Establishment of a Leukemic Cell Line MT-ALL With Multilineage Differentiation Potential*

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A. Introduction

Multiple steps are involved in hematopoietic differentiation and maturation from multipotent progenitor cells to terminally differentiated cells of each lineage. The availability of recombinant growth factors and of various clonogenic precursor cell assays has provided useful information about hematopoietic precursors, notably of the myeloid lineage [1–3]. However, the direct evaluation of primitive human lymphohematopoietic progenitor cells has been hampered by their low frequency in normal donors and the difficulties involved in expanding them in in vitro culture [4, 5]. Therefore, little is known about the events and mechanisms that result in the irreversible commitment of multipotent uncommitted progenitors to a specific cell lineage. Leukemias have been traditionally used as a model for lymphohematopoietic development and have been studied extensively for the expression of differentiation antigens [6, 7] and recently for rearrangements of genes of the immunoglobulin supergene family [8, 9]. They are considered to represent clonal expansions of lymphoid and myeloid progenitor cells and therefore provide an opportunity to study homogeneous populations of lymphohematopoietic progenitor cells. However, leukemic cell lines with the potential for multilineage differentiation that would allow the study of early events of lymphohematopoietic development are very rare [10, 11]. We report a novel acute lymphoblastic leukemia (ALL), MT-ALL, with a predominantly mature $CD3^+$ TCR α/β^+ phenotype, which in response to various growth factors displays the potential for multilineage differentiation in vitro. This leukemia may be instrumental in elucidating mechanisms involved in early lymphohematopoietic development.

B. Materials and Methods

Leukemic cell cultures were set up as shown in Fig. 1 and as previously reported [12]. Southern analysis was performed according to standard methods [12, 13]. The genomic probe pδ7 detects the constant region of the T-cell receptor (TCR) δ gene (J.M. Greenberg, C.W. Wilkowski, J.H. Kersey, unpublished results). pTγ1 is a cDNA probe derived from the leukemic cell line HPB-mlt [14], kindly provided by R. Holcombe. The Tβ probe is a cDNA probe kindly provided by T.W. Mak. Methods for the lymphokine-activated (LAK) and natural killer (NK) cell assays as well as the oxygen production assay have been described elsewhere [15, 16].

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Clinical Course of the Patient. The 15-year-old caucasian male presented with hemorrhagic diathesis, hepatosplenomegaly, and mediastinal mass. White blood cell count was 759000/μl, containing 99% lymphoblasts, which were $CD2^+$ TdT^+ $CD4^-$ $CD8^-$. Complete remission was achieved lasting for 5 months. The patient relapsed and attained unsustained remissions during which peripheral blood was obtained at three different times after informed consent. The patient died 1 year after diagnosis.

C. Results and Discussion

The leukemia presented in the patient with a predominantly mature T-lymphocyte phenotype ($CD2^+$ $CD3^+$ $CD4^-$ $CD5^+$ $CD7^+$ $CD8^-$ $TCR\alpha/\beta^+$) and a morphology typical for lymphoblasts (Fig. 2A). In the presence of different recombinant growth factors, various leukemic cell lines belonging to different cell lineages were established (Fig. 1). In the presence of interleukin 3 (IL-3) and/or granulocyte-macrophage colony-stimulating factor (GM-CSF), myeloid and monocytoid cells grew out, including terminally differentiated neutrophilic granulocytes (Fig. 2B). The cells were myeloperoxidase positive and expressed the myelomonocytic differentiation antigens CD13, CD14, MY8, and CD33 [17], while they were devoid of T-lineage associated antigens. The oxygen radical production, which is a typical feature of mature functional granulocytes, was assessed by measuring the reduction of the reporter substrate nitroblue tetrazolium (NBT) in the presence of various stimuli [16]. After stimulation with phorbol myristate (5 ng/ml), but not with the chemotactic oligopeptide F-met-leu-phe [16], NBT was reduced, proving that the myeloid cells were at least partially functional (data not shown).

A growth factor independent (GFI) leukemic cell line was established from the IL-3 cultures. Morphologically, these were undifferentiated blasts (Fig. 2C), coexpressing T-lymphoid (CD2, CD3, CD5, CD7, $TCR\alpha/\beta$) and myeloid lineage (CD13, CD14, CD33) associated differentiation antigens. In functional studies (oxygen radical production and NK and LAK activity; Fig. 3) these cells were inactive.

In the presence of IL-2, a cell line consisting of large lymphocytes with azurophilic granules (Fig. 2D) was established. In addition to the T-lineage associated antigens, the IL-2 dependent leukemic cells expressed CD8 and Leu-19, which can be found on cytotoxic T-lymphocytes [18, 19]. Functional studies confirmed that they represented the counterpart of MHC unrestricted cytotoxic T-lymphocytes [19]. The IL-2 de-

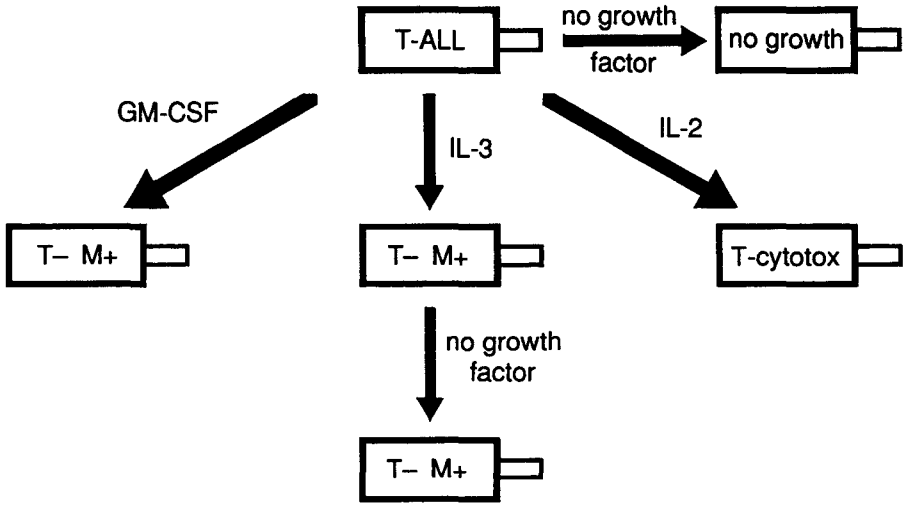

Fig. 1. Fresh peripheral blood leukemic blasts were cultured in the presence of rh GM-CSF (10–50 U/ml), IL-3 (10 U/ml), IL-2 (100 U/ml), or no growth factor. *T+/−*: positive/negative staining for T-lineage associated antigens; *M+/−*: positive/negative staining for myeloid lineage associated antigens and cytochemistry; *T-cytotox.*: expression of a phenotype consistent with cytotoxic T-lymphocytes (see text for details)

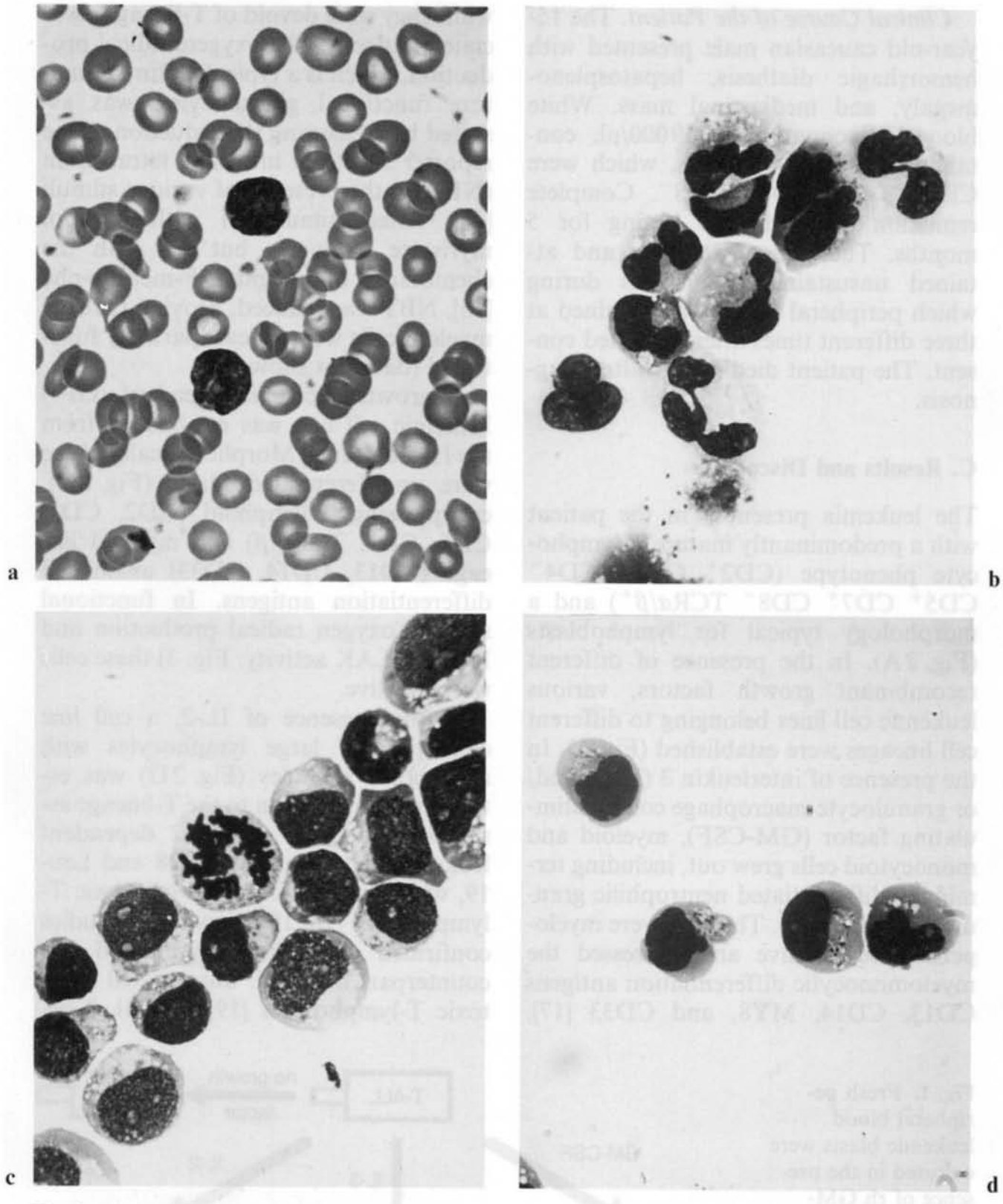

Fig. 2. a) Lymphoblasts with convoluted nuclear outlines in blood smear made from specimen from which the leukemic cell cultures were established. **b)** Maturing neutrophils with nuclear hypolobulation from the IL-3 cultured specimen. **c)** Leukemic cells with fine nuclear chromatin and prominent nucleoli from the growth factor independent leukemic cell cultures (see text and Fig. 1). **d)** IL-2 dependent leukemic cell cultures. The majority of these cells have distinct nucleoli and azurophilic granulation. (**a–d**, Wright's Giemsa, ×1200)

pendent leukemic cell cultures exerted high lytic activity against the NK sensitive target cell line K562 and the NK resistant cell line HL-60 [15] (Fig. 3). Surprisingly, the IL-2 dependent cell line co-expressed CD33 (data not shown).

Molecular genetic analysis demonstrated unambiguously that the various leukemic cell cultures were derived from the same malignant clone. Identical, unique rearrangements of the TCR δ (Fig. 4A, B), γ (Fig. 4C), and β (Fig. 4D) genes were demonstrated by Southern analysis. The TCR δ gene rearrangement, detected with the TCR δ constant region probe pδ7, most likely represents a Vδ3-Jδ1 rearrangement [12, 13, 19, 20]. Southern analysis using various restriction enzymes and a Jδ1 probe were consistent with this interpretation (data not shown). Cytogenetic analysis (Table 1) revealed a three way translocation (1;10;12) (q25;p13;p13) and a deletion of (6q15;q25) in all cell cultures analyzed, consistent with the molecular genetic findings.

In conclusion, we have characterized a novel leukemia which gives rise to cells belonging to different cell lineages, notably T-lymphoid and myeloid. We concluded that the cell lines were presumably derived from a putative multipotent leukemic progenitor. However, the exact pathway of multilineage differentiation remains to be elucidated. One possibility is that a minor population of multipotent leukemic progenitor cells which was present in the peripheral blood blasts of the patient, but undetectable by fluorescence-activated cell sorter (FACS) analysis, differentiated in response to various growth factors into the respective lineages. Alternatively, minor populations of lineage committed cells might have acquired a growth advantage under the different culture conditions which resulted in outgrowth of the different cell cultures. A third hypothesis suggests that due to posttransformational alterations of the genetic program, the mature $CD3^+TCR\alpha/\beta^+$ T-lymphoblasts were capable of undergoing lineage switch.

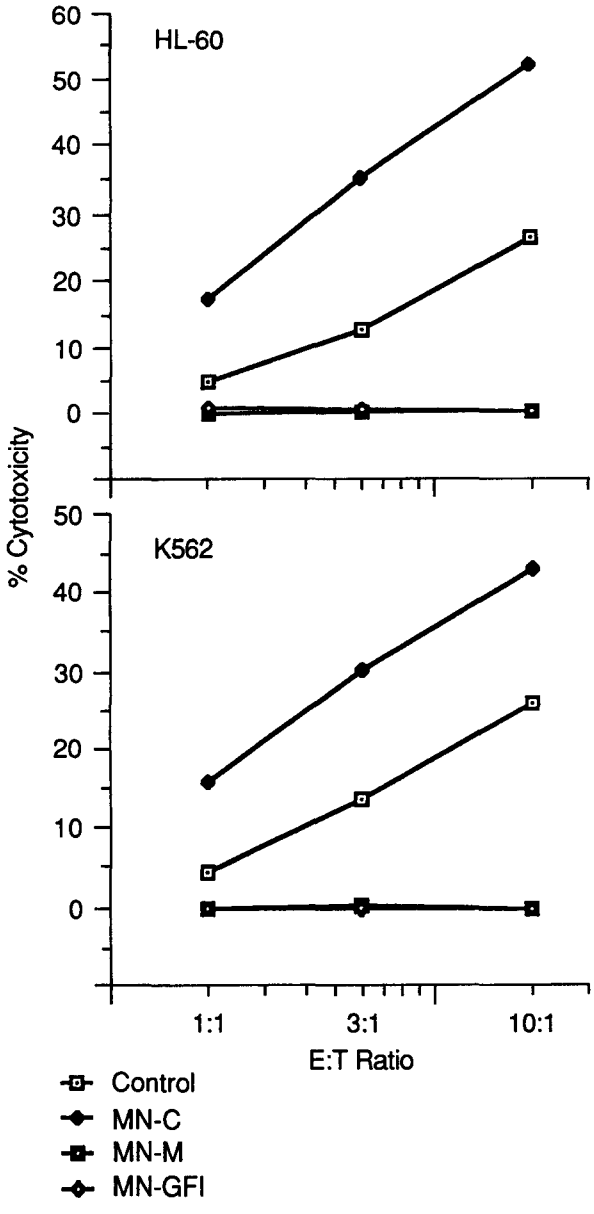

Fig. 3. Percent cytotoxicity of IL-2- (MN-C) and GM-CSF- (MN-M) dependent leukemic cell cultures, the growth factor independent leukemic cell cultures (MN-GFI) against the NK-sensitive target cell line K562 and the NK-resistant target cell line HL-60 in a chromium release assay. Control were peripheral blood mononuclear cells from a healthy donor which were cultured in the presence of IL-2 100 U/ml for 14 days. Mean values of triplicates are given, SEM was $<5\%$. Representative data of one experiment (which were reproduced in two additional experiments) are shown

Such a mechanism has recently been described in B- and pre-B-cell lymphomas which were derived from Eμ-*myc* transgenic murine bone marrow cells. After coinfection with the viral oncogene v-*raf* these B-lineage tumors displayed "lin-

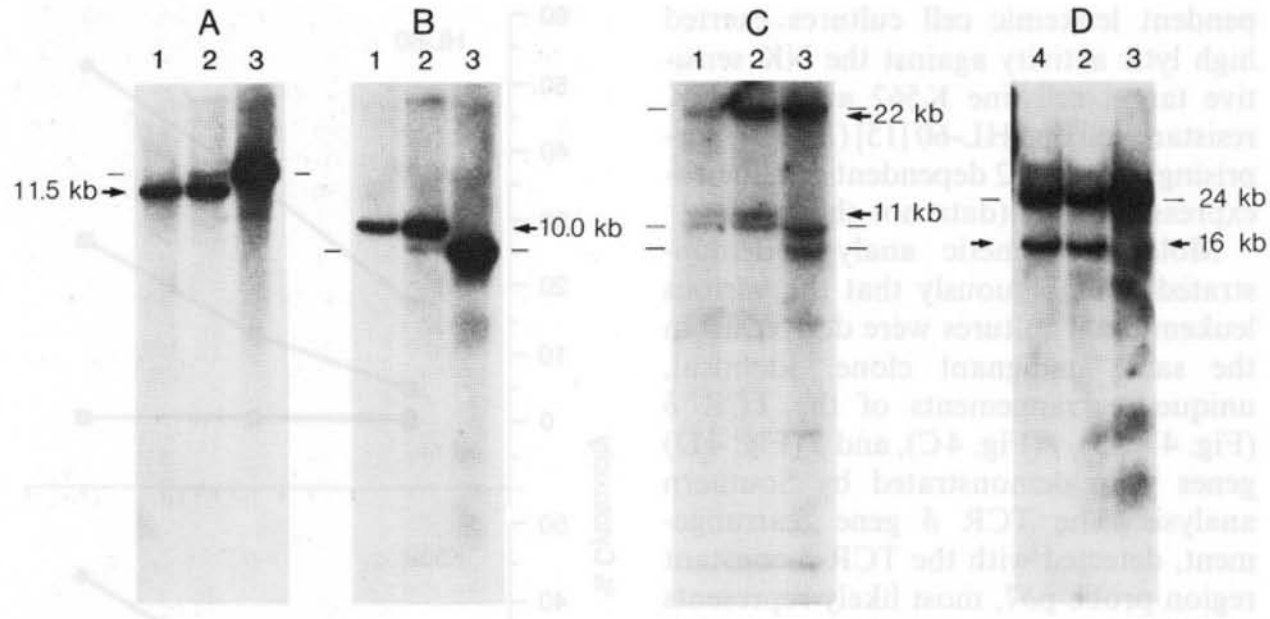

Fig. 4. High molecular weight DNA from IL-3-cultured (lane 1), IL-2-dependent (lane 4), uncultured leukemic cells (lane 2) and germline control (lane 3) were digested with Bam HI (Fig. 4A, D) and Xba I (Fig. 4B, C) and hybridized with the genomic TCR δ probe pδ7 (Fig. 4A, B), the TCR γ cDNA probe pTγ1 (Fig. 4C) and the TCR β cDNA probe Tβ. Germline bands are indicated with bars, rearranged bands with arrows. (Reproduced from the Journal of Experimental Medicine, 1989, vol. 169, pp. 1101–1122, by copyright permission of The Rockefeller University Press)

Table 1. Cytogenetic studies of cultured leukemic cells

Cultures	No. metaphases analyzed		G-banded karyotypes
	total	per clone	
Growth factor independent	10	7	47,XY,+19,del(6)(q15q25), t(1;10;12)(q25;p13;p13)
		2	47,XY,+19,del(6)(q15q25), dirdup(17)(q11.2→q23), t(1;10;12)(q25;p13;p13)
		1	48,XY,+19,del(6)(q15q25),+del(6)(q15q25), t(1;10;12)(q25;p13;p13)
GM-CSF	10	2	48,XY,+17,+del(6)(q15q25), t(1;10;12)(q25;p13;p13)
		8	49,XY,−7,+13,+19,+del(6)(q15q25), t(1;10;12)(q25;p13;p13),+der(7), t(7;17)(q36;21)
IL-3	10	5	47,XY,+19,del(6)(q15q25), t(1;10;12)(q25;p13;p13)
		2	48,XY,+19,del(6)(q15q25),+del(6)(q15q25), t(1;10;12)(q25;p13;p13)
		3	49,XY,+17,+19,+del(6)(q15q25), t(1;10;12)(q25;p13;p13)

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eage instability" and underwent "lineage switch" to macrophages [22]. Whether similar mechanisms are operational in our novel leukemic cell line is currently under investigation. Further studies will specifically address possible mechanisms involved in the process of multilineage differentiation in this instructive leukemia. We hope that these studies will be useful for a better understanding of

events of early lymphohematopoiesis, notably of the mechanisms that are involved in the irreversible commitment of multipotent progenitors to different cell lineages.

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