mice, the dramatic expansion of CD8(+) T and NK cells from birth gives rise to a very aggressive form of NK and T LGLL in approximately 30% of cases. Interestingly, approximately half of IL-15tg mice with this aggressive LGLL have the NK phenotype and the other half have the T cell phenotype, with virtually all T cell cases displaying the NK antigen NK1.1. All leukemias that were analyzed also had CD62L^{lo}CD69⁺ phenotype as found in

human LGLL,^{12,13} along with functional attributes often attributed to their human counterparts as noted below.^{14,15} Currently we do not have a clear understanding of the key molecular events that lead to the development of LGLL in IL-15tg mice, regardless of the cell type.

Previous studies indicate that human NK and T LGLL blasts retain the functional properties and immunophenotype of mature LGL, namely: (1) surface expression of CD56;16–18 (2) natural cytotoxicity towards tumor and virus infected cells¹⁹; and (3) production of inflammatory cytokines such as IFN- γ .²⁰ In this report, we have identified NK1.1 and the IL-15 receptor β chain, CD122, as common surface markers on all the NK and T LGLL arising in the IL-15tg mice. Additionally, these leukemias demonstrate spontaneous cytotoxicity toward YAC-1 targets and production of IFN- γ in response to monokine co-stimulation. Thus, these findings in our mouse model are consistent with human LGLL.^{14,15}

Remarkably, cytogenetic studies of human LGLL show few common recurrent chromosomal abnormalities associated with the disease.^{1,3,5} Our cytogenetic data demonstrate that trisomy 17 is a common abnormality in both NK and T LGLL arising in IL-15tg mice. Trisomy of chromosome 17 in murine leukemia is rare, and its occurrence in 100% of the NK and T LGLL examined in this study may attest to a pivotal role in leukemogenesis. It should be noted human chromosome 21 shows conserved syntenies to mouse chromosome 17 and as such shares a significant fraction of gene triplicates that are located on chromosome 21 in Down syndrome, where there is an increased incidence of acute myeloid and lymphoblastic leukemia. Thus it is conceivable, if not probable that the same extra copy of gene(s) on chromosome 21 in humans that are responsible for the acute leukemia in Down syndrome are contained within the mouse chromosome 17.^{21,22} Trisomy 15 is also found with variable frequency in murine models of acute myeloid leukemia (AML) and acute lymphoblastic leukemia, including those arising in the setting of the PML-RARA gene fusion, gamma irradiation, and Rauscher murine leukemia virus infection.^{23,24} Interestingly, trisomy of human chromosome 8, syntenic to mouse 15, is the most common single chromosomal aberration in both the myelodysplastic syndrome (MDS) and AML in humans, and has also been reported in human NK cell malignancies.²⁵ Spira and co-workers have hypothesized that trisomy is an early event in leukemogenesis which leads to increased oncogene dosage and thereby contributes to malignant transformation.²⁶ However, it remains to be determined if these recurrent chromosomal aberrations truly contribute to the onset or progression of LGLL in humans or in our animal model.

The utilization of a mouse with constitutive over-expression of the pro-inflammatory cytokine IL-15 to generate a model of the aggressive variant of NK or T LGLL is not in itself irrelevant to the human counterpart of this disorder. In many instances, the human NK or T LGLL cell lines that have been derived from patients with this disorder are dependent on either IL-15 or IL-2 *in vitro*, as both of these cytokines use the same β and γ_c receptor components to transmit their activation signals to the target cell.^{14,15,27} This *in vitro* dependence on IL-15 or IL-2 suggests comparable dependency on either cytokine *in vivo*, rather than an activating mutation in their receptor components that would render the cell line cytokine independent. Indeed, Waldmann and colleagues have designed and implemented an anti-IL-2/15R β mAb trial to treat T LGLL.²⁸ Finally, there is good evidence that IL-15 is critical for the development of human and murine NK cells and hepatic-derived NKT cells in mice, and for the homeostatic maintenance of NK/T-NK and CD8(+) memory T cells.²⁹ Collectively, with the evidence provided in this report, it appears that increased expression of IL-15 directly contributes to the malignant transformation of either NK cells, T-NK cells, T cells or their common precursor.

In summary, we have characterized a mouse model of spontaneous NK and T LGLL arising in the setting of increased expression of IL-15. This murine model most closely resembles the aggressive variant of human LGLL of the NK cell or T cell type, and may therefore prove to

be useful in order to develop novel treatment strategies for this rare disease that currently has no standard form of beneficial therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Pamela J Swiatek of the Van Andel Research Institute for her extensive help with FISH and REB experiments and helpful discussions. We also thank Michelle M. Le Beau of the University of Chicago Cancer Research Center for assistance with early mouse cytogenetics. We acknowledge the assistance of the following shared resources of The Ohio State University Comprehensive Cancer Center: Mouse Pathology, Flow Cytometry, Microscopy, and Statistics.

This work is supported by National Cancer Institute grants CA16058, CA95426, and CA68458 (M.A. Caligiuri).

References

1. Nakamura F, et al. Acute lymphoblastic leukemia/lymphoblastic lymphoma of natural killer (NK) lineage: quest for another NK-lineage neoplasm. *Blood* 1997;89:4665–4666. [PubMed: 9192799]
2. Alekshun TJ, Tao J, Sokol L. Aggressive T-cell large granular lymphocyte leukemia: a case report and review of the literature. *Am J Hematol* 2007;82:481–485. [PubMed: 17205534]
3. Matano S, Terahata S, Nakamura S, Kobayashi K, Sugimoto T. CD56-positive acute lymphoblastic leukemia. *Acta Haematol* 2005;114:160–163. [PubMed: 16227680]
4. Gentile T, et al. CD3+, CD56+ aggressive variant of large granular lymphocyte leukemia. *Blood* 1994;84:2315–2321. [PubMed: 7522625]
5. Johansson B, Mertens F, Mitelman F. Clinical and biological importance of cytogenetic abnormalities in childhood and adult acute lymphoblastic leukemia. *Ann Med* 2004;36:492–503. [PubMed: 15513300]
6. Fehniger TA, et al. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. *J Exp Med* 2001;193:219–231. [PubMed: 11208862]
7. Fehniger TA, et al. Fatal Leukemia in Interleukin-15 Transgenic Mice. *Blood Cells, Molecules, and Diseases* 2001;27:223–230.
8. Le Beau MM, Bitts S, Davis EM, Kogan SC. Recurring chromosomal abnormalities in leukemia in PML-RARA transgenic mice parallel human acute promyelocytic leukemia. *Blood* 2002;99:2985–2991. [PubMed: 11929790]
9. Graveel C, et al. Activating Met mutations produce unique tumor profiles in mice with selective duplication of the mutant allele. *Proc Natl Acad Sci U S A* 2004;101:17198–17203. [PubMed: 15557554]
10. Li XY, Matsuzaki G, Yoshikai Y, Muramori K, Nomoto K. T cells expressing both L-selectin and CD44 molecules increase in number in peritoneal exudate cells and in vitro-stimulated spleen cells from mice immunized intraperitoneally with *Listeria monocytogenes*. *Immunology* 1993;78:28–34. [PubMed: 7679661]
11. Natarajan K, Sawicki MW, Margulies DH, Mariuzza RA. Crystal structure of human CD69: a C-type lectin-like activation marker of hematopoietic cells. *Biochemistry* 2000;39:14779–14786. [PubMed: 11101293]
12. Yang J, et al. Antigen activation and impaired Fas-induced death-inducing signaling complex formation in T-large-granular lymphocyte leukemia. *Blood* 2008;111:1610–1616. [PubMed: 17993614]
13. Lima M, et al. TCRalpha β + / CD4+ large granular lymphocytosis: a new clonal T-cell lymphoproliferative disorder. *Am J Pathol* 2003;163:763–771. [PubMed: 12875995]
14. Robertson MJ, et al. Characterization of a cell line, NKL, derived from an aggressive human natural killer cell leukemia. *Exp Hematol* 1996;24:406–415. [PubMed: 8599969]

15. Gong JH, Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. *Leukemia* 1994;8:652–658. [PubMed: 8152260]
16. Kingreen D, Siegert W. Chronic lymphatic leukemias of T and NK cell type. *Leukemia* 1997;11:S46–S49. [PubMed: 9178839]
17. Prieto J, et al. Leukaemia of natural killer cell large granular lymphocyte type with HLADR-CD16-CD56bright+ phenotype. *J Clin Pathol* 1996;49:1011–1013. [PubMed: 9038741]
18. Macon WR, et al. Natural killer-like T-cell lymphomas: aggressive lymphomas of T-large granular lymphocytes. *Blood* 1996;87:1474–1483. [PubMed: 8608238]
19. Ohno T, et al. Fulminant clonal expansion of large granular lymphocytes. Characterization of their morphology, phenotype, genotype, and function. *Cancer* 1988;62:1918–1927. [PubMed: 3262409]
20. Itoh K, Tsuchikawa K, Awataguchi T, Shiiba K, Kumagai K. A case of chronic lymphocytic leukemia with properties characteristic of natural killer cells. *Blood* 1983;61:940–948. [PubMed: 6187398]
21. Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. *Blood* 2009;113:2619–2628. [PubMed: 19139078]
22. Hattori M, et al. The DNA sequence of human chromosome 21. *Nature* 2000;405:311–319. [PubMed: 10830953]
23. Herbst EW, Gropp A. Relevance of trisomy 15 and other chromosome abnormalities in spontaneous AKR leukemia of mice with and without Robertsonian rearrangement. *J Cancer Res Clin Oncol* 1982;104:207–218. [PubMed: 7161311]
24. Herbst EW, et al. Rauscher leukemia in the mouse in trisomy of chromosome 19. *Verh Dtsch Ges Pathol* 1982;66:122–127. [PubMed: 7184232]
25. Paulsson K, Johansson B. Trisomy 8 as the sole chromosomal aberration in acute myeloid leukemia and myelodysplastic syndromes. *Pathol Biol (Paris)* 2007;55:37–48. [PubMed: 16697122]
26. Spira J, Wiener F, Ohno S, Klein G. Is trisomy cause or consequence of murine T cell leukemia development? Studies on Robertsonian translocation mice. *Proc Natl Acad Sci U S A* 1979;76:6619–6621. [PubMed: 316545]
27. Di Noto R, et al. Characterization of two novel cell lines, DERL-2 (CD56+/CD3+/Tcr γ 5+) and DERL-7 (CD56+/CD3-/TCR γ delta-), derived from a single patient with CD56+ non-Hodgkin's lymphoma. *Leukemia* 2001;15:1641–1649. [PubMed: 11587224]
28. Morris JC, et al. Preclinical and phase I clinical trial of blockade of IL-15 using Mik1² monoclonal antibody in T cell large granular lymphocyte leukemia. *Proc Natl Acad Sci U S A* 2006;103:401–406. [PubMed: 16387851]
29. Ma A, Koka R, Burkett P. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. *Annual Review of Immunology* 2006;24:657–679.

Figure 1. Flow cytometry analysis of LGLL

(A) Splenocytes from leukemic mice were stained with anti-NK1.1, anti-CD3 ϵ and anti-CD49b, and analyzed by flow cytometry; isotype antibody control is in grey. (B) Expression of CD62L and CD69 on NK1.1+ cells is altered in leukemic mice versus WT controls.

Figure 2. Leukemia mice have reduced survival and enhanced lymphoid proliferation

(A) Blood smear staining with Wright-Giemsa stain shows a large number of leukemic blasts in peripheral blood of a representative moribund mouse. (B) Transmission electron micrograph (TEM) showing LGL leukemia within the spleen of leukemic mouse. The LGL leukemia cells show characteristic electron dense granules in the cytoplasm and LGL morphology. Bar = 2 μm. (C) H&E staining of bone marrow and spleen of leukemia mouse reveals massive infiltration of blasts in bone marrow and spleen (40X magnification).

(A)

(B)

Figure 3. SKY and FISH analysis reveal trisomy 15 and/or 17 in both NK and T LGL blasts
 (A) SKY analysis of T LGL leukemia reveals an extra copy of chromosome 9 (red oval), 15 (white oval) and 17 (blue oval). (B) Dual-color interphase FISH of independent NK (right) and T LGL (left) leukemia. Chromosome 15 probe (in green), chromosome 17 probe (in red), and DAPI counter-stain (blue) were used for detection of trisomies. The left image illustrates gain of both chromosomes 15 and 17, whereas the right image shows a gain of chromosome 17 only.

Figure 4. Cytotoxicity and IFN- γ release by NK and T LGL blasts

(A) Freshly isolated whole blood leukocytes from two mice with NK LGL ($\circ$) and T LGL ($\bullet$) leukemia that contained approximately 90% blasts were incubated with ^{51}Cr labeled YAC-1 target cells as described in 'Materials and Methods' at the indicated effector:target in 96-well plates in triplicate. The data represents mean $\pm$ SD of triplicates for all the effectors to target ratios. (B) IFN- γ production in leukemia mice was determined ($n = 6$) by overnight stimulation of total splenocytes with IL-12 and IL-18 *in vitro*. Supernatants were harvested and analyzed for IFN- γ production using an ELISA. Data are expressed as mean $\pm$ SD of duplicates and are pooled from four independent experiments.

Figure 5. Transplantability of primary leukemia cells *in vivo*

(A) Kaplan-Meier overall survival analysis shows a significant difference between WT controls (-*) and leukemia mice (NK LGL leukemia Δ and T LGL leukemia $\square$). (B) Kaplan-Meier survival curve of SCID mice after transfer of leukemia cells to secondary recipients. Groups of 4 mice were injected intravenously with 1×10^5 peripheral blood cells ($\square$) or injected with same number of spleen cells ($\diamond$) or bone marrow cells (Δ) on days 0. Mice transplanted with different sources of LGL leukemic blasts showed mortality within the same period of time irrespective of the source of LGL leukemic blasts. Results shown are representative of two independent experiments that were performed.

Table 1

Expression of surface antigens in NK and T LGL leukemia

Mouse number	Type of LGLL	NK1.1	CD122	DX5	CD3	TCR β	CD8	CD4	CD11b
1	NK	+	+	+	-	-	-	-	-
2	NK	+	+	-	-	-	-	-	-
3	NK	+	+	+	-	-	-	-	-
4	NK	+	+	+	-	-	-	-	-
5	NK	+	+	+	-	-	-	-	-
6	NK	+	+	+	-	-	-	-	-
7	NK	+	+	+	-	-	-	-	+
8	NK	+	+	+	-	-	-	-	+
9	NK	+	+	+	-	-	-	-	+
10	NK	+	+	-	-	-	-	-	-
11	NK	+	+	+	-	-	-	-	+
12	NK	+	+	+	-	-	-	-	-
13	NK	+	+	+	-	-	-	-	-
14	NK	+	+	-	-	-	-	-	-
15	NK	+	+	+	-	-	-	-	+
16	NK	+	+	+	-	-	-	-	+
17	NK	+	+	+	-	-	-	-	+
18	NK	+	+	+	-	-	-	-	+
19	NK	+	+	+	-	-	-	-	+
20	T	+	+	+	+	+	-	-	+
21	T	+	+	+	+	+	+	-	-
22	T	+	+	+	+	+	-	-	+
23	T	+	+	-	+	+	-	-	+
24	T	+	+	+	+	+	+	-	-
25	T	+	+	+	+	+	-	-	-
26	T	+	+	+	+	+	-	-	-
27	T	+	+	+	+	+	-	-	-

Mouse number	Type of LGLL	NK1.1	CD122	DX5	CD3	TCR β	CD8	CD4	CD11b
28	T	+	+	+	+	+	-	-	-
29	T	+	+	+	+	+	-	-	-
30	T	+	+	+	+	+	-	-	-
31	T	+	+	+	+	+	-	-	+
32	T	+	+	+	+	+	+	-	+
33	T	+	+	+	+	+	-	-	-
34	T	+	+	+	+	+	-	-	+
35	T	+	+	+	+	+	-	-	-
36	T	+	+	+	+	+	-	-	-
37	T	+	+	+	+	+	+	-	-
38	T	+	+	+	+	+	-	-	+
39	T	+	+	+	+	-	-	-	+